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ACRONYMS AND ABBREVIATION

Ac	Acetyl
AcOH	Acetic acid
Alloc	Allyloxycarbamoyl
Bn	Benzyl
Cbz	Carbobenzyloxy
DBU	Diazobicycloundecene
DCM	Dichloromethane
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMM	Dimethylmaloyl
DMP	Dess-Martin periodinane
EA	Ethyl Acetate
Et	Ethyl
IBX	Iodoxybenzoyc Acid
Me	Methyl
Nap	Naphthyilmethyl
Ph	Phenyl
Py	Pyridine
RT	Room temperature
PTSA	Para toluenesulfonic acid
PDT	Propanedithiol
T	Temperature
TBAF	Tert butyl ammonium fluoride
TBDMS	Tert butyl dimethyl silyl
Tf	Trifluoromethansulfonyl
TDS	Textyl dimethyl silyl
TEMPO	Tetramethylpiperidinyloxyl
THF	Tetrahydrofuran
TMSOTf	Trimethylsilyltriflate
Tol	Toluene

SYNTHESIS OF SACCHARIDE FRAGMENTS OF *Acinetobacter baumannii* ATCC17961 SURFACE POLYSACCHARIDE

INTRODUCTION

Immune system

The immune system is a network of biological structures and processes within an organism, with a protective function against disease-causing agents such as bacteria, viruses and parasites. It plays an important role in identifying and eliminating abnormal cells, including cancer cells. Fundamental are specialized cells, called antigen-presenting cells (APCs), which have the aim to display the antigen to lymphocytes and collaborate with them in the response to the antigen. Dendritic cells are a particular class of APCs, being responsible for the uptake of foreign antigens. Antigens include any molecule perceived by the immune system as foreign invader or simply potentially dangerous for the host.

Antigens that elicit immune responses are termed immunogens, usually they are protein. Not all antigens are naturally immunogenic. Small, non-immunogenic antigens are called haptens and must be coupled to larger immunogenic molecules, termed carriers, to stimulate a response. Large protein antigens usually contain epitopes equivalent to carriers and haptens and are therefore inherently immunogenic. Carbohydrates, by contrast, must often be coupled to proteins in order to be immunogenic, as is the case for the polysaccharide antigens used in the *Haemophilus influenza* type b vaccine.

There are two fundamentally different types of responses to invading microbes. The first line of defense is an immediate, rapid response called innate immune response which consists of all the immune defenses that lack immunologic memory. It defends the host from infection by other organism in a non-specific manner, so the innate system recognize and respond to pathogens in a generic way and it does not confer long-lasting or protective immunity to the host. Thus, a characteristic of innate responses is that they remain unchanged the antigen is encountered.

The other type of immunological response is due to the adaptive immune system, also known as the acquired immune system. It is slower to develop, but manifests an increased antigenic specificity and memory. The innate immune response makes a crucial contribution to the activation of adaptive immunity.

The molecular components of innate responses include complement, acute-phase proteins, and cytokines such as the interferons. The cellular components of this system include dendritic cells (DCs), macrophages, natural killer cells and granulocytes as basophils, eosinophils and neutrophils. The innate system responds to pathogen associated molecular patterns (PAMPs), which are compounds structurally and chemically different presents in pathogens, through stimulation of various pattern-recognition receptors (PRRs). Toll-like receptor (TLRs) are a class of PRRs which recognize a huge variety of PAMPs. They activate the maturation process of DCs, and involve production of cytokines and chemokines which are protein or glycoprotein able to modify and activate the cells involved in the immune response. This process allow the pro-inflammatory context essential for T-cells activation-differentiation and initiation of the adaptive (antigen-specific) immune response. When DCs encounter an antigen they undergo a transformation, being activated. They migrate in the draining lymph nodes where are present T-cells, which are activated by modified DCs, leading to the process that allows the onset of the immune response. Acquired responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. The initial stages of lymphocyte development do not require the presence of an antigen, but once these cells express a mature antigen receptor, their survival and further differentiation become antigen-dependent.

The cells involved in the acquired immune system are T and B lymphocytes which are a subset of leukocyte. B cells and T cells are the major types of lymphocytes.

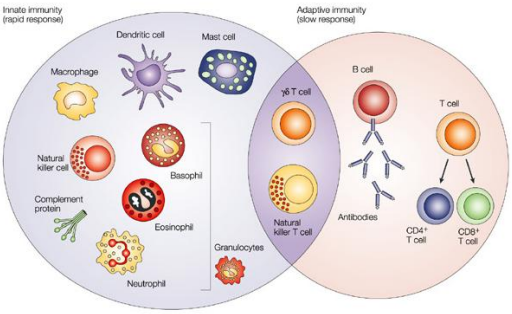

Fig. 1

When DCs encounter the antigen,they are able to process it, complexing with particular molecules called MHC (major-histocompatibility complex), present on the surface of DCs, which expressed the antigens to T-cells and ensure the recognition from them.

The crucial event of the T-cells activation - differentiation process is the immunological synapse, initiated by formation of a ternary complex MHC-antigen-T-cells receptor (TCR on T cell surface, followed by further specific interactions.

If the antigen is presenting by class II MHC, *helper T-cells* are activated (Th or CD4+). Class I MHC activated *cytotoxic T-cells* (CD8+ or CTL). Helper T-cells are fundamental to lead B lymphocytes to differentiate in memory cells or in plasma cells. B cells secrete immunoglobulins, the antigen-specific antibodies responsible for eliminating extracellular microorganisms. T cells help B cells to make antibody and can also eradicate intracellular pathogens by activating macrophages and by killing virally infected cells. While plasma cells produce low affinity IgM-type antibodies, which give an effective but short lasting response, memory cells enable a quantitatively and qualitatively superior secondary immune response to be mounted after a subsequent encounter with the same antigen, producing specific immunoglobulin (IgG antibodies). To allow this process of installation of immunological memory, the antigen has to be T-dependent, so produce B-memory cells through the interaction with Th-cells (MHC-II interaction). This kind of antigens are usually protein. Regarding T-independent antigens, they are able to

interact directly with B-cells, inducing an immunological response without the interaction with macrophages or T-cells. Without this cooperation is not possible the formation of memory cells. This process results in a lack of production of new memory B cells and a depletion of the memory B-cell pool, such that subsequent immune responses are decreased. Polysaccharides or proteic subunits usually found on the external coating of bacterial cells (capsule, flagella...) are example of T-independent antigens.

Antibodies recognizing an antigen don't interact and don't identify the entire molecule, but a specific portion of the surface called antigenic determinant or epitope.

The concept of vaccination is based on the fact that deliberate exposure to a harmless version of a pathogen generates memory cells but not the pathologic sequelae of the infectious agent itself. In this way, the immune system is primed to mount a secondary immune response with strong and immediate protection should the pathogenic version of the microorganism be encountered in the future.[1]

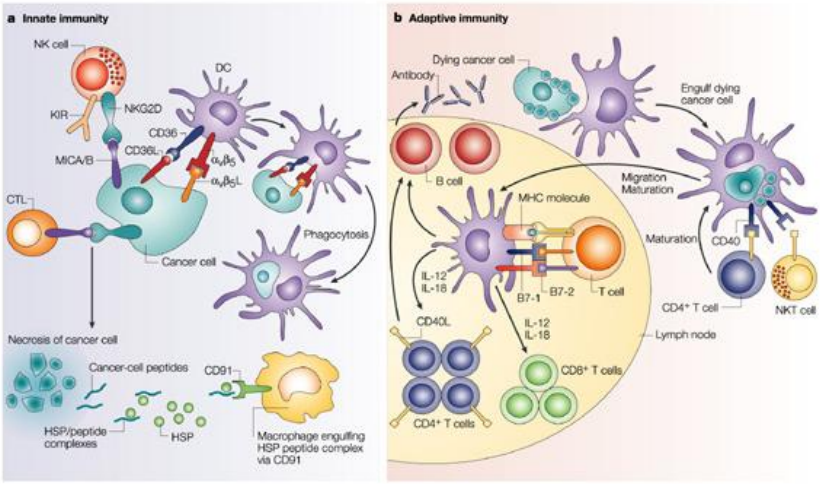

Fig. 2

Carbohydrates and immunology

Carbohydrates have been established as the most abundant – and arguably the most structural diverse – organic molecules found in nature. In contrast with proteins and nucleic acids, whose biological relevance has a longstanding reputation, carbohydrates have been considered always as poor-information and repetitive macromolecules with only structural functions. Only recently they have been recognized to play a crucial rule in biological recognition processes (e.g. bacterial and viral infection, cancer metastasis and inflammatory reactions). It's well known that oligosaccharides and glycoconjugates appear in all cells in different form, in particular they are present on the external layer. They can be found as peptido- and proteoglycans, glycoproteins, nucleic acids, lipopolysaccharides, or glycolipids. They dominate the landscape of the cell and are responsible for cell-cell interaction, the recognition of virus and bacteria, binding of toxins and are involved in cell growth, cell differentiation and cell motility. Depending on the type of bacterium carbohydrates may occur in the cell in different forms. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides (LPS) and lipoproteins which face into the external environment. As lipopolysaccharides are highly charged, the Gram negative cell wall has an overall negative charge. LPS of Gram-negative bacteria consists of three distinct structural components: species-specific repeating units of a polysaccharide (the O antigen); a conserved polysaccharide core with a glycosidic linkage to the O antigen; and an immunogenic component with a ketosidic linkage to the polysaccharide core (lipid A) The chemical structure of the outer membrane lipopolysaccharides is often unique to specific bacterial strains and is responsible for many of the antigenic properties of these strains. Moreover both Gram positive and negative bacteria could possess an additional polysaccharide coating called

capsule. It was demonstrated to be essential for the pathogenicity exerting a protective function against the host's immune defence.

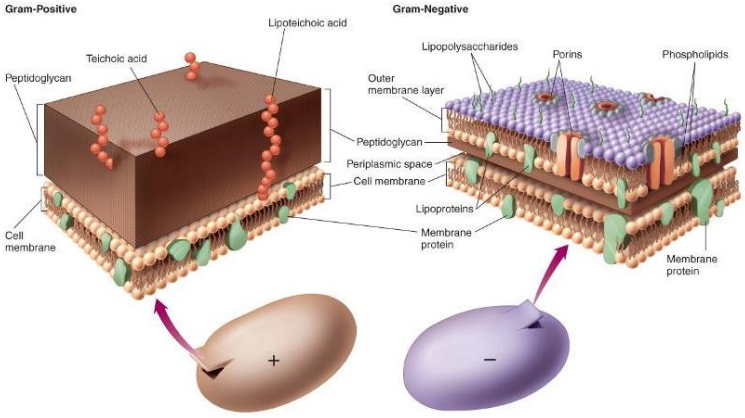

Figura 3

The exposure of carbohydrates on cells'surface enables them to interact with the immune system, acting as cell antigenic determinants. These features make carbohydrates attractive candidates as vaccine targets.

Polysaccharide antigens are mostly poor immunogens due to their B-lymphocyte independent (TI) nature. Often, anti-polysaccharide immune response is characterised by lack of T-lymphocyte memory, isotype restriction and delayed ontogeny. Children below 2 years of age and elderly respond poorly to polysaccharide antigens.

The conjugation of polysaccharides to proteins seems to allow carbohydrate-specific responses that elicit T-cell help, and this technique has been harnessed to improve the efficacy of vaccines.

In 1929 Avery and Goebel published an article in which they described that low molecular weight carbohydrates, similar to capsular bacterial polysaccharides epitopes (immunodeterminant portions) were able to stimulate the immune system through the production of specific antibodies, after the conjugation to a protein.[2]

Glycoconjugate vaccines have proven to be effective in the treatment of various diseases, caused by different kind of bacteria: *Haemophilus influenzae* type b,

Streptococcus pneumoniae (of the vaccine serotypes) and *Neisseria meningitidis*. The current understanding of the activation of the adaptive immune system by glycoconjugate vaccines is based on research with haptens conjugated to carrier proteins. The traditional explanation for the mechanism of induction of polysaccharide-specific antibodies by glycoconjugates is that these vaccines generate T cell help through several steps. Polysaccharide-protein conjugates bind to the B cell receptor (BCR) of polysaccharide-specific pre-B cells and are taken into the endosome. Once inside the cell, the protein portion is digested by proteases to release peptide epitopes, which bind to MHCII by replacing the self-peptide. The peptide from the vaccine carrier protein is presented to the αβ receptor of CD4+ T cells in the context of the MHCII molecule. Peptide/MHCII-activated T cells release cytokines to stimulate B cell maturation and induce immunoglobulin class switching from IgM to polysaccharide-specific IgG (**Fig. 4**).

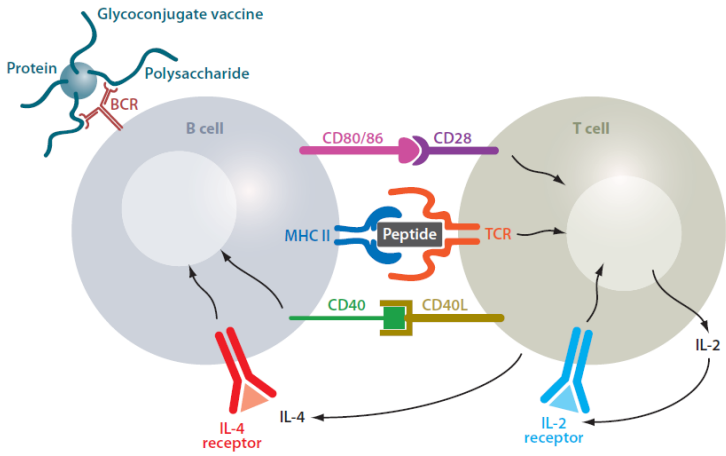

Fig. 4

Current conjugation chemistry requires polysaccharide modifications (e.g., random oxidation of the sugar chain) that alter natural epitopes, with consequent generation of low-affinity antibodies to the native polysaccharide. In addition, random conjugation between activated groups along the length of multiple repeating units

of a polysaccharide and random active sites on a protein (e.g., all lysine residues) is difficult to reproduce from batch to batch in a clinical vaccine preparation.

The empirical observation that covalent linkage of glycan to protein is important for their immunological activities has prompted in the last two decades carbohydrate chemists to synthesize oligosaccharides, which are relatively short in length in comparison to native polymers, mimicking the surface carbohydrates of different pathogens, and couple them to carrier protein. These studies have allowed to identify the minimal structural requirements for raising functional antibodies of a variety of microbial polysaccharides. Such minimal functional epitopes can be, in certain cases, even oligosaccharides as short as hexa-, tetra-,or even disaccharide.

To overcome the problems correlated to the preparation of glycoconjugate vaccines different methodologies have been investigated in recent years. One method is the achievement of glycoconjugate by chemically controlled coupling of the saccharide CPSs to carriers through their reducing ends via linker molecules. These conjugates have proven valuable not only as new vaccine candidates for clinical use but also as molecular probes used in the mapping of antigenic determinants of bacterial polysaccharide (to optimize vaccine configuration) and in studies of the molecular mechanisms of antibody and cellular responses to conjugate vaccines.

A second strategy, thanks to the development of carbohydrate chemistry, is the preparation of carbohydrate-based vaccine through the chemical synthesis. The vaccine against *Haemophilus influenzae type b* licensed in Cuba in 2003 is the first example of this new kind of vaccine.[3]

Acinetobacter baumannii

Acinetobacter baumannii is a nonfermentative Gram-negative cocco bacillus, isolated from soil and water which represent its natural habitats.

Fig. 5

It has emerged as one of the most troublesome pathogens for health care institutions globally, especially it is an important cause of nosocomial infections. This pathogen affects notably patients receiving mechanical ventilation, patients sustaining burns or trauma, and in surgical-site infections.

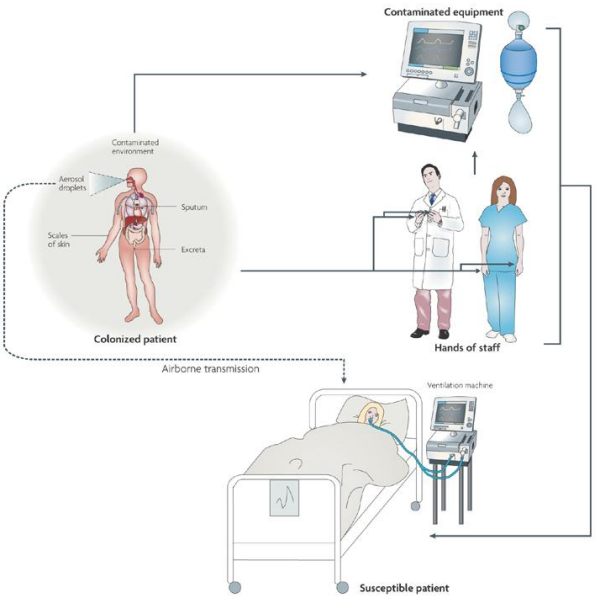

Fig. 6

The organism commonly targets the most vulnerable hospitalized patients, those who are critically ill with breaches in skin integrity and airway protection. Hospital acquired pneumonia is still the most common infection caused by this organism. However, in more recent times, infections involving the central nervous system, skin soft tissue, and bone have emerged as highly problematic for certain institutions. Furthermore *A. baumannii* has more recently caused a range of infectious syndromes in military personnel injured in the Iraq, Kuwait and Afghanistan conflicts, and this earned to it the name of "iraqibacteria".

As other nosocomial pathogens like *Pseudomonas aeruginosa*, *Clostridium difficile*, *Escherichia coli* and *Staphylococcus aureus* the clinical significance of *A. baumannii* has been propelled over the last 15 years for its remarkable ability to rapidly develop antibiotic resistance. Strains of this bacterium have demonstrated resistance to all known antibiotics. Acting in synergy with this emerging resistance profile is the uncanny ability of *A. baumannii* to survive for extended periods on environmental surfaces is notorious and is likely important for transmission within the health care setting. Multidrug resistance is common with health care-associated *A. baumannii* infections. The impressive number of acquired mechanisms of resistance makes selection of an appropriate empirical antimicrobial agent exceedingly difficult. Degradation enzymes against β-lactams, modification enzymes against aminoglycosides, altered binding sites for quinolones, and a variety of efflux mechanisms and changes in outer membrane proteins have been reported. Essentially, any and all of these elements can be combined to result in a highly drug-resistant, and at times panresistant, opportunistic pathogen. The consequences of these features are the increase of morbidity and mortality, as well an increased medical costs, indeed special procedure and isolation are required for patients wound by this pathogen. Here are shown the factors leading to the emergence and transmission of multidrug-resistant (MDR) *Acinetobacter* species.[4]

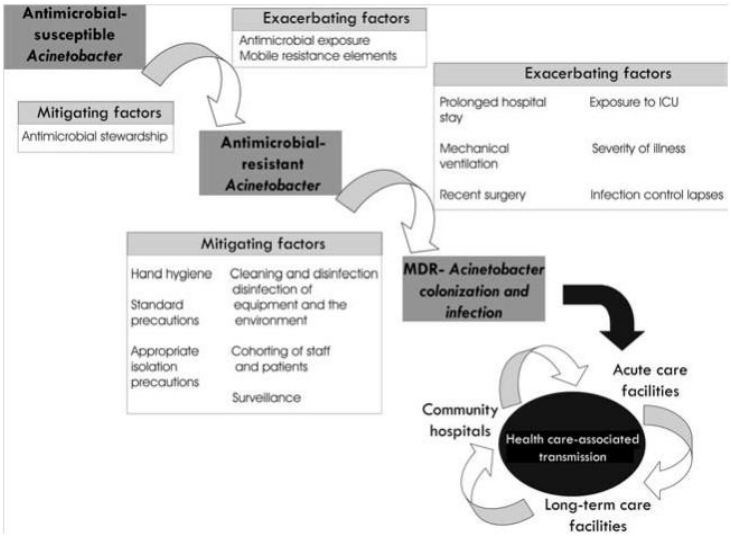

Fig. 7

In **Fig.8** are highlighted countries that have reported an outbreak of carbapenem-resistant *Acinetobacter baumannii*. Red color signifies outbreaks reported before 2006, and yellow signifies outbreaks reported since 2006. It can be noticed a broadcast of the infection due to this pathogen. This demonstrates why the attention of the biomedical research on this emerging bacterium is increased during recent years. Therefore there is an effort to find a solution for the treatment and the prevention of disease caused by this pathogen.[5]

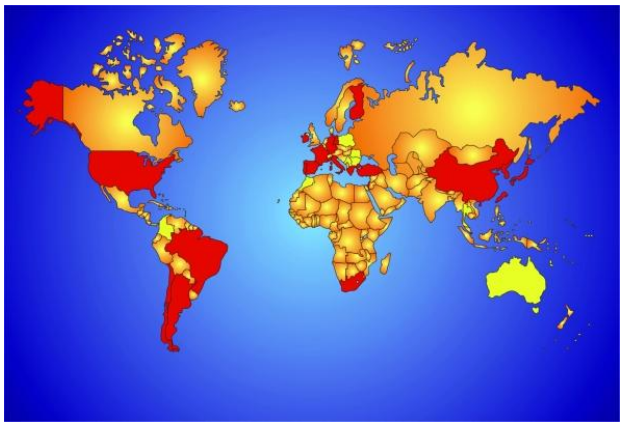

Fig. 8

Vaccine against *Acinetobacter baumannii*

Due to the difficulty in treating infections caused by multi-drug resistant *A. baumannii* through an antibiotic therapy, novel approaches are needed for the prevention of the disease induced by this pathogen. Targeted vaccination of individuals at risk could represents an effective and cost-efficient strategy to prevent outbreaks. Therefore, a broadly protective vaccine against *A. baumannii* may have a major impact on some high risk groups, including ICU patients, injured military personnel, patients undergoing elective surgery, diabetics and hemodialysis patients.

Despite these trends, no vaccines or antibody-based treatments for *A. baumannii* infections is currently on the market. The development of a vaccine for *A. baumannii* is complicated by the fact that no protective bacterial antigens have been identified and few virulence factors for this organism have been characterized.[6] Moreover the presence of different serotype of this bacterium makes the development of a specific vaccine a challenging task. Therefore, a rational research program was undertaken to identify a candidate antigen for an *A. baumannii* vaccine. Antigen discovery was based on identification of the immunodominant targets from *A. baumannii* membrane protein preparations following systemic infection. In this framework, during recent years different research group started to study different methodologies for the immunization against this bacterium.

The research group of Dr Michael McConnell exploited an inactivated whole cell (IWC), therefore (a classical approach), for active and passive immunization against *A. baumannii* in murine models. However the use of inactivated whole cells as a vaccine antigen may raise potential safety concerns over the possibility of incomplete inactivation.[7]

For this reason the same group investigated the use of outer membrane vesicles (OMVs) as a vaccine in a mouse model of disseminated sepsis. The use of OMVs avoids related to IWC, as they are non-viable particles and bacterial cells are

removed by filtration during the purification process. This approach produces a robust, protective immune response.[8]

That study, while promising, has the limitation of the undefined nature of the vaccine formulation and therefore the reproducibility on a large-scale preparation.

Outer membrane protein (OmpA) was identified as a promising candidate for active and passive immunization based on humoral immunodominance during infection in mice.

A. baumannii OmpA has been found to have a variety of interesting biological properties in *in vitro* model systems. For example, OmpA has been shown to bind to eukaryotic cells, translocate to the nucleus, and induce cell death. The research group of B. Spellberg isolated outer membrane protein of *A. baumannii* as antigenic material, and found that this material can be employed for active and passive immunization to prevent *A. baumannii* infections, but with this methodology only a single *A. baumannii* strain was evaluated, which limits the interpretation of these findings.[9]

In recent years different research group investigated other kind of candidates for a vaccine against this pathogen, as biofilm associated protein (Bap), and trimeric autotransporter protein (Ata), which are specific cell surface protein directly involved in biofilm formation of the bacterium. The authors argue that the remarkable capacity to acquire antimicrobial resistance is attributable to its biofilm formation ability.[10]

However, until now, these studies were conducted in vitro or in mouse model, so the formulation of a vaccine against *A. baumannii* still remains under investigation.

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RESULTS AND DISCUSSION

Aim of the work

In the framework of the synthesis of a candidate for a carbohydrate based vaccine against *Acinetobacter baumannii* we focused our attention on the virulent strain ATCC 17961.

In 2009 Vinogradov and coworkers identified the pentasaccharide reported in **Fig.1** as the repeating unit of the *O*-chain of the **LPS** belonging to this strain.[1] The peculiarities of this structure are the presence of a rare monosaccharide unit, namely 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid (**D**) and a tetraglycosidated galactoside (**C**). Since unusual saccharide and branched portions within pathogen-associated surface polysaccharides have usually strong antigenic properties and they are often essential components of the protective epitope, we focused our attention on three different fragments of the surface pentasaccharide, all of them containing the uncommon diacetamido residue **D**.

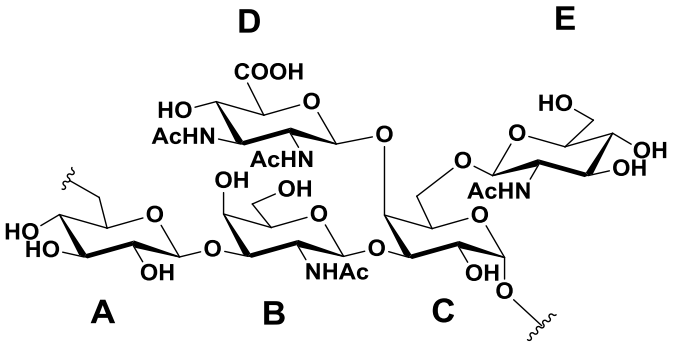


Fig. 1

The aim of this project is the synthesis of three fragments of the surface pentasaccharide of *Acinetobacter baumannii* ATCC 17961: the monomer **D**, the disaccharide **C**-**D** and the branched trisaccharide **C**-**D**-**E** (**Fig 2)**.

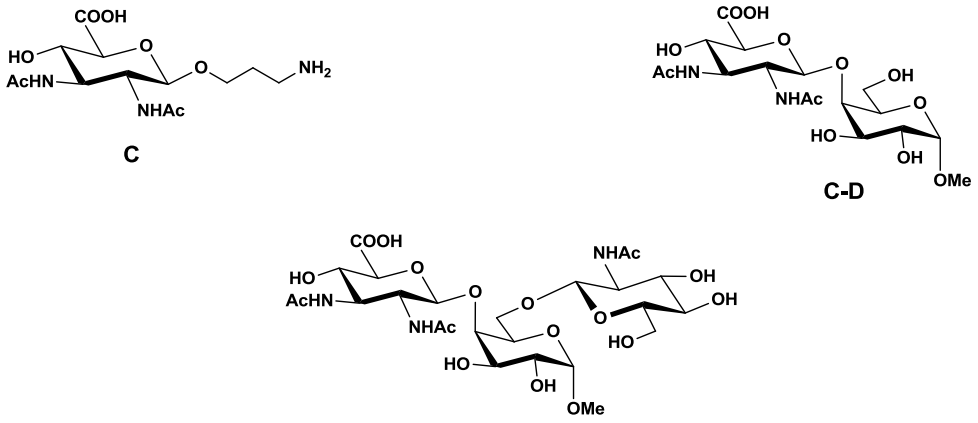
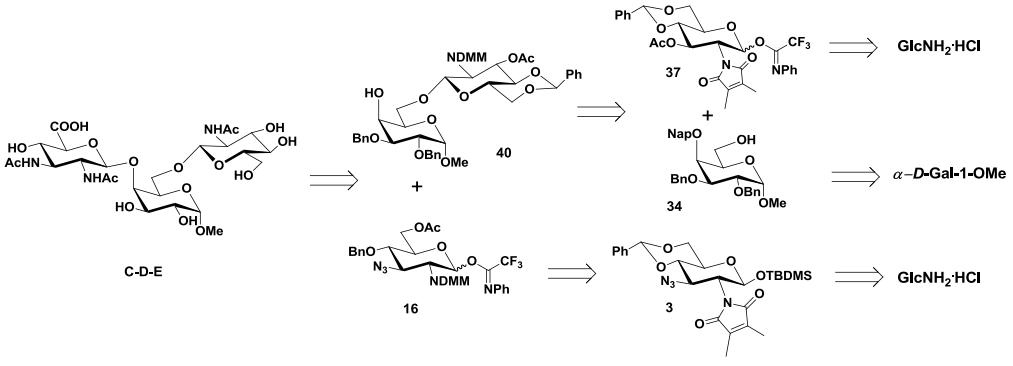


Fig. 2

The retrosynthetic approach is based on commercially available compounds such as glucosamine hydrochloride and α-methyl galactopyranoside. The retrosynthesis of the trisaccharide **C-D-E** is outlined in **Scheme 1**. Starting from glucosamine hydrochloride and α-methyl galactopyranoside. The crucial issues of the synthetic plan are the use of a *N*-participating protecting group to ensure the formation of β glycosidic bonds, a double inversion at C-3 to introduce two acetamido functions in trans-diequatorial arrangement for the synthesis of fragment **D** and exploit an orthogonal protection in at C-4 in the galactosyl moiety.



Scheme 1

The dimethylmaleimide (DMM) which ensures the exclusive formation of 1,2-trans glycosidic bonds has been employed as a participating protecting group of the amine, (**Fig.3**).

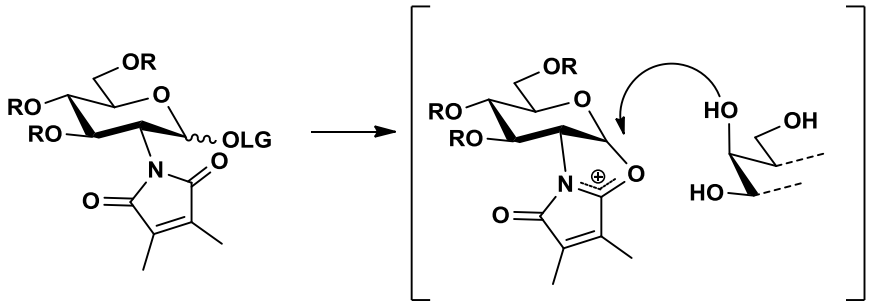
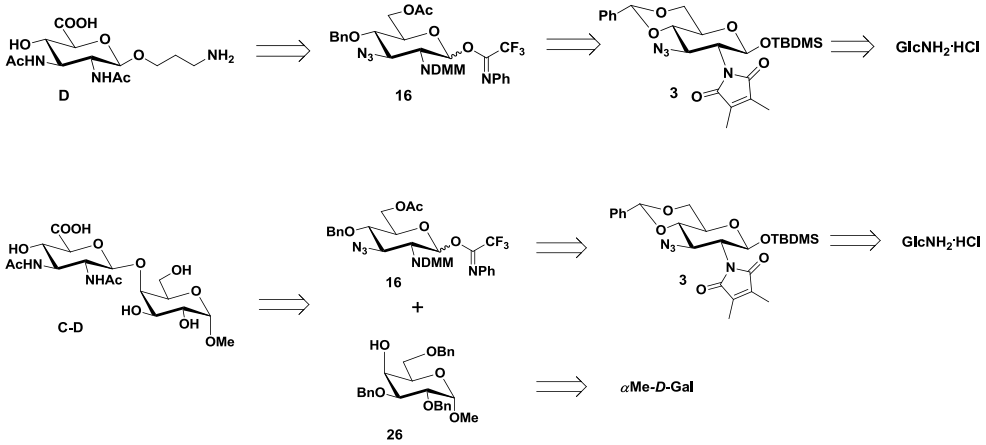


Fig. 3

A naphthylmethylether (Nap) orthogonal to benzyl ether and that can be easily cleaved by an oxidative treatment with DDQ, was installed at position 4 of the galactoside.

The same intermediates were used for the synthesis of the other two target compounds use. The monomer was obtained from the 2,3-"diamino" glycosyl donor **16**, while for the synthesis of the disaccharide. It was necessary to provide the acceptor with an orthogonal group in position 4 (**Scheme 2**).

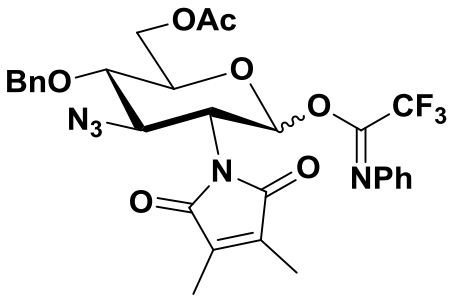


Scheme 2

Synthetic strategy

Synthesis of 2,3 “diamino” glycosyl donor 16

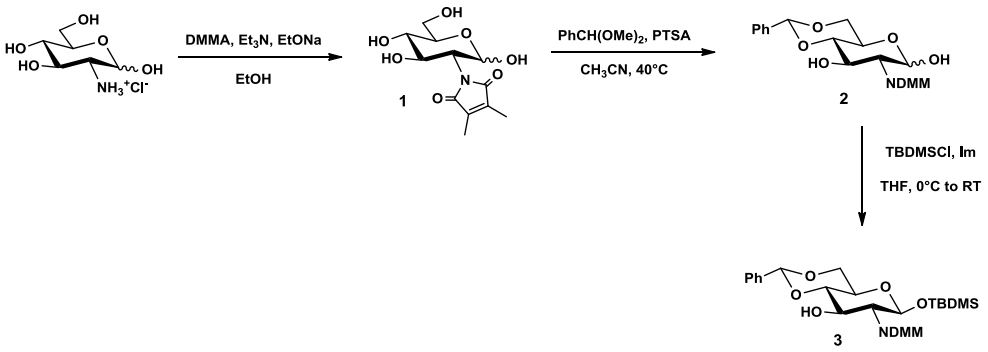
In a first attempt, a synthetic route able to provid in large scale glycosil donor **16**, (**Fig. 4**) precursor of the fragment **D** of the three compounds, was developed.



16

Fig. 4

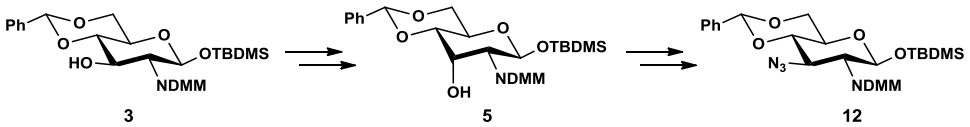
Starting from the commercially available D-glucosamine hydrochloride, the amino group was protected as dimethylmaleimide (DMM), using dimethylmaleic anhydride and TEA in a 1.0M solution of EtONa in EtOH. Without any further purification, positions 4 and 6 were protected as benzylidene acetal with benzaldehyde dimethylacetal, PTSA in CH_3CN at 40°C, giving compound **2** in 70% yield over two steps. Exploiting the higher reactivity of the anomeric hydroxyl, it was selectively silylated with tert-butyldimethylsilyl chloride and imidazole giving compound **3** in 84% yield (**Scheme 3**).



Scheme 3

Epimerization at the C-3

A crucial issue of the synthetic strategy was the introduction of a nitrogen functionality in position 3 in equatorial configuration. For this purpose it was necessary to perform a double inversion at this position The first step was the epimerization of C-3 to give the alloside **5**, then a S_N2 displacement with a suitable nucleophile as an azide furnished the 2-3 "diamino" compound **12** (**Scheme 4**).

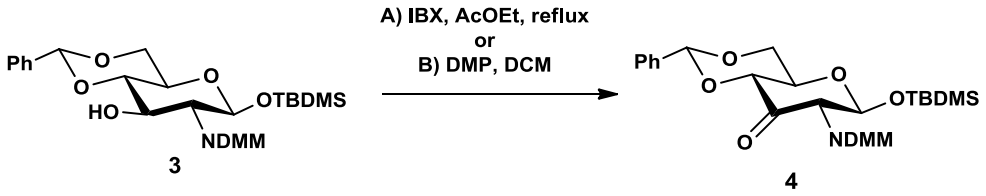


Scheme 4

In this case the first inversion of the hydroxyl was proved very challenging, so different methods described in the literature were investigated.

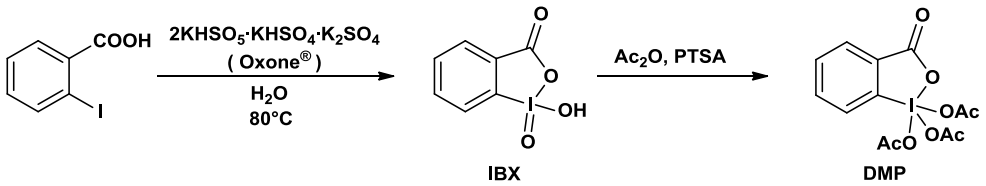
Oxidation of the equatorial alcohol, followed by the reduction of the corresponding ketone is a widely used method for epimerization of alcohols.

Oxidation with 2-iodoxybenzoic acid (**IBX**) in refluxing ethyl acetate gave **4** in 60% yield.[2] Better results were obtained performing the reaction using Dess-Martin periodinane (**DMP**) in dichloromethane, which gave ketone **4** in 90% yield. (**Scheme 5**).[3]



Scheme 5

These two oxidizing agents are commercially available, but due to their high cost, they were synthesized starting from a cheap starting material such as 2-Iodobenzoyc acid, and simple reagents as Oxone®, Ac_2O and PTSA. **IBX** can be obtained in 85% yield, while **DMP** with a 75% overall yield (**Scheme 6**).[4]

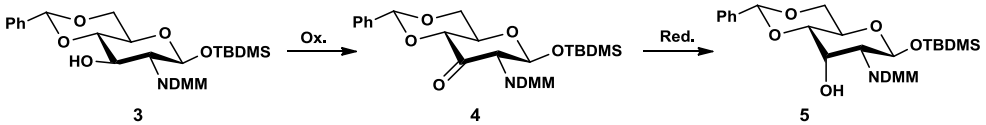


Scheme 6

The reduction of ketone **4** with $NaBH_4$ at room temperature or at 0°C led to the degradation of the molecule, due to the instability of the DMM group under reductive conditions.

The use of a milder reducing agent as $NaBH_3(CN)$ in acidic conditions allowed to obtain only trace of alloside **5**, with a large amount of byproducts.[5]

Only a reduction under carefully controlled conditions: using $NaBH_4$ in DCM:MeOH = 1:1, at -10°C, with a short and strictly controlled reaction time: (210s) led to the desired compound **5** in satisfactory yield (83%), without significant degradation (**Scheme 7**).[6]



Scheme 7

The inversion of configuration was checked by NMR. The chemical shift of some significant signal change, as the **H-1** of the alloside compound **5** shifts to lower fields. Moreover the pattern of the some signal change. **H-4** in the equatorial alcohol **3** is a pseudo-triplet while in the axial alcohol is a doublet of doublet, with a coupling constant between **H-4** and **H-3** of 2.4, in agreement with a *cis* constant. (**Fig.5**).

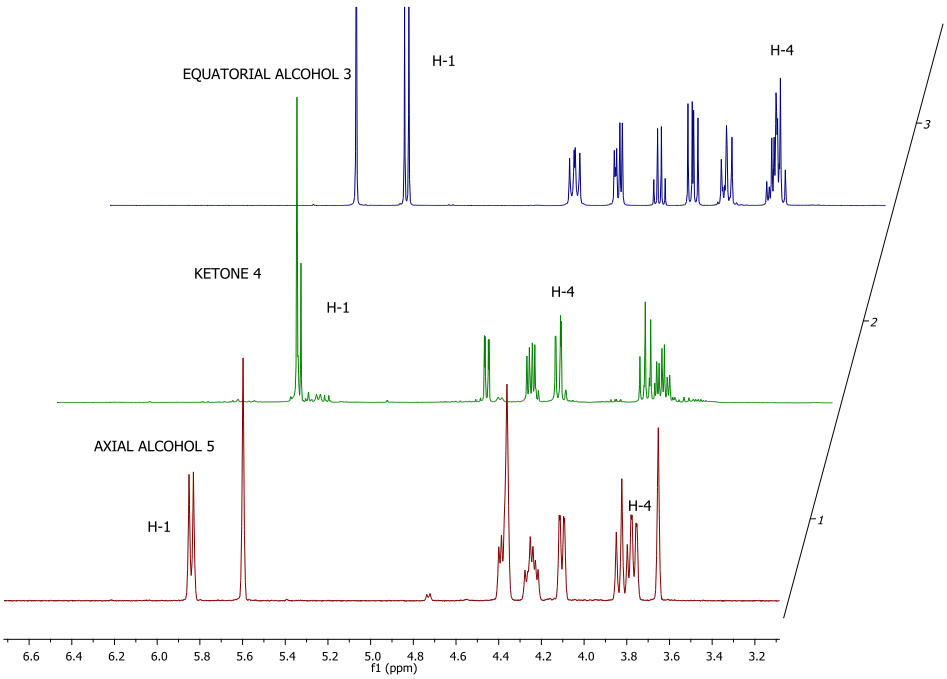
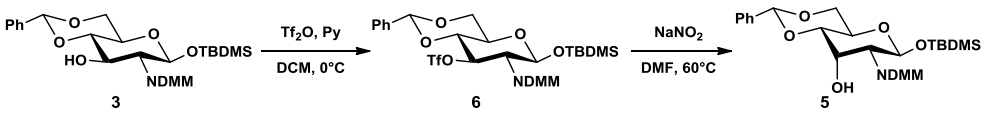


Fig. 5

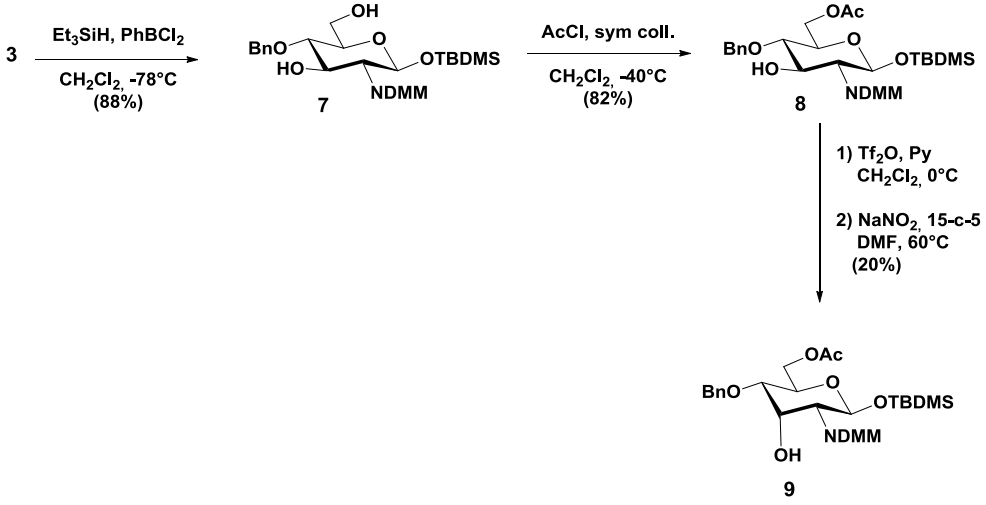
Before the optimization of this procedure other methods were investigated to invert the configuration of the OH.

The Lattrel-Dax reaction is a nitrite-mediated substitution of a triflate, to achieve the OH in the opposite configuration.[7] This method performed on this substrate gave poor results in terms of yield: 25% in 2 steps. Whereas the synthesis of the triflate **6** is almost quantitative, major issues were found in the reaction with $NaNO_2$ giving a complex mixture of compounds. (**Scheme 8**).



Scheme 8

We reasoned that the poor reactivity of triflate **6** could be due to the strain of the molecule. We therefore decided to remove first the benzylidene acetal by regioselective opening with Et_3SiH and $PhBCl_2$ (88% yield). The primary OH was then selectively acetylated, with AcCl and collydine at low temperature, and the epimerization was attempted via triflate on alcohol. Under the same conditions used for **3** this substrate gave also a large amount of byproducts, and only 20% yield of the desired product **9** (**Scheme 9**).

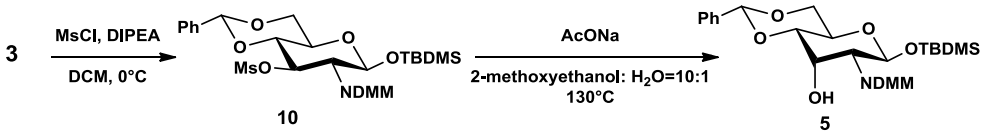


Scheme 9

These results are in agreement with recent studies carried out on this reaction with differently protected sugars. In these works is highlighted that the presence of an ester, as an acetate or a benzoyl group near the hydroxyl which will be reversed, is crucial to have good results. The presence of benzyl ether and benzylidene negatively affects the results of the reaction as in **8** and **3**.[8]

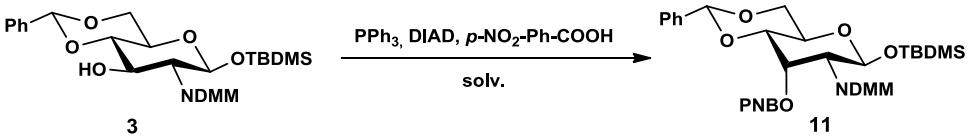
Another method for the epimerization can be performed via mesylate. The formation of this intermediate with mesyl chloride and DIPEA gave **10** in 85% yield. The inversion at C-3 can be achieved by sodium acetate in 2-methoxyethanol

and water at reflux. Also in this case, after 24 h under these harsh conditions, the mesylate was recovered unchanged. (**Scheme 10**).[9]



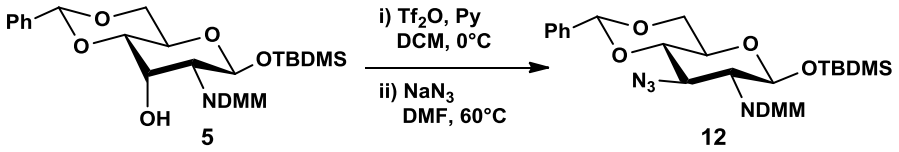
Scheme 10

A very useful, and widely used reaction to invert the configuration of a free OH with a suitable nucleophile is the Mitsunobu reaction, performed with PPh_3, DIAD and a nucleophile. Although para nitro benzoic acid is a good nucleophile, because it can be easily cleaved with TEA., glucoside **3** resulted unreact. The reaction was performed changing different parameters, such as solvent, temperature, order of addition of the reagents, but the starting material was always recovered unchanged, and product **11** was never observed. (**Scheme 11**).[10]



Scheme 11

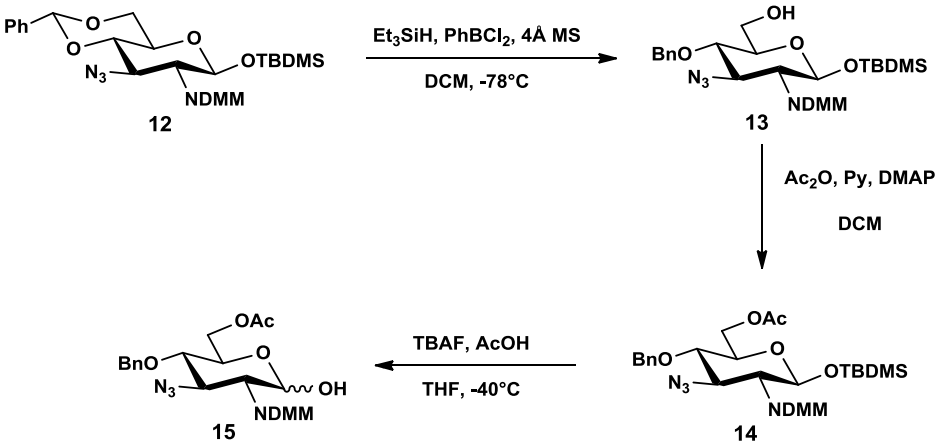
Once we obtained the alloside compound **5** in satisfactory yield through the oxidation-reduction protocol, the synthesis proceeded with the introduction of an amino functionality at position 3 in equatorial configuration. Starting from **5**, first the free OH was converted in a good leaving group as the triflate with triflic anhydride and pyridine. The reaction of this rather unstable intermediate with NaN_3 in moist DMF at 60°C gave compound **12** in 62% yield over two steps (**Scheme 12**).



Scheme 12

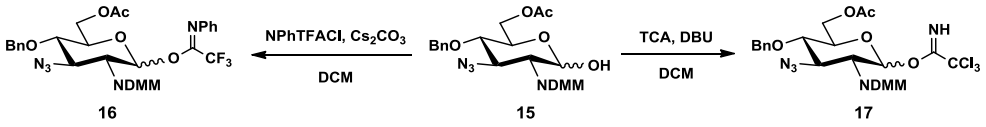
To introduce the azide we also tried the Mitsunobu reaction with DPPA, DIAD and PPh_3. As in the case of the equatorial alcohol **3**, also this substrate demonstrated to be unreactive in this conditions.[11]

Regioselective reductive opening of the benzylidene acetal with Et_3SiH and $PhBCl_2$, followed by acetylation of the primary OH provided compound **14** in 91% yield over 2 steps.[12] The anomeric hydroxyl was desilylated by treatment with TBAF 1.0 M in THF at -40°C to give **15** in 93% yield (**Scheme 13**).



Scheme 13

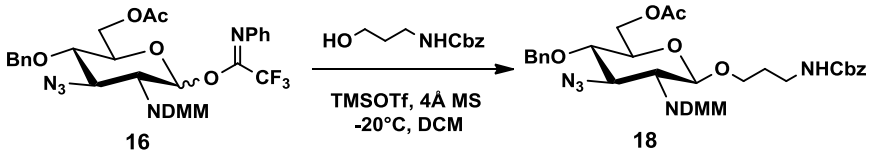
Hemiacetal **15** was transformed the *N*-phenyl trifluoroacetimidate **16** by reaction with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride and Cs_2CO_3 as a base (99% yield). The choice of this glycosyl donor was due to the low yield observed in the synthesis of the corresponding trichloroacetimidate **17** with trichloroacetonitrile and DBU (54% yield, **Scheme 14**).[13]



Scheme 14

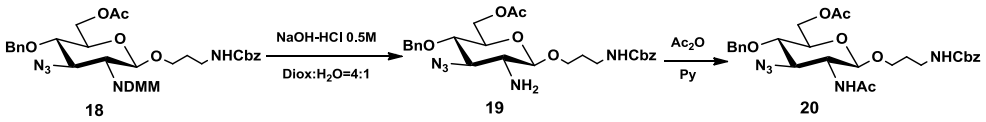
Synthesis of monomer D

For the synthesis of monomer **D**, glycosyl donor **16** was coupled with the commercially available 3-*N*-carbobenzyloxy-aminopropanol. This reaction was performed using TMSOTf as a promoter, and afforded protected monosaccharide **18** in 94% yield. Thanks to the presence of a participating protecting group as DMM in position 2, only the β anomer was obtained. (**Scheme 15**).



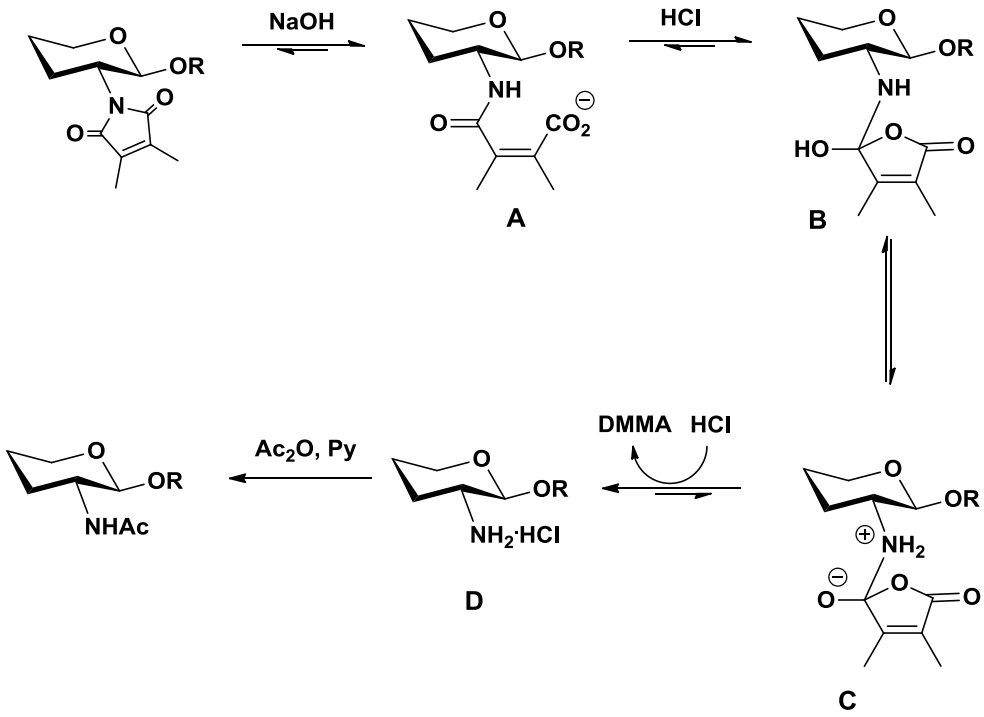
Scheme 15

Transformation of the DMM protected compound into the corresponding *N*-acetyl derivative was carried out under the following conditions: compound **18** was treated with sodium hydroxide until disappearance of the starting material, then addition of hydrochloric acid up to pH = 5 gave the free amine in position 2 (**19**). Acetylation with acetic anhydride in pyridine led to the acetamide **20** (91% yield over 2 steps) (**Scheme 16**).



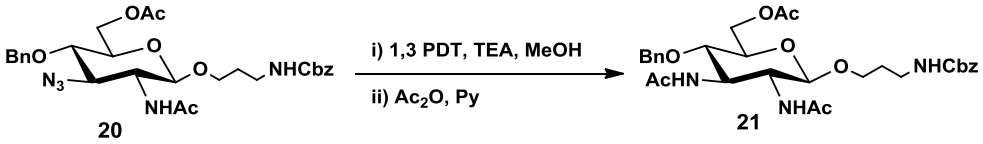
Scheme 16

The mechanism of DMM cleavage was illustrated by Schmidt and coworkers in 1998 and it is outlined in **Scheme 17**. Hydroxide addition leads to ring-opened intermediate **A**, which in the presence of acid is in equilibrium with butenolide **B**. Protonation of the basic nitrogen atom of the amide acetal moiety **C** leads to generation of DMMA and free amine hydrochloride **D** which is then transformed into the *N*-acetyl derivative (**Scheme 17**).[14]



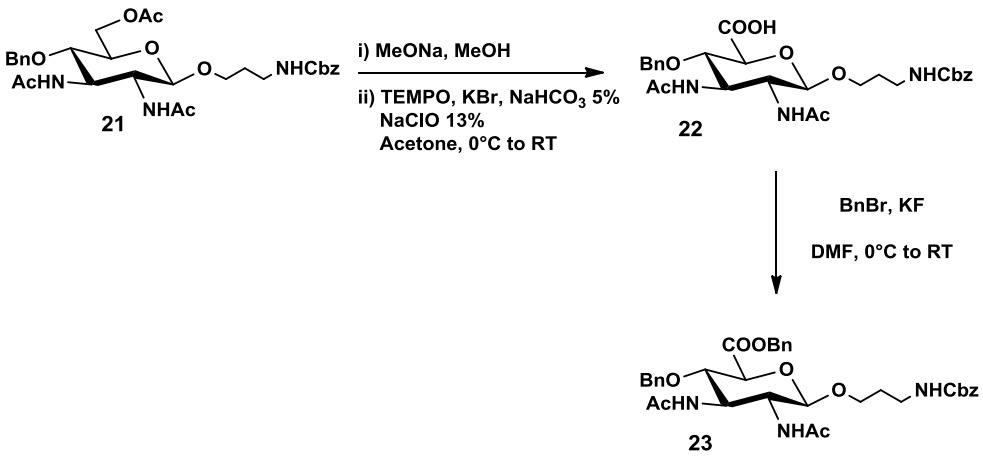
Scheme 17

The second acetamide function was introduced via azide reduction with 1,3-propanedithiol and TEA, and acetylation with acetic anhydride in pyridine. The 2,3-diacetamido compound **21** was obtained in 91% yield over 2 steps (**Scheme 18**).[15]



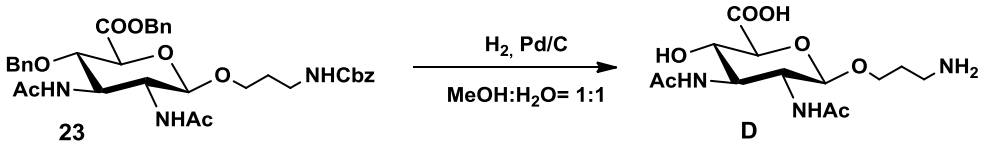
Scheme 18

The primary hydroxyl was deacetylated under Zemplen condition, and converted to carboxylic acid by a TEMPO mediated oxidation, followed by the addition of NaClO 13% aqueous solution, to give **22** In order to facilitate the purification via a flash chromatography, the acid was converted into the corresponding benzyl ester **23** with BnBr and KF as a base in 81% yield over 3 steps. (**Scheme 19**).



Scheme 19

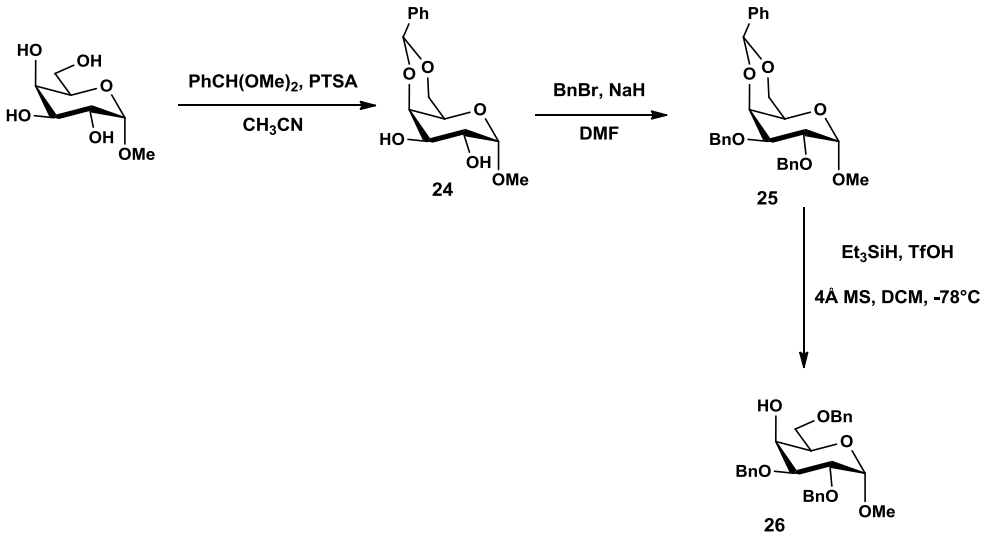
Catalytic hydrogenation of **23** removed the benzylic protecting groups, and afforded the monosaccharide **D**; that is the first target molecule provided with a suitable linker in anomeric position (**Scheme 20**).



Scheme 20

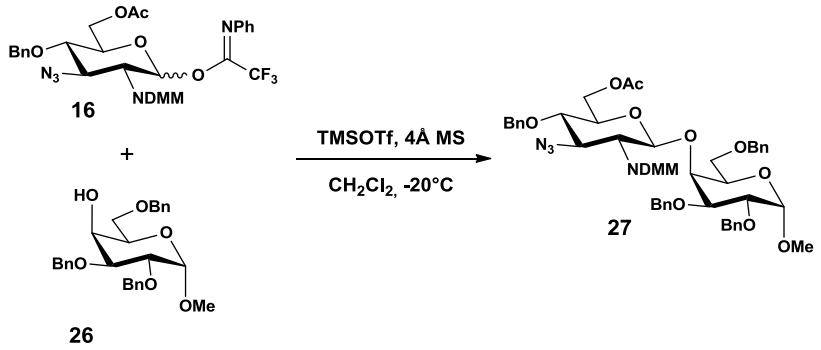
Synthesis of disaccharide C-D

Disaccharide fragment **C-D** was obtained from coupling of glycosil donor **16** and a suitable galactoside acceptor. This was easily synthesized in three steps from commercially available methyl-α-galactopyranoside. In a first attempt, positions 4 and 6 were protected as benzylidene acetal, then the free OH were benzylated obtaining **25** (85% yield over 2 steps). Regioselective reductive opening of the benzylidene carried out with Et_3SiH and TfOH at -78°C afforded the glycosyl acceptor **26** in 82% yield (**Scheme 16**).[12]



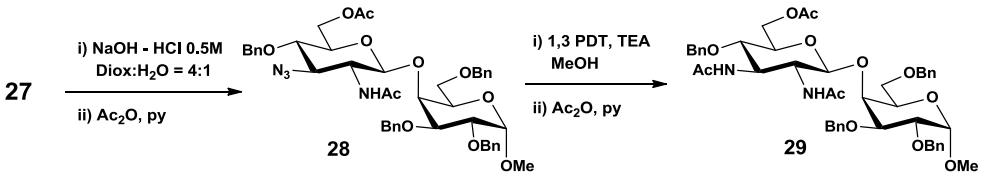
Scheme 21

Glycosylation of **26** with donor **16** in the presence of TMSOTf as a promoter provided the protected disaccharide **27** in 54% yield. (**Scheme 17**).



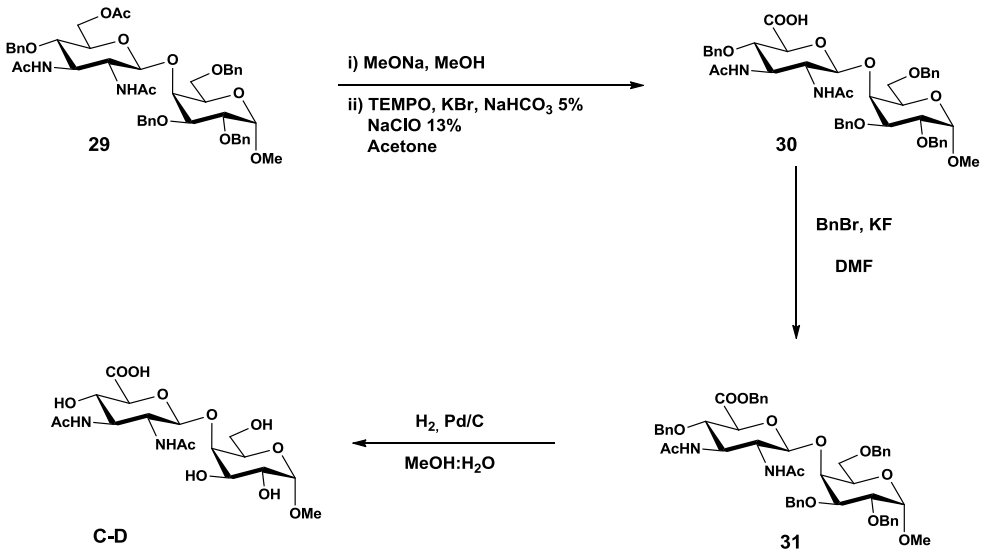
Scheme 22

The subsequent steps of deprotection and oxidation were performed following the same conditions previously described for the synthesis of monosaccharide **25**. First DMM group was removed and the free amine acetylated under standard condition. The azide was reduced with 1,3-propanedithiol and TEA and acetylated with acetic anhydride in pyridine, obtaining the diacetamido compound **29** (**Scheme 23**).



Scheme 23

Disaccharide **29** was deacetylated and oxidized to carboxylic acid **30** with TEMPO and NaClO 13% aqueous solution. The acidic functionality was converted in the corresponding benzyl ester **31** in order to facilitate the purification and the final hydrogenation led to disaccharide target molecule **C-D** (**Scheme 24**).

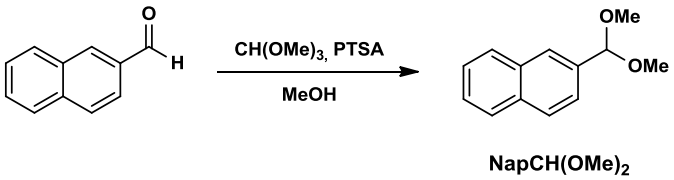

OAc
BnO
AcHN
NHAc
OBn
BnO
OBn
OMe
29
i) MeONa, MeOH
ii) TEMPO, KBr, NaHCO3 5%
NaClO 13%
Acetone
COOH
30
BnBr, KF
DMF
COOBn
31
H2, Pd/C
MeOH:H2O
HO
OH
C-D

Scheme 24

Synthesis of trisaccharide C-D-E

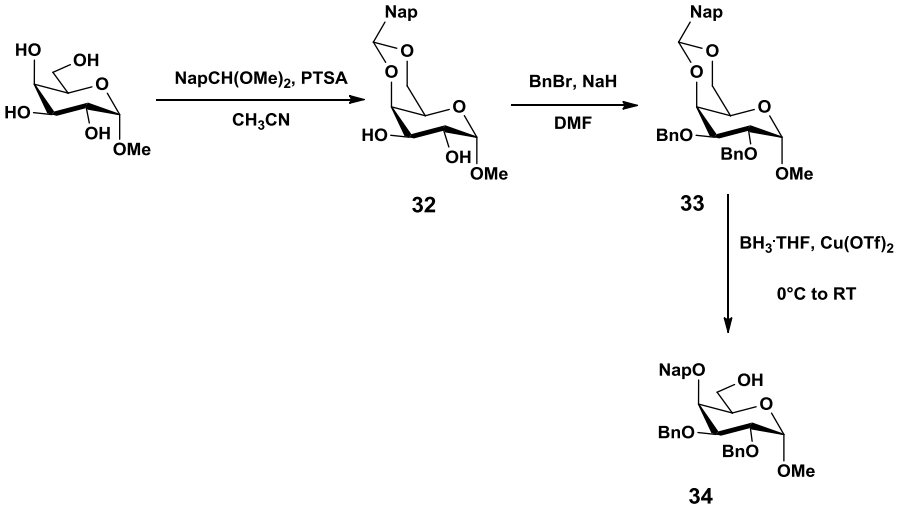
For the synthesis of the third target molecule it required the synthesis of a new galactosyl, bearing a protecting group at C-4 orthogonal to benzyl ether. For this goal the 2-naphtylmethil ether was chosen.

The starting material was, also in this case, the methyl-α-D-galactpyranoside. The first step was the installation of a naphthylidene acetal to protect positions 4 and 6. This reaction was performed with naphthaldehyde dimethylacetl, easily synthesized from naphthaldehyde in 85% yield (**Scheme 25**).[16]



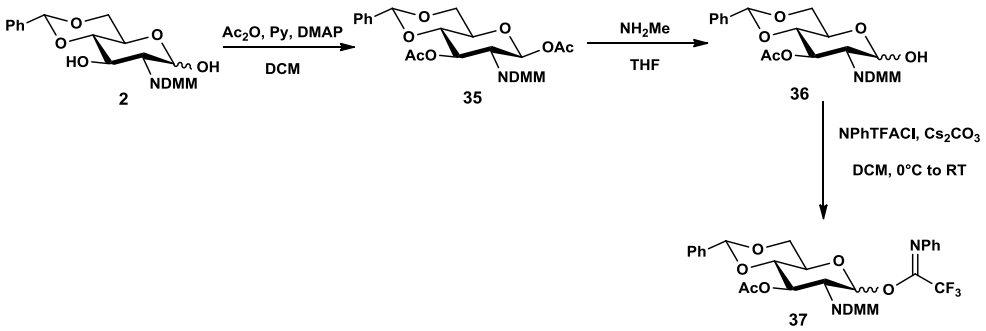
Scheme 25

Positions 2 and 3 were then benzylated, (95% yield over two steps) and the naphthylidene ring was regioselectively opened with BH_3·THF and $Cu(OTf)_2$ affording acceptor **34** (81% yield **Scheme 26**).[17]



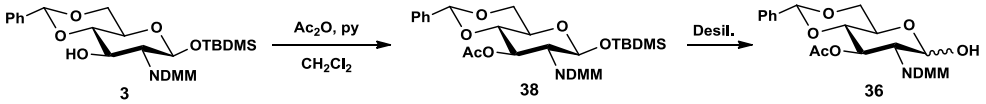
Scheme 26

Furthermore it was synthesized glycosyl donor **38**, precursor of fragment **E** of the trisaccharide. Starting from intermediate **2**, the two hydroxyls were acetylated, then the anomeric position was selectively deprotected with methylamine in THF, providing hemiacetal **36** in 74% yield over two steps. Glycosyl donor **37** was achieved as *N*-phenyl trifluoroacetimidate in 70% yield (**Scheme 27**).



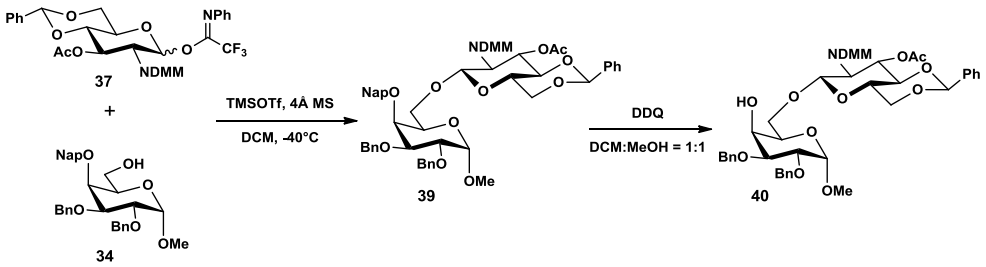
Scheme 27

The synthesis of **37** would be seemingly simpler from **3**, by 3-OH acetylation followed by 1-*O*-desilylation. This route was avoided for practical reasons. The benzylation of the free OH using NaH caused the degradation of the DMM. Moreover, the acetylation and subsequent desylilation provided the desired hemiacetal in only 50% yield, with the presence of unexpected byproduct, due to the migration of the acetate in anomeric position (**Scheme 28**). Desilylation was performed employing different reagents and condition as TBAF at low temperature or HF in pyridine, but the results were always unsatisfactory (from 25 to 40% yield).[18]



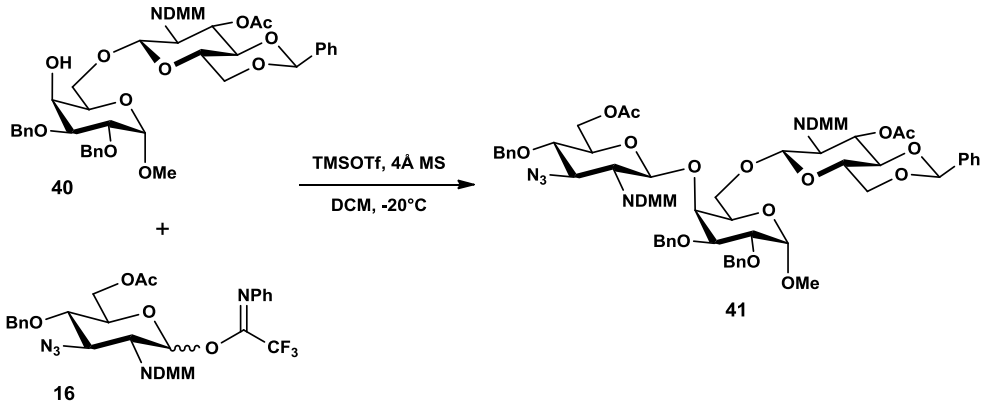
Scheme 28

The coupling of **37** with **34** using TMSOTf as a promoter afforded disaccharide **40** in 45% yield. Then Nap group was removed under oxidative condition, (DDQ) to provide disaccharide acceptor **41** in 80% yield (**Scheme 29**).



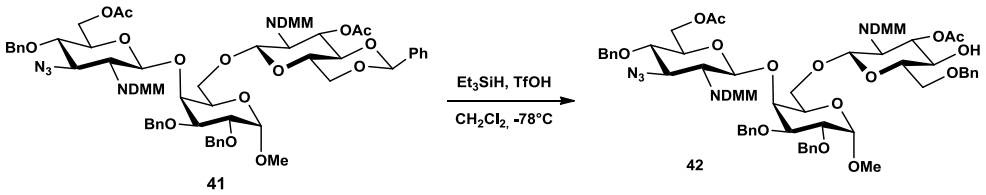
Scheme 29

The condensation between disaccharide acceptor **41** with *N*-phenyltrifloroacetimidate **16** furnished the protected trisaccharide **42** in 52% yield (**Scheme 30**).



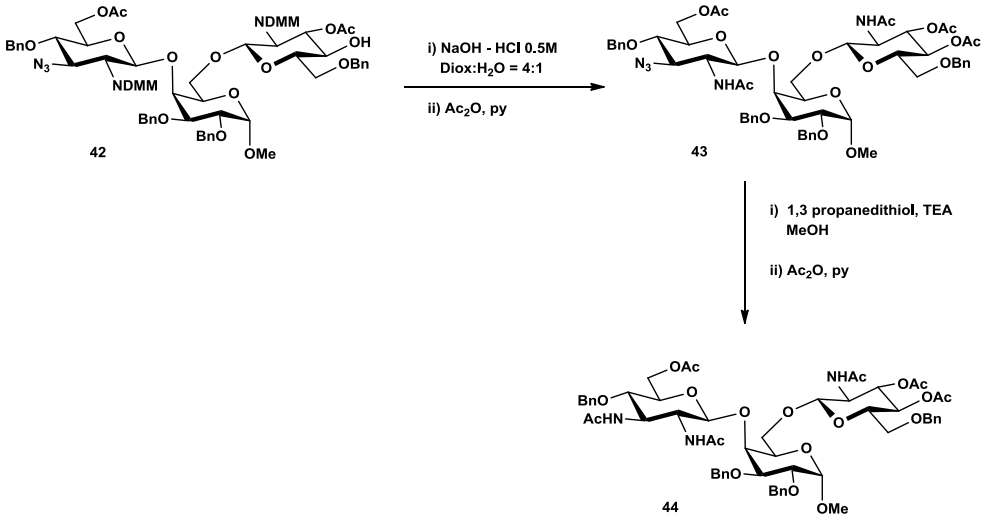
Scheme 30

Before deprotection of the DMM group it was necessary to open the benzylidene ring , because the acidic conditions during the DMM removal could decompose the molecule. This step was carried out with TfOH and Et_3SiH at -78°C, providing **43** in 83% yield (**Scheme 31**).



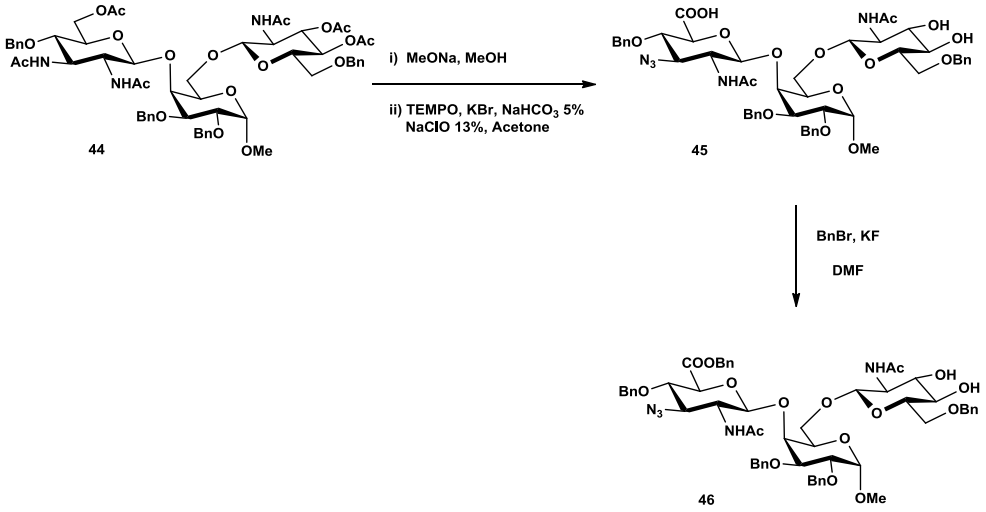
Scheme 31

Then the two DMM groups were removed under the conditions illustrated above, and the amines acetylated under standard condition giving **44** in 84% yield. The remaining azide was reduced with 1,3-propanedithiol and converted in the third acetamide function **45** in 80% yield (**Scheme 32**).



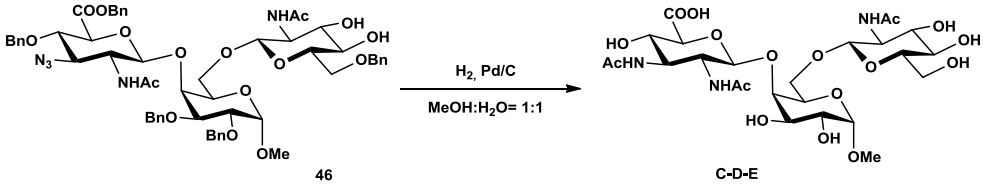
Scheme 32

Zemplen deacetylation removed both the acetates, but oxidation with TEMPO and NaClO to carboxylic acid is chemoselective involved and only the primary OH, so it is possible to obtain carboxylic acid **46** which was converted in the corresponding benzyl ester **47** in 79 % yield (**Scheme 33**).



Scheme 33

The removal of benzyl ethers and esters by catalytic hydrogenation furnished the third target molecule, trisaccharide **C-D-E**, in quantitative yield (**Scheme 34**).



Scheme 34

CONCLUSION & FUTURE WORKS

Three different fragments of the LPS O-chain moiety of *Acinetobacter baumannii* ATCC 17961 were synthesized. All this molecule contain in their structure a rare sugar namely 2,3-diacetamido-2,3-dideoxy-D-glucoronic acid.

The binding affinity of these fragments will be evaluated by competitive ELISA assay against the anti-LPS serum of *Acinetobacter baumannii* ATCC 17961 antibody.

The biological characterization of the synthetic compounds will be performed by Dr. Michael J. McConnell, from *Unit of Infectious Disease, Microbiology, and Preventive Medicine, Institute of Biomedicine of Sevilla* (**IBiS**).

The synthesis of the three fragments provided with a suitable linker in order to allow the conjugation with a carrier protein will be another issue of this project. For this purpose it will be necessary to furnish the galactoside fragment with a spacer. Moreover the synthesis of the pentasaccharide will be another tool. Also in this case the attention is focus on this fragment. Furthermore the linker in anomeric position a study on the use of protecting group has be done. A stable protection in position 2 is required, as a benzyl ether, while positions have to be provided with orthogonal functions.

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EXPERIMENTAL SECTION

General experimental methods

Thin Layer Chromatography

Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) were performed on Merck precoated 60F254 plates (0.25 mm and 0.2 mm thickness, respectively). Reactions were always followed by TLC-analysis; compounds were visualized, when appropriate, by UV light (254 nm) and always by spraying with:

- *Molybdic solution* [21 g of $(NH_4)_4Mo_4O_{24}$, 1g of $Ce(SO_4)_2$, 31 mL of H_2SO_4 98%, 970 mL water];

- *Sulphuric acid* [50 mL of H_2SO_4 98%, 450 mL of MeOH, 450 mL water];

- *Ninhydrin* [2.7g of 2,2-Dihydroxyindane-1,3-dione, 27 mL of AcOH, 900 mL of n-BuOH (or EtOH)];

with detection by charring at 200°C.

Flash chromatography

According to Still1 procedure, compounds were purified by flash chromatography, using Silica gel (SiO_2, high-purity grade (Merck Grade 9385), pore size 60 Å, 230-400 mesh particle size) by Sigma-Aldrich®.

In some cases it was also used a flash purification system, Biotage® SP1™:2 SP1 means a single-column flash purification system [with Touch Logic Control™ and TLC-to-gradient feature designed to accelerate and simplify the purification process]. Available in sizes from 10 g to 340 g with standard (50 μm) silica, Biotage SNAP cartridges were used to purify our compounds.

NMR analysis

NMR spectra were recorded in a Bruker Avance 400 instrument (400, 101 MHz for ^1H, ^{13}C), and all were run at room temperature (298K) if not specified. The samples were prepared using deuterated solvents, as $CDCl_3$, D_2O, CD_3OD and $(CD_3)_2SO$,

by Sigma-Aldrich®.

Chemical shifts (δ) are reported in ppm scale and the coupling constants (*J*) in Hz. The chemical shift displacement is based on the residual proton in the solvent (e.g. the $CHCl_3$, 0.01 % in 99.99 % $CDCl_3$). The multiplicity of signals has been described as: *s* (singlet), *d* (doublet), *t* (triplet), *m* (multiplet), *dd* (doublet of doublets), *dt* (doublet of triplets).

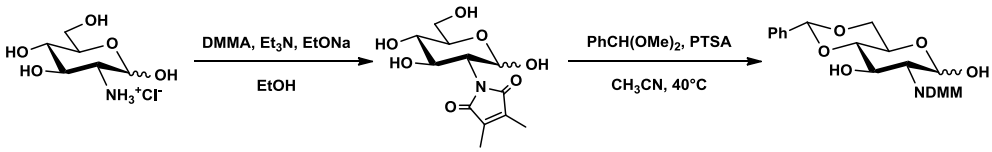
1H-NMR spectra were recorded for all the synthesized products. In the case of unknown structure, the characterization is here reported by 1H-NMR, 13C-NMR: the registration of bidimensional (2D-COSY, 2D-HETCOR) analyses was due to better assignment of peaks to the structure.

In the case of a dimer we called ring-A the sugar coupled with the spacer and ring-B the one at the non-reducing terminus.

Mass analysis

ESI mass were recorded in negative or positive modes on Jeol AX-505 and for high resolution on Q-TOF

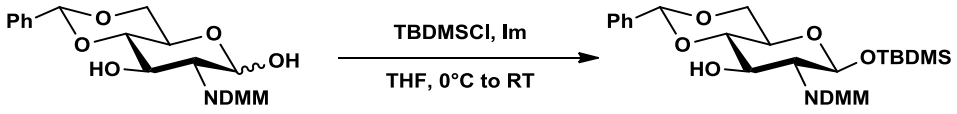
*4,6-O-Benzylidene-2-deoxy-2-dimethylmaleimido-α/β-*D*-glucopyranoside* (**1**)



D-Glucosamine hydrochloride (3.0 g, 13.91 mmol) was added to a sodium ethoxide solution (1.0 M in ethanol, 13.92 mL, 13.92 mmol). After 10 min, the mixture was treated with dimethylmaleic anhydride (0.88 g, 6.97 mmol) and stirred for 20 min. Triethylamine (1.4 mL, 13.91 mmol) was added and the reaction mixture was again treated with dimethylmaleic anhydride (0.88 g, 6.97 mmol). The reaction mixture was warmed to 60°C with stirring for 1.5 h then dried well in vacuo. The crude will be used as such in the next step.

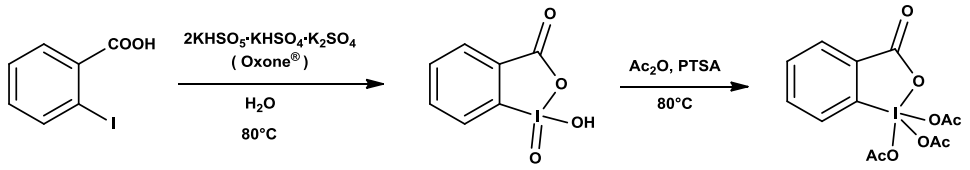
The residue was dissolved in dry CH_3CN (70 mL) then benzaldehyde dimethylacetal (0.32 g, 2.1 mmol, 0.32 mL) p-TsOH (0.028g, 0.14 mmol) were added. The solution was stirred at 40°C for 16 h then it was quenched by adding TEA until neutral pH and solvent removed in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate =1:1) to yield **1** (3.65g, 70%) as a yellow foam. - TLC (*n*-hexane/ethyl acetate =1:1): Rf = 0.29. - ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 – 7.34 (m, 5H, (Ph)), 5.59 (ss, 1H, CHPh), 5.30 (m, 1H, H-1), 4.75 – 4.59 (m, 1H, H-3), 4.56 (d, *J* = 4.4 Hz, 1H, H-2), 4.40 (dd, *J* = 10.4, 4.8 Hz, 1H, H-6), 4.29 (dd, *J* = 11.1, 3.5 Hz, 1H, H-4), 3.95 (dd, *J* = 10.5, 8.5 Hz, 1H, H-2), 3.81 (q, *J* = 9.8 Hz, 1H, H-6'), 3.71 – 3.52 (m, 2H, H-5, H-4), 3.18 (d, *J* = 8.5 Hz, 1H, OH), 2.52 (d, *J* = 3.1 Hz, 1H), 2.48 (d, *J* = 3.2 Hz, 1H), 2.09 – 1.94 (m, 1H).

*tert-Butyldimethylsilyl 4,6-O-Benzylidene-2-deoxy-2-dimethylmaleimido-β-*D-*glucopyranoside* (**2**)



1 (3.65 g, 9.73 mmol) and imidazole (1.32 g, 19,46 mmol) were dissolved in dry THF (50 mL) under N_2 atmosphere. The solution was cooled at 0°C and *tert*-butylchlorodimethylsilane (3.81 g, 25.29 mmol) was added. . After stirring at room temp for 24 h the reaction mixture was diluted with water (30 mL), then extracted with dichloromethane (2 x 50 mL). The organic phase were dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 4:1 to 3:1) to yield **2** (3,70g, 78%) as a white foam. - TLC (*n*-hexane/ethyl acetate =7:3): Rf = 0.36. - $[\alpha]_D$ = -32.2 (c=0.45, chloroform). - ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 – 7.34 (m, 5H, Ph), 5.56 (s, 1H, CHPh), 5.33 (d, *J* = 8.1 Hz, 1H, H-1), 4.53 (dt, *J* = 15.2, 7.6 Hz, 1H, H-3), 4.38 – 4.27 (m, 1H, H-6), 3.99 (dd, *J* = 10.7, 8.1 Hz, 1H, H-2), 3.89 – 3.76 (m, 1H, H-6'), 3.67 – 3.49 (m, 2H, H-5, H-4), 1.98 (s, 6H, $2CH_3$ (NDMM)), 0.87 – 0.73 (m, 9H, $SiC(CH_3)_3$), 0.15 – 0.04 (s, 3H, $Si(CH_3)$), 0.04 – -0.07 (s, 3H, $Si(CH_3)$). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.06 (2 CO (NDMM)), 137.24 (2 C-NDMM), 137.10 (C (Ar)), 130.26 – 125.70 (C-Ph), 101.98 (CH-Ph), 94.04 (C-1), 82.39 (C-4), 68.75 (C-6), 68.53 (C-3), 66.21 (C-5), 58.57 (C-2), 25.33 (t-Bu), 8.65 (CH3 (NDMM)), -4.23 (Si-CH3), -5.61 (Si-CH3). - EI-MS (positive mode); *m/z*: 512.6 [M + Na].

1,1,1-Triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one



Oxone (84.5 g, 137.5 mmol) was dissolved in a 1L flask, in water (456 mL). 2-Iodobenzoic acid (15.5 g, 62.5 mmol) was added, and reaction heat at 80°C for 3h, observing the formation of a white suspension. The temperature was cooled down at RT, and the reaction was put in a ice bath for 1h with the formation of a white precipitate.

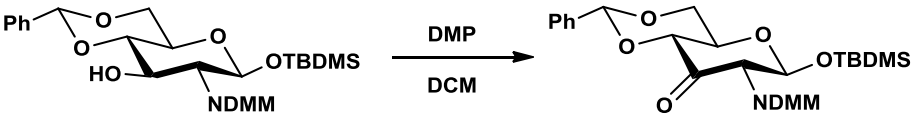
The solid was filtered off, and washed with small portions of cold water (3 X 10 mL) and cold acetone (3 X 10 mL). It was dried in vacuo for 1 night.

IBX (2-iodoxybenzoic acid) was suspended in 62 mL of Ac_2O, and a catalytic amount of PTSA was added (1% wt). The mixture was heated at 80°C for 3h, and the dissolution of the suspension was observed. The solution was cooled in an ice bath for 2h with the formation of a white solid. The cold mixture was filtered on Buckner, and washed with ether (5 x 5 mL). The resulting white crystalline solid (18.00 g, 42,4 mmol 68% yield on two steps) was dried in vacuo and stored at -20°C.

The spectroscopic data were in agreement with those reported in literature.[1]

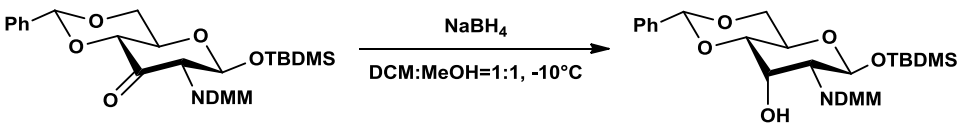
[1] Robert E. Ireland' and Longbin Liu, *J. Org. Chem.* **1993**,*58*, 2899

*tert-Butyldimethylsilyl 4,6-O-Benzylidene-2-deoxy-2-dimethylmaleimido-β-*D-*glucopyran-3-uloside* (**3**)



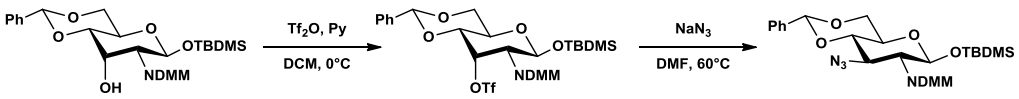
2 (3,70 g, 7,58mmol) was dissolved in dry DCM (70 mL) under N_2 atmosphere. A solution of DMP (6.42g, 15.16 mmol) in dry DCM (50 mL) was added dropwise, and stirred for 24h at room temperature. After completion of the reaction a 5% solution of thiosulfate (50 mL) was added and the mixture let stirred for 10' until two distinct phases were established. The organic layer was separated and washed with a saturated solution of $NaHCO_3$ (50 mL), and water (50 mL). The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 4:1) to yield **3** (3,32g, 90%) as a white foam. - TLC (*n*-hexane/ethyl acetate =7:3): Rf = 0.68. - 1H NMR (400 MHz, $CDCl_3$): δ 7.63 – 7.33 (m, 5H, Ph), 5.58 (ss, 2H, CHPh; H-1), 4.70 (dd, J = 7.3, 1.3 Hz, 1H, H-2), 4.50 (dd, J = 10.2, 4.6 Hz, 1H, H-6), 4.37 (dd, J = 9.8, 1.3 Hz, 1H, H-4), 3.96 (t, J = 10.1 Hz, 1H, H-6'), 3.92 – 3.84 (m, 1H, H-5), 2.01 (s, 6H, 2 CH_3 (NDMM)), 0.85 (s, 9H, $SiC(CH_3)_3$), 0.13 (s, 3H, $Si(CH_3)$), 0.04 (s, 3H, $Si(CH_3)$). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.31 (C-3), 171.00 (2 CO (NDMM)), 137.67 (2 C-(NDMM)), 136.38 (C-(Ar)), 127.64 (Ph), 102.08 (CHPh), 96.02 (C-1), 81.95 (C-4), 69.16 (C-6), 66.03 (C-5), 63.25 (C-2), 25.30 (tBu), 8.74 (CH3 (NDMM)), -4.40 (Si-CH3), -5.53 (Si-CH3). - HRMS-Q-TOF (positive mode); *m/z:* 510.1586 [M + Na]

*tert-Butyldimethylsilyl 4,6-O-Benzylidene-2-deoxy-2-dimethylmaleimido-β-*D-*allopyranoside* (**4**)



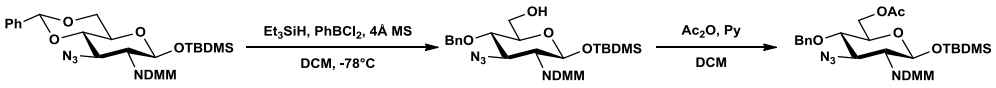
To a solution of **3** (3.32g, 6.82 mmol) in dry DCM:MeOH=1:1 (20 mL: 20 mL), under N_2 atmosphere, cooled at -10°C was added in one portion $NaBH_4$ (283mg, 7.5 mmol). The reaction was maintained under vigorous stirring for 210s, thus was added a saturated solution of NH_4Cl (10 mL) and acetone (10 mL). The reaction was stirred for 10 min, diluted with brine (30 mL) and extracted with DCM (3 x 50 mL). The organic phase were separated, dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 4:1) to yield **4** (2.77g, 83%) as a white foam. - TLC (*n*-hexane/ethyl acetate =7:3): Rf = 0.47. - 1H NMR (400 MHz, $CDCl_3$): δ 7.60 – 7.34 (m, 5H, Ph), 5.84 (d, *J* = 8.3 Hz, 1H, H-1), 5.60 (s, 1H, CHPh), 4.42 – 4.29 (m, 2H, H-3, H-6), 4.25 (td, *J* = 9.9, 5.1 Hz, 1H, H-5), 4.10 (dd, *J* = 8.2, 2.3 Hz, 1H, H-2), 3.82 (t, *J* = 10.3 Hz, 1H, H-6'), 3.77 (dd, *J* = 9.4, 2.4 Hz, 1H, H-4), 2.00 (s, 6H, $2CH_3$ (NDMM)), 0.79 (s, 9H, $SiC(CH_3)_3$), 0.11 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.00 (CO (NDMM)), 137.39(C-(NDMM)), 136.33 (C-Ar), 129.44, 128.32, 126.32 (Ph), 102.65 (CH-Ph), 91.01 (C-1), 84.87 (C-3), 76.17 (C-4), 69.58 (C-6), 64.10 (C-5), 54.98 (C-2), 25.38 (tBu), 8.83 (CH3-NDMM), -4.08 (Si-CH3), -5.55 (Si-CH3). - HRMS-Q-TOF (positive mode); *m/z:* 512.1528 [M + Na]

*tert-Butyldimethylsilyl 4,6-O-Benzylidene-2,3-dideoxy-3-azido-2-dimethylmaleimido-β-*D*-glucopyranoside* (**5**)



4 (2.77g, 5.66 mmol) was dissolved in dry DCM (25 mL) under N_2 atmosphere. Pyridine (1.37 mL, 16.98 mmol) was added and the solution was let stir at RT for 15'. The reaction was cooled at 0°C and triflic anhydride (1.43 mL, 8.49 mmol) was dropped. After 1.5 h the solution was diluted with DCM (20 mL), and washed with HCl 5% (30 mL), a saturated solution of $NaHCO_3$ (30 mL) and water (30 mL). The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The crude was let for 2 h in vacuo, then dissolved in humid DMF (30 mL). NaN_3 (1.1 g, 16.98 mmol) was added and the reaction let stir at 60°C over night. The solvent was removed under reduced pressure, the residue redissolved in DCM (40 mL) and washed with water (20 mL). The organic phase were dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 9:1) to yield **5** (1.89g, 65%) as a white foam. - TLC (*n*-hexane/ethyl acetate =7:3): Rf = 0.59. - 1H NMR (400 MHz, $CDCl_3$): δ 7.66 – 7.34 (m, 5H, Ph), 5.63 (s, 1H, CHPh), 5.30 (d, *J* = 8.0 Hz, 1H, H-1), 4.52 (dd, *J* = 11.2, 9.1 Hz, 1H, H-3), 4.37 (dd, *J* = 10.5, 4.5 Hz, 1H, H-6), 3.94 – 3.78 (m, 2H, H-2,H-6'), 3.78 – 3.58 (m, 1H, H-4,H-5), 1.99 (s, 6H, $2CH_3$ (NDMM)), 0.81 (s, 9H, $SiC(CH_3)_3$), 0.08 (s, 3H, $Si(CH_3)$), 0.00 (s, 3H, $Si(CH_3)$). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.06 (CO (NDMM)), 137.43 (C-(NDMM)), 136.71 (C-Ar), 129.08, 128.28, 126.01, (Ph) 101.46 (CH-Ph), 94.14 (C-1), 81.07 (C-4), 68.76 (C-6), 67.28 (C-5), 59.62 (C-3), 56.66 (C-2), 25.28 (tBu), 8.68 (CH3-NDMM), -4.26 (2 Si-CH3). - HRMS-Q-TOF (positive mode); *m/z:* 537.1710 [M + Na].

tert-Butyldimethylsilyl 6-O-acetyl-4-O-benzyl-2,3-dideoxy-3-azido-2-dimethylmaleimido-β-D-glucopyranoside (**6**)

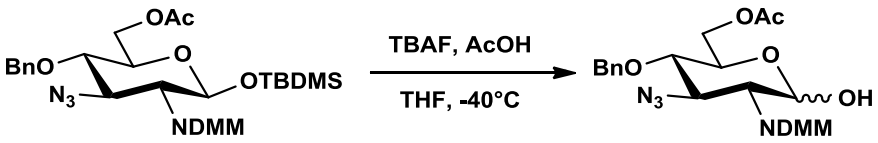


5 (1.89 g, 3.68 mmol) was dissolved in dry DCM (20 mL) under N_2 atmosphere. 4Å MS (5.00 g) were added and stir for 40' at RT. Et_3SiH (2.19 mL, 13.98 mmol) was added, the solution cooled at -78°C and $PhBCl_2$ (525 μL, 4.42 mmol) was added dropwise. After 10' the reaction was quenched with TEA until neutral pH, and filtered on celite pad. The solvent was removed under reduced pressure, the residue redissolved in DCM (30 mL) washed with a saturated solution of $NaHCO_3$ (20 mL) and water (20 mL). The organic phase was dried with sodium sulfate and the solvent removed in vacuo.

The crude was dissolved in dry DCM (20 mL) under N_2 atmosphere. Pyridine (1.2 mL, 14.72 mmol), acetic anhydride (695 μL, 6.44 mmol) and a catalytic amount of DMAP were added. After 2h the reaction was diluted with DCM (20 mL), and washed successively with HCl 5% (20 mL), a saturated solution of $NaHCO_3$ (20 mL) and water (20 mL). The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 9:1) to yield **6** (1.87g, 91%) as a white foam. - TLC (*n*-hexane/ethyl acetate =9:1): Rf = 0.43. - 1H NMR (400 MHz, $CDCl_3$): δ 7.51 – 7.20 (m, 5H, Ph), 5.20 (d, *J* = 8.0 Hz, 1H, H-1), 4.87 (d, *J* = 10.8 Hz, 1H, CH_2Ph), 4.62 (d, *J* = 10.8 Hz, 1H, CH_2Ph), 4.38 (ddd, *J* = 17.9, 10.3, 5.7 Hz, 2H, H-6, H-3), 4.19 (dd, *J* = 11.8, 5.6 Hz, 1H, H-6'), 3.82 (dd, *J* = 11.6, 8.0 Hz, 1H, H-2), 3.70 (ddd, *J* = 9.7, 5.6, 2.3 Hz, 1H, H-5), 3.46 (t, *J* = 9.4 Hz, 1H, H-4), 2.08 (s, 3H, CH_3CO), 2.00 (s, 6H, $2CH_3$ (NDMM)), 0.78 (s, 9H, $SiC(CH_3)_3$), 0.05 (s, 3H, $Si(CH_3)$), 0.00 (s,

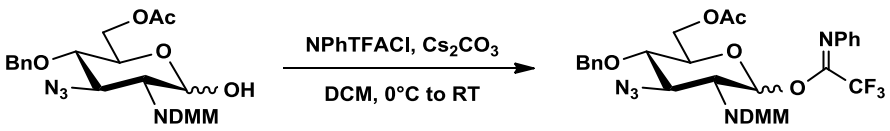
3H, Si(CH_3)).- ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.54 (2 CO (NDMM)), 137.47 (C-NDMM), 136.96 (C-Ar), 128.58, 128.32, 128.25 (Ph), 93.52 (C-1), 77.85 (C-4), 74.88 (CH2Ph), 73.83 (C-5), 63.29 (C-3), 62.84 (C-6), 56.53 (C-2), 25.31 (tBu), 20.76 (CH3CO), 8.69 (2 CH3 (NDMM)), -4.31 (Si-CH3), -5.62 (Si-CH3). - EI-MS (positive mode); *m/z*: 581.7 [M + Na].

6-O-acetyl-4-O-benzyl-2,3-dideoxy-3-azido-2-dimethylmaleimido-α/β-D-glucopyranoside (**7**)



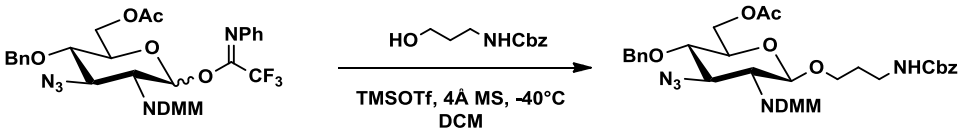
6 (1.87g, 3.35 mmol)was dissolved in dry THF (20 mL) under N_2 atmosphere. The solution was cooled at -40°C and TBAF 1.0 M in THF (5.02 mL, 5.02 mmol) was added dropwise and let stir for 2h. After completion of the reaction glacial acetic acid (100 μL, 1.67 mmol) was added, and the mixture warmed to r.t. The solution was diluted with brine (20 mL), and extracted with DCM (3 x 30 mL). The organic phase was separated, dried with sodium sulfate and solvent evaporated in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 1:1) to yield **7** (1.38g, 93%) as a white solid. - TLC (*n*-hexane/ethyl acetate =1:1): Rf = 0.38. - 1H NMR (400 MHz, $CDCl_3$) δ 7.37 (m, 5H, Ph), 5.21 (m, 2H, H-1-*α/β*), 4.93 – 4.78 (m, 2H, CHPh, H-3*α*), 4.61 (dd, J = 10.6, 4.3 Hz, 1H, CHPh), 4.49 – 4.34 (m, 2H, H-3*β*, H–6), 4.25 (m, 2H, H-6', H-5), 4.05 (m, 1H, H-2*α*), 3.82 – 3.68 (m, 2H, H-2*β*, H-5), 3.51 (m, 1H, H-4*α/β*), 2.99 (d, J = 7.0 Hz, 1H, OH), 2.10 (ss, 3H, CH3CO), 2.01 (ss, 6H, 2 CH3 (NDMM)). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.54 (2 CO (NDMM)), 137.47 (C-NDMM), 136.96 (C-Ar),129.31, 129.08, (Ph) 93.77 (C-1), 78.49 (C-4), 75.69 (CH2Ph), 74.69 (C-5), 63.60 (C-3), 63.23 (C-6), 56.56 (C-2), 21.57 (CH3CO), 9.55 (2 CH3 (NDMM)). - EI-MS (positive mode); *m/z*: 467.4 [M + Na].

6-O-acetyl-4-O-benzyl-2,3-dideoxy-3-azido-2-dimethylmaleimido-α/β-D-glucopyranosyl N-Phenyltrifluoroacetimidate (**8**)



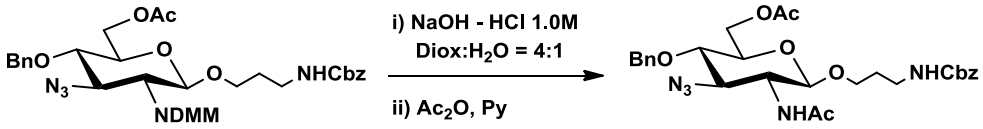
7 (1.38, 3.11 mmol) was dissolved in dry DCM (20 mL) under N_2 atmosphere. The solution was cooled at 0°C in an ice bath. 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (755 μL, 4.66 mmol) and cesium carbonate (1.52 g, 4.66 mmol) were added in sequence. The reaction was warmed to RT and stirred for 2h, then filtered on a celite pad and evaporated in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 9:1 + 1% TEA) to yield **8** (1.81g, 99%) as a white foam. - TLC (*n*-hexane/ethyl acetate =6:4): Rf = 0.66.

N-(Benzyloxycarbonyl)aminopropyl-6-O-acetyl-4-O-benzyl-2,3-dideoxy-3-azido-2-dimethylmaleimido-β-D-glucopyranoside (**9**)



8 (1.81 g, 3.07 mmol) and NHCbz-propanol (3.28 g, 15.35 mmol) were dissolved in dry DCM (20 mL) under N_2 atmosphere. 4Å MS (5.00 g) were added and stir for 40' at RT. The mixture was cooled at -40°C and a solution 0.1 M of TMSOTf (3.07 mL) in DCM was added dropwise. After 2h the reaction was quenched with TEA until neutral pH, than filtered on celite pad. The solvent was removed under reduced pressure. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 7:3) to yield **9** (1.77g, 91%) as a yellow oil. - TLC (*n*-hexane/ethyl acetate =1:1): Rf = 0.56. - 1H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.24 (m, 10H, Ph), 5.10 (s, 2H, CH2Ph(Cbz)), 5.01 (d, *J* = 8.4 Hz, 1H, H-1), 4.86 (d, *J* = 10.7 Hz, 1H, CHPh), 4.61 (d, *J* = 10.7 Hz, 1H, CHPh), 4.44 (d, *J* = 11.7 Hz, 1H, H-6), 4.30 (dd, *J* = 11.4, 9.3 Hz, 1H, H-3), 4.22 (dd, *J* = 12.0, 4.4 Hz, 1H, H-6'), 3.91 – 3.77 (m, 2H, H-2, H-7), 3.67 (ddd, *J* = 9.6, 4.3, 2.2 Hz, 1H, H-5), 3.52 (ddd, *J* = 18.9, 9.5, 7.3 Hz, 2H, H-7', H-4), 3.17 (dtt, *J* = 19.7, 13.2, 6.5 Hz, 2H, H-9, H-9'), 2.06 (s, 3H, CH3CO), 2.00 (s, 6H, 2 CH3 (NDMM)), 1.78 – 1.61 (m, 2H, H-8, H-8'). - 13C NMR (100 MHz, $CDCl_3$) δ 170.61 (2 CO (NDMM)), 156.38 (CO (NHCbz), 137.61 (C-NDMM), 136.86 (C-Ar), 128.60, 128.48, 128.37, 128.31, 128.07, 128.03 (Ph), 98.36 (C-1), 77.44 (C-4), 74.97 (CH2Ph), 73.86 (C-5), 66.97 (C-7), 66.51 (CH2Ph (NHCbz), 63.56 (C-3), 62.49 (C-6), 54.26 (C-2), 37.90 (C-9), 29.45 (C-8), 20.78 (CH3CO), 8.79 (2 CH3 (NDMM)). - HRMS-Q-TOF (positive mode); *m/z:* 658.2373 [M + Na]

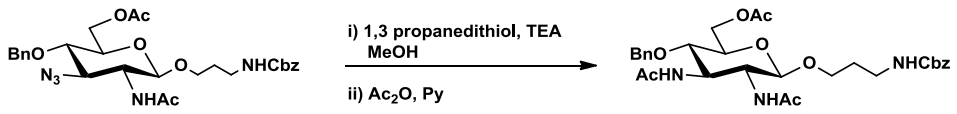
N-(Benzyloxycarbonyl)aminopropyl-6-O-acetyl-4-O-benzyl-2,3-dideoxy-2-acetamido-3-azido-β-D-glucopyranoside (**10**)



9 (1.77 g, 2.79 mmol) was dissolved in a dioxane:H_2O mixture (4:1, 10 mL), NaOH powder (2.80 g, 69.7 mmol) was added and let stirred for 24h at RT. Than the pH was adjusted at 5 by adding 1.0M HCl, and the solution was stirred for other 24h. After completion of the reaction, it was neutralized with potassium carbonate and dried well in vacuo. The residue was treated with pyridine (20 mL), acetic anhydride (10 mL) and stirred at RT for 16h. The solution was co-evaporated with toluene in vacuo. The residue was dissolved DCM (30 mL), and washed successively with HCl 5% (20 mL), a saturated solution of $NaHCO_3$ (20 mL), water (20 mL), dried over sodium sulfate and dried in vacuo. The residue was purified by flash chromatography (toluene/ethyl acetate = 6:4) to yield **10** (1.25g, 79%) as a yellow oil. - TLC (*n*-hexane/ethyl acetate =1:1): Rf = 0.27. - 1H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.16 (m, 10H, Ph), 6.52 (d, *J* = 7.8 Hz, 1H, NHAc), 5.20 – 5.08 (m, 2H, CH2Ph (Cbz)), 5.05 (s, 1H, NHCbz), 4.88 (d, *J* = 10.8 Hz, 1H, CHPh), 4.59 (d, *J* = 10.8 Hz, 1H, CHPh), 4.49 (d, *J* = 8.2 Hz, 1H, H-1), 4.36 (dd, *J* = 11.9, 1.7 Hz, 1H, H-6), 4.20 (dd, *J* = 12.0, 4.5 Hz, 1H, H-6'), 3.98 – 3.88 (m, 1H, H-7), 3.78 (t, *J* = 9.9 Hz, 1H, H-3), 3.54 (ddd, *J* = 13.8, 13.1, 6.1 Hz, 3H, H-2, H-5, H-9), 3.47 – 3.34 (m, 2H, H-4, H-7'), 3.19 – 3.05 (m, 1H, H-9'), 2.05 (s, 3H, CH3CO), 2.04 (s, 1H, CH3CO (NHAc)), 1.81 (ddd, *J* = 14.2, 9.5, 4.6 Hz, 1H, H-8), 1.65 (dd, *J* = 13.2, 9.1 Hz, 1H, H-8').^{13}C NMR (100 MHz, $CDCl_3$) δ 171.09 (COCH3-NHAc), 170.62 (CH3CO-Ac), 156.79 (CO (NHCbz), 137.19 (C-Ar), 136.69 (C-Ar), 128.60, 128.56, 128.31, 128.28, 128.19, 128.06 (Ph), 100.69 (C-1), 76.59 (C-4), 74.83 (CH2Ph), 73.70 (C-5), 66.81 (CH2Ph (NHCbz),66.71 (C-7), 66.64 (C-3), 62.74 (C-6), 55.49 (C-2), 37.33 (C-9), 29.74 (C-8), 23.25 (CH3CO (NHAc)), 20.79

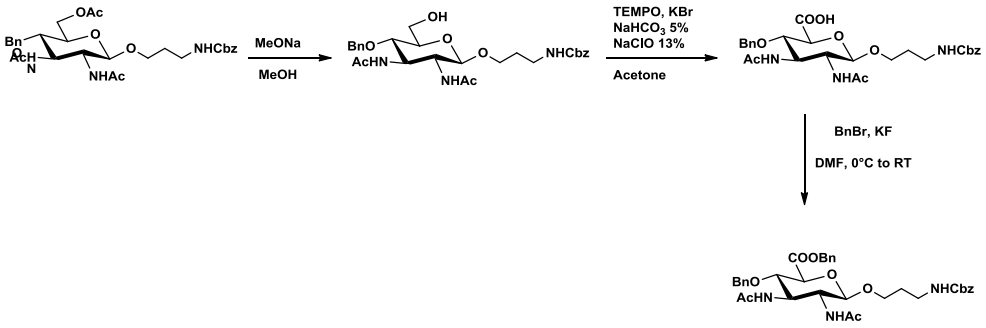
(CH3CO). - HRMS-Q-TOF (positive mode); *m/z:* 592.2842 [M + Na].

N-(Benzyloxycarbonyl)aminopropyl-6-O-acetyl-4-O-benzyl-2,3-dideoxy-2,3-diacetamido-β-D-glucopyranoside (**11**)



10 (1.25 g, 2.20 mmol) was dissolved in dry MeOH (15 mL) under N_2 atmosphere. Triethylamine (890 µL, 6.60 mmol) and 1,3-propanedithiol (660 µL, 6.60 mmol) were added in sequence and the solution was stirred for 24h at RT. After completion of the reaction the solvent was removed in vacuo. The residue was treated in pyridine (15 mL) with acetic anhydride (10 mL) and stirred at RT for 24h. The solution was coevaporated with toluene in vacuo. The residue was purified by flash chromatography (dichloromethane/methanol = 98:2) to yield **11** (1.16g, 91%) as a yellow oil. - TLC (dichloromethane/methanol = 95:5): Rf =0.15 - 1H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.25 (m, 10H, Ph), 7.17 (d, *J* = 8.1 Hz, 1H, NHAc (2)), 6.33 (d, *J* = 8.8 Hz, 1H, NHAc (3)), 5.21 – 5.02 (m, 3H, CH2Ph, NH, (Cbz)), 4.57 (dd, *J* = 24.9, 11.1 Hz, 2H, CH2Ph), 4.35 (dd, *J* = 11.9, 1.9 Hz, 1H, H-6), 4.23 (m, 2H, H-6', H-1), 4.15 (dd, *J* = 14.8, 5.5 Hz, 1H, H-3), 3.95 – 3.82 (m, 2H, H-2, H-3), 3.65 – 3.49 (m, 2H, H-5, H-9), 3.42 (t, *J* = 9.4 Hz, 1H, H-4), 3.38 – 3.29 (m, 1H, H-7'), 3.15 – 3.04 (m, 1H, H-9'), 2.06 (s, 3H, CH3CO), 1.97 (s, 3H, CH3CO (NHAc)), 1.92 (s, 3H, CH3CO (NHAc)), 1.78 (dd, *J* = 9.5, 5.1 Hz, 1H, H-8), 1.60 (dd, *J* = 12.1, 8.0 Hz, 1H, H-8'). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.96, 171.40 (2 CO (NHAc), 170.65 (CO (CH3CO), 156.88 (CO (NHCbz), 137.48 (C-Ar), 136.56 (C-Ar), 128.59, 128.53, 128.37, 128.20, 128.10 (Ph), 101.82 (C-1), 76.53 (C-4), 74.39 (CH2Ph), 74.23 (C-5), 66.89 (CH2Ph (NHCbz)), 66.32 (C-7), 63.16 (C-6), 56.03 (C-3), 54.13 (C-2), 36.96 (C-9), 29.86 (C-8), 23.37, 22.90 (2 CH3CO (NHAc)), 20.83 (CH3CO). - ESI (positive mode); *m/z:* 608.6 [M + Na].

Benzyl N-(Benzyloxycarbonyl)aminopropyl-4-O-benzyl-2,3-dideoxy-2,3-diacetamido-β-D-glucopyranuronate (**12**)



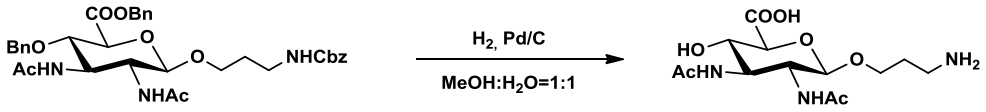
11 (1.16 g, 2.00 mmol) was dissolved in dry MeOH (10 mL) under N_2 atmosphere. A catalytic amount of a solution 0.1M MeONa in MeOH was added, and after 1h the reaction was neutralized with Amberlite IR-120. The resin was filtered off, and solvent evaporated in vacuo.

The crude was dissolved in acetone (15mL), and the solution was cooled at 0°C. Then were added successively: $NaHCO_3$ 5% in water (8.8 mL), KBr (475 mg, 4.00 mmol) and TEMPO (437 mg, 2.80 mmol). After 10' at 0°C NaClO 13% (0.74 mL) was added. After 30' TLC analysis (DCM:MeOH=95:5) shown the disappearance of the starting material, and a spot at Rf=0. The solvent was removed in vacuo, co-evaporated with toluene.

The residue was redissolved in dry DMF (15 mL) under N_2 atmosphere, and after the addition of KF (1.16 mg, 20 mmol) let stir for 5' at 0°C. Then BnBr (1.18 mL, 10 mmol) was added, letting warm at RT. After 16h. the solvent was removed in vacuo, the crude dissolved in DCM (20 mL) and washed with water (10 mL). The aqueous phase was extracted with DCM (3 X 10 mL). The combined organic layers were dried with sodium sulfate and evaporated in vacuo. The residue was purified by flash chromatography (dichloromethane/methanol = 98:2) to yield **12** (1.03g, 80%) as a white solid. - TLC (dichloromethane/methanol = 95:5): Rf =0.28. - ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.18 (m, 15H, Ph), 7.14 (d, *J* = 8.1 Hz, 1H,

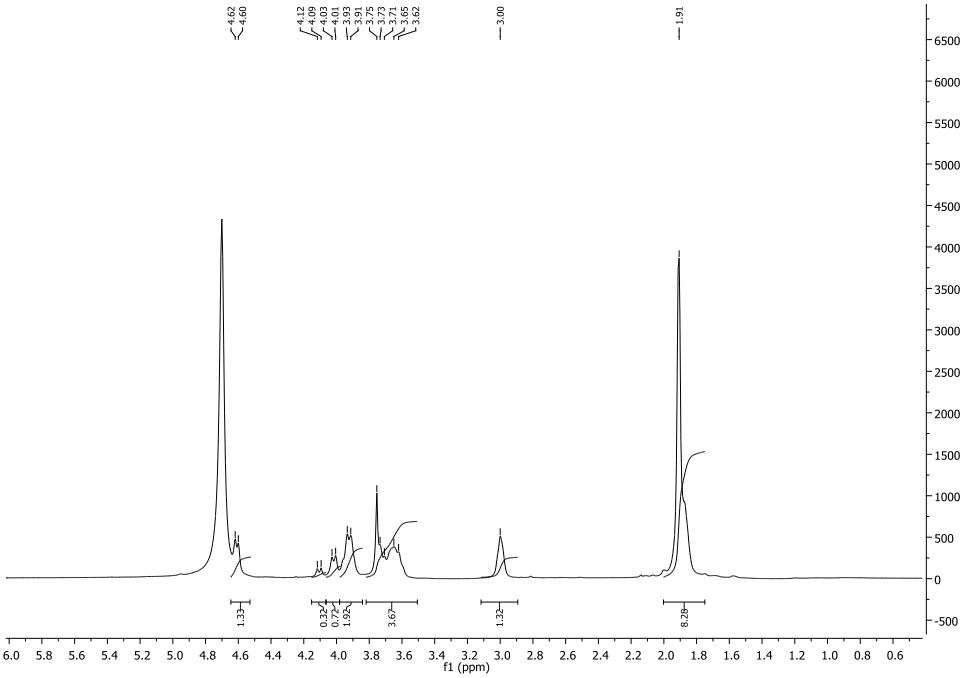
NHAc (2)), 6.30 (d, *J* = 8.6 Hz, 1H, NHAc (3)), 5.18 (q, *J* = 12.3 Hz, 2H, CH2Ph (COOBn)), 5.11 (s, 3H, CH2Ph, NH (Cbz)), 4.56 – 4.44 (m, 2H, CH2Ph), 4.36 (d, *J* = 6.8 Hz, 1H, H-1), 4.26 – 4.08 (m, 2H, H-3, H-5), 3.94 (dd, *J* = 16.3, 7.4 Hz, 2H, H-7, H-2), 3.82 (t, *J* = 8.0 Hz, 1H, H-4), 3.48 (dt, *J* = 17.4, 6.7 Hz, 1H, H-9), 3.37 – 3.26 (m, 1H, H-7'), 3.17 – 3.02 (m, 1H, H-9'), 1.92 (s, 3H, CH3CO (NHAc)), 1.85 (s, 3H, CH3CO (NHAc)), 1.82 – 1.70 (m, 1H, H-8), 1.68 – 1.54 (m, 1H, H-8'). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.32, 170.94 (2 CO (NHAc)), 168.77 (COOBn), 156.82 (CO (NHCbz)), 137.45, 136.59, 135.01 (3 C-Ar), 128.64, 128.60, 128.56, 128.51, 128.44, 128.23, 128.18, 127.97 (Ph), 101.65 (C-1), 76.94 (C-4), 75.08 (C-5), 73.79 (CH2Ph (NHCbz)), 67.36 (CH2Ph (COOBn)), 66.86 (CH2Ph (NHCbz)), 66.62 (C-7), 53.40 (C-3), 52.52 (C-2), 37.02 (C-9), 29.78 (C-8), 23.26, 22.94 (2 CH3CO (NHAc)). - HRMS-Q-TOF (positive mode); *m/z:*670.2637 [M + Na].

Aminopropyl-2,3-dideoxy-2,3-diacetamido-β-D-glucopyranuronic acid (**13**)

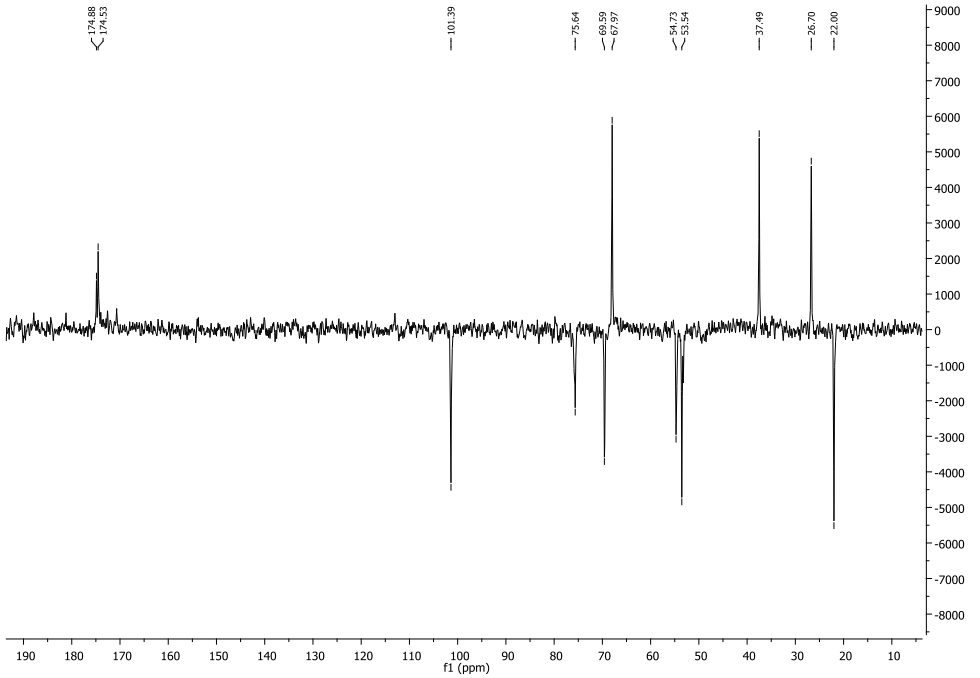


12 (1.03g, 1.6 mmol)was dissolved in MeOH:H_2O=1:1 (5mL:5mL), and a catalytic amount of Pd/C was added. The solution was put under H_2 atmosphere for 24h. The mixture was filtered off over a Celite pad and solvent removed under reduced pressure to yield **13** (0.53g, 100%) as a pale yellow solid. - ^{1}H NMR (400 MHz, D_2O) δ 4.63 (d, *J* = 8.4 Hz, 1H, H-1), 4.06 (d, *J* = 9.8 Hz, 1H, H-5), 4.00 – 3.87 (m, 2H, H-3, H-7), 3.80 – 3.56 (m, 3H, H-7', H-2, H-4), 3.02 (m, 2H, H-9, H-9'), 1.91 (b, 8H, CH3CO (NHAc), H-8, H-8'). - ^{13}C NMR (100 MHz, D_2O) δ 174.88 (CO (NHAc)), 174.56 (CO (NHAc)), 172.33 (COOH), 101.42 (C-1), 75.68 (C-5), 69.60 (C-4), 68.01 (C-7), 54.75 (C-3), 53.57 (C-2), 37.52 (C-9), 26.73 (C-8), 22.03 (2 CH3CO (NHAc)). - HRMS-Q-TOF (positive mode); *m/z:* 356.1548 [M + Na].

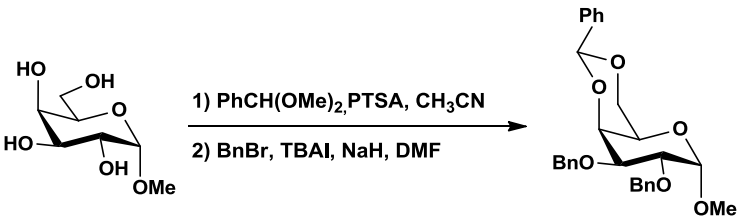
^{1}H-NMR compound **13**



^{13}C-NMR compound **13**



Methyl 2,3-di-O-benzyl-4,6-O-Benzylidene-α-D-galactopyranoside (**14**)

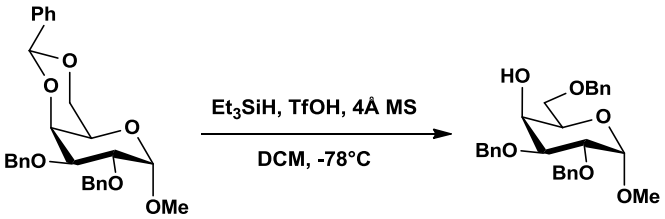


To a suspension of α-methyl galattose (1.00g, 5.2 mmol) in CH_3CN (25 mL), benzaldehyde dimethyl acetale (1.2 mL, 7.8 mmol) and a catalytic amount of p-toluenesulfonic acid were added. The reaction mixture was stirred at room temperature for 24h, then neutralized with TEA and evaporated in vacuo. The crude was purified by crystallization from ethyl acetate and *n*-hexane.

This compound was dissolved in dry DMF (25mL) under N_2 atmosphere BnBr (1.6mL, 13.5mmol) and TBAI (1.9g, 5.2mmol) were added. Then NaH (0.3g, 12.48 mmol) was added in small portion ensuring that the solution doesn't heat. After 2h the reaction was quenched adding MeOH, and the solvent removed in vacuo. The residue was dissolved in DCM (50 mL) and washed with brine (2 X 30mL), dried and evaporated. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 4:1) to yield **14** (2.36g, 98%) as a white solid. - TLC (*n*-hexane/ethyl acetate =1:1): Rf = 0.84.

The spectroscopic data were in agreement with those reported in literature.

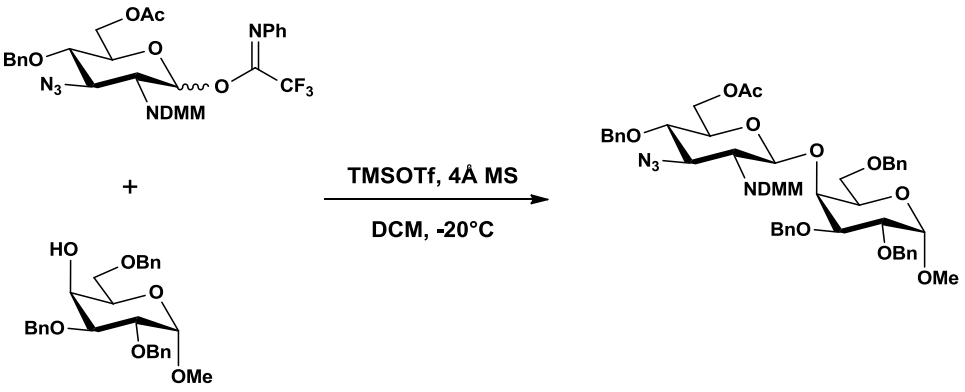
Methyl 2,3,4-tri-O-benzyl-α-D-galactopyranoside (**15**)



14 (2.36g, 5.1 mmol) was dissolved in dry DCM (40mL) under N_2 atmosphere. 3Å MS (3.0g) were added and stir for 40' at RT. Et_3SiH (2.6mL, 16.32.mmol) was added, the solution cooled at -78°C and then TfOH (1.26 mL, 14.3 mmol) was added dropwise. After 2h the reaction was quenched adding TEA until neutral pH and filtered on celite pad. The solvent was removed under reduced pressure, the crude was redissolved in DCM (50mL) washed with a saturated solution of $NaHCO_3$ (30mL) and water (30mL). The organic phase was dried with sodium sulfate and the solvent removed in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 4:1) to yield **15** as a yellow oil. (1.95g, 82%). TLC (*n*-hexane/ethyl acetate =7:3): Rf = 0.51.

The spectroscopic data were in agreement with those reported in literature.

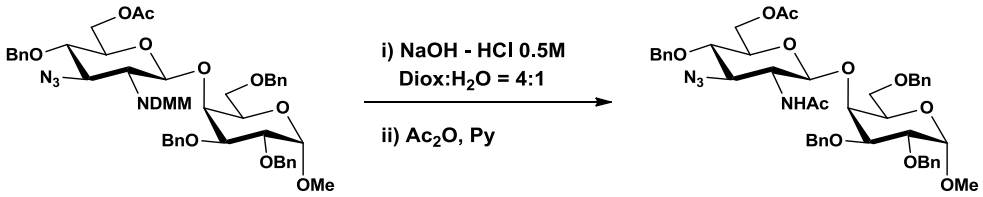
Methyl 6-O-acetyl-4-O-benzyl-2,3-dideoxy-3-azido-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-galactopyranoside (**16**)



A mixture of compound **8** (103 mg, 0.17 mmol) and compound **15** (65.7 mg, 0.14 mmol) was dissolved in dry DCM (8 mL) under Argon atmosphere. 4Å molecular sieves (200 mg) were added, and the suspension was stirred for 40' at RT. Then it was cooled at -20°C and a 0.1 M solution of TMSOTf (0.17 mL) was added dropwise. After 2h the reaction was quenched with TEA until neutral pH, than filtered on celite pad. The solvent was removed under reduced pressure. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 4:1) to yield **16** (67mg, 54%) as a yellow oil. - TLC (*n*-hexane/ethyl acetate =7:3): Rf = 0.34. - 1H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.13 (m, 20H, Ph), 5.11 (d, *J* = 8.2 Hz, 1H, H-1A), 4.88 (d, *J* = 10.6 Hz, 1H, CHPh), 4.68 – 4.52 (m, 6H, H-1B, H-3A, 2 CHPh, CH2Ph), 4.50 (d, *J* = 2.9 Hz, 2H, CH2Ph), 4.44 (d, *J* = 11.5 Hz, 1H, CHPh), 4.39 – 4.30 (m, 1H, H-6A), 4.16 (dt, *J* = 14.3, 5.5 Hz, 1H, H-6A'), 3.92 (dt, *J* = 14.0, 7.1 Hz, 1H, H-2A), 3.79 – 3.66 (m, 2H, H-4B, H-5B), 3.66 – 3.44 (m, 6H, H-5A, H-4A, H-2B, H-3B, 2 H-6B,), 3.33 (s, 3H, OCH3), 2.03 (s, 3H, CH3CO), 1.85 (s, 6H, 2 CH3 (NDMM)). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.38 (COCH3), 156.60 (), 138.32 (), 128.61, 128.43, 127.87, 127.80, 127.58, 127.45, 127.25 (Ph), 99.51(C-1A), 98.27 (C-1B), 77.54 - 77.18 (C-2B, C-3B, C-5A, C-4A), 74.90,

73.94, 73.43 (3 CH2Ph), 73.30 (C-5B), 73.09 (CH2Ph), 70.11 (C-6B), 69.13 (C-4B), 62.98 (C-3A), 62.40 (C-6A), 55.19 (OCH3), 54.51 (C-2A), 20.77 (CH3CO), 10.53 (2 CH3 (NDMM)). -- EI-MS (positive mode); *m/z:* .913.3 [M + Na].

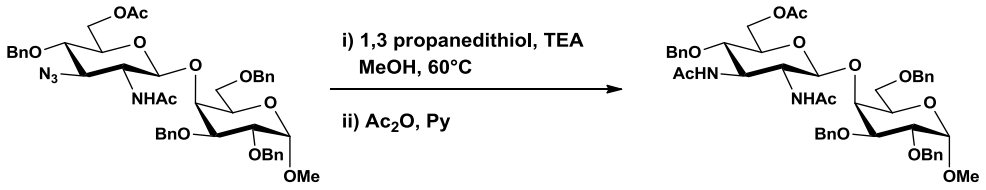
Methyl 6-O-acetyl-4-O-benzyl-2,3-dideoxy-2-acetamido-3-azido-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-galactopyranoside (**17**)



16 (67 mg, 0.075 mmol)was dissolved in a dioxane:H_2O mixture (4:1 = 10 ml), NaOH (75 mg, 1.88mmol)powder was added and let stirred for 24h at RT. Than the pH was adjusted at 5 by adding 1N HCl, and the solution was stirred for other 24h. After completion of the reaction, it was neutralized with potassium carbonate and the solvent evaporated in vacuo. The residue was treated in pyridine (5 mL) with acetic anhydride (0.5 mL, 4.8 mmol) and stirred at RT for 24h. The solution was co-evaporated with toluene in vacuo. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 6:4) to yield **17** (35mg, 56%) as a yellow oil. - TLC (*n*-hexane/ethyl acetate =1:1): Rf = 0.47. - ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.24 (m, 20H, Ph), 5.84 (d, *J* = 7.9 Hz, 1H, NHAc), 4.90 (dd, *J* = 18.1, 10.3 Hz, 2H, 2 CHPh), 4.74 (m, 2H, H-1A, CHPh), 4.65 (dd, *J* = 10.9, 3.7 Hz, 2H, 2 CHPh), 4.59 – 4.52 (m, 4H, 3 CHPh, H-1B), 4.36 – 4.27 (m, 1H, H-6A), 4.14 (dd, *J* = 12.0, 3.4 Hz, 1H, H-6A'), 4.02 (d, *J* = 3.4 Hz, 1H, H-4B), 3.96 (m, 2H, H-3B, H-5B), 3.85 – 3.76 (m, 2H, H-2A, H-2B), 3.76 – 3.65 (m, 1H, H-6B), 3.65 – 3.52 (m, 1H, H-6B'), 3.45 – 3.40 (m, 2H, H-5A, H-4A), 3.39 (s, 3H, OCH3), 3.18 (dd, *J* = 10.7, 9.1 Hz, 1H, H-3A), 2.11 (s, 3H, CH3CO (NHAc)), 2.01 (s, 3H (CH3CO)). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.44 (COCH3 (NHAc)), 170.39 (COCH3 (CH3CO)), 138.40, 138.12, 137.66, 137.06 (C-Ar), 128.99, 128.88, 128.68, 128.62, 128.54, 128.36, 128.30, 128.00, 127.71, 127.56, 127.42 (Ph), 102.56 (C-1B), 97.78 (C-1A), 77.78, 77.74, 77.67 (C-4B, C-2B, C-5B), 75.68 (C-4), 75.04 (CH2Ph), 74.66 (CH2Ph), 74.15 (C-5A), 73.27, 72.97 (2 CH2Ph), 69.57

(C-6B), 68.87 (C-3B), 68.79 (C-3A), 62.41 (C-6A), 55.37 (OCH3), 54.37 (C-2A), 23.01 (CH3CO (NHAc)), 20.74 (CH3CO). –ESI-MS (positive mode); *m/z:* 847.5 [M + Na].

*Methyl 6-O-acetyl-4-O-benzyl-2,3-diacetamido-2,3-dideoxy-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-galactopyranoside (***18***)*

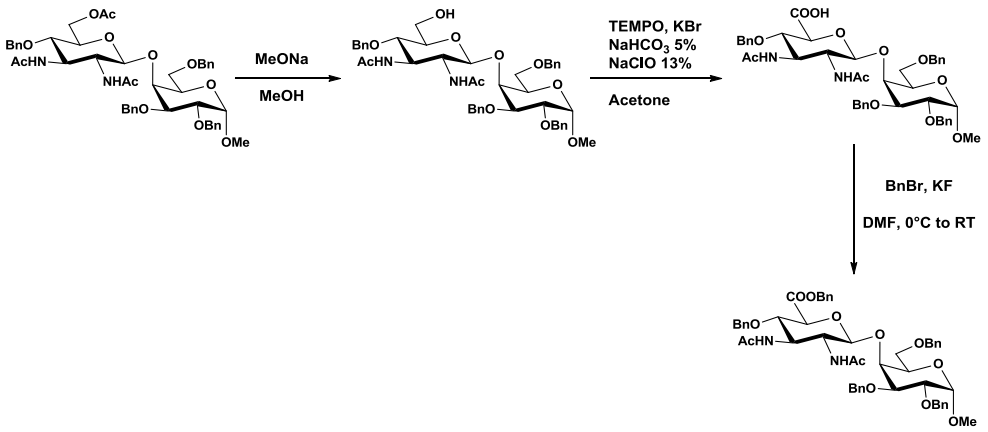


17 (35 mg, 0.042 mmol) was dissolved in dry MeOH (5 mL) under N_2 atmosphere. Triethylamine (41microL, 0.3 mmol) and 1,3-propanedithiol (30 microL, 0.3 mmol) were added in sequence and the solution warmed at 60°C for 24h. After completion of the reaction the solvent was removed in vacuo. The residue was treated in pyridine (5 mL) with acetic anhydride (0.273 mL, 2.7 mmol) and stirred at RT for 24h. The solution was co-evaporated with toluene in vacuo. The crude was purified by flash chromatography (DCM/MeOH 98:2) to yield **18** (23 mg, 65%) as yellow oil. Rf = 0.74 (DCM:MeOH = 9:1).

The residue was purified by flash chromatography (dichloromethane/methanol = 98:2) to yield **18** (23mg, 65%) as a yellow oil. - TLC (dichloromethane/methanol =9:1): Rf =0.74. - 1H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.09 (m, 20H, Ph), 6.49 (d, *J* = 6.8 Hz, 1H, NHAc), 6.20 (d, *J* = 8.3 Hz, 1H, NHAc), 5.10 (d, *J* = 12.2 Hz, 1H, CHPh), 4.85 (t, *J* = 10.5 Hz, 2H, 2 CHPh), 4.78 – 4.67 (m, 4H, H-1A, H-1B, 2 CHPh), 4.61 (d, *J* = 11.6 Hz, 1H, CHPh), 4.50 – 4.34 (m, 4H, 2 CHPh, H-1B, H-6A), 4.13 – 4.06 (m, 2H, H-6A', H-4B), 4.06 – 3.99 (m, 1H, H-3A), 3.97 (dd, *J* = 9.9, 3.1 Hz, 1H, H-3B), 3.94 – 3.86 (m, 2H, H-2A, H-5B), 3.79 (dd, *J* = 9.8, 3.5 Hz, 1H, H-2B), 3.74 (dd, *J* = 9.3, 7.0 Hz, 1H, H-6B), 3.67 (t, *J* = 9.2 Hz, 1H, H-4A), 3.55 (dd, *J* = 9.3, 5.5 Hz, 1H, H-6B), 3.38 (s, 3H, OCH3), 1.82 (s, 3H, CH3CO (NHAc)), 1.56 (s, 3H, CH3CO (NHAc)), 1.28 (s, 3H, CH3CO). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.44, 170.72 (COCH3 (NHAc)), 170.39 (COCH3 (CH3CO)), 138.40, 138.12, 137.66, 137.06 (C-Ar), 128.99, 128.88, 128.68, 128.62,

128.54, 128.36, 128.30, 128.00, 127.71, 127.56, 127.42 (Ph), 102.56 (C-1B), 97.78 (C-1A), 77.78, 77.74, 77.67 (C-4B, C-2B, C-5B), 75.68 (C-4), 75.04 (CH2Ph), 74.66 (CH2Ph), 74.15 (C-5A), 73.27, 72.97 (2 CH2Ph), 69.57 (C-6B), 68.87 (C-3B), 56.18 (C-3A), 62.41 (C-6A), 55.37 (OCH3), 54.37 (C-2A), 23.01, 22.55 (CH3CO (NHAc)), 20.74 (CH3CO). – ESI-MS (positive mode); *m/z:* .863.7 [M + Na].

Methyl (benzyl-(4-O-benzyl-2,3-diacetamido-2,3-dideoxy-β-D-glucopyranosyluronate)-(1→4)-2,3,6-tri-O-benzyl-α-D-galactopyranoside (**19**)



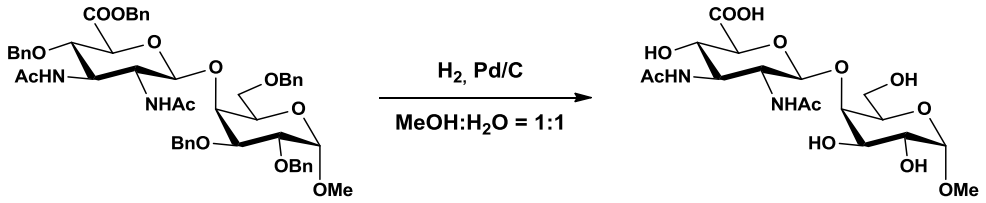
18 (23 mg, 0.027 mmol) was dissolved in dry MeOH (5 mL) under N_2 atmosphere. A catalytic amount of a solution 0.1M of MeONa in MeOH was added, and after 1h the reaction was neutralized with Amberlite IR-120. The resin was filtered off, and solvent evaporated in vacuo.

The crude was dissolved in acetone (4 mL), and the solution was cooled at 0°C. Then were added in successively $NaHCO_3$ 5% (1.3 mL), KBr (6.5 mg, 0.05 mmol) and TEMPO (6.0 mg, 0.04 mmol). After 10' at 0°C NaClO 13% solution (0.102 mL) was added. After 3h a TLC (DCM:MeOH=95:5) shown the disappearance of the starting material, and a spot at Rf=0. The solvent was removed in vacuo, stripping with toluene.

The crude was redissolved in dry DMF under N_2 atmosphere and BnBr (28 microL, 0.27 mmol) was added. The solution was cooled at 0°C, and KF (17 mg, 0.14 mmol) was added, letting warmed at RT. After stirring for 16h, the solvent was removed in vacuo, the crude dissolved in DCM (5 mL) and washed with water (3 mL). The aqueous phase was extracted with DCM (3 X 5 mL). The combined organic layers were separated, dried with sodium sulfate and evaporated in vacuo. The residue was purified by flash chromatography (dichloromethane/methanol =

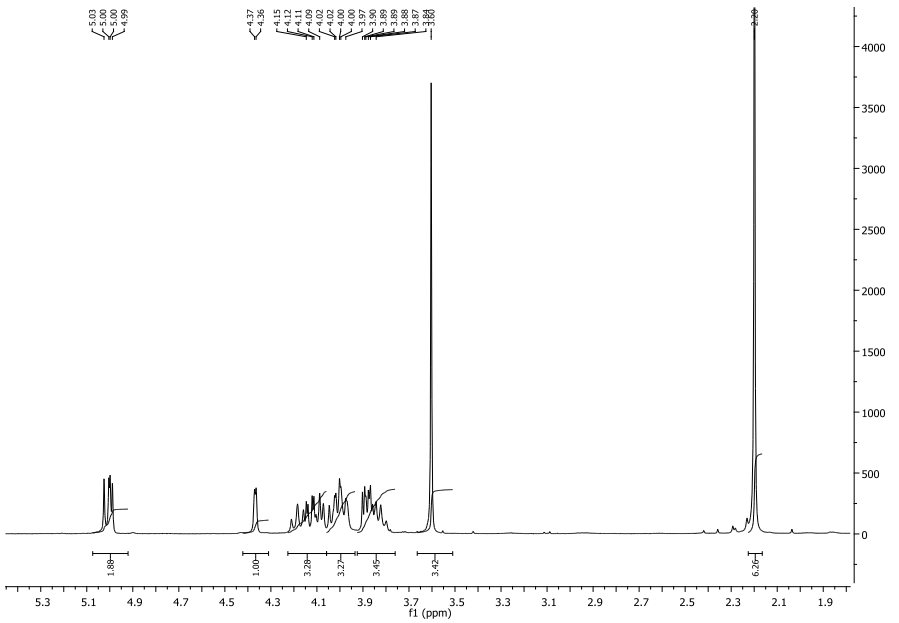
98:2) to yield **19** (20mg, 81%) as a white solid. - TLC (dichloromethane/methanol =95:5): Rf =0.35. - ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.06 (m, 25H, Ph), 6.50 (d, *J* = 6.9 Hz, 1H, NHAc), 6.18 (d, *J* = 8.2 Hz, 1H, NHAc), 5.10 (d, *J* = 12.2 Hz, 1H, CHPh), 4.85 (t, *J* = 10.6 Hz, 2H, 2 CHPh), 4.78 – 4.66 (m, 4H, H-1A, H-1B, 2 CHPh), 4.61 (d, *J* = 11.6 Hz, 1H, CHPh), 4.47 (d, *J* = 11.7 Hz, 1H, CHPh), 4.45 (s, 2H, CH2Ph), 4.39 (d, *J* = 11.6 Hz, 1H, CHPh), 4.10 (dd, *J* = 9.0, 5.6 Hz, 2H, H-4B, H-5A), 4.06 – 3.99 (m, 1H, H-3A), 3.97 (dd, *J* = 9.9, 3.1 Hz, 1H, H-3B), 3.94 – 3.83 (m, 2H, H-2A, H-5B), 3.82 – 3.75 (m, 1H, H-2B), 3.75 – 3.70 (m, 1H, H-6B), 3.67 (t, *J* = 9.2 Hz, 1H, H-4A), 3.55 (dd, *J* = 9.4, 5.5 Hz, 1H, H-6B), 3.37 (s, 3H, OCH3), 1.82 (s, 3H, CH3CO), 1.56 (s, 3H, CH3CO). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.41, 170.72 (COCH3 (NHAc), 168.31 (CO (COOBn)), 138.32, 138.00, 137.40, 137.09, 134.86 (C-Ar), 129.14, 128.85, 128.57, 128.48, 128.43, 128.39, 128.36, 128.03, 127.90, 127.75, 127.63, 127.59 (Ph), 103.40 (C-1B), 97.89 (C-1A), 78.14 (C-4A), 78.04 (C-4B), 77.83 (C-2B), 77.68 (C-3B), 76.12 (C-5A), 74.95, 74.21, 73.28, 73.15 (CH2Ph), 68.65 (C-6B), 68.42 (C-5B), 67.18 (CH2Ph (COOBn)), 56.18 (C-3A), 55.43 (OCH3), 53.59 (C-2A), 23.34, 22.55 (2 CH3CO (NHAc)). - - EI-MS (positive mode); *m/z:* 925.9 [M + Na].

*Methyl-2,3-diacetamido-2,3-dideoxy-β-D-glucopyranosyluronic-(1→4)-α-D-galactopyranoside (***20***)*

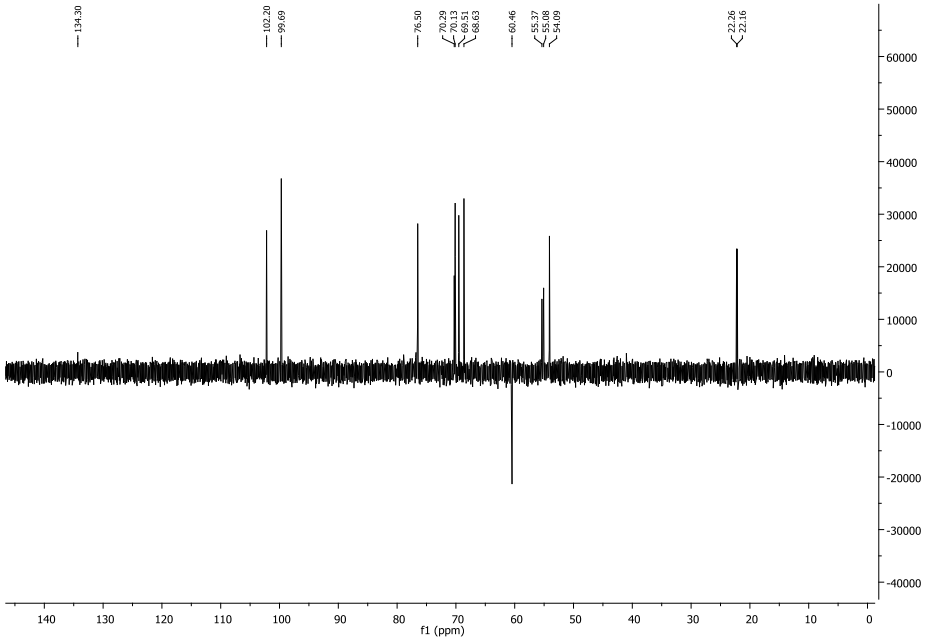


19 (19.7 mg, 0.021 mmol) was dissolved in MeOH:H_2O=1:1 (6 mL), and a catalytic amount of Pd/C was added. The solution was put under H_2 atmosphere for 24h. The mixture was filtered over a Celite pad and solvent removed under reduced pressure to yield **20** (9.5mg, 100%) as a pale yellow solid.(NMR analysis acquired at 323K) - ^{1}H NMR (400 MHz, D_2O) δ 5.00 (dd, 2H, H-1A, H-1B), 4.37 (d, *J* = 2.6 Hz, 1H, H-4B), 4.23 – 4.06 (m, 3H, H-3A, H-5A, H-5B), 4.06 – 3.94 (m, 3H, H-6B, H-3B, H-2A), 3.93 – 3.76 (m, 3H, H-6B', H-2B, H-4A), 3.60 (s, 3H, OCH3), 2.20 (s, 6H, 2 CH3CO (NHAc)). - ^{13}C NMR (100 MHz, D_2O) δ 134.32 (COOH), 102.20 (C-1B), 99.69 (C-1A), 76.50 (C-4B), 70.29 (C-5A), 70.13 (C-5B), 69.51 (C-3B), 68.63 (C-4A), 60.46 (C-6B), 55.37 (OCH3), 55.08 (C-3A), 54.09 (C-2A), 22.26 (2 CH3CO (NHAc)). – ESI-MS (positive mode); *m/z:* .475.4 [M + Na].

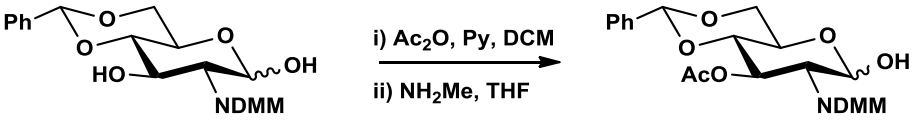
^{1}H-NMR compound **20**



^{13}C-NMR compound **20**



*3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (**21**)*



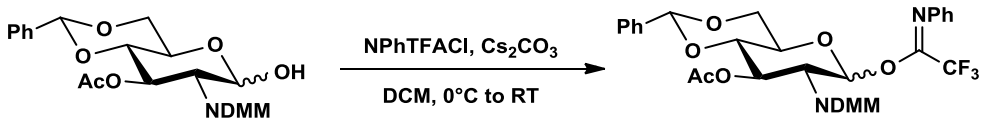
1 (1.0g, 2.6 mmol) was dissolved in dry DCM (15mL) under N_2 atmosphere. Pyridine (1.7 mL, 20.8 mmol), acetic anhydride (1.1 mL, 10.4 mmol) and a catalytic amount of DMAP were added. After 2h the reaction was diluted with DCM, and washed successively with HCl 5%, a saturated solution of $NaHCO_3$ and water. The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The residue was used in the next step.

It was dissolved in dry THF (25 mL) under N_2 atmosphere. A 2.0 M solution of NH_2Me in MeOH was added (1.3 mL, 2.6 mmol). After 2h the starting material persist in the reaction mixture, so other 0.7 mL of NH_2Me solution were added, letting stir overnight. The solvent was removed under reduced pressure and the residues redissolved in DCM (30mL). The organic layer was washed with a HCl 5% (15mL), and water (15mL). The organic phase was dried with sodium sulfate and the solvent removed under reduced pressure. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 7:3) to yield **21** (0.79g, 73%) as a yellow foam. - TLC (*n*-hexane/ethyl acetate =7:3): Rf = 0.25.

The spectroscopic data were in agreement with those reported in literature.[2]

[2] Dusan Hesek, Mijoon Lee, Ken-ichiro Morio, and Shahriar Mobashery, *J. Org. Chem.* **2004**, *69*, 2137-2146

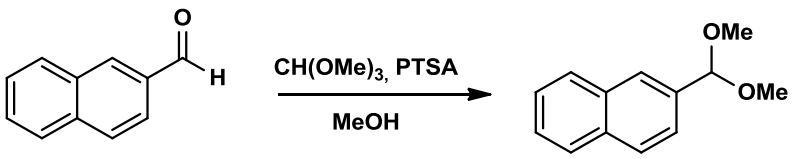
*3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside N-Phenyltrifluoroacetimidate (***22***)*



21 (0.79g, 1.9 mmol) was dissolved in dry DCM (20 ml) under N_2 atmosphere. The solution was cooled at 0°C in an ice bath. 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (0.46mL, 2.8 mmol) and cesium carbonate (0.91g, 2.8 mmol) were added in sequence. The reaction was warmed to RT and stirred for 2h, then filtered on a celite pad and evaporated in vacuo.

The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 9:1+ 1% TEA) to yield **22** (0.76g, 72%) as a yellow foam. - TLC (*n*-hexane/ethyl acetate =7:3): Rf = 0.40.

2-(dimethoxymethyl)naphthalene (**23**)

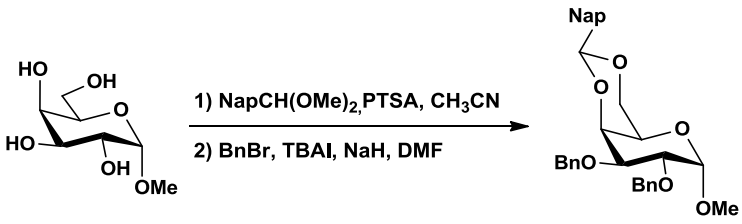


To a solution of 2-naphthaldehyde (15.6 g, 0.10 mol) in dry methanol (30 mL) trimethyl-orthoformate (15.6 mL, 0.15 mol, 1.5 equiv) and a catalytic amount of PTSA were added. The mixture was let stir 24h. The mixture was diluted with DCM (500 mL) and was washed with satd. $NaHCO_3$ solution (300mL) and water (300mL). The organic layer was dried and evaporated to yield **23** (18.1g, 0.09 mol) as a pale yellow oil. - TLC (*n*-hexane/ethyl acetate = 8:2): Rf = 0.67.

The spectroscopic data were in agreement with those reported in literature.[3]

[3] A. Borbàs et al. *Tetrahedron*, **2002**, *58*, 5723-5732

Methyl 2,3-di-O-benzyl-4,6-O-(2 naphtyl) methylene α-D-galactopyranoside (**24**)

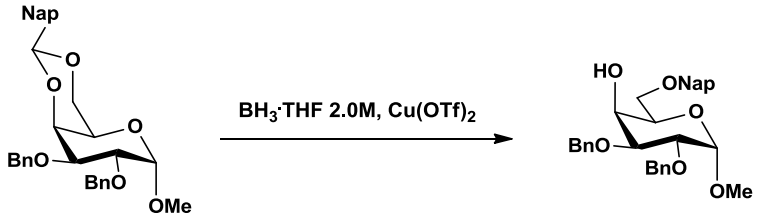


To a suspension of α-methyl galattoside (1.0g, 5.2 mmol) in CH_3CN (25mL), 2-naphthaldehyde dimethyl acetale (1.57g, 7.8 mmol) and a catalytic amount of p-toluenesulfonic acid were added. The reaction mixture was stirred at room temperature for 24h, then neutralized with TEA and evaporated in vacuo. The residue was purified by crystallization from ethyl acetate and *n*-hexane.

This compound was dissolved in dry DMF (25mL) under N_2 atmosphere BnBr (1.6mL, 13.5mmol) and TBAI (1.9g, 5.2mmol) were added. Then NaH (0.3g, 12.48 mmol) was added in small portion ensuring that the solution doesn't heat. After 2h the reaction was quenched adding MeOH, and the solvent removed in vacuo. The residue was dissolved in DCM (50 mL) and washed with brine (2 X 30mL), dried and evaporated. The residue was purified by flash chromatography (n-hexane/ethyl acetate = 7:3) to yield **24** (2.53g, 95%) as a white solid. - TLC (n-hexane/ethyl acetate =1:1): Rf = 0.6.

The spectroscopic data were in agreement with those reported in literature.[3]

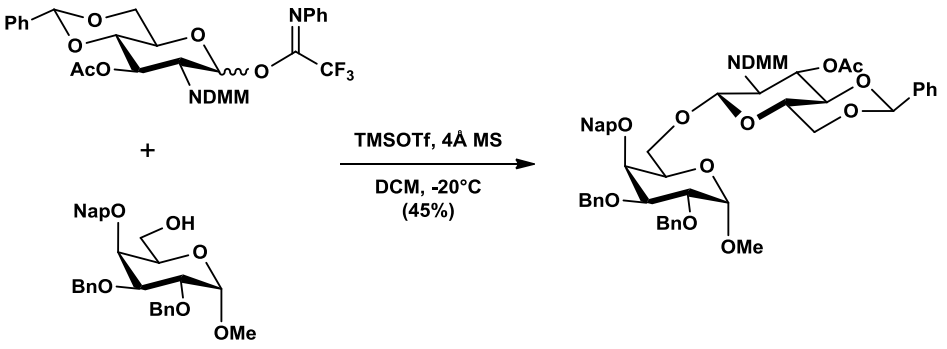
Methyl 2,3-di-O-benzyl-4-O-(2-naphtyl) methyl α-D-galactopyranoside (**25**)



24 (2.53g, 4.9 mmol) was dissolved in BH_3·THF complex 2.0M solution (25mL) and cooled at 0°C. A catalytic amount of $Cu(OTf)_2$:5% was added, the solution become dark. The temperature was allowed to warm at RT and let stir for 3h. The reaction was quenched with TEA and MeOH, and the solvent removed in vacuo. The residue was purified by flash chromatography (n-hexane/ethyl acetate = 1:1) to yield **25** (2.05g, 81%) as a colorless oil. - TLC (n-hexane/ethyl acetate =1:1): Rf = 0.21

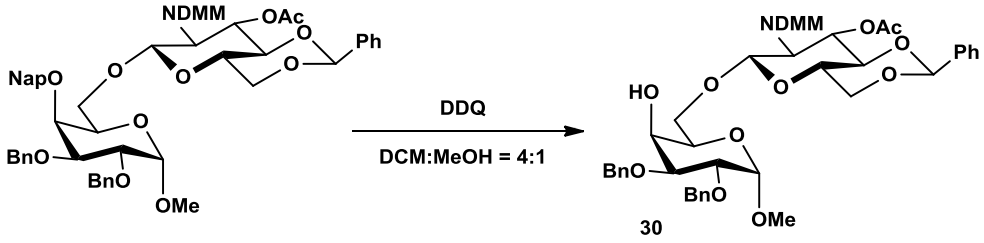
The spectroscopic data were in agreement with those reported in literature.[3]

Methyl 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-dimethylmaleimido-β- D-glucopyranosyl-(1→6)-2,3-dibenzyl-4-O-(2-naphtyl) methyl α-D-galactopyranoside(**26**)



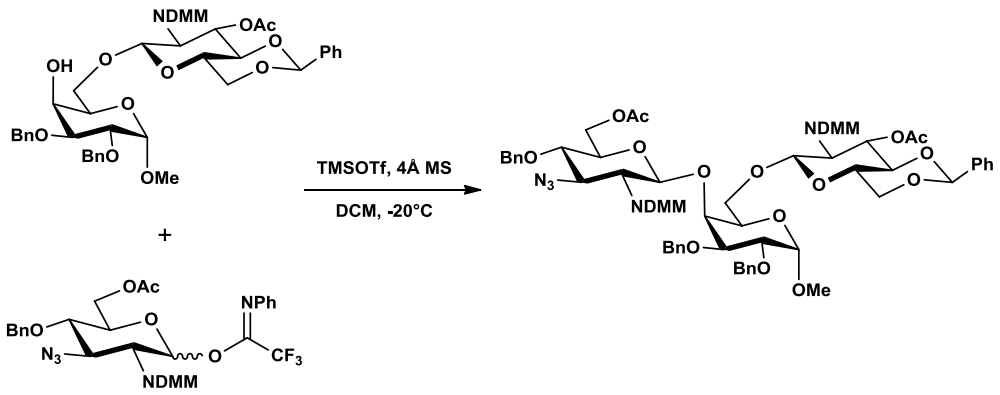
A mixture of compound **22** (114 mg, 0.2 mmol) and compound **25** (87 mg, 0.16 mmol) was dissolved in dry DCM (10 ml) under Argon atmosphere. 4Å molecular sieves (200 mg) were added, and the suspension was stirred for 40' at RT. Then it was cooled at -20°C and a 0.1 M solution of TMSOTf in DCM was added dropwise (0.2 mL). After 2h the reaction was quenched with TEA until neutral pH, than filtered on celite pad. The solvent was removed under reduced pressure. The residue was purified by flash chromatography (n-hexane/ethyl acetate = 7:3) to yield **26** (80mg, 53%) as a white foam. - TLC (n-hexane/ethyl acetate =7:3): Rf = 0.15. - ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.18 (m, 22H, Ph), 5.73 (t, *J* = 9.6 Hz, 1H, H-3C), 5.53 (s, 1H, CHPh), 5.31 (d, *J* = 8.4 Hz, 1H, H-1C), 4.81 (d, *J* = 12.0 Hz, 2H, 2 CHPh), 4.73 – 4.61 (m, 2H, 2 CHPh), 4.54 (d, *J* = 2.8 Hz, 1H, H-1B), 4.44 – 4.33 (m, 1H, H-5B), 4.11 – 4.01 (m, 1H, H-2C), 4.01 – 3.93 (m, 1H, H-5C), 3.82 (m, 4H, H-6B, H-6'B, H-6C, H-2B), 3.72 (m, 3H, H-4B, H-6'B, H-3B), 3.26 (s, 3H, OCH3), 1.95 (ss, 9H, CH3CO, 2 CH3 (NDMM)). EI-MS (positive mode); *m/z:* 936.4[M + Na].

Methyl 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→6)-2,3-dibenzyl-α-D-galactopyranoside (**27**)



To a solution of **27** (80 mg, 0.086 mmol) in dry DCM:MeOH=4:1 (2.5 mL:0.5 mL), under N_2 atmosphere, was added DDQ (freshly crystallized from chloroform) (30.mg, 0.12 .mmol). After 2h since the starting material didn't disappear, another portion of DDQ was added. (30.mg, 0.12 mmol). After 1h the reaction was diluted with DCM (10 mL), and washed with a saturated solution of $NaHCO_3$ (3 X 10 mL). Organic phases were dried on Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (n-hexane/ethyl acetate = 6:4) to yield **27** (50.7mg, 80%) as a white foam. - TLC (n-hexane/ethyl acetate =1:1): Rf = 0.42. - ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.18 (m, 15H, Ph), 5.73 (t, *J* = 9.6 Hz, 1H, H-3C), 5.53 (s, 1H, CHPh), 5.31 (d, *J* = 8.4 Hz, 1H, H-1C), 4.81 (d, *J* = 12.0 Hz, 2H, 2 CHPh), 4.73 – 4.61 (m, 2H, 2 CHPh), 4.54 (d, *J* = 2.8 Hz, 1H, H-1B), 4.44 – 4.33 (m, 1H, H-5B), 4.11 – 4.01 (m, 1H, H-2C), 4.01 – 3.93 (m, 1H, H-5C), 3.82 (m, 4H, H-6B, H-6'B, H-6C, H-2B), 3.72 (m, 3H, H-4B, H-6'B, H-3B), 3.26 (s, 3H, OCH3), 1.95 (ss, 9H, CH3CO, 2 CH3 (NDMM)). EI-MS (positive mode); *m/z:* 796.3 [M + Na].

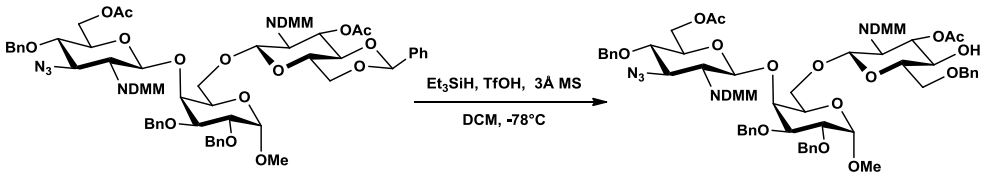
Methyl 6-O-acetyl-4-O-benzyl-2,3-dideoxy-3-azido-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→6)-2,3-dibenzyl-α-D-galactopyranoside (**28**)



A mixture of compound **8** (0.203 g, 0.33 mmol) and compound **27** (208 mg,.27 mmol) was dissolved in dry DCM (10 ml) under Argon atmosphere. 4Å molecular sieves (400.mg) were added, and the suspension was stirred for 40' at RT. Then it was cooled at -20°C and a 0.1 M solution of TMSOTf in DCM (0.208 mL) was added dropwise. After 2h the reaction was quenched with TEA until neutral pH, than filtered on celite pad. The solvent was removed under reduced pressure. The residue was purified by flash chromatography (n-hexane/ethyl acetate = 6:4) to yield **28** (162 mg, 50%) as a white foam. - TLC (n-hexane/ethyl acetate =1:1): Rf = 0.62. - ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.30 (m, 20H, Ph), 5.80 – 5.70 (m, 1H, H-3C), 5.52 (s, 1H, CHPh), 5.22 (d, *J* = 8.4 Hz, 1H, H-1C), 4.97 (d, *J* = 8.3 Hz, 1H, H-1A), 4.90 (d, *J* = 10.7 Hz, 1H, CHPh), 4.68 (d, *J* = 10.7 Hz, 1H, CHPh), 4.65 – 4.50 (m, 4H, H-6A, H-3A, CH2Ph), 4.42 (m, 3H, CHPh, H-1B, H-6C), 4.05 (dd, *J* = 11.9, 3.7 Hz, 1H, H-6'A), 3.98 (dd, *J* = 10.2, 8.4 Hz, 1H, H-2C), 3.86 (dd, *J* = 11.5, 8.4 Hz, 1H, H-2A), 3.81 – 3.42 (m, 11H, H-4A, H-5A, 2 H-6B, H-5B, H-4B, H-3B, H-2B, H-3C, H-4C, H-6'C), 3.39 (d, *J* = 2.7 Hz, 1H, H-5C), 3.15 (s, 3H, OCH3), 2.15 (s, 6H, 2 CH3CO), 1.94 (s, 12H, CH3 (NDMM)). - HRMS-Q-TOF

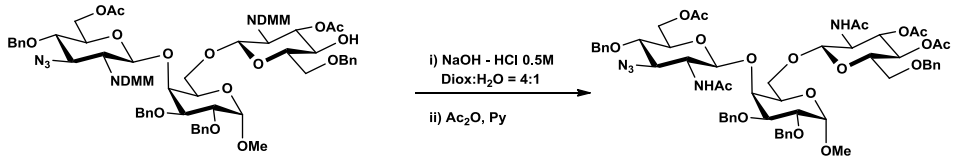
(positive mode); *m/z:* .1222.4551 [M + Na].

Methyl 6-O-acetyl-4-O-benzyl-2,3-dideoxy-3-azido-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-4-O-benzyl-2-deoxy-2-dimethylmaleimido-β- D-glucopyranosyl-(1→6)-2,3-dibenzyl-α-D-galactopyranoside (**29**)



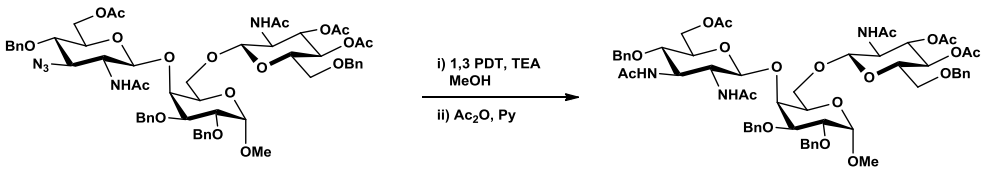
28 (162 mg, 0.135 mmol) was dissolved in dry DCM (10 mL) under N_2 atmosphere. 3Å MS (200 mg) were added and stir for 40' at RT. Et_3SiH (.ml, .mmol) was added, the solution cooled at -78°C and then (.ml, .mmol) TfOH was added dropwise. After 2h the reaction was quenched adding TEA until neutral pH and filtered on celite pad. The solvent was removed under reduced pressure, the crude was redissolved in DCM washed with a saturated solution of $NaHCO_3$ and water. The organic phase was dried with sodium sulfate and the solvent removed in vacuo. The residue was purified by flash chromatography (n-hexane/ethyl acetate = 6:4) to yield **29** (134 mg, 83%) as a white foam. - TLC (n-hexane/ethyl acetate =1:1): Rf = 0.54. - ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.21 (m, 19H), 5.53 (dd, *J* = 10.7, 8.1 Hz, 1H), 5.10 (d, *J* = 8.5 Hz, 1H), 4.95 (d, *J* = 8.4 Hz, 1H), 4.88 (d, *J* = 10.7 Hz, 1H), 4.68 – 4.48 (m, 8H), 4.41 (dd, *J* = 7.6, 3.9 Hz, 2H), 4.04 – 3.97 (m, 1H), 3.96 – 3.81 (m, 3H), 3.81 – 3.68 (m, 4H), 3.62 (dd, *J* = 9.9, 2.9 Hz, 2H), 3.54 – 3.42 (m, 4H), 3.39 (d, *J* = 2.4 Hz, 1H), 3.12 (s, 3H), 2.12 (s, 3H), 2.00 (s, 3H), 1.91 (s, 7H).

Methyl 6-O-acetyl-4-O-benzyl-2,3-dideoxy-2-acetamido-3-azido-β-D-glucopyranosyl-(1→4)-3,4-di-O-acetyl-4-O-benzyl-2-deoxy-2-acetamido-β–D-glucopyranosyl-(1→6)-2,3-dibenzyl-α-D-galactopyranoside (**30**)



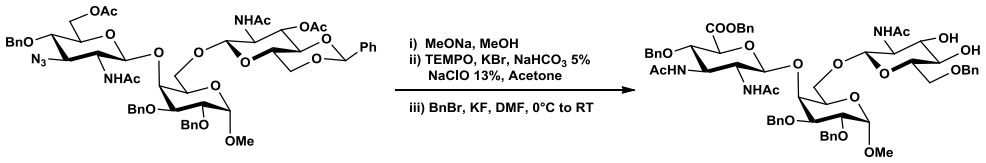
29 (134 mg, 0.11 mmol) was dissolved in a dioxane:H_2O mixture (4:1, 5ml). NaOH powder (220 mg, 5.5 mmol) was added and let stirred for 24h at RT. Than the pH was adjusted at 5 by adding 1N HCl, and the solution was stirred for other 24h. After completion of the reaction, it was neutralized with potassium carbonate and the solvent evaporated in vacuo. The residue was treated in pyridine (5mL) with acetic anhydride (1.1mL, 11 mmol) and stirred at RT for 24h. The solution was co-evaporated with toluene in vacuo. The residue was purified by flash chromatography (dichloromethane/methanol = 98:2) to yield **30** (102 mg, 84%) as a white foam. - TLC (dichloromethane/methanol = 95:5): Rf = 0.34.

Methyl 6-O-acetyl-4-O-benzyl-2,3-dideoxy-2,3-diacetamido-β-D-glucopyranosyl-(1→4)-3,4-di-O-acetyl-4-O-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2,3-dibenzyl-α-D-galactopyranoside (**31**)



30 (102 mg, 0.09 mmol) was dissolved in dry MeOH (5 mL) under N_2 atmosphere. Triethylamine (0.087 mL, 0.63 mmol) and 1,3-propanedithiol (0.065mL, 0.63 mmol) were added in sequence and the solution stirred at RT for 24h. After completion of the reaction the solvent was removed in vacuo. The residue was treated in pyridine (5 mL) with acetic anhydride (0.46 mL, 4.5 mmol) and stirred at RT for 24h. The solution was co-evaporated with toluene in vacuo. The residue was purified by flash chromatography (dichloromethane/methanol = 98:2) to yield **31** (92.5mg, 80%) as a white foam. - TLC (dichloromethane/methanol = 95:5): Rf = 0.30.

Methyl (benzyl-(4-O-benzyl-2,3-diacetamido-2,3-dideoxy-β-D-glucopyranosyluronate)-(1→4)-3,4-di-O-acetyl-4-O-benzyl-2-deoxy-2-acetamido-β- D-glucopyranosyl-(1→6)-2,3-dibenzyl-α-D-galactopyranoside (**32**)

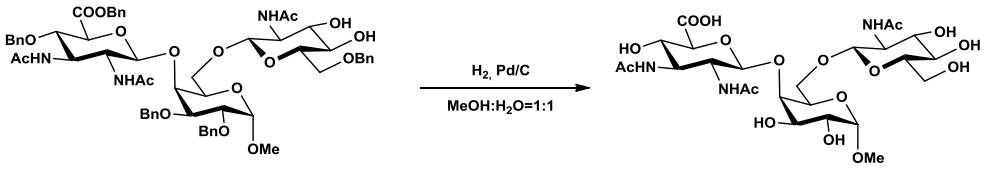


31 (92.5mg, 0.082 mmol) was dissolved in dry MeOH (5 mL) under N_2 atmosphere. A catalytic amount of a solution 0.1M of MeONa in MeOH was added, and after 1h the reaction was neutralized with Amberlite IR-120. The resin was filtered off, and solvent evaporated in vacuo.

The crude was dissolved in acetone (4 mL), and the solution was cooled at 0°C. Then were added in successively $NaHCO_3$ 5% (3.5mL), KBr (13 mg, 0.13 mmol) and TEMPO (16.6 mg, 0.10 mmol). After 10' at 0°C NaClO 13% solution (0.27 mL) was added. After 3h a TLC (DCM:MeOH=95:5) shown the disappearance of the starting material, and a spot at Rf=0. The solvent was removed in vacuo, stripping with toluene.

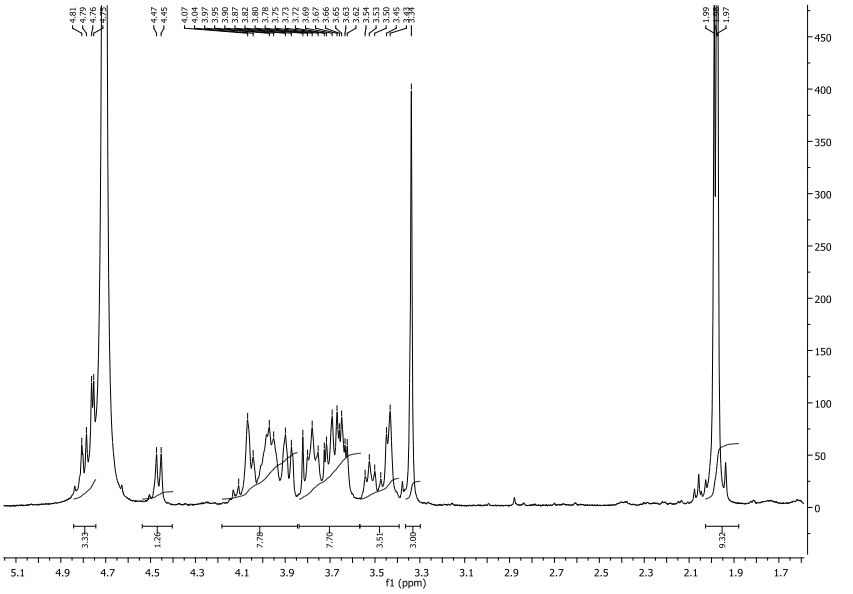
The crude was redissolved in dry DMF (5mL) under N_2 atmosphere and BnBr (0.09mL, 0.72 mmol) was added. The solution was cooled at 0°C, and KF (45 mg, 0.37 mmol) was added, letting warmed at RT. After stirring for 16h, the solvent was removed in vacuo, the crude dissolved in DCM (5 mL) and washed with water (3 mL). The aqueous phase was extracted with DCM (3 X 5 mL). The combined organic layers were separated, dried with sodium sulfate and evaporated in vacuo. The residue was purified by flash chromatography (dichloromethane/methanol = 98:2) to yield **19** (77mg, 79%) as a white solid. - TLC (dichloromethane/methanol =95:5): Rf =0.44.

Methyl (4-O-benzyl-2,3-diacetamido-2,3-dideoxy-β-D-glucopyranosyluronate)-(1→4)-3,4-di-O-acetyl-4-O-benzyl-2-deoxy-2-acetamido-β- D-glucopyranosyl-(1→6)-2,3-dibenzyl-α-D-galactopyranoside (**33**)

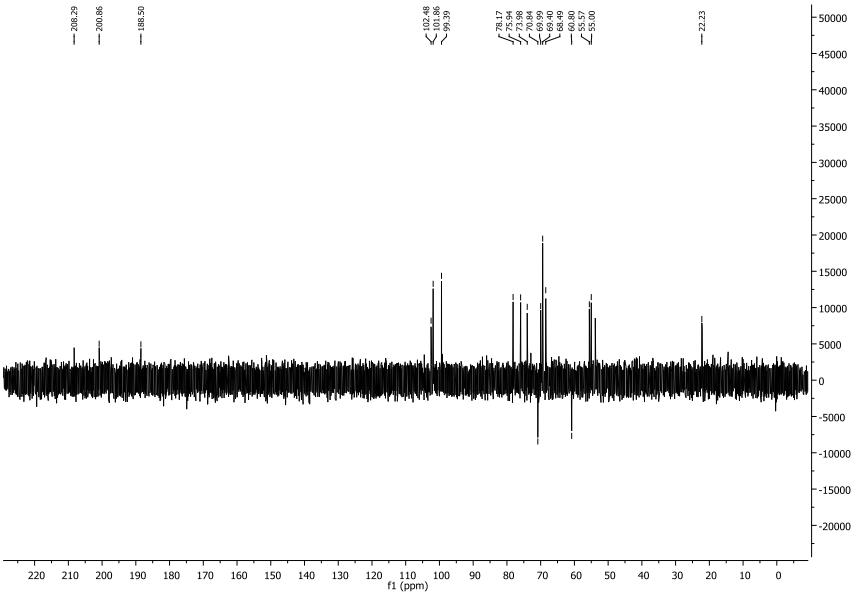


32 (77mg, 0.064 mmol) was dissolved in MeOH:H_2O=1:1 (5mL:5mL), and a catalytic amount of Pd/C was added. The solution was put under H_2 atmosphere for 24h. The mixture was filtered off over a Celite pad and solvent removed under reduced pressure to yield **13** (41mg, 100%) as a pale yellow solid. - ^{1}H NMR (400 MHz, D_2O) δ 4.80 (d, *J* = 8.3 Hz, 1H), 4.76 (d, *J* = 3.8 Hz, 1H), 4.46 (d, *J* = 8.2 Hz, 1H), 4.20 – 3.85 (m, 6H), 3.85 – 3.58 (m, 6H), 3.59 – 3.39 (m, 3H), 3.34 (s, 2H), 2.03 – 1.92 (m, 7H). - ^{13}C NMR (100 MHz, D_2O) δ 208.29, 200.86, 188.50, 102.48, 101.86, 101.86, 99.39, 99.39, 78.17, 78.17, 75.94, 75.94, 73.98, 70.84, 69.99, 69.40, 69.40, 68.49, 68.49, 60.80, 55.57, 55.00, 22.23. – ESI-MS (positive mode); *m/z:* 656.6 [M + 1H].

^{1}H-NMR compound **33**



^{13}C-NMR compound **33**



SYNTHESIS OF BIFUNCTIONAL SUGAR-BASED ORGANOCATALYSTS

INTRODUCTION

Organocatalysis

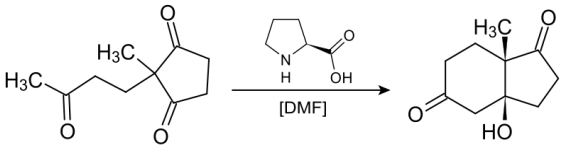
Organocatalysis uses metal-free low molecular weight organic molecules predominantly composed of C, H, O, N, S and P to accelerate chemical reactions. Most of the attention is focused on this type of molecules especially for their use in asymmetric catalysis, with regard to compounds of pharmaceutical interest.

Despite the large consensus by the chemical community of the important role of metal catalysts in synthesis and the ongoing search for new systems, the apparent drawbacks cannot be ignored anymore. These include the high cost and effort for the preparation of the catalysts, the use of noxious metals, which, although present in trace amounts, contaminate the final organic product, the lack of orthogonality with a wide range of functional groups, and in some cases the need to operate under rigorously anhydrous or anaerobic conditions.

The advent of organocatalysis brought the prospect of a complementary mode of catalysis, with the potential for savings in cost, time and energy, an easier experimental procedure, and reductions in chemical waste. These benefits arise from three factors. First, organic molecules are generally insensitive to oxygen and moisture in the atmosphere, so there is no need for special reaction vessels, storage containers and experimental techniques, or for ultra-dry reagents and solvents. Second, a wide variety of organic reagents such as amino acids, carbohydrates and hydroxy acids are naturally available from biological sources as single enantiomers. Simple organocatalysts are therefore usually cheap to prepare and readily accessible in a range of quantities, suitable for small-scale reactions to industrial-scale reactions. Third, small organic molecules are typically non-toxic and environmentally friendly, increasing the safety of catalysis in both biological and chemical research across all research settings, including industry and academic institutions. The combination of these factors substantially lowered the entry costs for researchers who were interested in developing enantioselective catalysts. With no need for gloveboxes, inert gases, ultra-dry solvents or even high levels of experimental expertise, it is not surprising that the field quickly became flooded

with research groups from around the globe.[1]

The first enantioselective organocatalytic reactions had already been described at the beginning of the 20th century, and some astonishing, selective reactions such as the proline-catalyzed synthesis of optically active steroid partial structures by Hajos, Parrish, Eder, Sauer and Wiechert had been reported in 1971.(**Scheme 1)**[2]



Scheme 35

After these "pioneering" works, other example of organocatalysts that have been very successful in asymmetric synthesis were developed and numerous research groups around the world are now exploring the potential of the method. A variety of key asymmetric carbon–carbon and carbon–heteroatom bond-forming reactions (such as, Diels–Alder and 1,3-dipolar cycloadditions, direct aldol condensation, Mannich and Michael reactions, epoxidation, hydride transfer, nitroalkane addition to enones, α-halogenation, and amination of aldehydes) can be carried out by using organocatalytic methods.

In the framework of the asymmetric catalysis a particular class of compounds that has been utilized successfully in a large variety of organocatalytic transformations is that of thioureic organocatalyst.

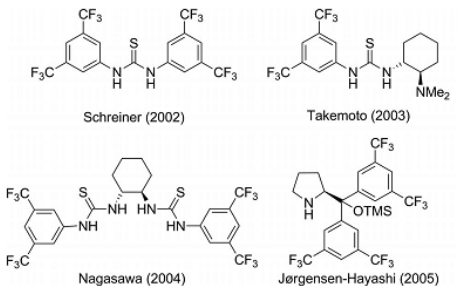


Fig. 6

They are able to accelerate and stereochemically control organic transformations through predominantly double hydrogen-bonding interactions with the respective substrate(s) (non-covalent organocatalysis). It should be noticed that most of these compounds have the CF_3 group in their structure. Recent studies, through modeling and NMR experiments, demonstrated that the presence of these groups increase the Lewis acidity of the catalytic sites, therefore the ability to coordinate the substrates of the reactions, consequentely the activity of the molecule.[3]

Jacobsen and coworkers developed a chiral thiourea (**Fig.2**) derivative used in enantioselective nucleophilic additions of HCN and ketene silyl acetals to imines (Strecker and Mannich reactions), obtaining good results in terms of chemical yield and stereoselection. This compound consists in *t*-leucine residue, linked with a derivative of 1,2-trans-diamino cyclohexane.[4]

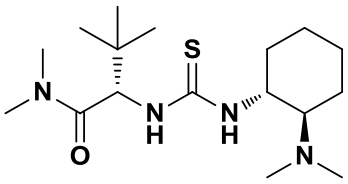


Fig. 7

The same compound and its derivatives have been successfully used also in aza-Baylis-Hillman reactions, addition of acetophenone and acetylacetone to nitrostyrene and addition of diethyl malonate to imine differently substituted. Moreover the addition of nitroester to imine gave good results.

For this kind of bifunctional system were hypothesized different mechanism where two transition states were proposed.

In the case of neutral catalytic species (**A**) the thiourea group coordinates the carbobenzyloxy group of the imine, the basic dimethylamino group have the role to deprotonate the malonate and promote its addition to the carbon-nitrogen double bond. However, the presence of a basic group is not strictly necessary for carry out the reaction. In the case of a protonated form of the catalytic species it is possible to hypothesize a transition state **B** in which the amino group coordinates the

protonated nucleophile in its conjugated form, through hydrogen bonds in a system that would work only by the establishment of a network of hydrogen bonds.

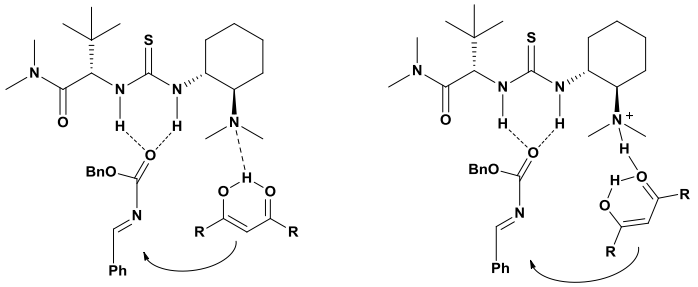


Fig. 8

Another bifunctional thioureic system was developed by Takemoto and his team and it is shown in **Fig.4**.[5]

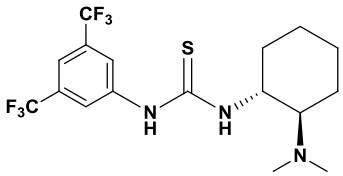


Fig. 9

Also in this case a 1,2-trans-diamino cyclohexane is employed as chiral scaffold, bearing a tertiary amine and 3,5-bis(trifluoromethyl)phenyl thioureic group in trans configuration. This catalyst gave good results in different reactions, as aza-Henry, Michael addition, and addition of nitroalkane to imine. Since the catalysis proceed through the formation of hydrogen bonds, polar solvents inhibit their formation, decreasing the yield and the stereoselection. Best results were obtained with apolar solvents, in particular DCM or toluene, depending on the reaction.

Taking into consideration the addition reaction of diethylmalonate to β-nitrostyrene, the mechanism hypothesized proceeds through a double activation of both the electrophile (β-nitrostyrene) and the nucleophile (diethylmalonate), by the two functionalities of the molecule. As shown in **Fig.5**, the thioureic moiety interacts with the nitro group of the nitroolefin increasing its electrophilicity, while

the tertiary amine coordinates the enolic form of the malonate.

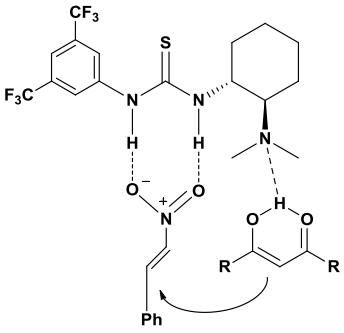


Fig. 10

Carbohydrate-based organocatalyst

Carbohydrates have been established as the most abundant and arguably the most structural diverse organic molecules found in nature.

They are chiral molecules readily available in nature in a variety of diastereomeric forms. Their conformational rigidity provides a well defined three-dimensional spatial arrangement of substituents with various multi-configured hydroxyl groups for chemical modification.

Furthermore, thanks to their flexibility, they are attractive as molecular scaffolds, both as ligands for enantioselective catalysis or themselves as metal-free catalysts, for their low cost, potential polyfunctionalization, and easy possibility of different modifications for fine tuning of steric, electronic and solubility properties.

The first employment of carbohydrates in asymmetric homogeneous catalysis dates back to the early ’80, in which sugars have been used as ligands for transition metals. Different research groups used diphophonate moieties on saccharide scaffold as chiral ligand to coordinate a Rh atom. This organo-metallic compounds were used in hydrogenation of (Z)-α-acetamido-cinnamic acid to L-phenylalanine.[6]

The first paper where a sugar was used as organocatalyst was published in 2006 from the research group of Kunz, relying on Jacobsen work.[7] Starting from glucosamine as readily accessible chiral scaffold they synthesized a library of catalyst, bearing different protecting group, with the ureic and the imine functionality in different position of the sugar. These compounds gave good results in asymmetric Mannich reaction. The same catalysts were used to promote Strecker reactions, but in this case there was a decrease of yield and e.e., probably due to the lower basicity of *N*-acyl-immine which reduced the affinity with N-H of the urea, a prerequisite for the reaction.(**Fig.6**).

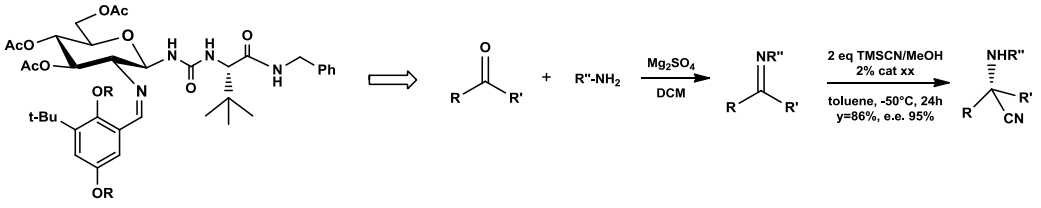


Fig. 11

More recently, based on the works of Takemoto and Jacobsen, different catalyst based on saccharide scaffold were developed to promote the addition of acetylacetone to nitroolefine.

Tang in 2008 coupled a saccharide provided with an isothiocyanate in anomeric position with a N-[(1R,2R)-Z-aminocycloesil]-N,N-dimethylamine.(**Fig.7**).[8]

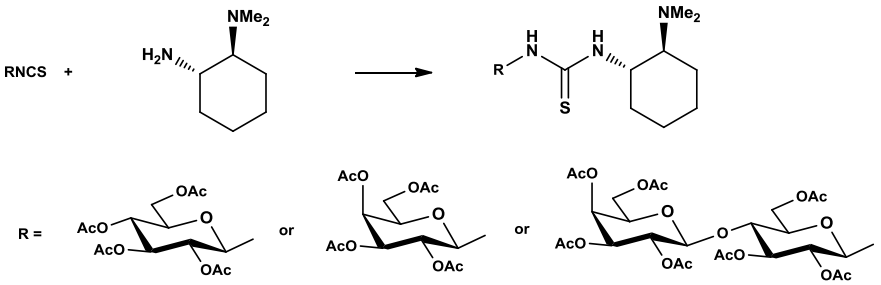


Fig. 12

Exploiting this synthetic strategy, Shao coupled the sugar with an aminoacidic diamino derivative.[9] In this case the absolute configuration of the product is modulated by the stereocenter of the aminoacid. In both these cases the authors obtained under optimize conditions good chemical yield (90 %) and e.e. of 85-96%. (**Fig.8**).

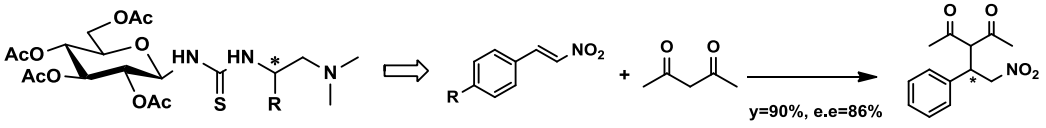


Fig. 13

Davis and his group synthesized a series of chiral ketones derived from glucosamine, bearing an acetamido group in position 2, and employed these compound in asymmetric epoxidation of alkenes.[10] This reaction allows to obtain chiral epoxide, a useful class of compounds for the pharmaceutical industry. Despite the conversion of the substrates were usually satisfactory, the e.e. were good only with some substrates, under the optimized condition.(**Fig.9**).

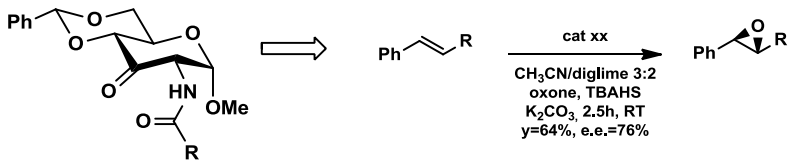


Fig. 14

The research group of Peddinti in 2010 synthesized prolinamidic catalyst on a saccharide scaffold, starting from commercially available L-proline and D-glucosamine, in aldol reactions, and in the asymmetric Michael addition of cyclohexanone to trans-β-nitrostyrene.[11] The same group in 2011 developed a glucosamine-based primary amines as organocatalysts for the asymmetric aldol reaction.[12]

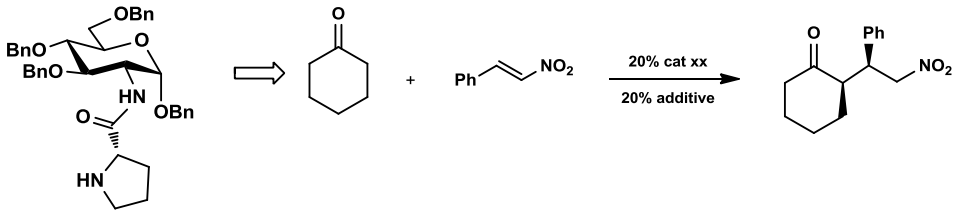


Fig. 15

Wang and coworkers in 2010 developed a new type of sugar-based pyrrolidine organocatalyst, which is capable of catalyzing Michael addition reaction of ketones to nitrostyrenes. A remarkably better catalytic performance was provided by the reactions in terms of productivity (up to 98%), diastereoselectivity (syn/anti 99:1), enantioselectivity (up to 99%) under solvent-free reaction conditions at room temperature.[13]

In a modular and more efficient approach, the sugarbased pyrrolidine is achieved through a click reaction, which introduces a chiral pyrrolidine moiety into a common and inexpensive carbohydrate.

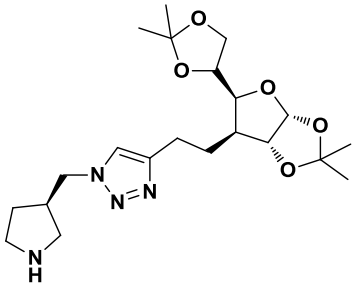


Fig. 16

Despite these compounds show a good catalytic activity, and give promising results usually on specific substrates, and under particolar reactions condition, the use of sugar as scaffold for asymmetric organocatalysis still remains under investigation by the scientific community. They remain attractive for the possibility to modulate the functional groups on the molecule. Thanks to this features, we are able to install different catalytic functionalities: thioureic group, amide, carbonyl groups, and moreover with the manipulation of the other functions of the molecule is possible to change the physical properties of the compounds, as the solubility, or the possibility to be supported on resin or silica.

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RESULTS AND DISCUSSION

Aim of the work

In 2011 the research groups of Benaglia and Lay, inspired by the seminal work by Kunz, decided to investigate the synthesis of a new family of (thio)urea–amine organocatalysts on the model of Takemoto. Both catalytic residues were connected by an enantiomerically pure saccharide-based scaffold, as a chiral skeleton alternative to diamino cyclohexane. They functionalized a derivative of D-glucosamine hydrochloride with a (thio)ureic moiety in anomeric position, and a tertiary, secondary or primary amine in position 2 of the sugar, while the other hydroxyls were differently protected. (**Fig.1**).[1]

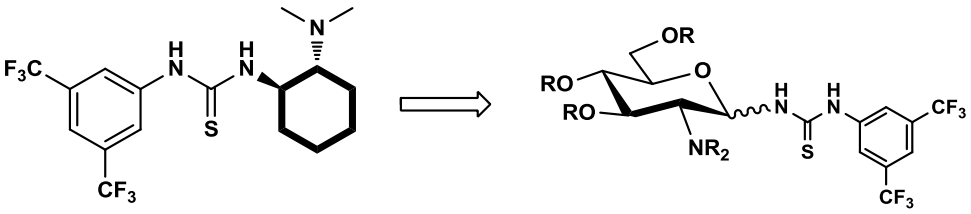
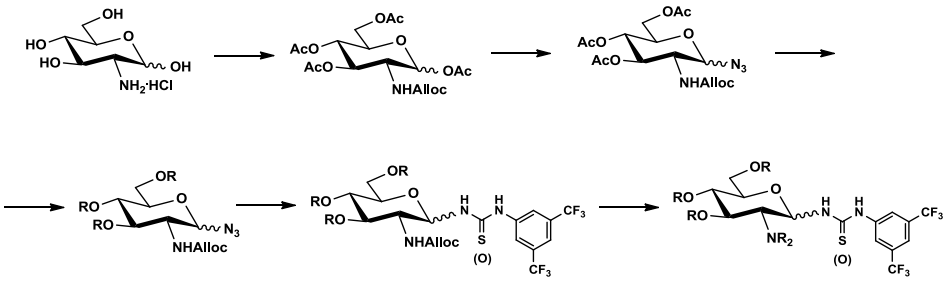


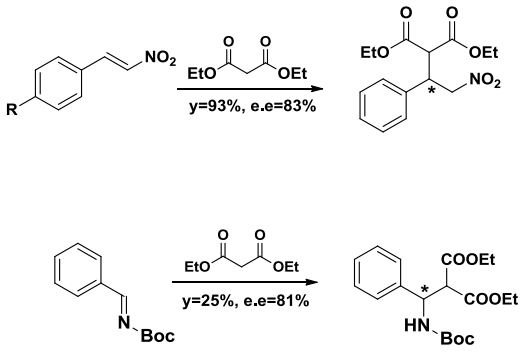
Fig. 1

The synthetic pathway is briefly illustrated in **Scheme 1**. After protection of the amine and acetylation of the free hydroxyls, an azide was introduced in anomeric position using $SnCl_4$. After deacetylation the hydroxyls at C-3, C-4 and C-6 were protected, and the azide was converted in iminophosphorane through an aza-Wittig-Staudinger reaction, which was coupled with an arylisothiocyanate to form the thioureic function. The final steps are the deprotection of the amine, followed by the conversion in a secondary or tertiary amine through reductive amination to afford different types of catalysts. (**Scheme 1**).



Scheme 1

These organocatalysts were tested in different reactions as the addition of acetylacetone to differently substituted β-nitrostyrenes, and in Mannich reaction between diethyl malonate with the *N*-Boc imine of benzaldehyde (**Scheme 2**).



Scheme 2

The best results were obtained using silyl ethers as protecting groups (R=Et_3Si), but also with acetate, benzyl, or benzylidene acetal these compounds showed an interesting activity.

Preliminary experiments demonstrated the importance of the presence of a tertiary amine, because primary and secondary amines did not show any catalytic activity.

A major drawback of these organocatalysts is that they are configurationally instable due to the presence of an anomeric emyaminal which caused easy anomerization leading to an α/β mixture of the catalyst.

For these reasons it was thought to install the thiourea on a different ring position, maintaining the trans-diequatorial configuration of the two amino functions. Also in this case D-glucosamine was also employed as starting material, while the

tertiary amine was introduced in position 2 and the thiourea in position 3 of the sugar in equatorial orientation. In this way the stability of the compound is substantially increased. A future development could also be the inversion of the two catalytic sites of the molecule, as in structure **B** (**Fig 2**).

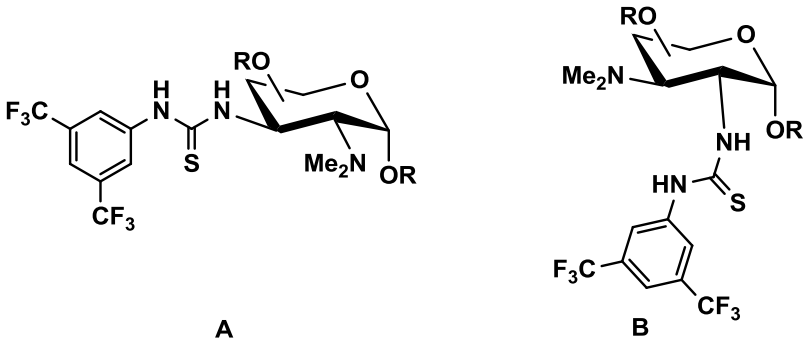
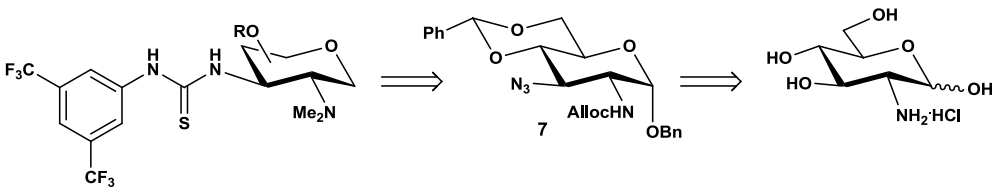


Fig. 2

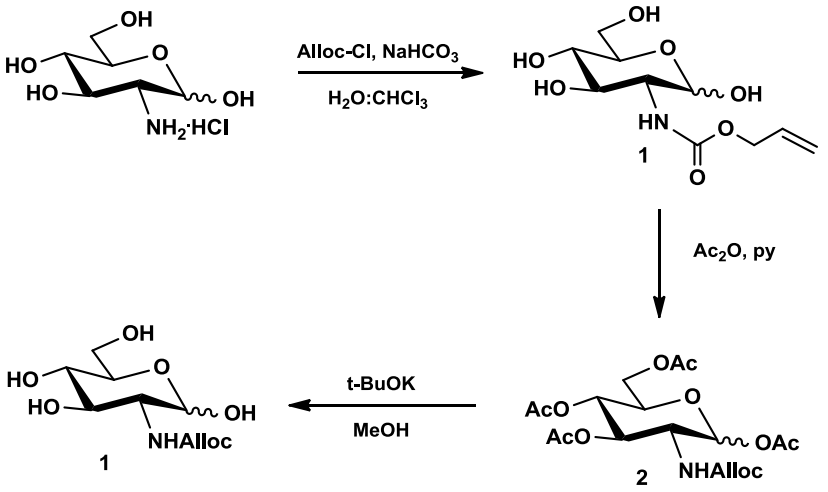
Synthetic strategy

The retrosynthetic strategy show in **Scheme 3** highlights the presence of a suitably protected building-block **7**, provided with the two nitrogen functionality in position 2 and 3 in trans-diequatorial configuration. The amine at C-2 is protected as allyloxycarbamoyl (Alloc) group, while an azide is installed at C-3. The anomeric position is protected with a stable group as a benzyl ether in α configuration, while 4-OH and 6-OH are protected as a benzylidene acetal.



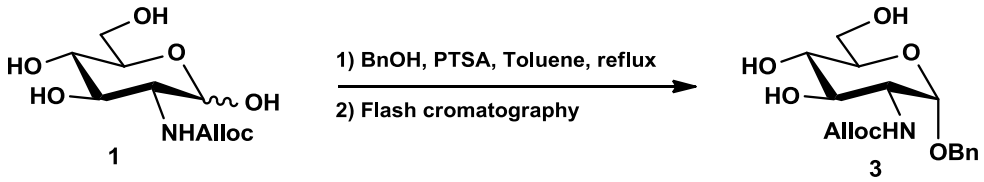
Scheme 3

The synthesis started from commercially available D-glucosamine hydrochloride. First the Alloc group was introduced to protect the amine. This reaction was performed in a biphasic solvents mixture ($CHCl_3$-H_2O). The starting material was first dissolved in a water solution of $NaHCO_3$ to release the amine from the hydrochloride. Then a solution of allylchloroformiate in chloroform was added. This compound resulted contaminated by a large amount of inorganic salts, and therefore difficult to purify or to manipulate as a crude residue. For this reason it was acetylated in pyridine and acetic anhydride, chromatographically purified and then deacetylated to give pure compound **1** in 53% yield over 3 steps (**Scheme 4**).[2]



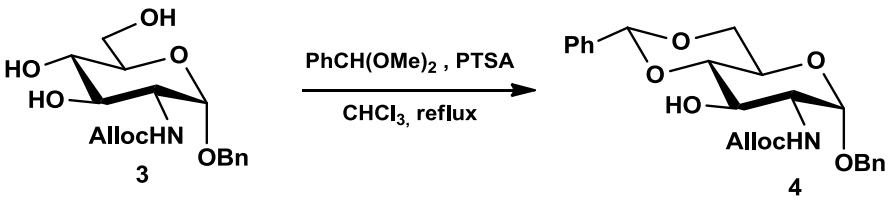
Scheme 4

Compound **1** was selectively benzylated in anomeric position through a Fischer glycosylation with BnOH and PTSA. This reaction was performed under thermodynamic control in order to obtain the most stable α-anomer in large amount. Using toluene as a solvent and heating to reflux for several hours, a 9:1 α:β ratio was obtained. Moreover Dean-Stark apparatus was used to distil off the water formed during the reaction, favouring the formation of the product. The two anomers were easily separated by flash chromatography, and the α compound **3** was achieved in 62% yield (**Scheme 5**).



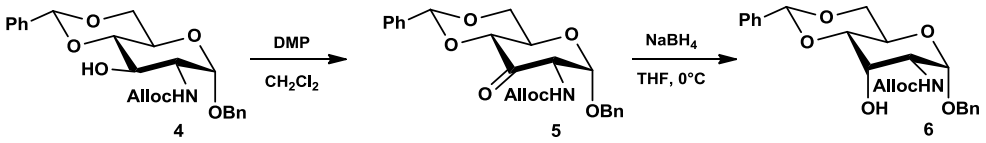
Scheme 5

The next step is the protection of positions 4 and 6 as benzylidene acetal with benzaldehyde dimethylacetal, PTSA in chloroform under reflux. This conditions are employed to remove the methanol formed during the reaction by a Dean-Stark apparatus, to shift the equilibrium to the product **4** (**Scheme 6**).[3]



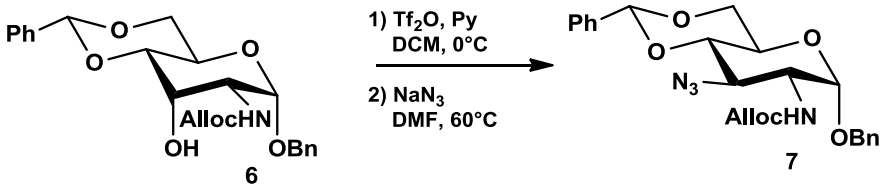
Scheme 6

The equatorial alcohol **4** was converted in the corresponding ketone **5**, by oxidation with Dess-Martin periodinane (DMP) synthesized in our laboratory. Reduction of ketone **5** with $NaBH_4$ in THF at 0°C afforded the alloside **6** in 52% yield over 2 steps (**Scheme 7**).



Scheme 7

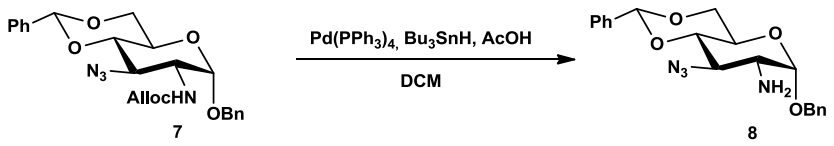
To introduce the azide in equatorial configuraton the hydroxyl group was converted in a good leaving group as the triflate. The subsequent reaction with sodium azide in moist DMF at 60°C proceeded via a S_N2 mechanism providing the 2,3 "diamino" building block **7** in 47% yield over 2 steps (**Scheme 8**).



Scheme 8

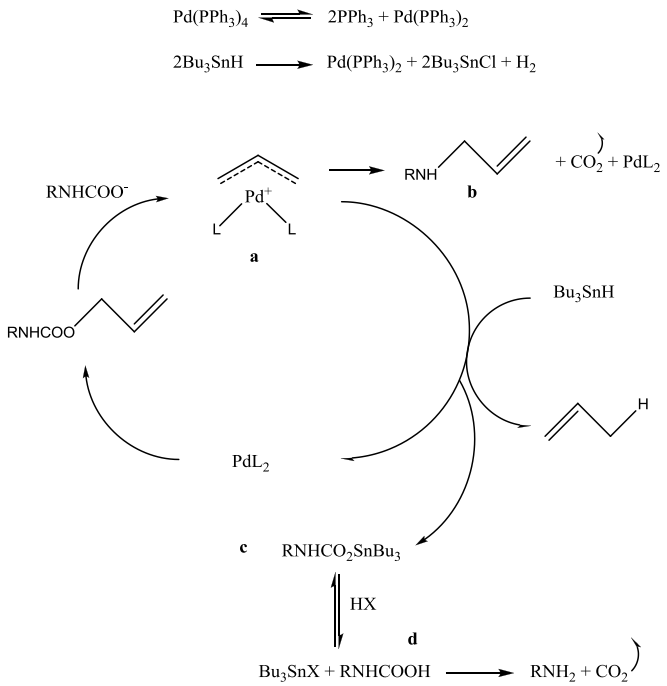
The subsequent steps were the transformation of the two nitrogen moiety in the catalytic sites of the organocatalyst.

In a first attempt, the allyloxycarbonyl group was removed with Bu_3SnH, AcOH, and a catalytic amount of $Pd(PPh_3)_4$ (**Scheme 9**).



Scheme 9

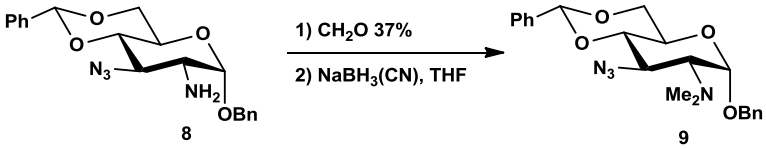
The general mechanism of this reaction is illustrated in **Scheme 10**. Generally assumed for most palladium-catalyzed allylic alkylation reactions, the true catalytic species is believed to be the coordinatively unsaturated bis (tripheny1phosphine) palladium(0) complex**,** which is formed either by ligand dissociation from tetrakis (triphenylphosphine) palladium(0). This reaction proceeds thanks to Pd^{II} formed upon reduction by Bu_3SnH. The species of Pd^{II} coordinate to the olefin of the carbamate, this complex can eliminate CO_2 and form a secondary amine, or react with another moecule of hydride, regenerating the catalyst, forming propylene, tributhyltin carbamate. The latter treated with acid (H-X) eliminates a tributhyltin specie (Bu_3SnX) and compound **d**, that decarboxylates and allows the formation of the free primary amine.[4]



Scheme 10

Product **8** wasn't purified and used directly in the next step. As demonstrated by the previous work, the presence of a tertiary amine in position 2 of the sugar is fundamental to have a good catalytic activity of the compound. For this reason a reductive amination was carried out.

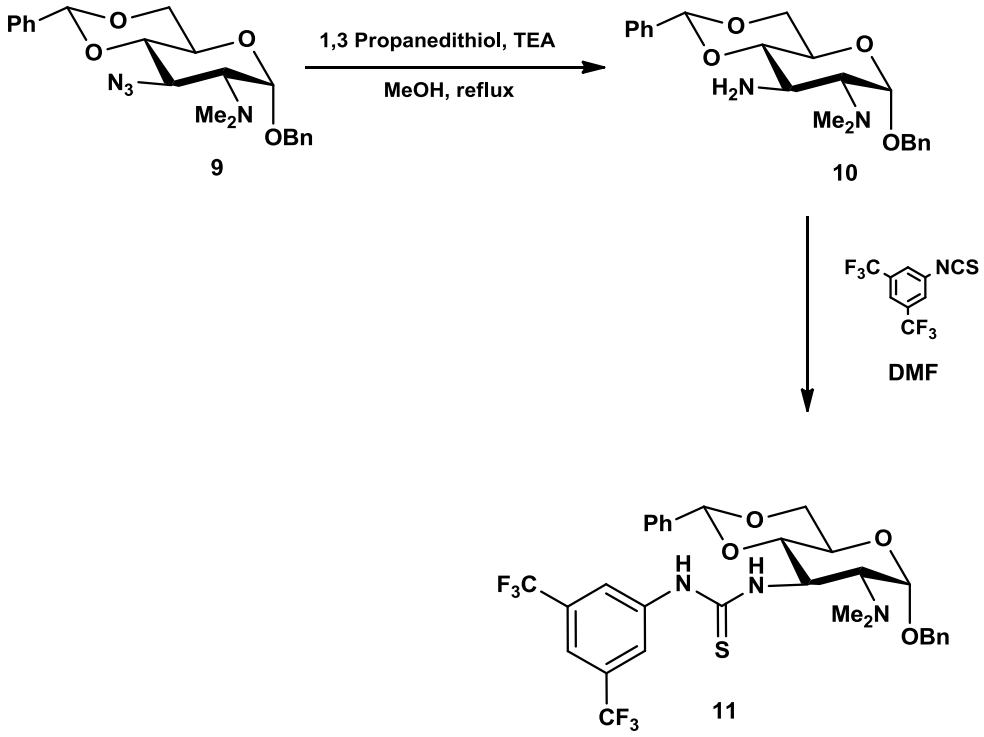
First the primary amine was treated with an aqueous solution (37%) of formaline, to give the corresponding imine. The reduction with $Na(CN)BH_3$, afforded the desired tertiary amine **9** in 42% yield (**Scheme 11**).



Scheme 11

The next step is the reduction of the azide with 1,3-propanedithiol to give the primary amine (**10**), which was treated with 3,5-bis-trifluoromethyl

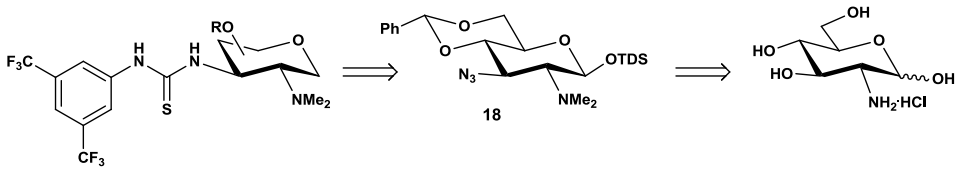
phenylisothiocyanate to give the target thioureic organocatalyst **11** in 40% yield over 2 steps (**Scheme 12**).



Scheme 12

As can be noticed from the synthetic strategy described above, the overall yield is not satisfactory. In order to increase the chemical yield of some crucial steps a second synthetic strategy was designed.

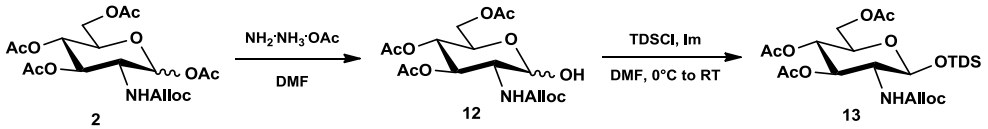
As shown in **Scheme 13**, a second building-block was identified, that, unlike the previous one, bears a silyl group in anomeric position in β configuration.



Scheme 13

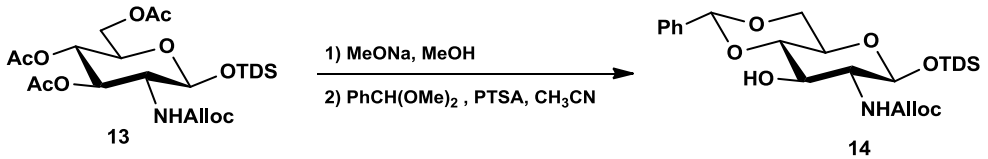
It is clear that some of the synthetic steps are similar to those described before for the synthesis of **11**.

Starting from **2** as common intermediate, the anomeric position was selectively deacetylated with a solution of hydrazine acetate in DMF, and the free OH was silylated with thexyl dimethyl silyl chloride to achieve **13** in 76% yield overall yield from glucosamine hydrochloride (**Scheme 14**).



Scheme 14

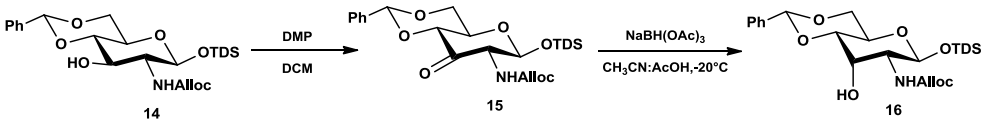
The treatment under Zemplen condition released the hydroxyl groups, and a benzylidene acetal was installed in positions 4 and 6 with benzaldehyde dimethylacetal and PTSA in acetonitrile at RT, providing the equatorial alcohol **14** in 87% yield over 2 steps (**Scheme 15**).



Scheme 15

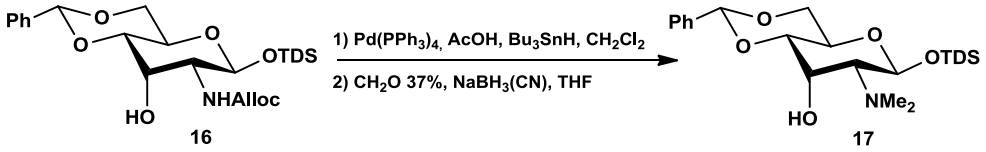
The double inversion at C-3 to introduce the azide in equatorial configuration was performed as described above by an oxidation-reduction procedure, followed by a S_N2 reaction.

First the ketone **15** was synthesized with DMP and the reduction was carried out with $NaBH(OAc)_3$ in a mixture of CH_3CN:AcOH 1:1 at -20°C. The major steric hindrance of the H^- in this raegent allowed to obtain a higher ratio of the axial compound **16** in 64% yield over 2 steps. In this case the use of $NaBH_4$ furnished an equimolar mixture of the two diastereoisomers **14** and **16** (**Scheme 16**).



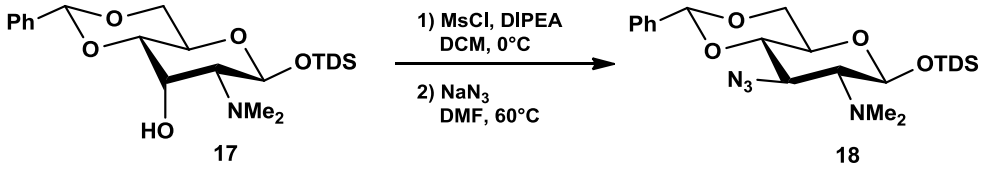
Scheme 16

Unlike the previous synthetic route, the subsequent step was the removal of the Alloc group and the installation of the tertiary amine in the same conditions described before (**Scheme 17**). This was required because the transformation of the free OH in a good leaving group as a triflate or a mesylate under different conditions never afforded the desired products. The reaction with triflic anhydride and pyridine led to the degradation of the product, or the formation of the triflate in low amount, while the treatment with .mesyl chloride and DIPEA didn't give any result and the starting material was recovered unchanged.



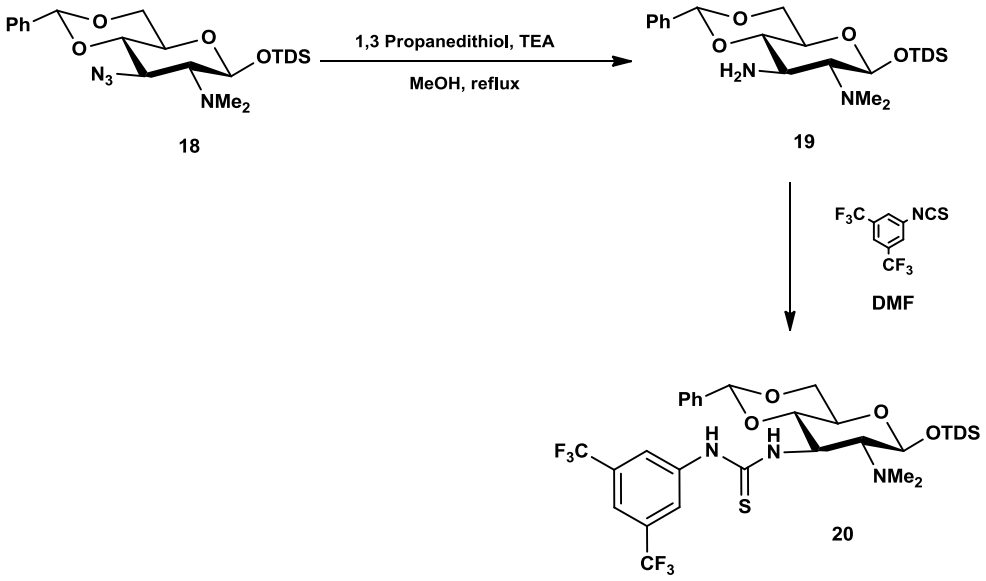
Scheme 17

The next step was the introduction of the azide. Also in this case the treatment with triflic anhydride led to the formation of byproducts, while the mesylation gave good results. S_N2 displacement with NaN_3 afforded the 2,3 "diamino" building block (**18**) in 30% yield over 2 steps (**Scheme 18**).



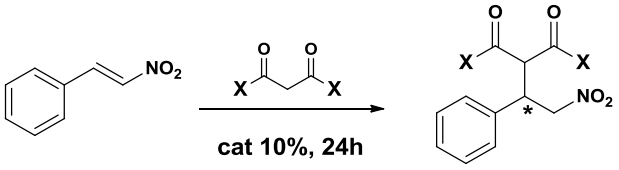
Scheme 18

The thioureic moiety was introduced in compound **18** after the reduction of the azide with 1,3-propanedithiol and TEA, followed by the coupling with the aryl isothiocyanate to give the second organocatalyst **20** in 35% yield over 2 steps (**Scheme 19**).



Scheme 19

Catalysts **11** and **20** were used as "mimicks" of the Takemoto's catalyst in Michael addition between acetylacetone or diethylmalonate to β-nitrostyrenes (**Scheme 20**). In preliminary catalyst test DCM or toluene were employed as solvent, and the reactions were carried out at RT or at 0°C, while the catalyst loading and the reaction time were maintained constant.



Scheme 20

The results of the test are illustrated in **Table 1**.

	CAT	X	Solv.	T	Yield (%)	e.e (%)
1	**11**	**CH_3**	**CH_2Cl_2**	RT	>99%	73%
2	**11**	**CH_3**	**CH_2Cl_2**	0°C	>99%	53%
3	**11**	**CH_3**	**Tol.**	RT	30%	73%
4	**11**	**OEt**	**CH_2Cl_2**	RT	10%	81%
5	**20**	**CH_3**	**Tol.**	RT	--	--
6	**20**	**CH_3**	**CH_2Cl_2**	RT	--	--

Tab. 1

It should be noticed that organocatalyst **11** showed a very good chemical activity in the conversion of acetylacetone in DCM, with a moderate enantioselection, that was reduced at 0°C. Furthermore the e.e was maintained in toluene as solvent, or using diethylmalonate as substrate, but in this two cases the chemical activity was much lower.

On the contrary, catalyst **20** didn't show any conversion of the substrate neither in toluene nor in DCM.

We tried to find a convincing reason to explain the enourmously different bahaviour of the two catalyst. A reasonable hypothesis is that the β configuration

and the steric hindrance of the silyl ether impose some conformational bias to catalyst **20** preventing the correct and ideal coordination of the electrophile and nucleophile.

A detailed computational study will be helpful to identify the preferred conformations of the two catalyst and to highlight their expected different mode of coordination of the substrates.

Bibliography

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[3] Andrej Babica, Slavko Pecar, *Tetrahedron: Asymmetry*, **2008**, *19*, 2265–2271
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EXPERIMENTAL SECTION

General experimental methods

Thin Layer Chromatography

Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) were performed on Merck precoated 60F254 plates (0.25 mm and 0.2 mm thickness, respectively). Reactions were always followed by TLC-analysis; compounds were visualized, when appropriate, by UV light (254 nm) and always by spraying with:

- *Molybdic solution* [21 g of $(NH_4)_4Mo_4O_{24}$, 1g of $Ce(SO_4)_2$, 31 mL of H_2SO_4 98%, 970 mL water];

- *Sulphuric acid* [50 mL of H_2SO_4 98%, 450 mL of MeOH, 450 mL water];

- *Ninhydrin* [2.7g of 2,2-Dihydroxyindane-1,3-dione, 27 mL of AcOH, 900 mL of n-BuOH (or EtOH)];

with detection by charring at 200°C.

Flash chromatography

According to Still procedure, compounds were purified by flash chromatography, using Silica gel (SiO_2, high-purity grade (Merck Grade 9385), pore size 60 Å, 230-400 mesh particle size) by Sigma-Aldrich®.

In some cases it was also used a flash purification system, Biotage® SP1™:2 SP1 means a single-column flash purification system [with Touch Logic Control™ and TLC-to-gradient feature designed to accelerate and simplify the purification process]. Available in sizes from 10 g to 340 g with standard (50 μm) silica, Biotage SNAP cartridges were used to purify our compounds.

NMR analysis

NMR spectra were recorded in a Bruker Avance 400 instrument (400, 101 MHz for ^1H, ^{13}C), and all were run at room temperature (298K) if not specified. The samples were prepared using deuterated solvents, as $CDCl_3$, D_2O, CD_3OD and $(CD_3)_2SO$,

by Sigma-Aldrich®.

Chemical shifts (δ) are reported in ppm scale and the coupling constants (*J*) in Hz. The chemical shift displacement is based on the residual proton in the solvent (e.g. the $CHCl_3$, 0.01 % in 99.99 % $CDCl_3$). The multiplicity of signals has been described as: *s* (singlet), *d* (doublet), *t* (triplet), *m* (multiplet), *dd* (doublet of doublets), *dt* (doublet of triplets).

1H-NMR spectra were recorded for all the synthesized products. In the case of unknown structure, the characterization is here reported by 1H-NMR, 13C-NMR: the registration of bidimensional (2D-COSY, 2D-HETCOR) analyses was due to better assignment of peaks to the structure.

In the case of a dimer we called ring-A the sugar coupled with the spacer and ring-B the one at the non-reducing terminus.

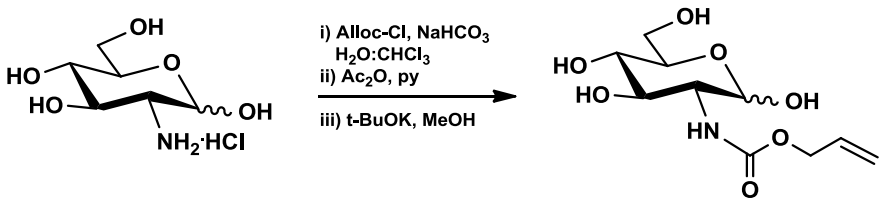
Mass analysis

ESI mass were recorded in negative or positive modes on Jeol AX-505.

HPLC analysis

The HPLC analysis were performer with: Agilent 1100 series. The chiral columnn used was Daicel Chiralpak AD.

*2-N-allyloxycarbamoyl-2-amino-2-deoxy-D-glucopyranoside (**1**)*

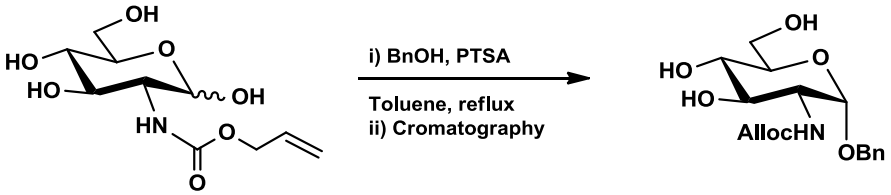


D-Glucosamine hydrochloride (10.0 g, 46.4 mmol) was dissolved in H_2O (150 mL), then $NaHCO_3$ was added (14.8 g, 176 mmol). After 10' a 0.4M solution of allylchloro formiate in $CHCl_3$ was added dropwise. (7.3 mL in 174 mL of $CHCl_3$). The biphasic solution was let vigorously stir for 24h. the solvents were evaporated in vacuo, and the residue was dissolved in pyridine (175 mL), and Ac_2O (37.8 mL, 360 mmol) was added. After 16h the solvent was removed in vacuo. The residue was dissolved in AcOEt (200 mL), and washed successively with HCl 5% (100 mL), a saturated solution of $NaHCO_3$ (100 mL) and water (100 mL). The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex: AcOEt = 7:3) to yield the product acetylate as a solid. This one was dissolved in dry MeOH under N_2 atmosphere. A catalytic amount of t-BuOK was added, and after 1h the reaction was neutralized with Amberlite IR-120. The resin was filtered off, and solvent evaporated in vacuo to yield **1** as (6.46g, 53%). TLC (dichloromethane:methanol=9:1) Rf=0.37.

The spectroscopic data were in agreement with those reported in literature[4]

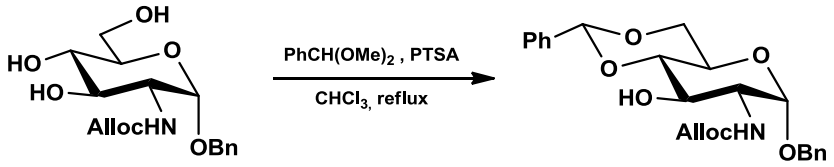
[4] E. Kamst et al. *Carbohydrate Research*, **1999**, *321*, 176–189

*Benzyl-2-N-allyloxycarbamoyl-2-amino-2-deoxy-α-D-glucopyranoside (***2***)*



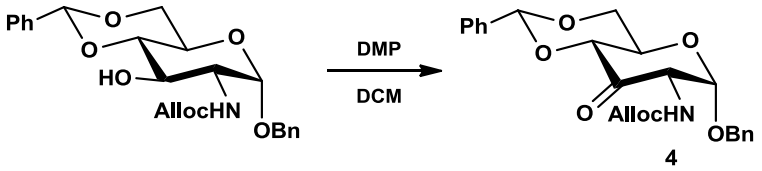
1 (6.46 g, 24.6 mmol) was suspended in toluene (100 mL) and BnOH (30 mL, 380 mmol). A catalytic amount of PTSA was added and the reaction was heated at reflux for 4h. A Dean-Stark apparatus was installed in order to eliminate the water formed during the reaction. The reaction was quenched with TEA, and cooled at RT. The solvent was evaporated in vacuo, and the residue purified by a flash chromatography. First the large amount of BnOH was removed with DCM as eluent, then the product was purified with DCM:MeOH = 95:5, to yield **2** as a brown solid 68% yield with an α:β ratio of 9:1.Rf = 0.39 (DCM:MeOH = 9:1) $[\alpha]_D$ = 88.58 (c = 0,01 g/ml; $CHCl_3$). - ^{1}H-NMR (400 MHz, $CDCl_3$): 7.33 (5H, m, (Ph)), 5.90 (1H, ddd, H9, J(H9-H10) = 22.4 Hz, J(H9-H10') = 10.7 Hz, J(H9-H8,H8') = 5.50 Hz), 5.50 (1H, d, NH, J(NH-H2) = 9.00 Hz), 5.25 (2H, m, H10, H10'), 4.92 (1H, d, H1, J(H1-H2) = 3.3 Hz), 4.70 (1H, d, H7, J(H7-H7') = 12.1 Hz), 4.56 (2H, 47 m, H8, H8'), 4.46 (1H, d, H7', J(H7-H7') = 12.1 Hz), 3.94-3.56 (9H, m, H2, H3, H4, H5, H6, H6', OH). - ^{13}C-NMR (100 MHz, $CDCl_3$): 156.97 (CO), 137.03 (Cq(Ph)), 132.58 (C9), 128.53-127.96 (CH(Ph)), 117.98 (C10), 97.17 (C1), 73.23-70.69 (C5, C3, C4), 69.93 (C7), 66.10 (C8), 61.62 (C6), 55.29 (C2). - ESI-HRMS (positive mode) *m/z*: 376,1366 g/mol [M + Na].

*Benzyl-2-N-allyloxycarbamoyl-2-amino-2-deoxy-4,6-O-benzylidene-α-D-glucopyranoside (**3**)*



2 (5.3 g, 15.0 mmol) was dissolved in $CHCl_3$ (100 mL). $PhCH(OMe)_2$ (3.4 mL, 22.5 mmol) and a catalytic amount of PTSA were added A Dean-Stark apparatus was installed in order to eliminate the methanol formed during the reaction and the temperature was heated at reflux. After 2h the reaction was quenched by adding TEA, and cooled at RT. The solvent evaporated in vacuo, and the residue purified by a flash chromatography (Hex:EA = 4:1) to yield **3** as yellow solid (5.50 g, 84%) Rf = 0.38 (Tol:EA = 7:3) $[\alpha]_D$ = 57.88 (c = 0,005 g/ml; $CHCl_3$). - ^{1}H-NMR (400 MHz, $CDCl_3$): 7.63-7.17 (10H, m, H(Ph)), 5.94 (1H, ddd, H9, J(H9-H10) = 17.2 Hz, J(H9-H10') = 10.9 Hz, J(H9-H8,H8') = 5.6 Hz), 5.59 (1H, s, H11), 5.34 (1H, dd, H10, J(H10-H9) = 17.2 Hz, J(H10-H10') = 1.5 Hz), 5.25 (1H, dd, H10', J(H10'-H9) = 10.9 Hz, J(H10'-H10) = 1.5 Hz), 5.11 (1H, d, NH, J(NH-H2) = 5.1 Hz), 4.98 (1H, d, H1, J(H1-H2) = 2.6 Hz), 4.76 (1H, d, H7, J(H7-H7') = 11.9 Hz), 4.61 (2H, d, H8, H8', J(H8,8'-H9) = 5.6 Hz), 4.55 (1H, d, H7', J(H7'-H7) = 11.9 Hz), 4.27 (1H, dd, H6, J(H6-H6') = 10.1 Hz, J(H6-H5) = 4.7 Hz), 3.98 (2H, m, H2,H3), 3.90 (1H, ddd, H5, J(H5-H4) = 11.6 Hz, J(H5-H6) = 8.1 Hz, J(H5-H6') = 4.7 Hz), 3.79 (1H, dd, H6', J(H6'-H6) = 10.1 Hz, J(H6'-H5) = 8.1 Hz), 3.62 (1H, dd, H4, J(H4-H5) = 11.6 Hz, J(H4-H3) = 6.6 Hz). - ^{13}C-NMR (100 MHz, $CDCl_3$): 136.81-134.46 (Cq(Ph)), 132.43 (C9), 129.76-126.30 (CH(Ph)), 118.18 (C10), 102.02 (C11), 97.06 (C1), 73.83-71.48 (C3, C4, C5), 70.02 (C7), 66.22 (C8), 62.12 (C6), 55.23 (C2). - ESI-HRMS (positive mode) *m/z:* 464.1791 g/mol [M + Na]

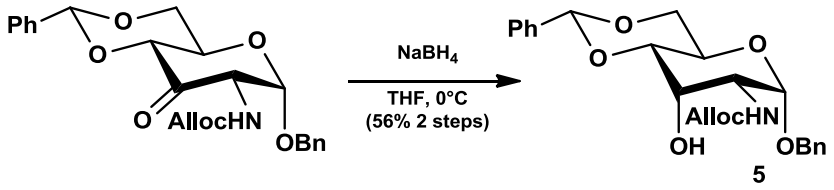
*Benzyl-2-N-allyloxycarbamoyl-2-amino-2-deoxy-4,6-O-benzylidene-α-D-glucopyran-3-uloside (***4***)*



4

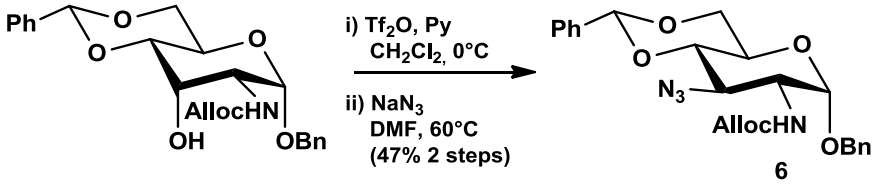
3 (5.5 g, 12.6 mmol) was dissolved in dry DCM (30 mL) under N_2 atmosphere. A solution of DMP (10.6 g, 25.2 mmol) in dry DCM (30 mL) was added dropwise, and stirred for 24h at room temperature. After completion of the reaction a 5% solution of thiosulfate (50 mL) was added and the mixture let stirred for 10' until two distinct phases were established. The organic layer was separated and washed with a saturated solution of $NaHCO_3$ (50 mL), and water (50 mL). The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Tol:EA = 4:1) to yield **4** as a white foam. (4.09 g, 74%). Rf = 0.64 (Tol:EA = 7:3) $[\alpha]_D$ = 103.3 (c = 0,005 g/ml; $CHCl_3$) - ^{1}H-NMR (400 MHz, $CDCl_3$): 7.68-7.14 (10H, m, H(Ph)), 5.92 (1H, ddd, H9, J(H9-H10) = 16.2 Hz, J(H9-H10) = 10.8 Hz, J(H9-H8) = 5.6 Hz), 5.55 (2H, m, NH, H11), 5.42 (1H, d, H1, J(H1-H2) = 4.4 Hz), 5.34 (1H, dd, H10, J(H10-H9) = 16.2 Hz, J(H10-H10') = 1.1 Hz), 5.24 (1H, dd, H10', J(H10'- H9) = 10.8 Hz, J(H10'-H10) = 1.1 Hz), 4.80-4.64 (2H, m, H2, H7), 4.57 (3H, m, H10, H10', H7'), 4.39 (1H, d, H4, J(H4-H5) = 7.8 Hz), 4.33 (1H, dd, H6, J(H6-H6') = 10.9 Hz, J(H6-H5) = 4.7 Hz), 4.14 (1H, ddd, H5, J(H5-H6) = 17.0 Hz, J(H5-H6') = 10.9 Hz, J(H5-H4) = 7.8 Hz), 3.94 (1H, dd, H6', J(H6-H6') = 10.9 Hz, J(H6'-H5) = 4.7 Hz). - ^{13}C-NMR (100 MHz, $CDCl_3$): 195.37 (C3), 156.33 (CO(Alloc)), 136.94-136.82 (Cq(Ph)), 133.04 (C9), 130.42-127.04 (CH(Ph)), 118.65 (C10), 102.62 (C11), 100.19 (C1), 83.21 (C4), 71.07 (C7), 70.02 (C6), 66.91 (C5), 66.76 (C8), 60.47 (C2). - ESI-HRMS (positive mode) *m/z:* 462.1523 g/mol [M + Na].

*Benzyl-2-N-allyloxycarbamoyl-2-amino-2-deoxy-4,6-O-benzylidene-α-D-allopyranoside (***5***)*



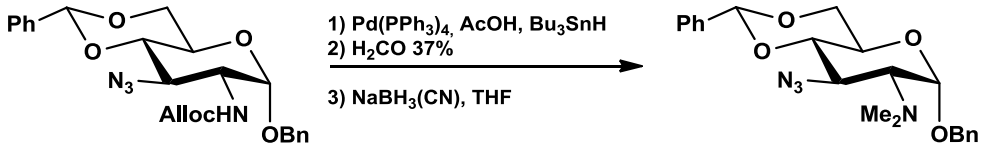
To a solution of **4** (4.09g, 9.32 mmol) in dry THF:EtOH=4:1 (20 mL: 5 mL), under N_2 atmosphere, cooled at 0°C was added in small portion $NaBH_4$ (517 mg, 14 mmol). After 2h the reaction was quenched with water (20 mL) and extracted with DCM (2 X 30 mL) The organic phase were separated, dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex:EA = 4:1) to yield **5** as a white foam. (2.87 g, 70%). Rf = 0.56 (Tol:EA = 7:3) $[\alpha]_D$ = -0.62 (c = 0,004 g/ml; $CHCl_3$). - ^{1}H-NMR (400 MHz, $CDCl_3$): 7.68-7.22 (10H, m, H(Ph)), 5.95 (1H, ddd, H9, J(H9-H10) = 17.2 Hz, J(H9-H10') = 10.8 Hz, J(H9-H8) = 5.6 Hz), 5.61 (2H, m, NH, H11), 5.34 (1H, dd, H10, J(H10-H9) = 17.2 Hz, J(H10-H10') = 1.2 Hz), 5.26 (1H, dd, H10', J(H10'-H9) = 10.8 Hz, J(H10'-H10) = 1.2 Hz), 5.00 (1H, d, H1, J(H1-H2) = 3.9 Hz), 4.79 (1H, d, H7, J(H7-H7') = 11.9 Hz), 4.59 (3H, m, H7', H8, H8'), 4.34 (1H, dd, H6, J(H6-H6') = 10.2 Hz, J(H6-H5) = 5.1 Hz), 4.26 (1H, m, H3), 4.20 (1H, m, H5), 4.01 (1H, m, H2), 3.80 (1H, m, H6'), 3.67 (1H, dd, H4, J(H4-H5) = 9.7 Hz, J(H4-H3) = 2.6 Hz). - ^{13}C-NMR (100 MHz, $CDCl_3$): 134.40-132.61 (Cq(Ph)), 130.88 (C9), 129.33-125.14 (CH(Ph)), 120.85 (C10), 101.41 (C11), 97.99 (C1), 79.74 (C4), 73.47 (C7), 68.65 (C8), 68.41(C3), 62.75 (C6), 57.76 (C5), 51.92 (C2). - ESI-HRMS (positive mode) *m/z:* 464.1678 g/mol [M + Na].

*Benzyl-2-N-allyloxycarbamoyl-2-amino-3-azido-2,3-dideoxy-4,6-O-benzylidene-α-D-glucopyranoside (***6***)*



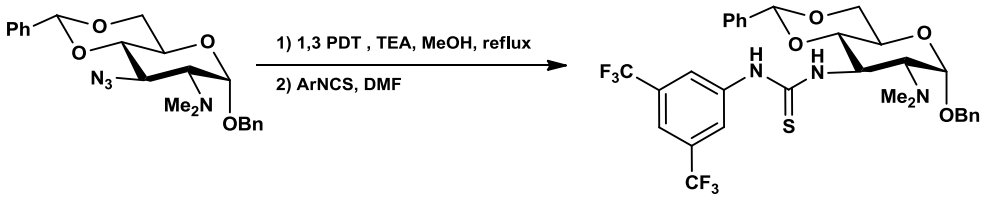
4 (2.87g, 6.52 mmol) was dissolved in dry DCM (25 mL) under N_2 atmosphere. Pyridine (1.57 mL, 19.56 mmol) was added and the solution was let stir at RT for 15'. The reaction was cooled at 0°C and triflic anhydride (1.61 mL, 9.78 mmol) was dropped. After 1.5 h the solution was diluted with DCM (20 mL), and washed with HCl 5% (30 mL), a saturated solution of $NaHCO_3$ (30 mL) and water (30 mL). The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The crude was let for 2 h in vacuo, then dissolved in humid DMF (30 mL). NaN_3 (1.27 g, 19.56 mmol) was added and the reaction let stir at 60°C over night. The solvent was removed under reduced pressure, the residue redissolved in DCM (40 mL) and washed with water (20 mL). The organic phase were dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex:EA=9:1) to yield **6** (1.42 g, 47%) as a white foam. Rf = 0.44 (Tol:EA = 7:3) $[\alpha]_D$ = 21.36 (c = 0,004 g/ml; $CHCl_3$). - ^{1}H-NMR (400 MHz, $CDCl_3$): 7.58-7.20 (10H, m, H(Ph)), 5.95 (1H, ddd, H9, J(H9-H10) = 16.1 Hz, J(H9-H10') = 10.9 Hz, J(H9-H8,H8') = 5.7 Hz), 5.61 (1H, s, H11), 5.36 (1H, dd, H10, J(H10-H9) = 16.1 Hz, J(H10-H10') = 1.2 Hz), 5.27 (1H, dd, H10', J(H10'-H9) = 10.9 Hz, J(H10'-H10) = 1.2 Hz), 5.08 (1H, m, NH), 4.94 (1H, d, H1, J(H1-H2) = 3.5 Hz), 4.76 (1H, d, H7, J(H7-H7') = 11.6 Hz), 4.61 (2H, m, H8,H8'), 4.56 (1H, d, H7, J(H7'-H7) = 11.6 Hz), 4.26 (1H, m, H6), 3.96 (2H, m, H5, H2), 3.82 (2H, m, H6', H3), 3.67 (1H, t, H4, J(H4-H3,H5) = 9.4 Hz). - ^{13}C-NMR (400 MHz, $CDCl_3$): 129.09-125.96 (CH(Ph)), 118.03 (C10), 101.45 (C11), 97.23 (C1), 80.53 (C4), 70.16 (C7), 68.87 (C6), 66.10 (C8), 63.22 (C5), 61.65 (C3), 53.81 (C2). - ESI-HRMS (positive mode) *m/z:* 489.1748 g/mol [M + Na].

*Benzyl-3-azido-2,3-deoxy-4,6-O-benzylidene-2-N,N-dimethylamino-α-D-glucopyranoside (**7**)*



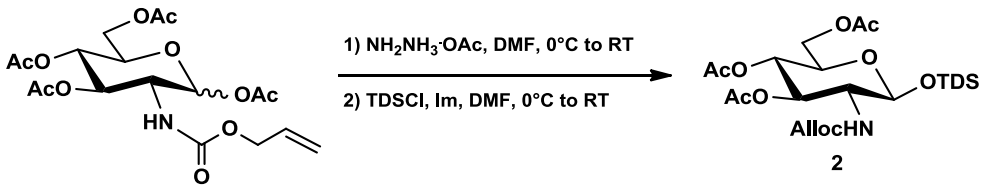
6 (1.17g, 3.06 mmol) was dissolved in dry DCM (25 mL) under N_2 atmosphere. A actalytic amount of $Pd(PPh_3)_4$, glacial AcOH (0.45 mL, 7.34 mmol) and Bu_3SnH (0.9mL, 3.36 mmol) were added in succession. The mixture was let stir at RT for 24h. The solvent was removed under reduced pressure and the residue dried in vacuo. This one was redissoved in THF, then a solution of H_2CO 37% in water (7.8 mL, 107 mmol) was added and let stir for 24h. $NaCN(BH)_3$ was slowly added and stirred for 2h. Then the reaction was treated with AcOH until pH = 4-5, and the mixture was let stir for 3h. Then the reaction was cooled at 0°C with an ice bath, and the pH adjusted slowly at 9 by a cold 5% solution of NaOH. Then the solution was extracted with AcOEt (2 X 30 mL) and the organic phase washed with brine (20 mL) and water (20 mL). The organic phase were dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex:EA) to yield **7** (0.52 g, 42%) as a white foam. Rf = 0.43 (Tol:EA = 7:3) $[\alpha]_D$ = 0.9 (c = 0,002 g/ml; $CHCl_3$). - ^{1}H-NMR (400 MHz, $CDCl_3$): 7.62-7.24 (10H, m, H(Ph)), 5.63 (1H, s, H8), 5.11 (1H, bs, H1), 4.75 (1H, d, H7, J(H7-H7') = 11.5 Hz), 4.60 (1H, d, H7', J(H7'-H7) = 11.5 Hz), 4.28 (1H, dd, H6, J(H6-H6') = 10.2 Hz, J(H6-H5) = 4.8 Hz), 4.19 (1H, dd, H3, J(H3-H2) = 11.2 Hz, J(H3-H4) = 9.5 Hz), 3.93 (1H, td, H5, J(H5-H4) = 9.9 Hz, J(H5-H6,H6') = 4.8 Hz), 3.77 (1H, t, H6', J(H6'-H5,H6) = 10.3 Hz), 3.65 (1H, t, H4, J(H4-H3,H5) = 9.4 Hz), 2.78 (1H, dd, H2, J(H2-H3) = 11.2 Hz, J(H2-H1) = 3.3 Hz), 2.58 (6H, s, H(Me)). - ^{13}C-NMR (400 MHz, $CDCl_3$): 136.98 (C(Ph)), 129.02-126.00 (CH(Ph)), 101.45 (C8), 98.22 (C1), 81.89 (C4), 69.81 (C7), 69.13 (C6), 65.45 (C2), 63.00 (C5), 58.56 (C3), 41.98 (CH3). - ESI-HRMS (positive mode) *m/z:* 433.1851 g/mol [M + Na].

*Benzyl-4,6-O-benzylidene-2,3-deoxi-2-N,N-dimethylamino-3-N-3,5-bis (trifluoromethyl-thiourea-α-D-glucopyranoside (**8**)*



7 (0.52g, 1.28mmol) was dissolved in dry MeOH (25mL) under N_2 atmosphere. TEA (1.2mL, 8.96 mmol) and 1,3-PDT (0.9mL, 8.96 mmol) were added and the solution was warmed at 60°C and let stir for 24h. The reaction was cooled at RT, and the solvent removed in vacuo. The residue was redissolve in dry DMF (15mL), under N_2 atmosphere. 3,5-bis (trifluoromethy) isothiocyanate (1.17ml, 6.4mmol) was added and the solution let stir for 24h. Then the solvent was removed in vacuo, and the residue was purified by flash chromatography (Tol:EA = 9:1) to yield **8** (0.31mg, 40%) as a white foam. Rf = 0.49 (Tol:EA =7:3). - ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.55-7.27 (13H, m, H(Ph)), 5.54 (1H, s, H_8), 4.93 (1H, d, H_1 J(H_1-H_2) = 3.7 Hz), 4.83 (1H, d, H_7, J(H_7-$H_{7'}$) = 11.7 Hz), 4.59 (1H, d, $H_{7'}$, J($H_{7'}$-H_7) = 11.7 Hz), 4.24 (1H, dd, H_6, J(H_6-$H_{6'}$) = 9.9 Hz, J(H_6-H_5) = 4.4 Hz), 3.81 (2H, m, H_4, H_5), 3.72 (1H, t, $H_{6'}$, J($H_{6'}$-H_5,H_6) = 10.1 Hz), 3.65 (1H, m, H_3), 2.35 (1H, m, H_2), 1.27 (6H, s, H(Me)). - ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.58 (C=S), 129.09-126.17 (CH(Ph)), 101.74 (C8), 97.48 (C_1), 76.36 (C_3), 69.89 (C_7), 69.34 (C_6), 67.66 (C_4), 64.34 (C_5), 48.05 (C_2), 29.70 (CH3). - ESI-HRMS (positive mode) *m/z:* 656.2 g/mol [M + H].

*Thexyl-dimethylsilyl-3,4,6-tri-O-acetyl-2-N-allyloxycarbamoyl-2-amino-2-deoxy-α-D-glucopyranoside (**9**)*

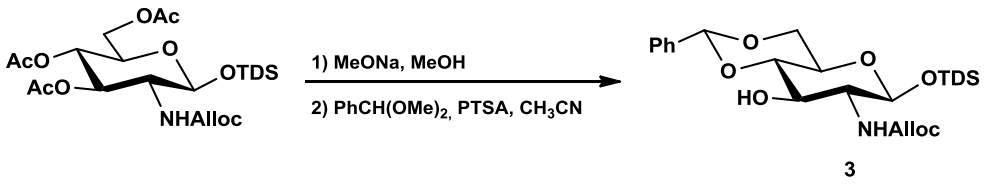


Hydrazine monohydrate (0.85mL, 14 mmol) was dissolved in MeOH (7mL), and the mixture coled at 0°C. AcOH (1.2mL, 21 mmol) was slowely added dropwise and the reaction was let stir at 0°C for 30'. In a second flask, the acetylated compound (5.00g, 11.6 mmol) was dissolved in DMF (50mL), cooled at 0°C, and was added dropwise the solution of hydrazine acetate, letting warm at RT. After 2h the solvent was removed under reduced pressure, the residue dissolved in EA (100mL), and washed with a satured solution of NaHCO3 (3 X 30mL). The organic phases were dried with sodium sulfate and the solvent evaporated in vacuo.

The residue was dissolved in dry DMF (50mL) under N_2 atmosphere. Imidazole (2.36g, 34.8 mmol) was added and the temperature cooled at 0°C. Thexil-dimethyl silyl chloride (3.0mL, 15.1 mmol) was added dropwise and the mixture was let stir at RT for 16h. The solvent was removed under reduced pressure, and the residue redissolved in DCM (100mL), washed with water (2 X 40mL). The aquose phases were extracted with DCM (3 X 100mL) and the combined organic phases were dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex:EA=8:2) to yield **9** (4.18g, 76%) as a white foam. Rf = 0.43 (Hex:EA = 7:3).

The spectroscopic data were in agreement with those reported in literature.

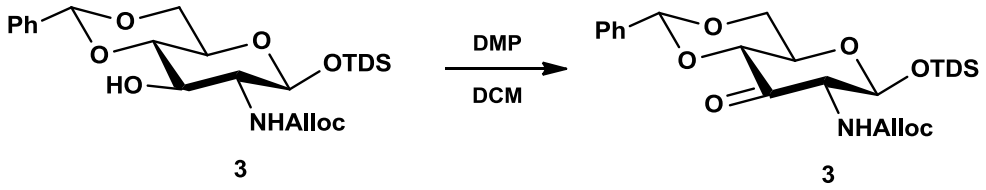
*Thexyl-dimethylsilyl-2-N-allyloxycarbamoyl-2-amino-2-deoxy-4,6-O-benzylidene-α-D-glucopyranoside (**10**)*



3

9 (4.18g, 8.9mmol) was dissolved in dry MeOH (50mL) under N_2 atmosphere. A catalytic amount of a solution 0.1M of MeONa in MeOH was added, and after 2h the reaction was neutralized with Amberlite IR-120. The resin was filtered off, and solvent evaporated in vacuo. The residue was dissolved in CH_3CN (50mL) dry under N_2 atmosphere. $PhCH(OMe)_2$ (2.0mL, 13.35 mmol)and a catalytic amount of PTSA were added, and the mixture let stir at RT for 4h. The reaction was quenched adding TEA until neutral pH, and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex:EA=8:2) to yield **10** (3.77g, 86%) as a white foam. Rf = 0.56 (Hex:EA = 7:3). - ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.56 – 7.24 (m, 5H, (Ph)), 5.94 (m, J = 17.2, 10.4 Hz, 1H, H-10), 5.57 (s, 1H, (CHPh)), 5.34 (dd, J = 17.2, 1.5 Hz, 1H, H-11), 5.28 – 5.20 (dd, 1H, H-11'), 4.85 (s, 1H, H-1), 4.59 (dd, J = 5.7, 1.2 Hz, 2H, H-9-9'), 4.31 (dd, J = 10.5, 4.9 Hz, 1H, H-6), 3.81 (t, J = 10.2 Hz, 1H, H-2, H-6'), 3.59 (t, J = 9.2 Hz, 1H, H-4), 3.54 – 3.45 (m, 2H, H3, H-5), 1.71 – 1.60 (m, 1H, H-Si), 0.95 – 0.85 (m, 12H, H-Thexil), 0.22 – 0.14 (ss, 6H, 2 (CH3Si)). - ^{13}C-NMR (100 MHz, $CDCl_3$): δ 137.13 (C10), 127(Ph), 117.99 (C-11), 101 (CHPh), 91.1 (C-1), 77.01 (C-6), 76.69 (s), 68.72 (C-9), 65.94 (C-2, C-3,C-4, C-5), 24.82 (C-Thexyl).

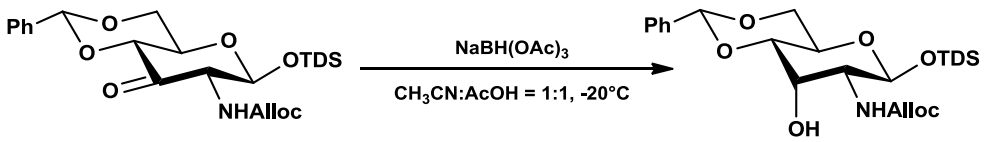
*Thexyl-dimethylsilyl-2-N-allyloxycarbamoyl-2-amino-2-deoxy-4,6-O-benzylidene-α-D-glucopyran-3-uloside (***11***)*



3 **3**

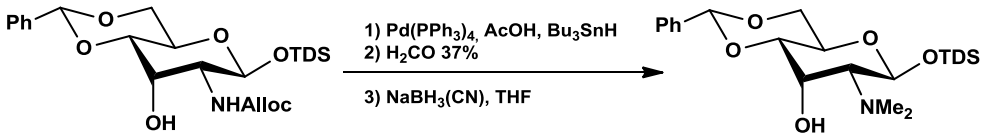
10 (3.77g, 7.65 mmol) was dissolved in dry DCM (30 mL) under N_2 atmosphere. A solution of DMP (9.7g, 23 mmol) in dry DCM (30 mL) was added dropwise, and stirred for 24h at room temperature. After completion of the reaction a 5% solution of thiosulfate (50 mL) was added and the mixture let stirred for 10' until two distinct phases were established. The organic layer was separated and washed with a saturated solution of $NaHCO_3$ (50mL), and water (50mL). The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex:EA = 4:1) to yield **15** as a white foam. (2.64 g, 71%). Rf = 0.34 (Hex:EA = 7:3). - ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.56 – 7.27 (m, 5H, (Ph)), 6.02 – 5.87 (m, 1H, H-10), 5.60 (s, 1H, CHPh), 5.35 (ddd, J = 17.2, 3.0, 1.5 Hz, 1H, H-11), 5.25 (ddd, J = 10.4, 2.6, 1.3 Hz, 1H, H-11'), 4.77 (d, J = 7.4 Hz, 2H, H-9-9'), 4.67 – 4.49 (m, 2H, H-1, H-2), 4.44 (ddd, J = 11.3, 10.2, 3.1 Hz, 2H, H4, H-6), 3.95 (t, J = 10.2 Hz, 1H, H-6'), 3.61 (td, J = 9.9, 4.9 Hz, 1H, H-5), 1.65 (ddd, J = 16.8, 11.9, 4.9 Hz, 1H, H-Si), 0.95 – 0.82 (m, 12H, H- Thexyl), 0.24 – 0.14 (m, 6H, 2 (CH3Si)).

*Thexyl-dimethylsilyl-2-N-allyloxycarbamoyl-2-amino-2-deoxy-4,6-O-benzylidene-α-D-allopyranoside (***12***)*



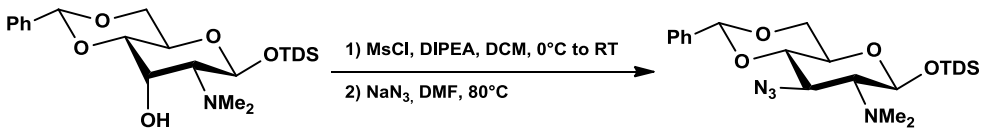
15 (2.64g, 5.4 mmol) was dissolved in a mixture of CH_3CN:AcOH=1:1 (50mL) under N_2 atmosphere and the reaction cooled at -20°C in an dry ice-acetone bath. $NaBH(OAc)_3$ (3.4g, 16.2 mmol) was slowely added dropwise and the mixture was let stir for 2h. The solvent was removed under reduced pressure, and the residue redissolved in DCM (50mL), washed with water (2 X 30mL). The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex:EA = 4:1) to yield **16** as a white foam (2.42 g, 91%). Rf = 0.33 (Hex:EA = 7:3). - ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.62 – 7.18 (m, 5H, (Ph)), 5.95 (ddd, J = 16.1, 10.9, 5.7 Hz, 1H, H-10), 5.61 (s, 1H, CHPh), 5.34 (ddd, J = 17.3, 3.1, 1.5 Hz, 1H, H-11), 5.29 – 5.18 (m, 1H, H-11'), 4.91 (d, J = 8.0 Hz, 1H, H-1), 4.59 (d, J = 4.8 Hz, 2H, H9, H-9'), 4.36 (dd, J = 10.3, 5.0 Hz, 1H, H-6)), 4.29 (d, J = 1.5 Hz, 1H, H-3), 3.98 (td, J = 10.0, 5.0 Hz, 1H, H-5), 3.81 (t, J = 10.3 Hz, 2H, H-2, H-6), 3.69 (dd, J = 9.4, 2.5 Hz, 1H, H-4), 1.65 (dt, J = 13.7, 6.9 Hz, 1H, H-Si), 0.95 – 0.79 (m, 12H, H-Thexil), 0.18 (dd, J = 6.1, 3.0 Hz, 6H, 2 (CH3Si)). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.09 (C-10), 127(Ph), 117 (C-9), 101 (CHPh), 96 (C-1), 77.33 (C-4), 79 (C-3), 69.17 (C-6), 65.5 (C-9), 62 (C-5), 24.80 (C- Thexyl).

*Thexyl-dimethylsilyl-2-deoxy-4,6-O-benzylidene-2-N,N-dimethylamino-α-D-allopyranoside (***13***)*



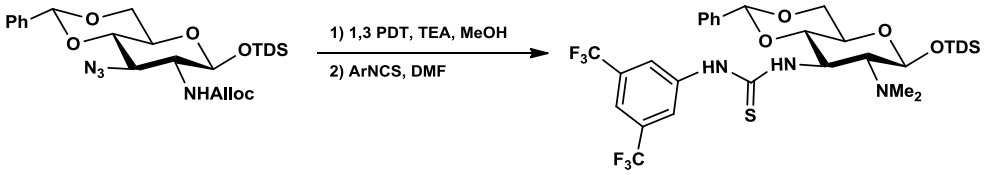
16 (2.42g, 4.9 mmol) was dissolved in dry DCM (30mL) under N_2 atmosphere. A catalytic amount of $Pd(PPh_3)_4$, glacial AcOH (0.67mL, 11.7 mmol) and Bu_3SnH (1.46mL, 5.39 mmol) were added in succession. The mixture was let stir at RT for 24h. The solvent was removed under reduced pressure and the residue dried in vacuo. This one was redissoved in THF, then a solution of CH_2O 37% in water (12.7 mL, 171 mmol) was added and let stir for 24h. $NaCN(BH)_3$ (2.5g, 39.2 mmol) was slowly added and stirred for 2h. Then the reaction was quenched with AcOH until pH = 4-5, and the mixture was let stir for 3h. Then the reaction was cooled at 0°C with an ice bath, and the pH adjusted slowly at 9 by a cold 5% solution of NaOH. Then the solution was extracted with AcOEt (2 X 30 mL) and the organic phase washed with brine (20 mL) and water (20 mL). The organic phases were dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (DCM:MeOH=95:5) to yield **17** (1.04g, 49%) as a white solid. Rf = 0.43 (DCM:MeOH=95:5). - ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.20 (m, 5H, (Ph)), 5.58 (s, 1H, CHPh), 5.24 (d, J = 7.9 Hz, 1H, H-1), 4.35 (m, 1H, H-6), 3.98 (dd, J = 10.0, 5.0 Hz, 1H, H-5), 3.74 (t, J = 10.3 Hz, 2H, H-3, H-6), 3.57 (dd, J = 9.4, 2.6 Hz, 1H, H-4), 2.6 (d, J = 3.2 Hz, 6H, 2 (NCH3)), 2.35 (dd, J = 7.9, 2.8 Hz, 1H, H-2), 1.71 (dt, J = 13.7, 6.9 Hz, 1H, H-Si), 1.03 – 0.79 (m, 12H, H-Thexil), 0.31 – 0.09 (m, 6H, 2 (CH3Si)). - ^{13}C NMR (100 MHz, $CDCl_3$): δ132.52 – 131.59 (Ph), 101.89 (CHPh), 95.38 (C-1), 79.47 (C-4), 69.60 (C-3), 68.27 (C-6), 67.5 (C-2), 63.47 (C-5), 43.94, 33.79, 24.80 (C- Thexyl). - ESI-MS (positive mode) *m/z:* 438.4 g/mol [M + Na].

*Thexyl-dimethylsilyl-3-azido-4,6-O-benzylidene-2-N,N-dimethylamino-2,3-deoxy-α-D-allopyranoside (***14***)*



17 (1.04g, 2.4 mmol) was dissolved in dry DCM (20mL) under N_2 atmosphere. DIPEA (2.53mL, 14.4 mmol) was added and the solution let stir 15'. The temperature was cooled at 0°C in an ice-bath and mesyl chloride (0.56mL, 7.2 mmol) was added dropwise. The temperature was raised at RT and the mixture let stir for 2h. The reaction was diluted with DCM and the organic phase washed with a solution of HCl 5%. The organic phase was dried with sodium sulfate and the solvent evaporated in vacuo. The crude was let for 2 h in vacuo, then dissolved in humid DMF (30 mL). NaN_3 (0.47g, 7.2 mmol) was added and the reaction let stir at 80°C over night. The solvent was removed under reduced pressure, the residue redissolved in DCM (40 mL) and washed with water (20 mL). The organic phase were dried with sodium sulfate and the solvent evaporated in vacuo. The residue was purified by flash chromatography (Hex:EA=9:1) to yield **18** (277 mg, 25%) as a white foam. Rf = 0.80 (Hex:EA = 8:2). - ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.66 – 7.13 (m, 5H, Ph)), 5.57 (s, 1H, CHPh), 4.86 (d, J = 7.9 Hz, 1H, H-1), 4.29 (dd, J = 10.5, 4.9 Hz, 1H, H-6), 3.73 (dt, J = 27.0, 10.2 Hz, 2H, H6', H-4), 3.51 (t, J = 9.4 Hz, 1H, H-3), 3.37 (td, J = 9.6, 4.9 Hz, 1H, H-5), 2.60 – 2.46 (m, 7H, H-2, 2 (NCH3)), 1.70 (dq, J = 13.9, 6.9 Hz, 1H, H-Si), 0.98 – 0.85 (m, 12H, H- Thexyl), 0.19 (t, J = 8.0 Hz, 6H, 2 (CH3Si)). - ESI-MS (positive mode) *m/z:* 463.5 g/mol [M + H].

*Thexyl-dimethylsilyl-4,6-O-benzylidene-2,3-deoxi-2-N,N-dimethylamino-3-N-3,5-bis-(trifluoromethyl-thiourea-α-D-glucopyranoside (***15***)*



18 (277 mg, 0.6 mmol) was dissolved in dry MeOH (5 mL) under N_2 atmosphere. TEA (0.58mL, 4.2 mmol) and 1,3-PDT (0.43mL, 4.2 mmol) were added and the solution was warmed at 60°C and let stir for 24h. The reaction was cooled at RT, and the solvent removed in vacuo. The residue was redissolve in dry DMF, under N_2 atmosphere. 3,5-bis (trifluoromethy) isothiocyanate (0.55mL, 3.0 mmol) was added and the solution let stir for 24h. Then the solvent was removed in vacuo, and the residue was purified by flash chromatography (Tol:EA=9:1) to yield **19** (127 mg, 30%) as a colorless oil. Rf = 0.15 (Hex:EA =9:1). - ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.65 – 7.11 (m, 8H, (Ph)), 5.62 (s, 1H, CHPh), 5.00 (d, *J* = 7.7 Hz, 1H, H-1), 4.36 (dd, *J* = 10.7, 4.9 Hz, 1H, H-6), 3.7 (t, *J* = 9.3 Hz, 2H, H-6, H-3), 3.60 (t, 1H, H-4), 3.58 (m, 1H, H-5), 2.38 (m, 7H, H-2, 2 (NCH3)), 1.72 (dt, *J* = 13.8, 6.9 Hz, 1H, H-Si), 0.97 – 0.89 (m, 12H, H-Thexil), 0.23 (d, *J* = 3.3 Hz, 6H, 2 (CH3Si)). - ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.37 (C=S), 128.86 (d, *J* = 38.9 Hz, (Ph)), 103.54 (CHPh), 96.63 (C-1), 81.74 (C-4), 69.62 (C-6), 67.15 (C-5), 56.50 (C-3), 41.35, 33.80, 20.81 (C- Thexyl). - ESI-MS, (positive mode) *m/z*: 730.4 g/mol [M + H].

Catalytic tests

All the catalytic tests were performed with a catalyst loading of 10%, for 24h, with 1mL of solvent 1 eq. of β-nitrostyrene and 1.2 eq of acetylacetone or diethylmalonate were used. The products were isolated by flash chromatography (Hex:EA=9:1), and the e.e. was evaluated by HPLC on Chiralpak AD. As mobile phase was used Hex/iPrOH=85:15, with P=15bar.