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Synthesis of fragments of *Salmonella typhi* capsular polysaccharide and their zwitterionic analogues

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Abbreviations and acronyms

Ac	Acetyl
ACN	Acetonitrile
AcOEt	Ethyl acetate
AcOH	Acetic acid
AgOTf	Silver triflate
APC	Antigen-presenting cell
BAIB	Bis(acetoxy)iodobenzene
BCR	B-cell receptor
CPS	Capsular polysaccharide
CRM_{197}	Cross-reactive material from diphtheria toxin
CSA	10-Camphorsulfonic acid
CTL or CD8+	Cytotoxic T lymphocytes
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DC	Dendritic cell
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	*N*,*N*-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMP	Dess-Martin periodinane
DT	Diphtheria toxoid
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
Et	Ethyl
FTMS	Fourier transform mass spectroscopy
Gal	Galactose
Glc	Glucose
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography

IBX	2-Iodoxybenzoic acid
IC_{50}	Half maximal inhibitory concentration
ICR	Ion cyclotron resonance
Ig	Immunoglobulin
Im	Imidazolyl
ImH	Imidazole
KLH	Keyhole limpet hemocyanin
Lev	Levulinoyl
Man	Mannose
Me	Methyl
MHC	Major histocompatibility complex
MPLA	Monophosphoryl lipid A
NBS	*N*-Bromosuccinimide
NK	Natural killer
NMR	Nuclear magnetic resonance
OMP	Outer membrane protein from *N. meningitidis* serogroup B
OTf	Triflate
Pam_3Cys	Tripalmitoyl-*S*-glyceryl-cysteine lipopeptide
PAMP	Pathogen-associated molecular pattern
Ph	Phenyl
PMB	*p*-Metoxybenzyl
PRR	Pattern recognition receptor
PS	Polysaccharide
PTFAI	*N*-Phenyltrifluoroacetimidate
pTSA	*p*-Toluenesulfonic acid
Py	Pyridine
rEPA	Recombinant exoprotein from *Pseudomonas aeruginosa*
Rha	Rhamnose
rt or r.t.	Room temperature
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide

TBDMS	*t*-Butyldimethylsilyl
tBuOH	*t*-Butanol
TCA	Trichloroacetimidate
TCR	T cell receptor
TDS	Textyldimethylsilyl
TEMPO	2,2,6,6-Tetramethylpiperidine *N*-oxide
TFA	Trifluoroacetic acid
Th or CD4+	Helper T cells
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TLR	Toll-like receptor
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TOF	Time-of-flight
Tol	Toluene
TT	Tetanus toxoid
Tyv	Tyvelose
WHO	World Health Organization
ZPS	Zwitterionic polysaccharide

1

Introduction

1.2 Fundamentals of immunology

The immune system is the body's defense against infectious organisms and other invaders.[1] It is made up of a network of cells, tissues, molecules and organs that work together, through a series of steps called immune response, to protect the body against the invasion of organisms and substances that can cause diseases. Molecules which are perceived by the immune system as a foreign invader or simply as potentially dangerous for the host are referred to as antigens. The first barrier against external agents is physical and includes mechanical defenses, such as epithelial tissues (skin and mucous), and their secretions.

1.2.1 Specific and nonspecific immunity

The immune system can react against pathogens by the integration of two distinct arms of the immune response: the innate and the adaptive responses (Figure 1). The former is rapid and aspecific, and establishes the first line of immune defense, acting during the early stages of infection (within minutes). Inflammation is a sign of the activation of this defensive line: it is initiated by antigen-presenting cells (APCs) present in all tissues, mainly resident macrophages, dendritic cells (DCs), histiocytes, Kupffer cells, and mastocytes. These cells present receptors named pattern recognition receptors (PRRs), which can be stimulated by molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs). At the onset of an infection, these cells undergo activation and release soluble chemical factors, including specialized chemical mediators, called cytokines, which recruit specialized white blood cells (monocytes, macrophages, phagocytes, dendritic cells, NK cells) to sites of infection. Moreover, they activate the complement system, a biochemical cascade that helps, or "complements", the ability of antibodies to clear pathogens or mark them for destruction by other cells. The cascade is composed of many plasma proteins, synthesized in the liver, primarily by hepatocytes.

On the contrary, the adaptive response is slower (typically takes days to weeks to develop) but recognizes pathogens with high affinity, providing the fine antigenic

specificity required for complete elimination of the infective agent and the generation of the immunological memory. Antigen-presenting cells, and in particular dendritic cells, provide a crucial bridge between the innate and adaptive responses.

The immunological response causes the production of specific proteins (commonly known as antibodies) and the participation of many different types of cells called lymphocytes, which belong to the leukocyte class. In detail, the specific immunity is different from the nonspecific one by four main characteristics:

- specificity: is the ability of the immune system to recognize, in an extremely selective way, and destroy particular antigens by producing specialized lymphocytes and specific antibodies. The antigens which are able to provoke such a response are viruses, bacteria, fungi, protozoa and parasitic worms.
- diversity: it is derived from the ability of the specific system to respond to millions of different antigens. Such a variety of reactions is made possible by the presence of a great number of lymphocytic cells, each specific to a particular immunological agent.
- recognition of *self*/*non-self*: this characteristic refers to the skill of the immune system to distinguish own-body molecules from the foreigners (antigens). Anomalies which affect this recognition ability can cause severe diseases, called autoimmune, in which the immune system attacks tissues of its own organism, misidentifying them as external.
- memory: it is related to the faculty of the specific system to "remember" antigens which has already encountered. As a result, the reaction is faster and more effective when successive exposures to the same antigen occur. The practice of vaccination is founded upon this characteristic.

Immunity acquired through vaccination is called artificial immunity, as opposed to the active one which naturally develops in patients recovered from an infectious illness. Vaccines consist of a large variety of microorganisms or parts of them (living attenuated bacteria, killed germs, toxoids, microbial products) deprived of their power of infection but not of their immunogenicity. Moreover, antibodies can be transmitted from a person to another: this is the passive immunity. Such an

immunity can be natural, as from mother to son through the placenta, or artificial, through the direct administration of antibodies from an animal or from a human being already immune against the disease of interest.

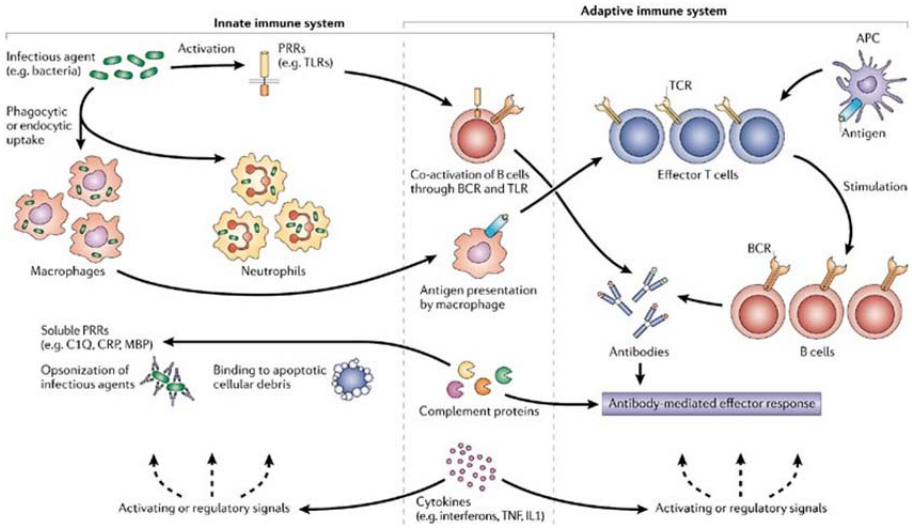


Figure 1: the immune system.

1.2.2 B and T Lymphocytes and immunoglobulins

Lymphocytes play a key role in the immunological response. In vertebrate organisms there are two different kinds of lymphocytes: the B ones, responsible for the humoral response, and the T ones, which take part in the cell-mediated reaction (Figure 2a). As mentioned above, the APCs surface is plenty of pattern-recognition receptors (PRRs), including the recently discovered Toll-like receptor family (TLRs), that can recognize a huge variety of PAMPs, leading to full maturation of DCs, antigen uptake and intracellular processing (Figure 2a). The triggering and amplification of the adaptive arm of the immune response initiates when mature DCs migrate to the draining lymph nodes, where they prime naïve T cells. The crucial event for T cell activation/differentiation is the immunological synapse, initiated by the formation of a ternary complex between MHC (major histocompatibility complex, class I or II) on DC surface, antigen and T cell receptor (TCR) on the T cell surface. Depending on the antigen exposed on the DC surface,

the immunological synapse might induce the activation of cytotoxic T lymphocytes (CTL, or CD8+ T cells, in cases in which the antigen is presented by class I MHC), effector T cells that destroy target cells infected by intracellular viruses or bacteria, and/or the proliferation of helper T cells (Th, or CD4+ T cells, in cases in which the antigen is presented by a class II MHC). Furthermore, T and B cells can cooperate in a so-called T cell-dependent immune response mechanism. Activated Th cells interact with resting B cells through a class II MHC and driving their proliferation and differentiation into plasma cells (antibody-forming cells, mainly producing low-affinity IgM-type antibodies) and memory B cells. Unlike plasma cells, memory B cells survive for a long time in the body and respond rapidly to subsequent exposures of antigen by secreting high-affinity IgG antibodies.

Antibodies are a class of proteins named immunoglobulins (Ig) possessing particular combinatory sites that can bind structural-complementary particles (antigens). Antibodies consist of two different parts: the constant region, which is in common to all immunoglobulins belonging to the same class, and a variable region, which includes the site of interaction with the antigen. From a structural point of view, immunoglobulins consist of four glycopeptide chains (tetramers): two light chains identical to each other and two heavy chains also identical to each other. Tetramers are tied to each other (in a Y-shape) by 3 to 14 disulphide bond, depending on the immunoglobulin class. The heavy chains, next to each other, are the trunk of the Y, while the light chains are the arms. There are five classes of human immunoglobulins: IgG, IgA, IgM, IgD, IgE.

Several antigens are protein structures or polysaccharide molecules, often components of the external coatings of the immunogenic agent. Usually, antibodies are not able to recognize the whole molecule. In fact, they can recognize only a small superficial part of it called antigenic determinant or epitope.

As above mentioned, activation of B cells in order to create memory B cells is a process that requires participation of T-helper cells. Antigens which are able to stimulate this cooperative response are called T-dependent. Conversely, T-independent antigen can provoke humoral immunological reaction without collaboration with T-helper cells. Carbohydrates, such as the polysaccharide chains

found in capsule of bacteria, are typical T-independent antigenic molecules. They stimulate B cells by cross-linking the B-cell receptors (BCRs) and drive the production of IgM immunoglobulins. This process results in a lack of production of new memory B cells and a depletion of the memory B cell pool, so that subsequent immune responses are decreased (Figure 2b).

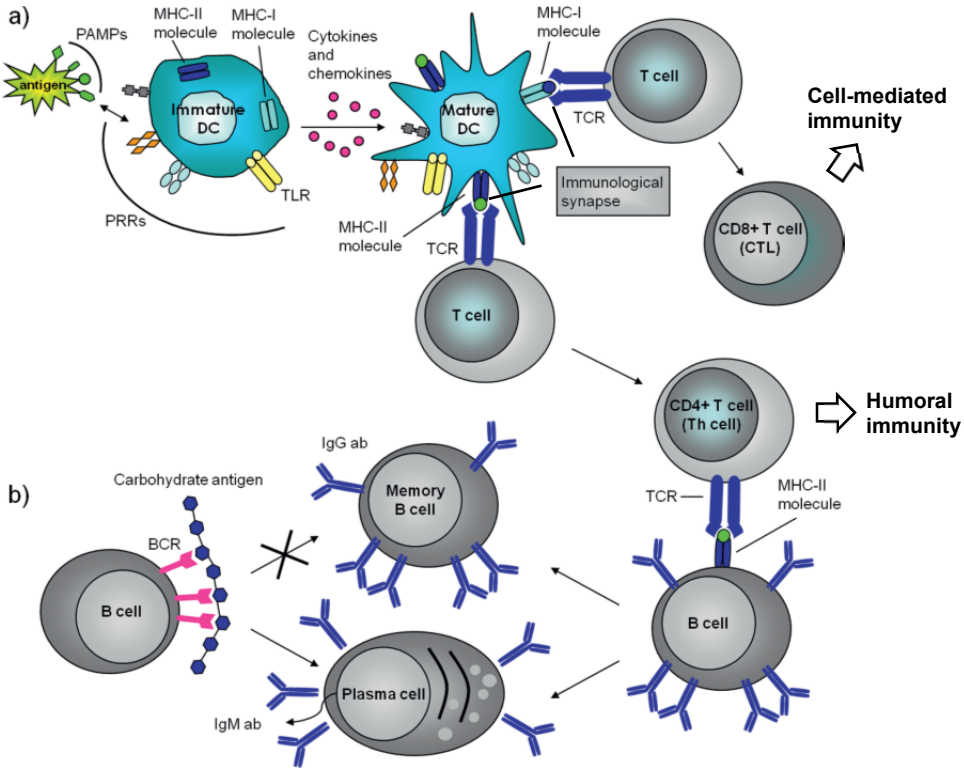


Figure 2: diagramatic representation of the immune response.

1.3 Carbohydrates and immunological response

The first communication about polysaccharides in immunology dates back to 1917, when Dochez and Avery published an article which dealt with a "specific soluble substance" secreted by Pneumococcal bacterium during its growth.[2] Few years later, in 1923, Heidelberger and Avery demonstrated that the soluble substance was simply a polysaccharide.[3,4] This fundamental discovery had a double result: it was the first example of the correlation between chemical structure and immune specificity of antigens and, on the other hand, denied the belief that only proteins could be antigens. Immunological properties of polysaccharides were found in their abilities to induce immunological response and to subsequently create specific antibodies (globulins). In the following years, the concept that only a particular small part of the polysaccharide (determinant zone or epitope) is essential in the antigen-antibody interaction and, as a result, for the antigenic specificity, has been developing. There are two categories of epitopes: sequential epitopes, characterized by a specific linear sequence, and conformational epitopes with a particular 3D structure. Moreover, an antigen molecule may contain several epitopes, each recognized by different antibodies.

1.3.1 Anatomy of the bacterial cell

In order to examine in detail the immunology of polysaccharides, it is useful to briefly describe the anatomy of the microorganisms which are the objects of the immunological response. From a structural point of view, bacteria (unicellular prokaryotic microorganisms) are primarily composed of a plasma membrane which encloses the cytoplasm. This membrane, made of phospholipids and proteins, is a semipermeable barrier that acts as a support platform for enzymes of the respiratory chain, of the biosynthesis of membrane phospholipids, of wall polymers and of DNA. Outside the plasma membrane there is the cell wall, which gives structural rigidity to the cell. In prokaryotes, this element mainly protects the internal osmotic pressure caused by the great discrepancy between protein (or other molecules) concentration inside and outside the cell. Bacterial cells are different from all other

cells in nature because of the presence of peptidoglycans near the cytoplasmic membrane. The layer of complex peptides (peptidoglycans) consists of a skeleton composed of alternate units of *N*-acetylglucosamine and *N*-acetylmuramic acid, to which peptide chains are linked by amide bonds between L-alanine units and COO^- moiety of the muramic acid. In addition, the peptide sequence creates cross-links with polyglycines by $–NH_2$ group in lysine. All these interactions result in the formation of a narrow network structure which is able to give rigidity to the wall of the cell. Even though all the bacterial cell walls contain peptidoglycans not all of them have the same structure: this diversity, which reflects in permeability differences, makes it possible to classify bacterial microorganisms in Gram-negative and Gram-positive. In the latter the peptidoglycan layer is relatively thick (200-800 Å) and, in addition, there are teichoic and lipoteichoic acids. On the contrary, in Gram-negative bacteria the peptidoglycan layer is thinner (50-100 Å). Furthermore, there is an additional outer membrane made by lipopolysaccharides, lipoproteins and phospholipids (Figure 3). The chemical structure of the lipopolysaccharide membrane (also called endotoxin) is often specific for any bacterial strain and is one of the main factors of immunogenicity in these bacteria.

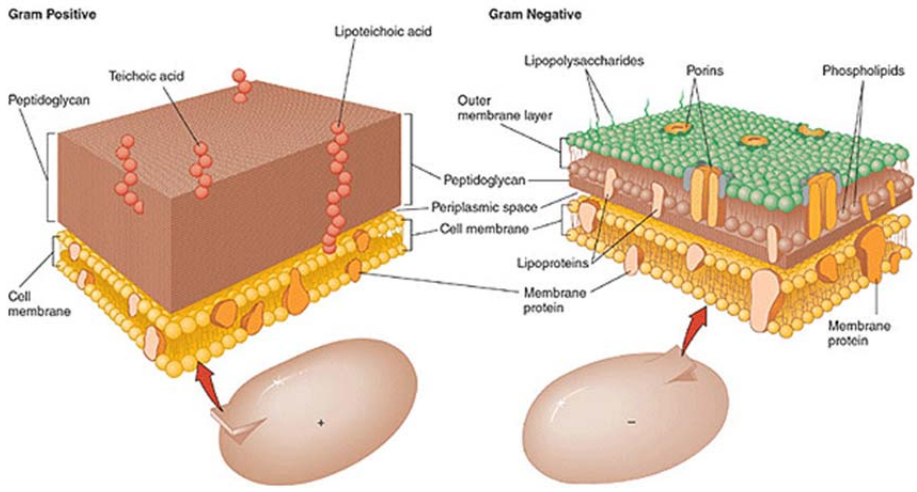


Figure 3: cross section of bacterial cell walls.

1.3.2 Bacterial capsule

Regardless of being Gram-negative or Gram-positive, several bacteria possess one more outer membrane called capsule, and so they are referred to as encapsulated bacteria. It is composed of polysaccharide chains, homo- or heteropolymers usually consisting of small oligosaccharide repeating units (1-6 monosaccharide elements, often D-glucose, D-galactose, D-mannose and L-fucose), but with a great variability of functionalizations. The capsule is considered a virulence factor because it enhances the ability of bacteria to cause disease. The capsule can protect bacterial cells from engulfment by eukaryotic cells, such as macrophages and delays the protective action of the specific immune system. Currently, encapsulated bacteria are one of the main causes of the high mortality rate owed to bacterial infections in several geographic areas.

As previously described, interactions between antigen and antibody do not involve the whole polysaccharide chain, but only small portions are responsible for the recognition phenomenon. A logic consequence is that both primary and secondary structures of the polysaccharide capsule are significant. The former is responsible for the serological characteristic of the bacterium, whereas the latter determines the physical and biological properties. As well as proteins, polysaccharides can adopt particular 3D structures using inter- or intramolecular interactions which are sensible to changes in temperature. It is this ordered conformation that makes it possible the interaction between bacteria and the host.

1.3.3 Immunogenic polysaccharides and cellular response

As already highlighted in 1.2.2, the cooperation between T and B cells is essential for the establishment of the immunological memory. However, immune responses to polysaccharides are typically T cell-independent. Because of their polymeric structures, polysaccharides bind several B cell receptors (BCRs) simultaneously, leading to direct activation of B cells without cooperation with T cells. As a result, the hallmark of the immune response to carbohydrates is an exclusively primary immune response with low-affinity IgM production and no class switch to high-

affinity IgG antibodies. Moreover, pure polysaccharides cause immune responses of relatively short duration, and they do not induce immunological memory (*i.e.*, they fail to evoke a booster effect). This effect is even more evident in immunologically immature individuals, such as infants and children, who do not have sufficiently differentiated G and M immunoglobulins able to provide an immunological protection.

1.3.4 Glycoconjugate vaccines

The use of polysaccharide-based vaccines as prophylactic method has started in the middle of last century. However, due to the T-independent response raised by polysaccharides, vaccines based on CPS have limited clinical usefulness. In 1929 Avery and Goebel reported for the first time the use of low molecular weight carbohydrates (containing the epitope of capsular bacterial polysaccharides) linked to carrier proteins.[5] These constructs were able to stimulate the response of the immune system through the production of specific antibodies. These findings demonstrated the possibility to enhance the immunogenicity of T cell-independent polysaccharides by conjugation to an immunogenic carrier protein, generating T cell-dependent glycoconjugate antigens able to induce immunological memory. The protein carrier incorporates T cell epitope peptides, which facilitate uptake and processing of the glycoconjugate by APCs, enhancing the presentation of the carbohydrate antigen for activation of helper T cells.[6] In this way immunological memory is established, raising a strong, durable and protective immune response from early childhood. Carrier proteins must be non-toxic, non reactogenic and should not share T cell epitopes with relevant capsular pathogens. Diphtheria toxoid (DT), CRM_{197}, keyhole limpet hemocyanin (KLH), tetanus toxoid (TT), recombinant exoprotein from *Pseudomonas aeruginosa* (rEPA), outer membrane protein from *N. meningitidis* serogroup B (OMP) and more recently protein D derived from non-typeable *H. influenzae* are among the most used proteins.

Commonly employed conjugation methods make use of chemoselective reactions between suitable functional groups available both on the protein and on the

carbohydrate moiety. Anchoring sites on the protein can be lateral amines (lysine residues), carboxylic acids (aspartic or glutamic acid) or sulfhydrils (cysteine). In addition, specific functional groups can be introduced on the protein through site selective modification techniques. On the contrary, attachment sites on the carbohydrate portion can be the intrinsic carbonyl group at the reducing end or aldehyde groups inserted by random periodate oxidation of oligo- or polysaccharides, whereas the use of synthetic carbohydrates provided with a suitable linker ending with amino, carboxylic, sulfhydrilic or olefinic groups ensures to preserve the structural integrity of the saccharide moiety (Figure 4).[7]

Even if glycoconjugate vaccines are among the most used worldwide in current routine immunization programs, they suffer from some problems. First of all, the random strategies do not allow an accurate control of the conjugation reaction. Moreover, the precise structure of the carbohydrate portion eliciting the immune response cannot be established *a priori*, leading to an increase in manufacturing cost due to low batch-to-batch reproducibility and difficult characterization of the products. Additionally, in some cases the immunological response of the protein can interfere with that of the saccharide moiety. This is especially true for glycoconjugates of short chain oligosaccharides. Lastly, care must be taken in the choice of the linker. In fact, it has been shown that these moieties could be highly antigenic and therefore suppress antibody responses to weakly immunogenic saccharide antigens such as *self*-antigens.[8,9] For all these reasons, the identification of alternatives to glycoconjugate constructs is becoming nowadays a relevant task in the vaccinology research field.

Furthermore, in order to achieve optimal host protection, a vaccine setting should include a component (adjuvant) capable of amplifying the immune response. In particular, because saccharide antigens are often poorly immunogenic, carbohydrate-based vaccines need adjuvants to improve their efficiencies and the quality and specificity of their immune responses. Typical immunoadjuvants widely explored in vaccine settings are complete and incomplete Freund's adjuvants, Detox, QS-21 (a saponin extracted from the bark of the Quillaja saponoria tree), and monophosphoryl lipid A (MPLA).

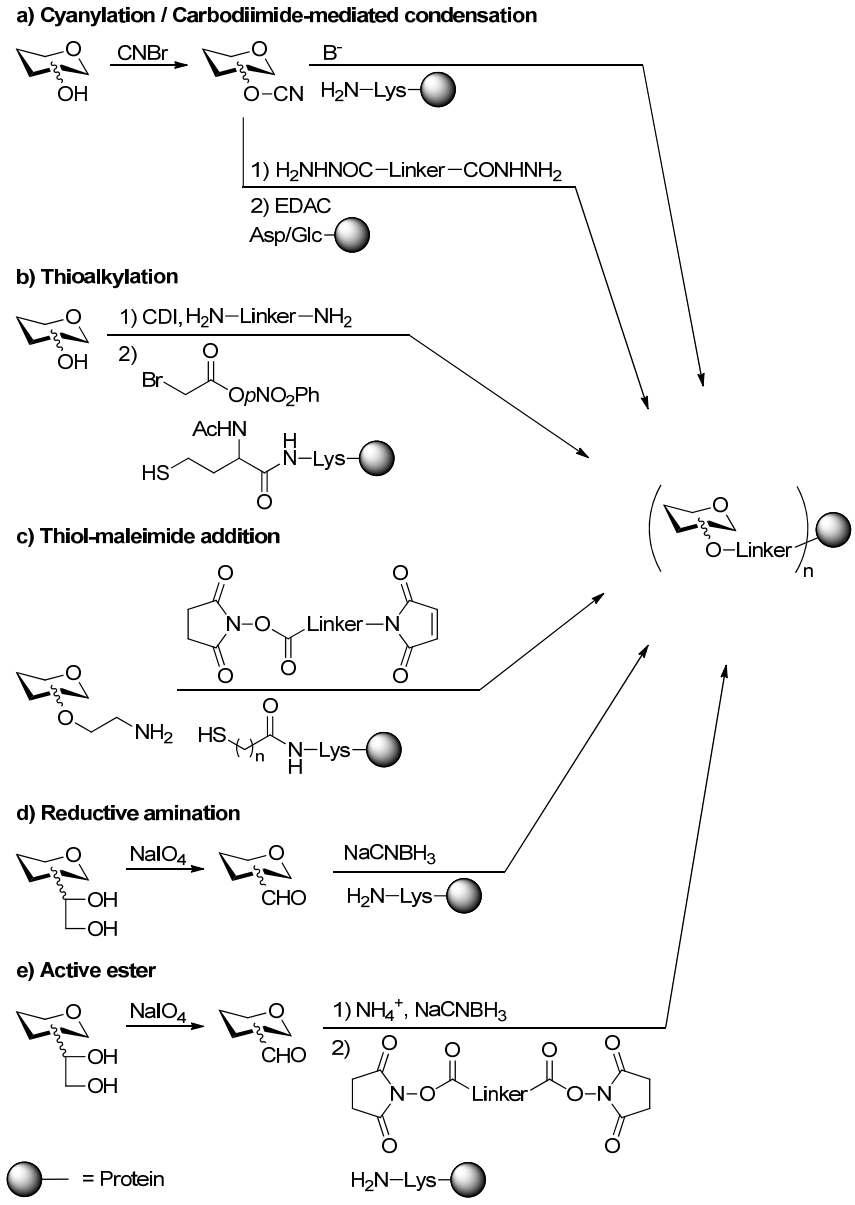


Figure 4: commonly employed strategies for the conjugation of carbohydrate antigens to carrier proteins.

The administration of these strong immunoactive species can cause undesired side effects, however, and milder and safer lipopeptide-based immunoadjuvants have been employed in many vaccine candidates. In particular, a number of derivatives of the lipopeptide tripalmitoyl-S-glyceryl-cysteine (Pam_3Cys) have been covalently linked to other components and included as "built-in" immunoadjuvants in vaccine

constructs (Figure 5). Adjuvants are perceived as "danger signals" after binding to PRRs, and stimulate the activation and maturation process of APCs, thus enhancing the speed and duration of both the innate and the adaptive immune responses. In particular, adjuvants function as immune potentiators, providing the pro-inflammatory context necessary for optimal antigen-specific immune activation and amplifying the innate immune response. On the other hand, adjuvants can also act as delivery systems to localize vaccine components and to target them to APCs.

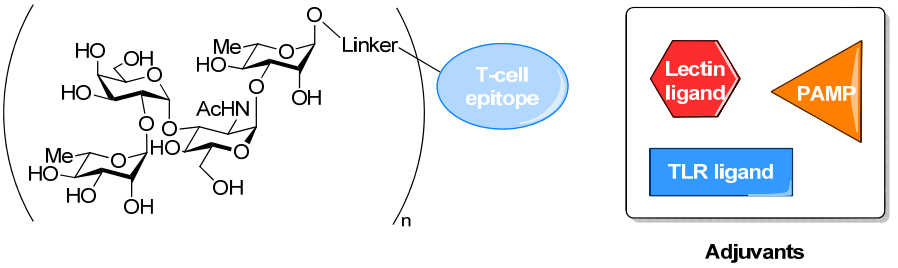


Figure 5: composition of glycoconjugate vaccines.

1.3.5 Multivalency

Biological interactions involving carbohydrates have an extremely low glycan-receptor affinity, often in the mM range. In nature, this issue is circumvented by multivalent receptor-ligand interactions, where multiple ligands interact simultaneously with multimeric receptors inducing an increase of the binding affinities of several order of magnitude. This is called "multivalent effect" or just "multivalency". When this phenomenon is referred to carbohydrate-receptor interactions, it is commonly named "cluster glycoside effect".[10] It is a widespread opinion that this effect is essential for many fundamental biological interactions. Generally speaking, the valency of a system is the number of connections of the same type it can generate during the interaction process with a receptor. The main difference between mono- and multivalent interactions has thermodynamic causes. The association constant of a polyvalent interaction k_{poly} is higher than that of the monovalent interaction k_{mono}. This difference depends on the structure and on the geometry of the receptor, as well as on the disposition of the ligands on the surface.

As far as the immune system is concerned, it is well known that all the classes of antibody have two receptor sites. Polyvalent bond with recognition patterns seems to be a peculiar characteristic of the immune system. A major goal pursued by the vaccinology research is therefore to reproduce the multivalency effect into fully synthetic systems using polyfunctional scaffolds capable of displaying multiple copies of carbohydrate antigens. Under this point of view, the traditional glycoconjugate vaccines can be considered as an example of replication of the cluster glycoside effect, since they contain a large number of carbohydrate antigens exposed on the protein surface that are collectively much more immunogenic than the corresponding monovalent forms. Fully synthetic vaccines are based on a modular architecture and incorporate into the same molecule different functional units: a synthetic glycan or a multivalent glycocluster, a synthetic T cell epitope and an adjuvant, such as a ligand of TLR or C type-lectin receptor onto dendritic cells surface. Each of them is synthesized independently, and then chemically conjugated in a convergent way, providing total control on the chemical structure (Figure 6).

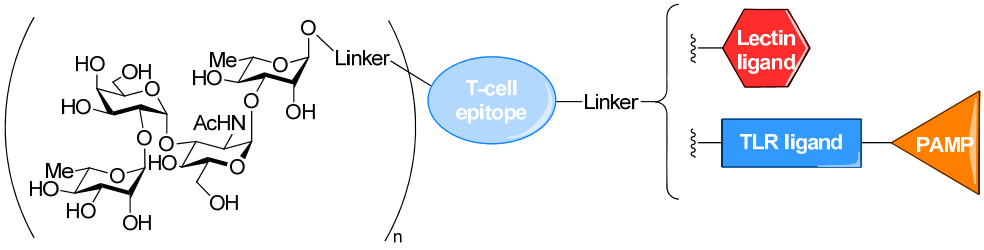


Figure 6: composition of a fully synthetic vaccine.

During the last years, a wide range of synthetic clustered glycosides has been designed to interfere in an array of immune-related biological processes. Among them, glycopeptides,[11-14] glycodendrimers,[15-20] glycopolymers,[21-25] glyconanoparticles,[26,27] glycofullerenes,[28-30] glycocalixarenes,[31-35] and sugar-functionalized carbon nanotubes[36] and quantum dots[37-39] were shown to be efficient biomimetics of natural glycoclusters, often reaching low nM activities.

1.3.6 Zwitterionic polysaccharides

Zwitterionic polysaccharides (ZPS) are a class of structurally distinct bacterial polysaccharides that possess a zwitterionic charge motif distributed along the chain (*i.e.* they contain both positive and negative charge centers within a repeating unit structure). Typical examples are the Sp1 capsular polysaccharide from *S. pneumoniae* type 1 and capsular polysaccharides A1, A2 and B from *Bacteroides fragilis* (Figure 7).

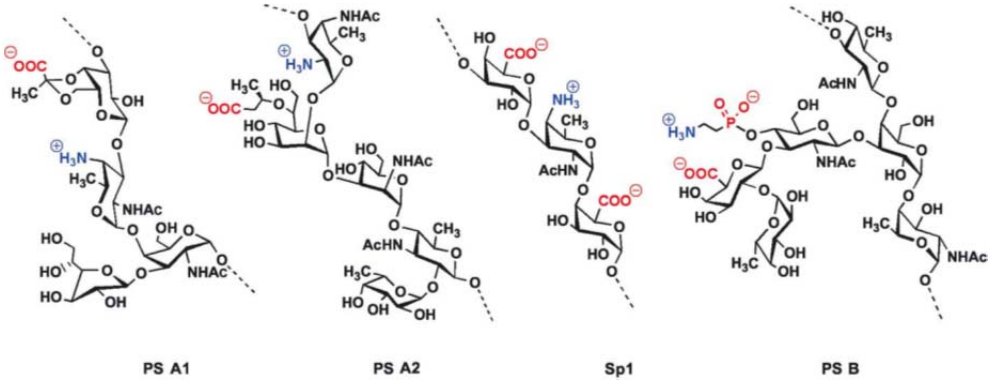


Figure 7: examples of zwitterionic polysaccharides.

ZPSs display a unique behavior among bacterial polysaccharides. A number of recent reports described the ability of such compounds to act like traditional T cell-dependent antigens, activating CD4+ T cells both *in vitro* and *in vivo* through the traditional MHC-dependent mechanism, without protein conjugation.[7,40-44] More interestingly, it seems that also the introduction of a zwitterionic motif into a naturally non-zwitterionic polysaccharide induces the activation of T cells.[45,46] The immunogenic activity of ZPS is strictly related to their three dimensional conformation and overall spatial charge organization: it has been proposed that they assume an helical conformation, where all the charges are exposed on the outer surface of the polymer, in a regularly spaced pattern.[47,48] Based on these findings, ZPS might offer previously unrecognized opportunities for the design of new classes of vaccines:

- the use of ZPSs as scaffolds, acting both as carrier and adjuvant (as shown by Andreana and co-workers).[49-51]

- the artificial introduction (by chemical and/or chemo-enzymatic methods) of a zwitterionic charge motif onto the surface glycans of selected pathogens.

The latter methodology could represent a novel and valuable alternative to currently employed glycoconjugate vaccines (see 1.3.4), and could afford vaccines useful for the prevention of a variety of infectious diseases, especially in pediatric area at high risk of infections.

However, a better understanding of how ZPS antigens stimulate the host immune system and a correlation of the ZPS structural properties with their biological activity are required.

2

Salmonella typhi and typhoid fever

2.1 Salmonella typhi

Salmonella enterica serovar typhi, briefly *Salmonella typhi*, is a Gram-negative bacterium belonging to the family of Enterobacteriaceae (Figure 8).

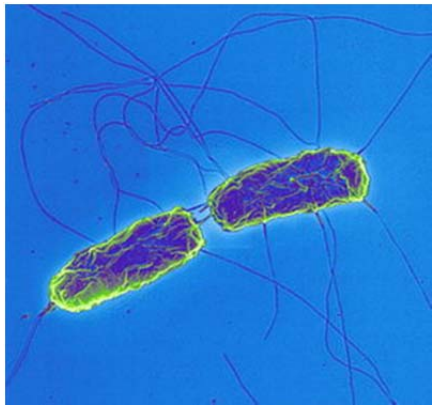
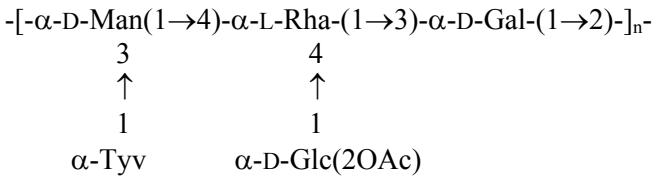


Figure 8: *Salmonella typhi*.

It is a highly invasive bacterium that passes through the intestinal mucosa to reach the reticuloendothelial system. After 8-14 days of incubation, it precipitates a systemic illness.

S. typhi has three antigens (Figure 9):

- the H antigen is a heat sensitive protein of the peritrichous flagellae.
- the O or somatic antigen is a cell-wall lipopolysaccharide.[52] Its repeating unit is the following:



- the Vi antigen is the capsular polysaccharide which overlies the O antigen. Its presence prevents the O-specific antibodies from binding the O antigen.

The Vi antigen is present in strains freshly isolated from patients. Felix and Pitt, from both epidemiologic observations and clinical studies, found out that this antigen enhances the pathogenicity of the bacterium and for this reason they called it "Vi", abbreviation of virulence.[53,54]

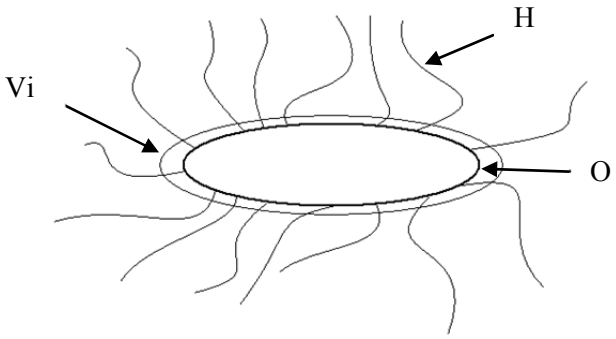
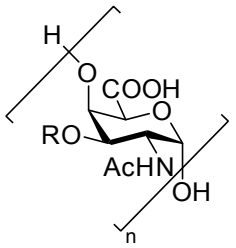


Figure 9: H, O and Vi antigens from *Salmonella typhi*.

Vi antigen is an anionic homopolymer composed of *N*-acetylgalactosaminuronic acid repeating units predominantly *O*-acetylated at position 3 (Figure 10).[55] The degree of 3-*O*-acetylation ranges from 60% to more than 90% in some strains. The immunogenicity of Vi antigen is closely related to its degree of *O*-acetylation. Partial *O*-deacetylation slightly increase immunogenicity, whereas complete *O*-deacetylation eliminate the immunogenicity of Vi.[56]



R = Ac or H

Figure 10: chemical structure of the Vi antigen.

2.2 Typhoid fever

2.2.1 Symptoms and transmission

Typhoid fever (caused by *Salmonella typhi*) is a systemic infection characterized by high fever (39-40 °C), abdominal pain, nausea, loss of appetite and headache. In the early stage of the illness a bronchitic cough is common and in several patients liver dysfunctions can been detected. In more severe form of typhoid fever cerebral dysfunction, such as delirium or coma and shock may be present. This disease may have complications which involve any organ; an example is intestinal perforation followed by hemorrhage.[57] Other kinds of complications are: central nervous system (3-35%), cardiovascular system (1-5%) and pulmonary system (1-6%).[58] This infection lasts for numerous weeks and sometimes may last for months. However, not all cases are hospitalized, only 10-40% of them. Humans are the only natural hosts of *Salmonella typhi* and transmission can occur by consume of polluted water or infected food. Indeed, human fecal material can contaminate water in areas where sanitation is primitive and water supplies are not treated. People can transmit typhoid fever as long as the bacteria remain in their body. Most people are infectious during the first week of convalescence but also up to three months if not treated. Furthermore, 2-5% of untreated patients can become permanent carriers of the bacterium. Anyway, diffusion of the illness from person to person by contact is uncommon. Studies showed that the intensity of the consequences of the infection depends on the concentration of the bacterium in the vehicle.[57] Moreover, the incidence of typhoid fever seems to follow seasonal patterns. In some endemic areas its peak can be seen in the hot, dry months because of the lack of rain which increase the concentration of the organisms in water supplies. Instead, in other places, a peak occurs in the rainy seasons because of the breakdown in the system that separate sewage from drinking water.[58]

2.2.2 Epidemiology

Because of the ways of transmission, typhoid fever is mostly spread in developing countries where health conditions are inadequate, hygiene is poor, and lack of drinking water occurs. This situation was present at the end of the 19th century in most large cities in the USA and western Europe, where water supplies were available but the water wasn't treated, so that the transmission was amplified and the disease was highly endemic in those areas. With the introduction of water treatments in the 20th century, the incidence of typhoid fever decreased enormously. Even though the infection almost disappeared from industrialized cities, nowadays it remains in most of the less-developed areas, such as Africa, Asia and South-America (Figure 11). According to the World Health Organization (WHO), in 2000, typhoid fever caused an estimated 21.7 million illnesses and 217,000 deaths, which makes typhoid fever a serious global public health issue.[59]

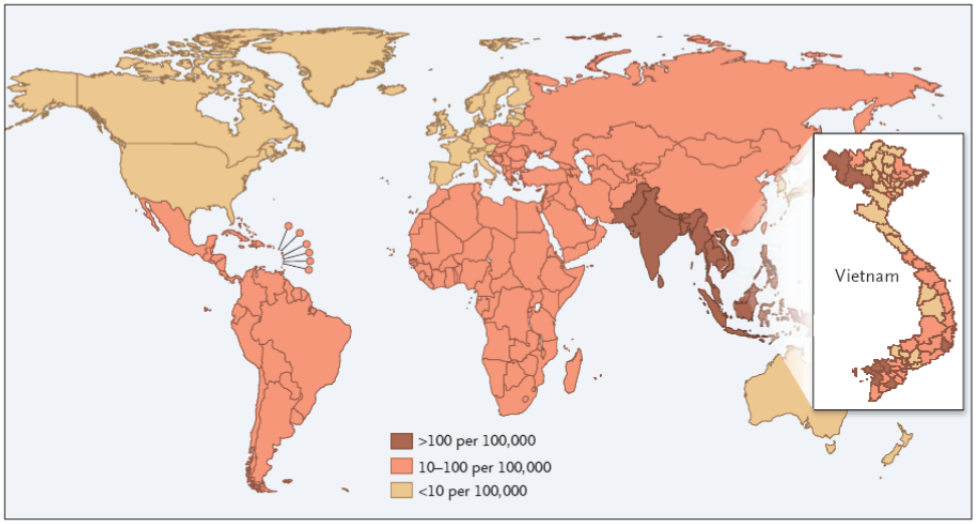


Figure 11: geographical distribution of typhoid fever.

Its incidence is highest in children and young adults between 5 and 19 years old (Figure 12). These cases as of 2010 caused about 190,000 deaths up from 137,000 in 1990. Infants, children, and adolescents in South-central and South-eastern Asia experience the greatest burden of illness. Nonetheless, outbreaks of typhoid fever are frequently reported from sub-Saharan Africa and countries in South-eastern

Asia. Historically, in the pre-antibiotic era, the case fatality rate of typhoid fever was 10–20%. Today, with prompt treatment, it is less than 1%. However, about 3-5% of individual who are infected will develop a chronic infection in the gall bladder. Since *Salmonella typhi* is human-restricted, these chronic carriers become the crucial reservoir and can persist for decades, further spreading the disease and complicating the identification and treatment of the disease.[60-64]

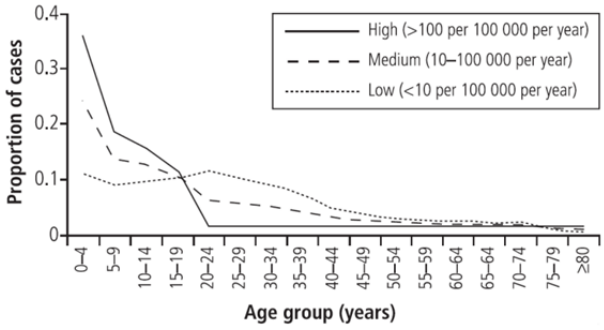


Figure 12: distribution of typhoid fever, by age group, at various incidences.

The global population which may be exposed to typhoid fever infection can be divided into three main groups which, for their higher probability to be infected, are called "risk groups". They are:

- people living in the endemic area;
- travellers;
- clinical microbiological technicians.

The first class is the most obvious. Among all the indigenous populations, children are at the higher risk. Moreover, travellers and military personnel from industrialized countries are probably at special risk in the endemic areas. Indeed, they do not have the background immunity which most of the indigenous population has acquired as a consequence of the numerous infections. However the most unusual class at risk of exposure is that of clinical microbiological technicians. A laboratory is supposed to be safe from infections, especially concerning typical vehicles of typhoid fever, contaminated water and food. But probably in the special conditions of a laboratory the disease may be spread by aerosol or contact which is extremely in contrast whit the usual way of transmission.[57]

2.3 Diagnosis, treatment and immunization

2.3.1 Diagnosis: a challenge

It is not easy to diagnose typhoid fever. The most commonly employed method is the blood culture, but it is positive in only 40-60% of cases, usually early in the course of the disease. Moreover, in much of the developing world, widespread antibiotic availability and prescribing is another reason for the low sensitivity of blood cultures. Stool and urine cultures become positive after the first week of infection but their sensitivity is much lower whereas bone marrow cultures are more sensitive but difficult to obtain, relatively invasive, and of little use in public health settings. The classic Widal test measures antibodies against O and H antigens of *S. typhi* and is more than 100 years old. Although robust and simple to perform, this test lacks sensitivity and specificity, and reliance on it alone in areas where typhoid fever is endemic may lead to overdiagnosis. Newer diagnostic tests, such as the Typhidot or Tubex, which directly detect IgM antibodies against a host of specific *S. typhi* antigens have been developed, but these have not proved to be sufficiently robust in large scale evaluations in community settings. Despite all these new developments, the diagnosis of typhoid fever in much of the developing countries is made on clinical criteria. This poses problems, since typhoid fever may mimic many common febrile illnesses without localizing signs and, subsequently, the differential diagnosis includes malaria; sepsis with other bacterial pathogens; infections caused by intracellular organisms such as tuberculosis; and viral infections such as dengue fever, acute hepatitis, and infectious mononucleosis.[65]

2.3.2 Treatment

Early diagnosis of typhoid fever and prompt institution of appropriate antibiotic treatment are essential for optimal management, especially in children. Typhoid fever started to be treated with antibiotics in the mid-20th century. The first antibiotic used was chloramphenicol which could decrease the severity of the illness and the fatality rate. Subsequently, between 1970 and 1985 sporadic

outbreaks of chloramphenicol-resistant typhoid fever occurred. As a result, other antibiotics were introduced such as ciprofloxacin which was highly effective and amoxicillin which was useful in developing countries, where the cost of therapy is a critical factor. The emergence of multidrug resistant typhoid fever in the 1990s led to widespread use of fluoroquinolones as the treatment of choice for suspected typhoid fever, especially in South Asia and South East Asia where the disease was endemic. Standard treatment with chloramphenicol or amoxicillin is associated with a relapse rate of 5-15% or 4-8% respectively, whereas the newer quinolones and third generation cephalosporins are associated with higher cure rates. In recent years, however, the emergence of resistance to quinolones has placed tremendous pressure on public health systems in developing countries as treatment options are limited. Studies of short course antibiotic treatment for multidrug resistant typhoid fever have shown that fluoroquinolones can achieve satisfactory cure rates, but parenteral ceftriaxone was associated with higher rates of relapse.[65]

2.3.3 Immunization

Until proper sanitary conditions and clean drinking water are guaranteed in developing countries, vaccination against typhoid fever remains the most cost-effective defense for susceptible populations and is recommended by the WHO.[66] Up to now, only three licensed vaccines are available on the market.[67] First generation inactivated whole-cell vaccine were introduced in 1896 but their efficacy was proved only in 1960. The cells were inactivated in different ways: by heat, alcohol, formalin or acetone. The latter was the most used method because preserved the Vi antigen and improved the stability of the vaccine on long-term storage. Trials in different endemic areas showed that two doses of this type of vaccine resulted in 73% efficacy over three years (95% confidence interval 65% to 80%), but also that the inactivated whole-cell vaccines caused fever and systemic reaction in 9% to 34% of recipients.[67] Therefore, albeit still licensed, this kind of vaccine is no longer available for routine vaccinations.[67]

Consequently, only two newer types of vaccine are commercially available: orally administrated Ty21a and parenteral Vi polysaccharide.

Ty21a consists of an attenuated strain of *S. typhi* in which multiple genes, including the genes responsible for the production of Vi antigen, have been mutated chemically so as to render it harmless but nevertheless immunogenic. This vaccine exists in two different oral formulations which need to be kept refrigerated: liquid or enteric-coated capsule. There are no differences in activity between them. Three doses are required to make the Ty21a vaccine effective. Protection starts 10-14 days after the third dose. Vaccination has to be repeated every year for travelers and every 3 years for those living in endemic areas in order to boost the protection against infection. This vaccine is licensed in 56 countries in Asia, Africa, Europe, South America and USA only for people 6 years and older.[68] A theoretical problem is the reversion of the mutated strain in the virulent *S. typhi*. However, this possibility has never been detected in all the trials carried out.

The Vi polysaccharide is a parenteral vaccine made of purified natural Vi antigen. The extraction procedure results in a non-denatured polysaccharide that preserves all *N*- and *O*- acetyl moieties. Only a single parental dose is required. Protection begins 7 days after the administration, while the maximum protection is achieved 28 days later when the highest concentration of antibodies is present. Revaccination is suggested every three years. The only adverse effect detected is a local pain in the area of injection, but it is mild and of short-time. This vaccines is licensed in Australia and in 92 countries in Africa, Asia, the Americas and Europe for adults and children 2 years and older.[69]

Even though they have fewer adverse effects than the previous one, they are both less effective over a three-years period (cumulative efficacy is 51% for Ty21a and 55% for Vi) and the duration of protection is not well determined, with estimates of five to seven years for the Ty21a vaccine and three years for Vi vaccination.[70] Moreover, they cannot be administered to newborns and pre-school aged children. That's why *only* two countries, China and Vietnam, have incorporated typhoid fever vaccination into their routine immunization programs. In order to overcome the inherent limitations associated with a T-independent antigen-based vaccine,[71,72]

Vi has been conjugated to carrier proteins such as tetanus toxoid (Pedatyph) and the nontoxic recombinant exotoxin A of *Pseudomonas aeruginosa* (Vi-rEPA),[73,74] giving rise to the third generation of typhoid vaccine. Although no efficacy data are available for Pedatyph, which is a licensed vaccine only in India,[75] Vi-rEPA was shown to be safe in all ages including infants and provided excellent and long lasting immunity with 92% protection over two years post vaccination in a randomized, two-dose placebo controlled trial in 2 to 5-year-old children in Vietnam, and with 89% protection over 46 months.[76-80] More recently, Novartis Vaccines reported about the development of a conjugate vaccine suitable for infant immunization.[81] They conjugated the Vi polysaccharide to CRM_{197}, a non-toxic variant of diphtheria toxin. The source of the Vi antigen is a low risk and high Vi-yielding organism, *Citrobacter freundii* WR7011. Vi polysaccharide from *Citrobacter* is structurally similar and immunologically indistinguishable to Vi from *S. typhi*.[82] The immunogenicity and safety of Vi-CRM_{197} conjugate was assessed in two randomized phase 2 trials in Pakistan, India, and Philippines. Vi-CRM_{197} was found to be safe and well tolerated and one dose of Vi-CRM_{197} significantly increased concentrations of anti-Vi antibody in adults, children, and older infants. However, in children and older infants, a second dose of conjugate vaccine had no incremental effect on antibody titres and, at all ages, concentrations of antibodies increased substantially 6 months after vaccination.[83]

3

The subject

3.1 Introduction

As already mentioned, typhoid fever is a public health concern because of the growing resistance of *Salmonella typhi* against the antibiotics used for the treatments and the absence of a routine immunization. The population which is mainly at risk consists of pre-school- and school aged children in the endemic areas. For all these reasons it is necessary to find a vaccine that possess two characteristics:

- it must contain T-dependent antigens so that it can induce the immunological memory;
- it must be effective in children.

The new conjugated Vi vaccines seem to fulfill all these requirements but they are still under development (see 2.3.3). Moreover, their production has some drawbacks. The biological extraction of Vi antigen from *Salmonella typhi* (controlled depolymerization) is complicated and does not lead to a product with an high degree of purity, thus resulting in a decrease of the efficacy. In addition, it is difficult to obtain large amounts of the antigen from the causative agent. In order to overcome these difficulties, it would be interesting to find an efficient synthetic route to Vi fragments of variable length. In addition, we aim at investigating if the introduction of the zwitterionic motif into a naturally non-zwitterionic polysaccharide confers to the resulting ZPS the ability to activate T cells without protein conjugation (see 1.3.6). The structure of the Vi CPS makes it an ideal candidate for a careful investigation of the biological behavior of synthetic zwitterionic oligomers. In fact, Vi fragments can be easily converted into zwitterionic derivatives by formal *N*-deacetylation, without introducing huge structural modifications. With these compounds in hand it would be possible to correlate the structural and conformational properties of the ZPS with their biological activity, both in terms of charge pattern and minimum molecular weight required for immunogenicity.

3.2 State of the art

Only two syntheses of Vi antigen oligomers have been reported in the literature so far. The first one dates back to 1999, from Sinaÿ and co-workers.[84] They synthesized Vi oligosaccharides up to hexasaccharide bearing a methyl group both at the anomeric position of the reducing end and at position 4 of the non-reducing end. The starting point was the glycal **1**. The azido functionality was introduced by azidonitration reaction, leading to the formation of both the α- and β-glycosylnitrate in moderate yield. The former was converted into donor **2**, bearing a xanthate as the leaving group, and the latter into acceptor **3**. Glycosylation reaction afforded both α and β anomer (Figure 13).

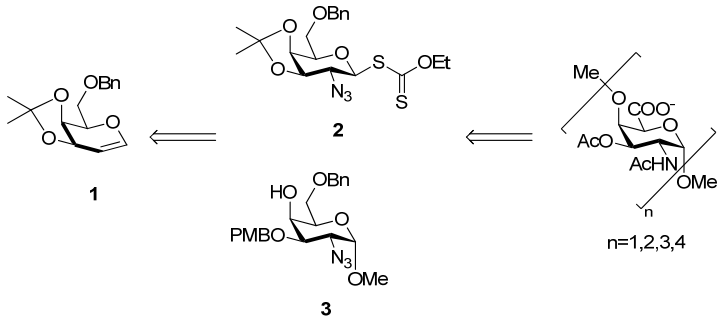


Figure 13: retrosynthetic analysis.

The drawbacks of this synthetic procedure are:

- the azidonitration reaction is a laborious reaction that leads to the production of both anomers in only moderate yield;
- the glycosylation reaction was not stereoselective and provided a mixture of anomers (α/β = 3.3:1). Purification of anomeric mixtures is often difficult and causes a significant drop of the yield;
- the oligosaccharides bear a non-natural 4-*O*-methyl group at the non-reducing end;
- the methyl group at C-1 of the reducing end does not allow the conjugation of Vi fragments to a protein

Another synthesis was reported recently by Ye and co-workers.[85] They prepared fragments up to the tetrasaccharide carrying a methyl group at the reducing end and a non-natural 4-*O*-acetyl group at the non-reducing end (Figure 14). The synthesis was characterized by the use of *N*-acetyloxazolidinone-protected thioglycoside donor **4** and acceptor **5**. Oxazolidinone acts as a non-participating group in glycosylation reaction enhancing the α stereoselectivity and leading, in this case, to the formation of only the α anomer.

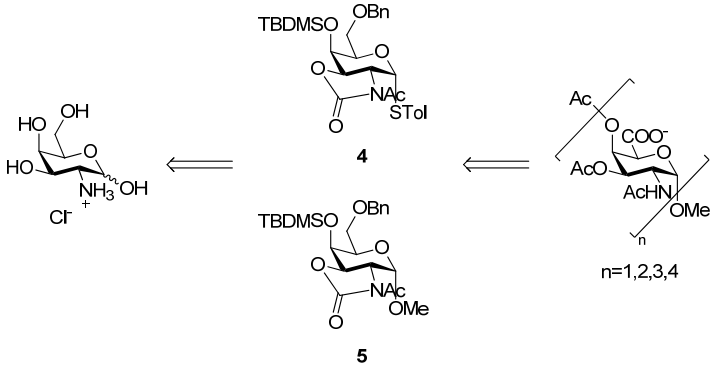


Figure 14: retrosynthetic approach.

The drawbacks of this synthetic strategy are:

- the presence of an unnatural acetyl group at C-4 of the non-reducing end that cannot be selectively removed.
- the methyl group at C-1 of the reducing end does not allow the conjugation to multivalent scaffolds, including proteins.

3.3 Aim of the work

Our work is aimed at finding an alternative, more efficient and versatile synthesis of Vi fragments, that could circumvent the main drawbacks and challenges evidenced by Sinaÿ's and Ye's approaches. In particular:

- it should ensure the exclusive formation of α-glycosidic bonds;
- it should provide versatile oligosaccharide building blocks in order to allow protein conjugation at the reducing-end as well as chain elongation at C-4 of the non-reducing end;
- it should allow direct access both to natural Vi fragments and their zwitterionic analogues.

With the final aim to perform comprehensive biological and immunological studies, we directed our attention on the synthesis of two distinct series of Vi-related oligosaccharides: the one corresponding to the natural structure and their zwitterionic derivatives. Moreover, the role of 3-*O*-acetylation will be also taken into account by the synthesis of both fully 3-*O*-acetylated and fully non-3-*O*-acetylated oligosaccharides. In addition, all the oligosaccharides will be endowed with a suitable linker at C-1 of the reducing end to facilitate subsequent conjugation to multivalent scaffolds (Figure 15).

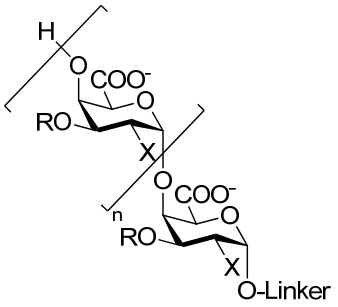


Figure 15: structure of the target Vi oligomers.

4

3-*non*-*O*-Ac (3-OH) oligomers

4.1 First synthetic route

In the first part of the work, we focused on the synthesis of oligomers non acetylated at position 3. We designed a flexible synthetic strategy based on versatile intermediates suitably protected for the systematic introduction of charge centers on each repeating unit, and containing non-participating protecting groups onto 2-amino functions to allow the formation of 1,2-*cis* glycosidic linkages. The use of the azide group at C-2 allows the formation, in the late stages of the synthesis, of either an amino group (like in the ZPS) or an acetamido function (as in the natural Vi CPS). Moreover, the acceptor contains a suitable linker at the anomeric position in order to facilitate subsequent conjugation to multivalent scaffolds. Both intermediates are easily attainable from commercially available galactosamine hydrochloride (Figure 16).

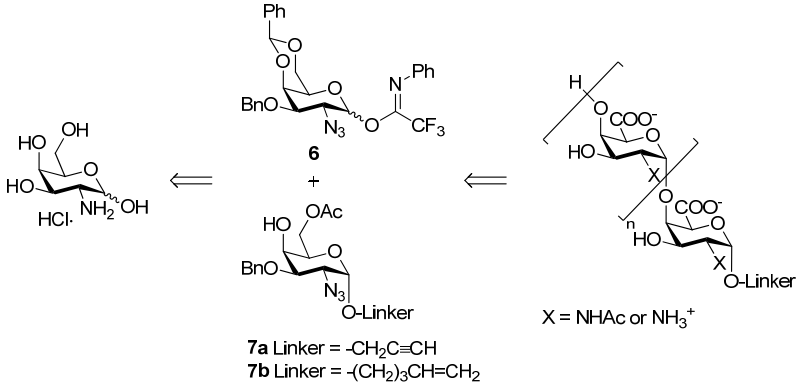
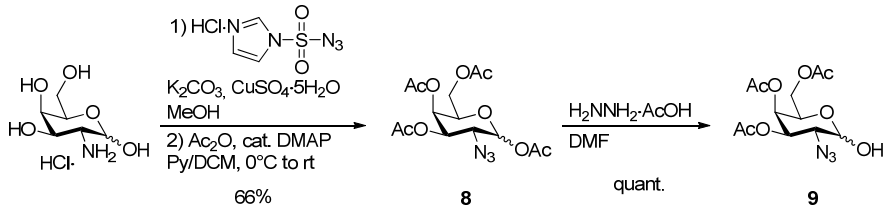


Figure 16: retrosynthetic analysis.

Initially, we selected a propargyl group as the linker moiety and, after some preliminary optimization, we identified the *N*-phenyltrifluoroacetimidate moiety as the best leaving group, as it was less prone to give rearrangement by-products during the glycosylation reaction.[86] The synthetic strategy has been optimized on both gram and multi-gram quantities. The synthesis of the two building blocks started from galactosamine hydrochloride, which was subjected to one-pot copper-catalyzed diazotransfer reaction and acetylation using imidazolyl sulfonyl azide hydrochloride as the azide source. This type of reagent is less expensive and safer

than the classical triflic azide.[87,88] Compound **8** was then selectively deprotected at the anomeric position, furnishing intermediate **9** (Scheme 1).



Scheme 1: preparation of common intermediate **9**

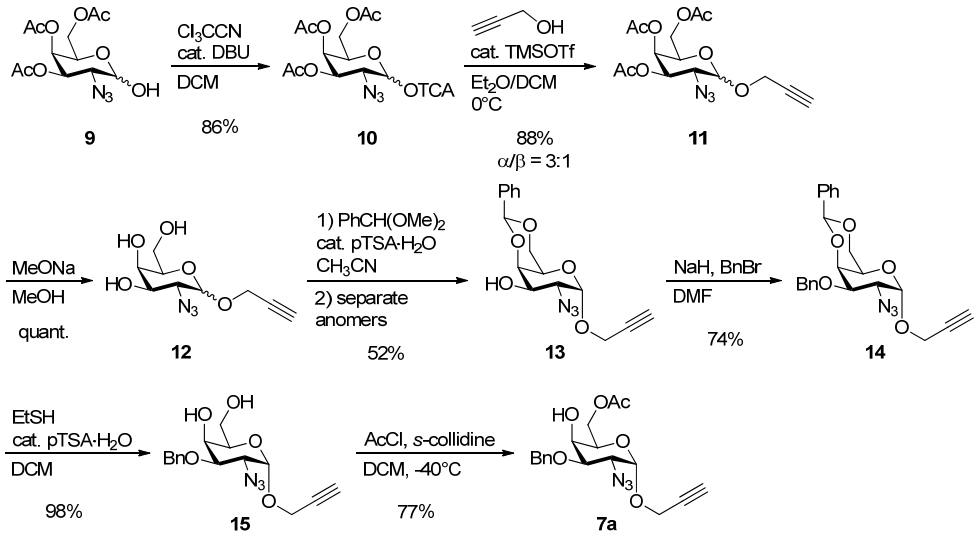
4.1.1 Acceptor synthesis

The synthesis of the acceptor started from compound **9**, which is activated as trichloroacetimidate (**10**). Glycosylation with propargyl alcohol and catalytic TMSOTf afforded a mixture of the corresponding glycosides **11**. The α/β ratio ranged from a good 9:1 to a moderate 3:1 when the reaction was performed on small and large batches, respectively. The two anomers were inseparable, so they were subjected to Zemplén deacetylation and benzylidene formation and, at this stage, they could be easily separated by flash chromatography. The 3-OH of the pure α anomer **13** was then benzylated. The benzylidene was cleaved by means of a transacetalization with ethanethiol and selective acetylation with acetyl chloride and *sym*-collidine at low temperature afforded acceptor **7a** in 22% overall yield over 7 steps (Scheme 2). We also tried to recycle the undesired beta anomer, but the removal of the propargyl group by treatment with potassium *tert*-butoxide followed by catalytic osmium tetroxide and *N*-methylmorpholine *N*-oxide did not take place.[89]

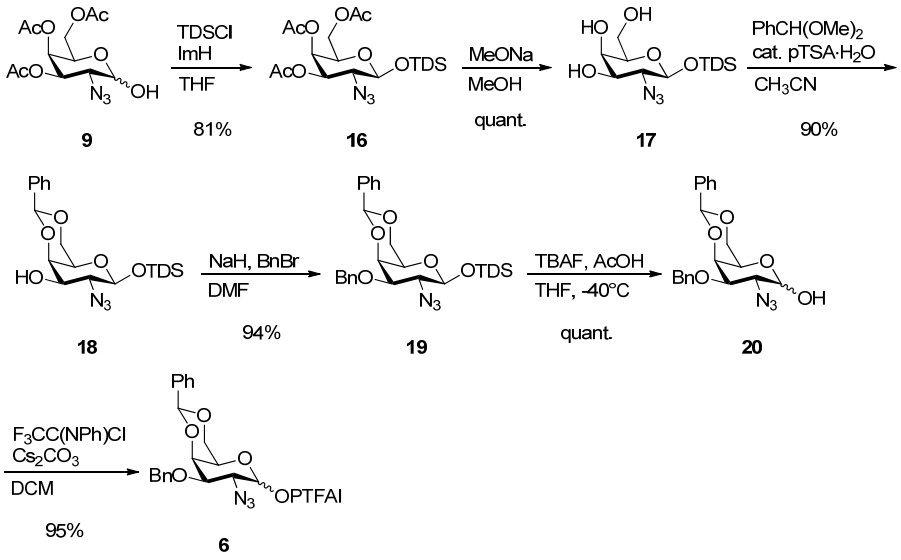
4.1.2 Donor synthesis

The synthesis of the donor started from compound **9**, which was protected at the anomeric position as a silyl ether, then subjected to Zemplén deacetylation and installation of 4,6-*O*-benzylidene, affording compound **18**. The 3-OH was then

benzylated and the anomeric silyl group was cleaved. The last step was the formation of the imidate by the treatment with cesium carbonate and *N*-phenyltrifluoroacetimidoyl chloride, furnishing the desired donor **6** in good 65% overall yield over 6 steps (Scheme 3).



Scheme 2: synthesis of acceptor **7a**.

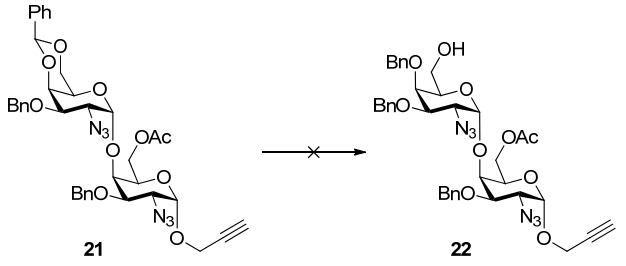


Scheme 3: synthesis of donor **6**.

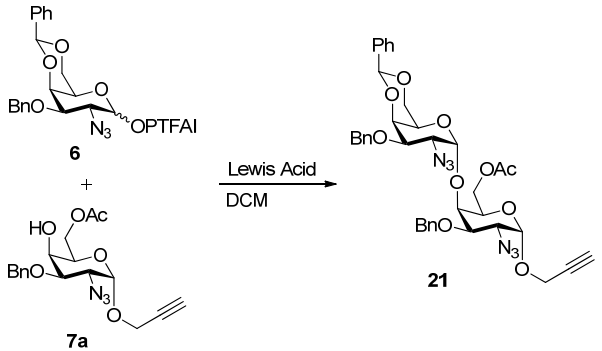
4.1.3 Glycosylation and deprotection

Then, we optimized the glycosylation between these two building blocks (Scheme 5). As we had in hand a reactive donor and a less reactive acceptor, we decided to use an excess of the former to promote the reaction. Table 1 summarizes the results obtained. Gratifyingly, all the tested reaction conditions gave stereoselectively the desired α product. The first attempt was performed using 1.5 eq of donor and 0.2 eq of TMSOTf as the acidic promoter at -20 °C but the yield was low. We thought that this result could be explained by the high acidity of the reaction medium. So we tried a weaker Lewis acid like AgOTf, obtaining only traces of the product. The next attempt was made employing only 0.1 eq of TMSOTf and increasing the amount of donor up to 2 equivalents. In this condition the yield was good, 58% of the α disaccharide **21**. We explored also an "inverse glycosylation" procedure,[90] but we could detect the α-(1→6)-dimer as by-product, probably deriving from 6-OH to 4-OH acetyl migration in the acceptor under prolonged acidic conditions. The best result were obtained by a slow addition *via* a syringe pump, over 1 hour, of a diluted solution (0.055 M) of the Lewis acid. This methodology permitted the use of only 1.5 eq of donor, instead of 2, with comparable yield and the same stereoselectivity.

In order to deprotect the primary positions the benzylidene acetal must be regioselectively opened and the acetyl group must be removed. Unfortunately, when we attempted regioselective reductive opening of the benzylidene ring on the acetylated dimer **21** (Scheme 4) we obtained complex reaction mixtures with various reagents combinations and no product was formed (Table 2).[91-94]



Scheme 4: regioselective reductive benzylidene ring opening on dimer **21**.



Scheme 5: glycosylation reaction.

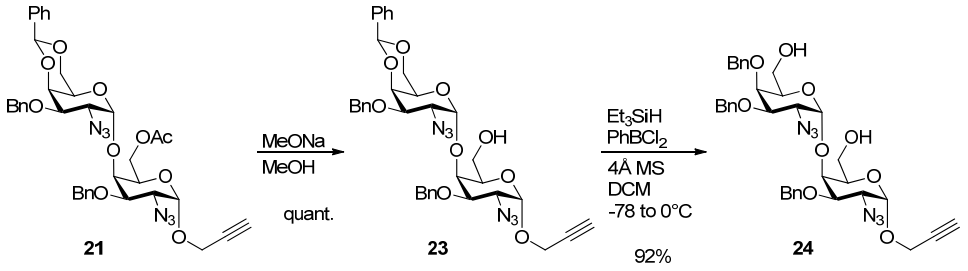
	Conditions	**Don. eq.**	**Acc. eq.**	**Lewis acid**	**T (ºC)**	**Yield**
1	Direct glycosylation	1.5	1	TMSOTf, 0.2 eq.	-20	16%, α
2		1.5	1	AgOTf, 0.6 eq.	-10	trace, α
3		2	1	TMSOTf, 0.1 eq.	0	58%, α
4	"Inverse glycosylation"	2	1	TMSOTf, 0.1 eq.	0	45% α-(1→4) + 8% α-(1→6)
5	Slow addition of diluted L.A.	1.5	1	TMSOTf, 0.1 eq.	0 → rt	71%, α

Table 1: glycosylation reaction conditions.

Reagents	T (°C)	Yield
CuOTf, BH_3·THF	15	/
CuOTf, BH_3·THF	-15	/
$CoCl_2$, BH_3·THF	rt	/
Et_3SiH, $PhBCl_2$	-78 → -40 -78 → 0 -78 → rt	/ / /

Table 2: conditions for the regioselective reductive benzylidene ring opening on dimer **21**.

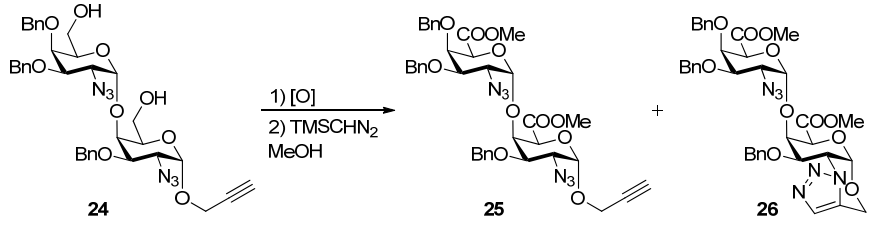
Surprisingly, when we tried the same reaction on the deacetylated dimer **23**, we obtained smoothly the desired product **24** in 92% yield (Scheme 6).



Scheme 6: deprotection of the primary positions.

4.1.4 Oxidation

The following step is the oxidation of the primary positions. To facilitate the purification and the characterization of the product during the initial optimization of the reaction conditions we decided to isolate the corresponding *bis*-methyl ester (Scheme 7).



Scheme 7: oxidation to methyl uronates.

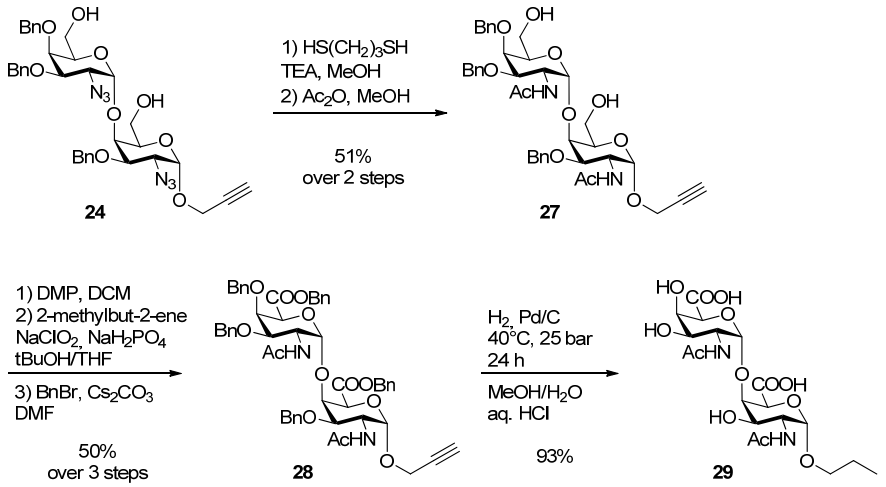
Several methodologies were tested on compound **24**, and the results are summarized in Table 3. The oxidation with $RuCl_3$ gave a complex mixture of inseparable products, probably due to oxidation of the multiple bond and of the benzyl ethers. Also the application of the classical TEMPO-mediated reaction conditions gave unsatisfactory results. The use of catalytic TEMPO and BAIB as the stoichiometric oxidant resulted in a complex reaction mixture, presumably due to degradation of the triple bond. On the contrary, the reaction did not occur using sodium hypochlorite as the oxidant. Only a two-step procedure involving Dess-Martin periodinane (DMP) and Pinnick oxidation[95] gave the desired product in 39% yield, along with another major product **26** derived from intramolecular Huisgen cycloaddition under basic conditions. The use of IBX instead of DMP furnished only product **26** in low yield.

	Oxidizing agents	**Temp.**	**Yield**
1	$RuCl_3 \cdot 2H_2O$, $NaIO_4$	rt	/
2	TEMPO, BAIB	rt	/
3	TEMPO, NaOCl, KBr, $NaHCO_3$	0ºC	n.r.
4	DMP then $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene	rt	39% **25** + 11% **26**
5	IBX then $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene	80ºC, rt	22% **26**

Table 3: oxidation conditions.

4.1.5 Natural Vi disaccharide

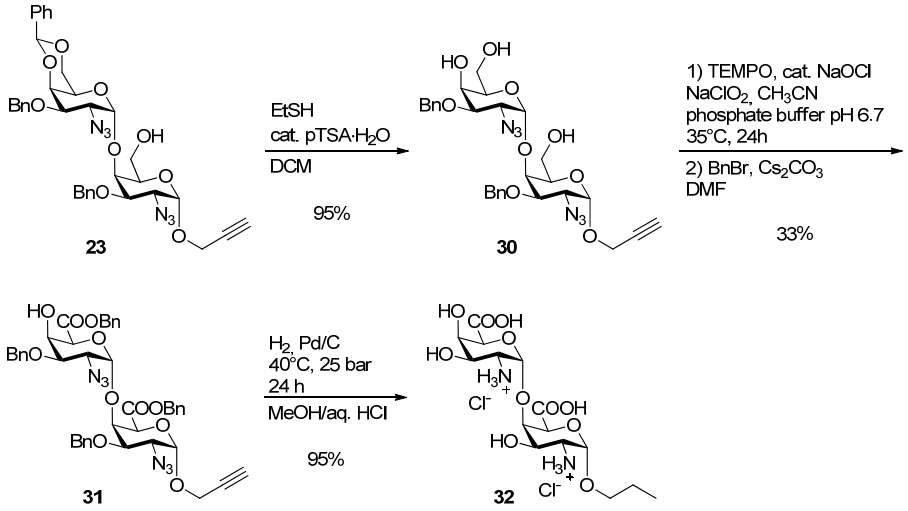
In order to overcome the issue related to intramolecular "click reaction", compound **24** was subjected to azide reduction, acetylation and deacetylation affording compound **27**, suitable for the synthesis of the natural Vi disaccharide. In this way, due to the presence of the acetamido function at position 2, compound **27** could be oxidized by the DMP/Pinnick protocol, as the intramolecular cyclization could not take place. The oxidation, followed by protection of the carboxylic acids as benzyl esters to facilitate purification of the product, furnished the desired uronate **28** in moderate and non-reproducible 50% yield. Full deprotection was finally achieved by high pressure hydrogenolysis (Scheme 8).



Scheme 8: synthesis of the natural Vi disaccharide.

4.1.6 Zwitterionic disaccharide derivative

The synthesis of the zwitterionic disaccharide derivative required the application of special reaction conditions for the oxidation step, in order to avoid the formation of undesired side-products. We found in the literature a procedure specifically developed for the oxidation of C-C multiple bond-containing molecules, the so-called Zhao's modification of the TEMPO oxidation.[96] The reaction takes place under strictly controlled buffered conditions (phosphate buffer pH 6.7) and employs sodium chlorite as the stoichiometric oxidant and only a catalytic amount of sodium hypochlorite. We employed compound **30** obtained from **23** by benzylidene cleavage. The oxidation under buffered conditions, followed by esterification, gave only the desired product **31**, although in modest yield. Finally, full deprotection by high pressure hydrogenation furnished the zwitterionic derivative **32**, isolated as the corresponding dihydrochloride (Scheme 9).



Scheme 9: synthesis of the zwitterionic disaccharide derivative.

4.1.7 Biological assay

The binding affinities of compounds **29** and **32** were preliminary evaluated by ELISA competitive assay using natural Vi polysaccharide as positive control. The results showed that both disaccharides are recognized by the antibody in a dose-dependent manner (Figure 17).

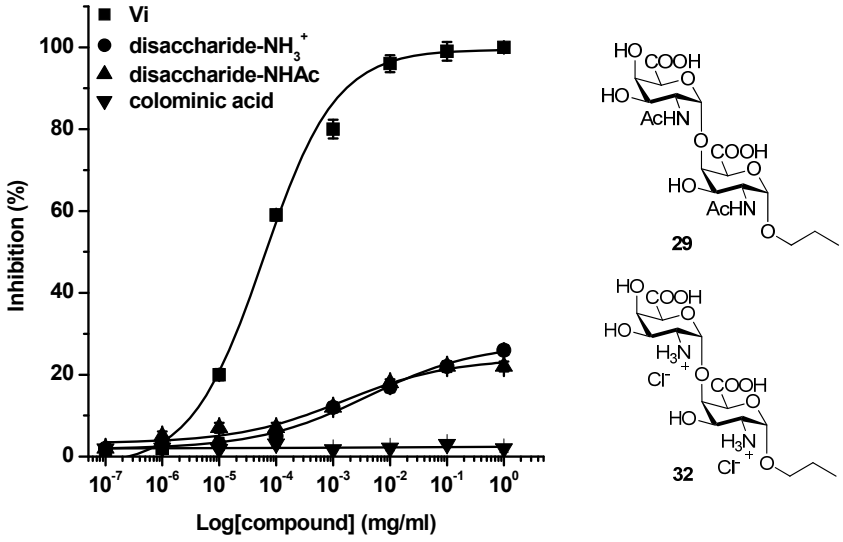


Figure 17

4.1.8 Disaccharide donors

However, the elongation strategy so far described is suitable only for the synthesis of very short oligomers. We therefore explored a new and more efficient oligomerization strategy based on disaccharide donors. We designed two new disaccharide donors: compound **33** is the elongation block since, after the glycosylation, the benzylidene group can be easily manipulated in order to obtain a new glycosyl acceptor. Compound **34** is the capping block, as the 4-OH is protected with a permanent benzyl group. The use of these building blocks, along with compound **7** and **35** as acceptors, permits the elongation of the chain by either the "2n+1" or the "2n+2" approaches, two monosaccharide units at a time (Figure 18). The synthesis started from the common intermediate **19**. The monosaccharide acceptor **37** was obtained in two step by benzylidene cleavage and selective acetylation at low temperature. The monosaccharide donor **39** was obtained by regioselective reductive benzylidene ring opening, acetylation, deprotection of the anomeric position and formation of the imidate (Scheme 10).

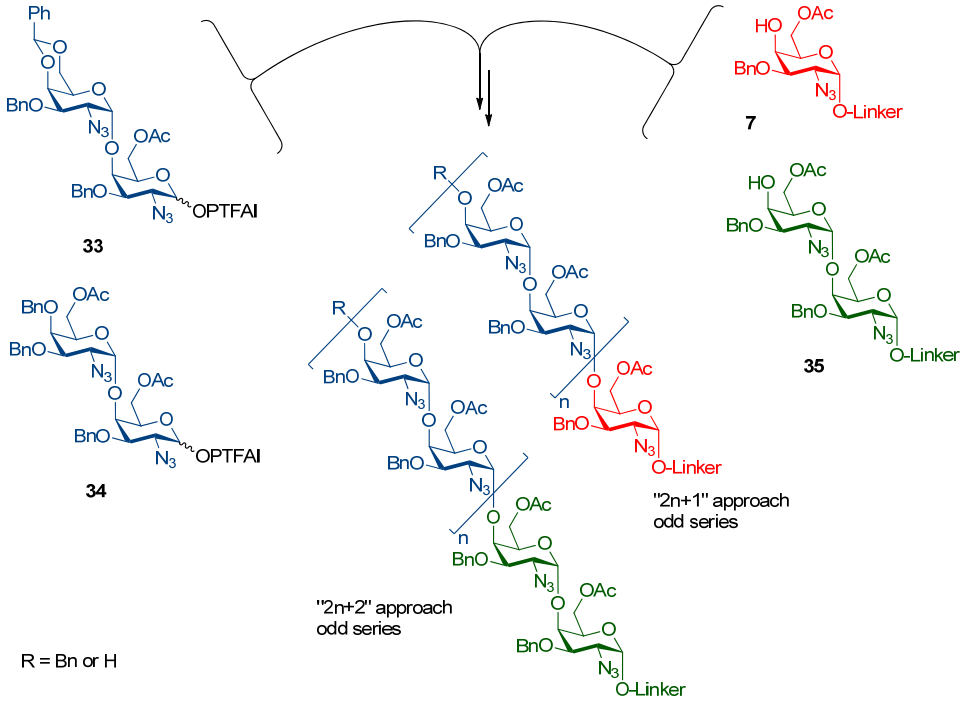
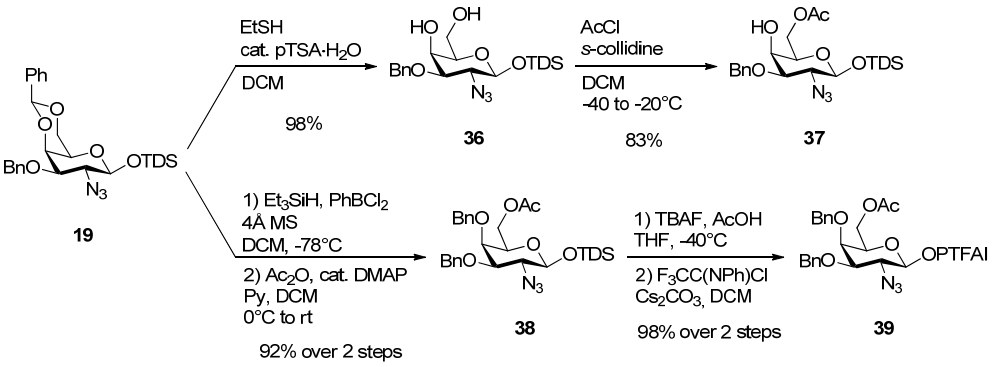
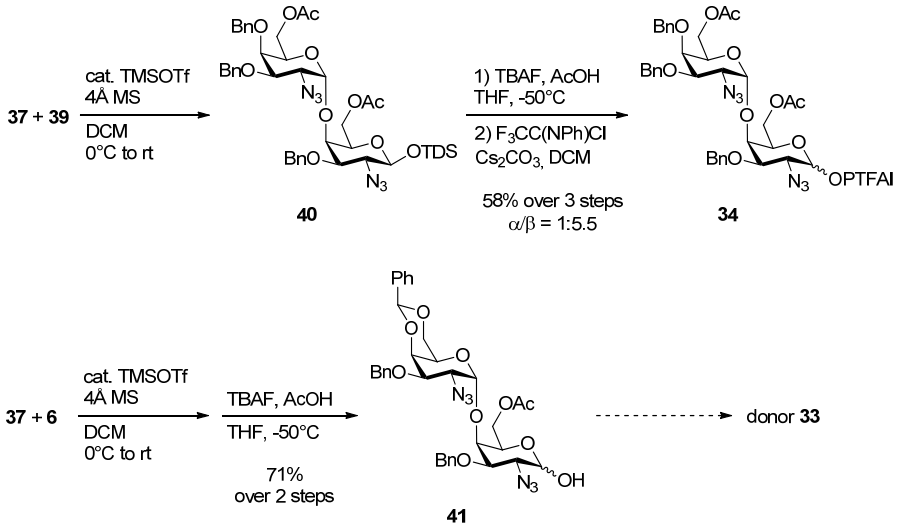


Figure 18: glycosylation approach based on disaccharide donors.



Scheme 10: synthesis of the monosaccharide donor and acceptor.

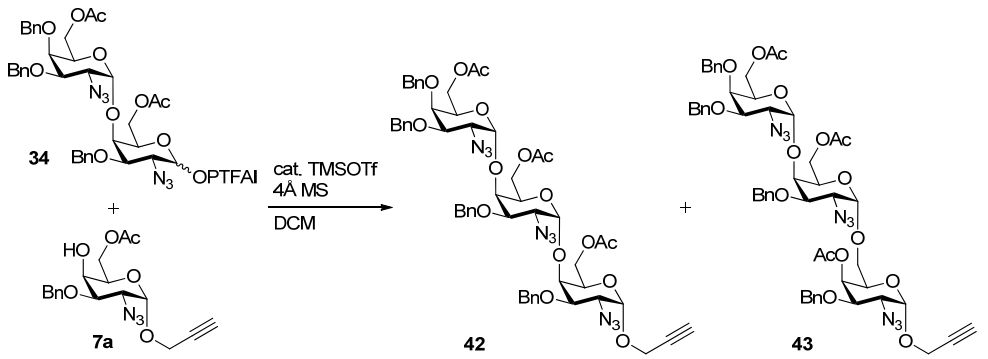
Glycosylation of **37** and **39** with 0.1 eq of TMSOTf afforded precursor **40**, which was converted into donor **34** by desilylation and formation of the corresponding imidate in good overall yield. The same steps were applied to the synthesis of compound **41**, precursor of the disaccharide donor **33** (Scheme 11).



Scheme 11: synthesis of the disaccharide donors.

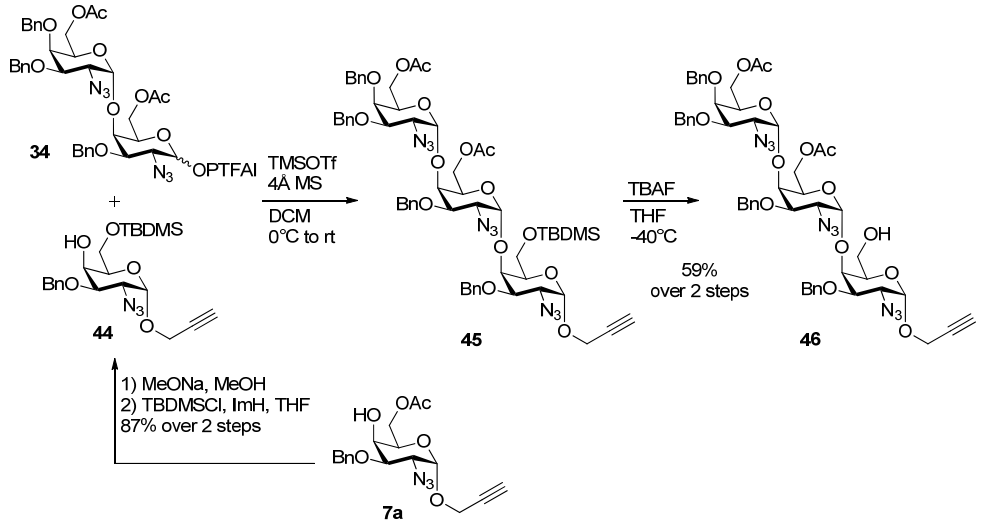
4.1.9 Trisaccharide derivatives

Then, as a proof of concept, we tested disaccharide donor **34** in the synthesis of the trisaccharide but unfortunately, under standard glycosylation conditions, at -40 and 0°C, we got an inseparable mixture of the α-(1→4) and α-(1→6) regioisomers, probably due to acetyl migration, as deduced by analysis of the NMR spectra (Scheme 12).



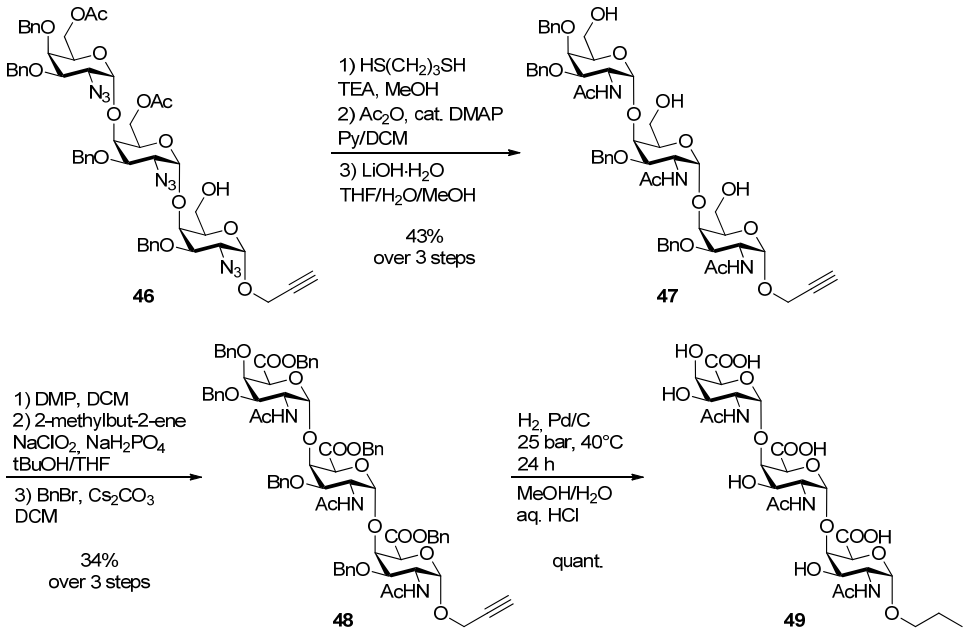
Scheme 12: glycosylation with disaccharide donor.

To overcome this problem we replaced the acetyl group at position 6 in the acceptor with a silyl ether, thus obtaining only the desired product **46** in 59% yield after desilylation (Scheme 13).



Scheme 13: optimized glycosylation with disaccharide donor.

Firstly, we tried Zhao's TEMPO oxidation on the triol derived from deacetylation of compound **46** in order to obtain the zwitterionic derivative, but the reaction did not take place. Thus, compound **46** was subjected to azide reduction and acetylation, deprotection of the primary positions and oxidation with the DMP/Pinnick protocol followed by benzyl esters formation to obtain the protected Vi trisaccharide **48**, in 34% yield. Also in this case, the final deprotection was achieved by high pressure hydrogenolysis (Scheme 14).



Scheme 14: synthesis of the natural Vi trisaccharide.

4.2 Second synthetic route

The encouraging results of the ELISA test on the two disaccharide derivatives (see 4.1.7) prompted us to improve our synthetic strategy. In particular, several drawbacks derived from the use of the propargyl group as the linker moiety, that caused a drastic drop of the overall yield during the oxidation step. We therefore switched to a 4-pentenyl linker (Figure 19). One advantage of this linker is that it is easily converted into various functional groups that can be used in the conjugation chemistry (such as aldehydes, amino groups and carboxylic acids).

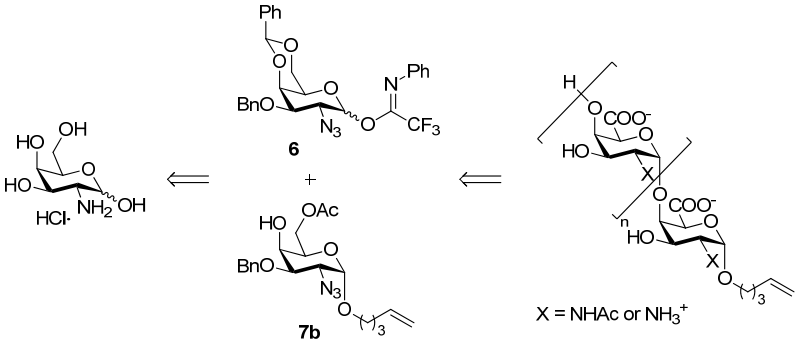
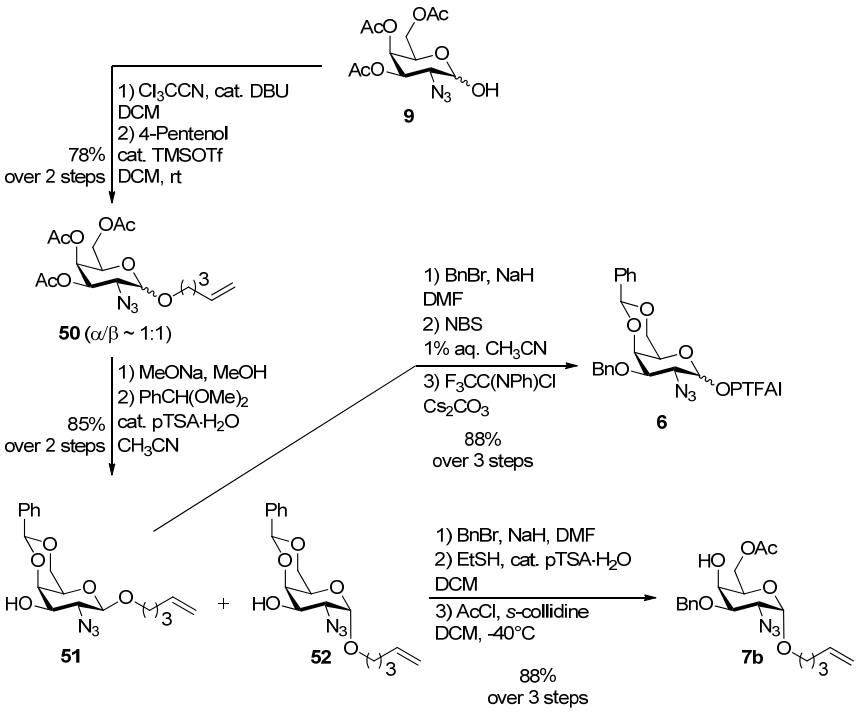


Figure 19: retrosynthetic approach.

4.2.1 Building blocks synthesis

The synthesis of the new building blocks started from the common intermediate **9**. The anomeric position was activated as a trichloroacetimidate and glycosylation with 4-pentenol using catalytic TMSOTf afforded a nearly 1:1 mixture of the corresponding glycosides **50**. The two anomers could be easily separated by flash chromatography after Zemplén deacetylation and installation of a 4,6-*O*-benzylidene. The key advantage of the use of the pentenyl moiety is the possibility to exploit both anomers. In particular, the pure α anomer **52** can be converted into acceptor **7b** by benzylation, benzylidene removal and selective acetylation at position 6. On the other hand, the pure β anomer **51** can be recycled through a halocyclization reaction followed by *in situ* hydrolysis and converted into donor **6** (Scheme 15).[97] This possibility contributes to keep the whole strategy as much

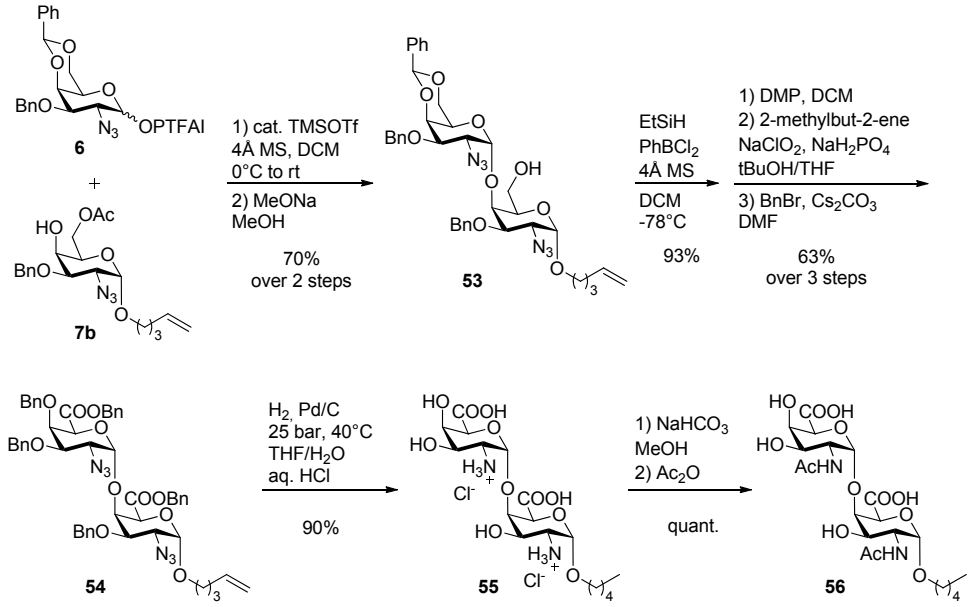
economical as possible. By comparison, in the previous strategy, the α/β ratio of the propargyl glycosides was higher, but the β anomer has to be discarded after benzylidene formation, leading to a loss of about 30% of product (see 4.1.1).



Scheme 15: synthesis of donor **6** and acceptor **7b**.

4.2.2 Disaccharide derivatives

The glycosylation between **6** and **7b** was performed under the previously optimized conditions, by a slow addition of the Lewis acid, affording compound **53** with complete stereoselectivity, in 70% yield after deacetylation. Compound **53** was subjected to regioselective reductive benzylidene ring opening and then oxidized by the DMP/Pinnick protocol, followed by protection of the carboxylic acids as benzyl esters, thus obtaining the desired uronate **54** in 63% yield. The zwitterionic derivative **55** was simply obtained after full deprotection by hydrogenolysis. The disaccharide corresponding to the natural Vi structure **56** was obtained from **55** by neutralization of the hydrochloride and selective *N*-acetylation (Scheme 16).



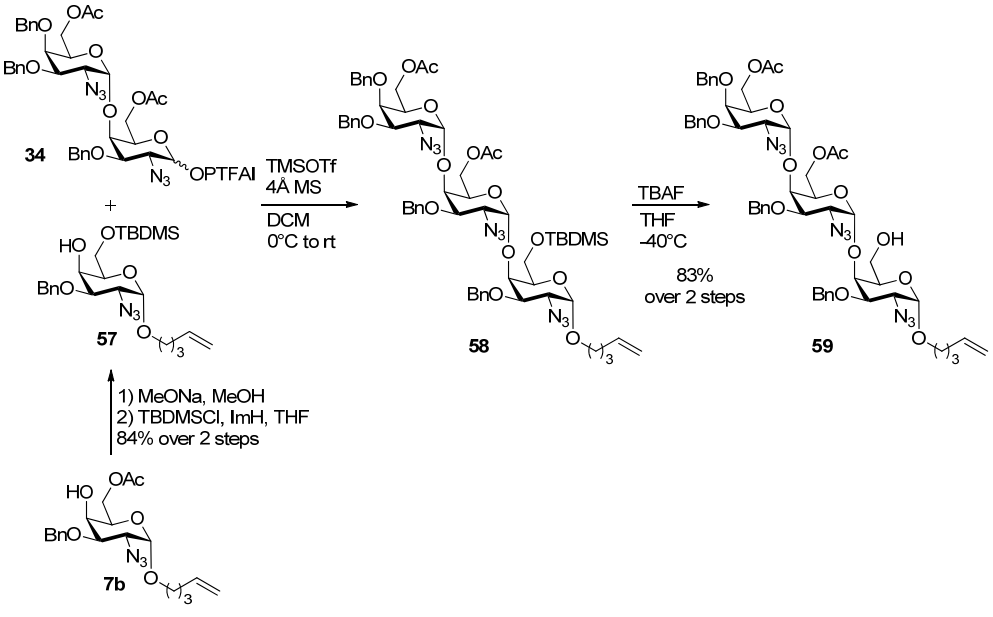
Scheme 16: synthesis of the natural Vi disaccharide and its zwitterionic derivative.

4.2.3 Glycosylation with disaccharide donor

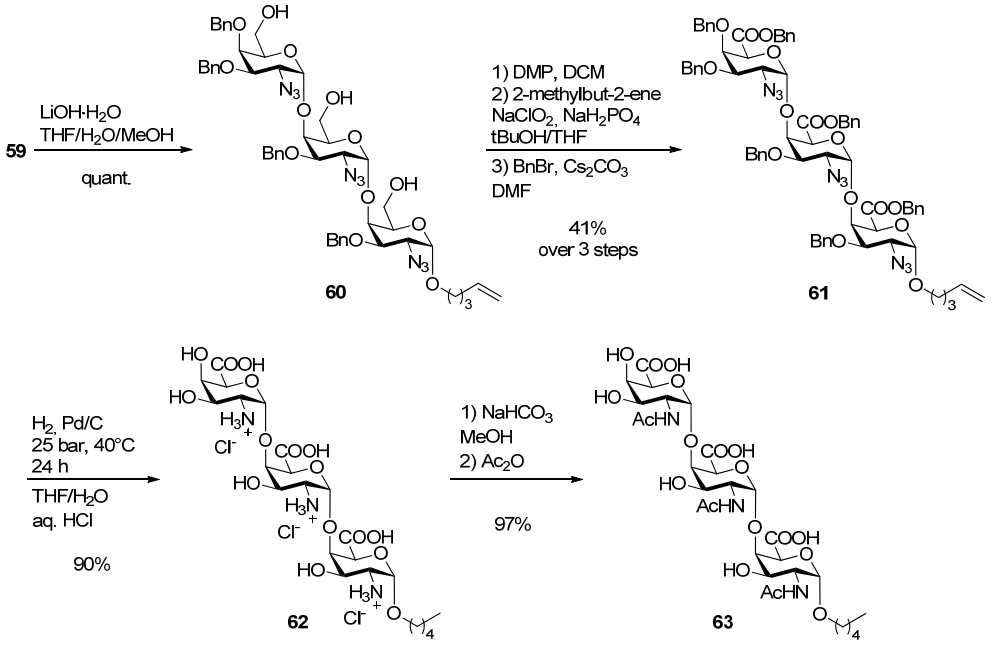
Given the above mentioned acetyl migration issue during the glycosylation with disaccharide donor (see 4.1.8), we decided to replace the acetyl group at position 6 with a silyl ether also in acceptor **7b**. The new acceptor **57** was then glycosylated with disaccharide donor **34**, smoothly obtaining the desired protected trisaccharide **59** in 83% yield after desilylation (Scheme 17).

4.2.4 Trisaccharide derivatives

Compound **59** was subjected to deprotection of the primary positions by saponification and then oxidized with the DMP/Pinnick protocol followed by benzyl esters formation to obtain the protected uronate **61**, but the yield of the oxidation step dropped to a moderate 41%. Also in this case the zwitterionic derivative **62** was obtained by high pressure hydrogenolysis and the Vi trisaccharide **63** was obtained from **62** by selective *N*-acetylation (Scheme 18).



Scheme 17: glycosylation with disaccharide donor.



Scheme 18: synthesis of the natural Vi trisaccharide and its zwitterionic derivative.

4.2.5 Biological assay

The biological behavior of the four compounds was evaluated by ELISA competitive assay using horse anti-Vi polyclonal antibodies and natural Vi polysaccharide as the positive control. The results showed that the oligosaccharides are all recognized by the antibody in a dose-dependent manner (Figure 20 and Figure 21). In particular, both trisaccharides have a slightly higher affinity than the disaccharides and their IC_{50} values are in the same order of magnitude as that of the natural polysaccharide. But it is also important to notice that the zwitterionic derivatives have comparable values as their natural Vi counterpart, suggesting that the *N*-acetylation could not be a structural prerequisite for antibody recognition. However, all four compounds have a low avidity (as judged by their maximum inhibition values), but this could be explained by their short chain length. In fact, they are too small to compete efficiently with the natural polysaccharide (Table 4).

Compound	**IC_{50} (mg/ml)**	**Maximum Inhibition[a] (%)**
Vi CPS (~ 4000 residues)	6.1×10^{-5}	100 ± 2
Vi disaccharide **56**	1.5×10^{-4}	28 ± 4
Zwitterionic disaccharide **55**	7.2×10^{-4}	30 ± 5
Vi trisaccharide **63**	2.9×10^{-5}	35 ± 3
Zwitterionic trisaccharide **62**	1.3×10^{-5}	34 ± 7

Table 4: [a] the maximum inhibition elicited by each compound at 1 mg/ml

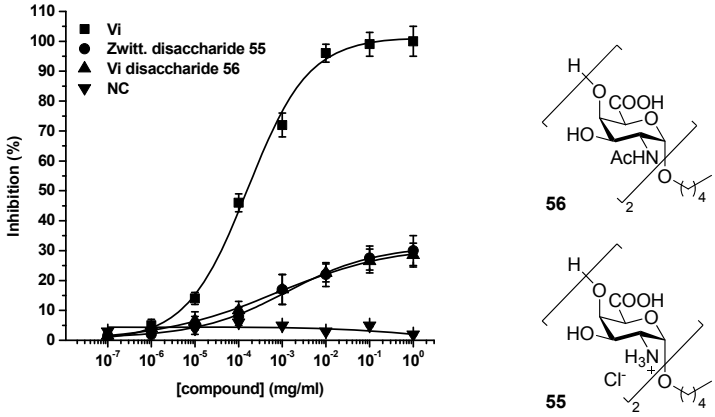


Figure 20

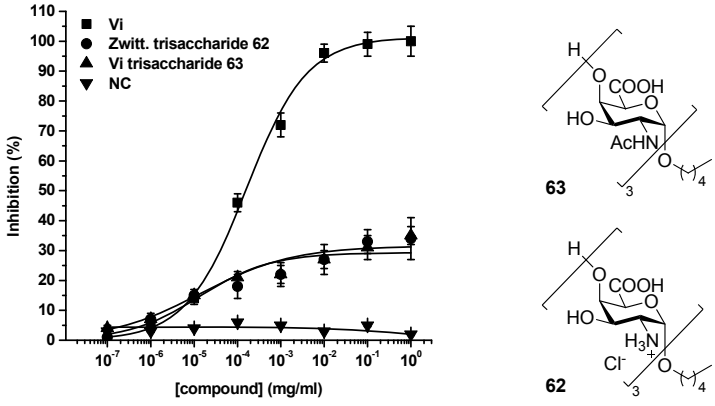


Figure 21

5

3-*O*-Ac oligomers

5.1 First route

As previously mentioned in chapter 2.1, the immunogenicity of Vi antigen is closely related to its degree of 3-*O*-acetylation. Accordingly, in the second part of this work we investigated a different synthetic strategy in order to obtain the natural Vi oligomers and their zwitterionic analogues fully acetylated at C-3. Initially, we decided to introduce the acetate ester in the early steps of the synthesis by acetylation of glycosides **51** and **52** (Figure 22).

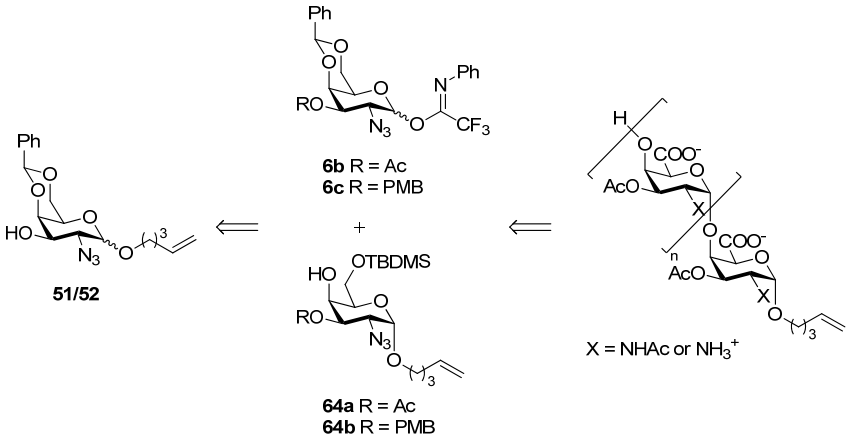
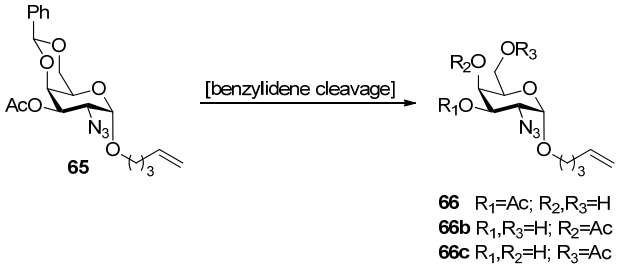


Figure 22: retrosynthetic analysis.

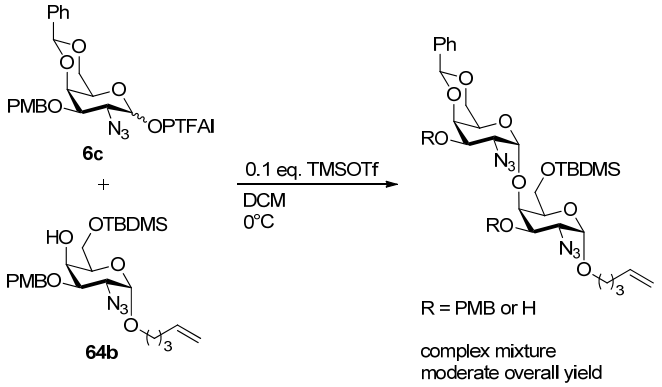
Disappointingly, we got extensive acetyl migration during the benzylidene cleavage step (Scheme 19) under different reaction conditions (Table 5). We then explored the use of the *p*-methoxybenzyl (PMB) as a temporary protection of 3-OH, but it was found very labile under the acidic conditions of the glycosylation reaction, leading to an inseparable mixture of partially protected disaccharides in moderate overall yield (Scheme 20).



Scheme 19: acetyl migration issue during the benzylidene cleavage step.

Conditions	Yield
EtSH, cat. *p*TSA, dry DCM, rt	60% + 15% **b**, **c**
EtSH, cat. *p*TSA, dry DCM, 0°C	20% + 40% **b**
80% TFA, DCM, -20°C to rt	50% + 30% **b**, **c**
80% AcOH, 70°C	40% + 20% **b**, **c**

Table 5: benzylidene removal conditions.



Scheme 20: glycosylation with PMB-protected building blocks.

5.2 Second route

Taking into account the previous issues, we decided to move to a totally different approach based on the use of uronate building blocks following an oxidation *pre*-glycosylation approach (Figure 23). In this way, besides overcoming the problem related to protection at C-3, we also aimed at improving the dramatic drop of the overall yield occurring when the oxidation step is performed in the late stages of the synthesis.

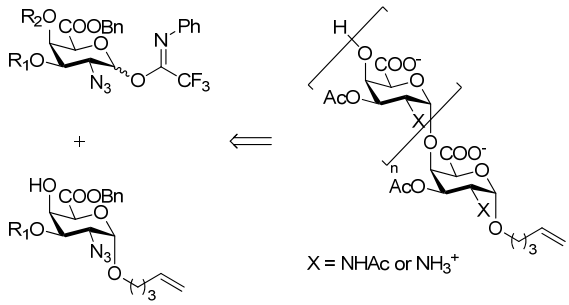
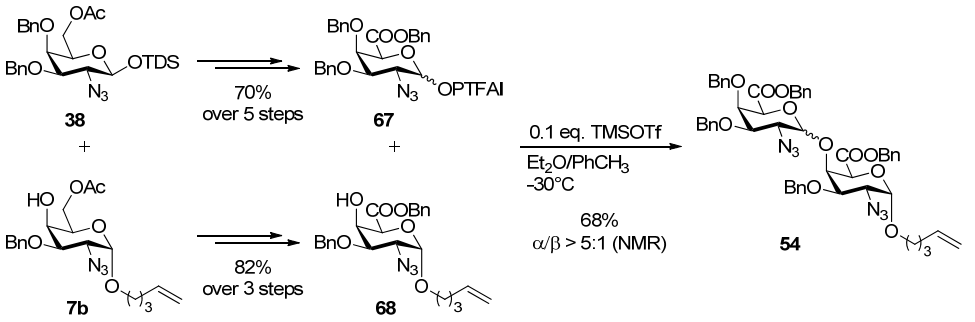


Figure 23: uronate building blocks.

The presence of the electron-withdrawing ester function at C-5 makes the uronic acids less reactive glycosylation partners than their unoxidized counterparts. In particular, uronate donors are known to possess poor reactivity and/or lack of selectivity and therefore require excellent leaving groups for their activation.[98,99] In order to test the feasibility of this new strategy, we carried out a preliminary glycosylation attempt with two readily prepared building blocks **67** and **68**, and, although not completely stereoselective, the reaction furnished disaccharide **54** in good 68% yield with an α/β ratio higher than 5 to 1 (estimated by NMR, Scheme 21). Literature data show that the α stereoselectivity in the glycosylation with galacturonate donors can be improved by the presence of 4-*O*-acyl groups, an effect known as remote anchimeric assistance.[100-102] Therefore we designed the new strategy based on donor **96** and acceptor **70**. Both compounds contain an acetate group at position 3 and the donor bears an orthogonal acyl group at position 4, allowing easy chain growth. Moreover, due to the high cost of the starting material (galactosamine hydrochloride), we decided to synthesize the new galacturonate

building blocks *via* inversion of C-4 configuration of glucosamine hydrochloride (Figure 24).



Scheme 21: preliminary glycosylation attempt.

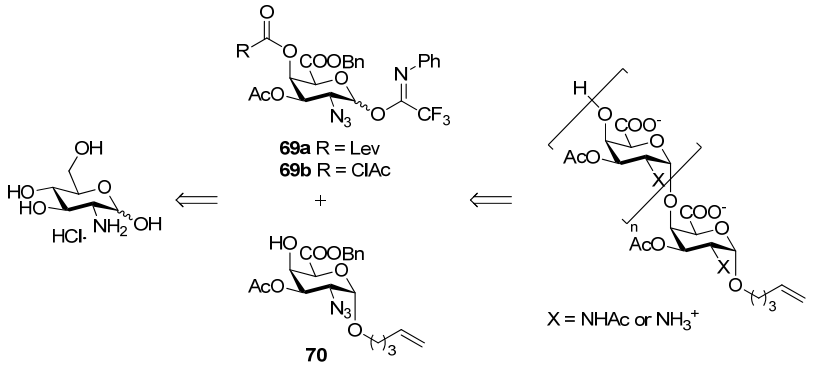
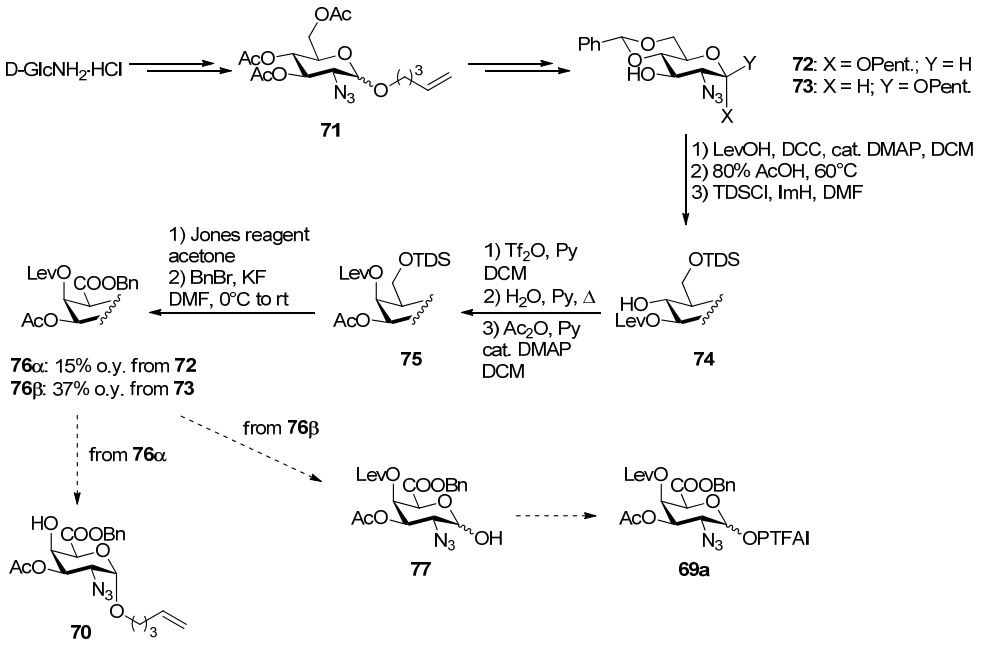


Figure 24: retrosynthetic strategy.

5.2.1 Building blocks synthesis

Initially, we selected a Lev moiety as orthogonal acyl protecting group at C-4. The early steps of this new synthetic strategy are the same previously described for galactosamine, but performed on glucosamine, and led to obtain the two anomers **72** and **73**. They were separated by crystallization and used independently in the following steps. The two compounds were protected at position 3 with the Lev group, followed by benzylidene cleavage and silylation of the primary position. The inversion with concomitant Lev migration was obtained by treatment with triflic anhydride followed by addition of water and heating.[103] Acetylation followed by simultaneous desilylation/oxidation of the primary position with Jones reagent

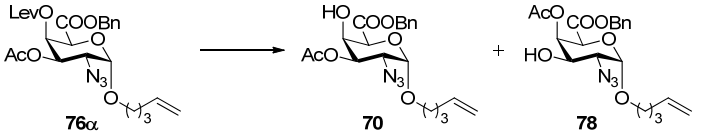
afforded uronates **76** in satisfactory yields. At this stage the pure α anomer was planned to be converted into acceptor **70** by selective removal of the Lev group, whereas the pure β anomer transformed into donor **69a** through pentenyl hydrolysis and formation of the imidate (Scheme 22).



Scheme 22: synthesis of donor **69a** and acceptor **70**.

Disappointingly, when we attempted Lev removal on precursor **76α** (Scheme 23) we obtained, under different reaction conditions, an inseparable mixture of the desired product **70** and a variable amount of the glycoside **78** derived from acetyl migration from C-3 to C-4 (Table 6). We ascribed this result to the rather basic conditions required for Lev removal. We reasoned that we could circumvent the acetyl migration by replacing the levulinoyl group with the chloroacetyl ester, that can be removed under strictly neutral conditions. As a proof of concept, we tested this reaction on galactoside **83**. This compound was obtained in good yield starting from intermediate **79**, which was subjected to installation of a 3,4-*O*-isopropylidene, oxidation with BAIB and catalytic TEMPO under two-phases conditions, hydrolysis of the acetal, borinic ester-catalyzed regioselective acetylation[104] at position 3 and chloroacetylation at position 4. ClAc removal was

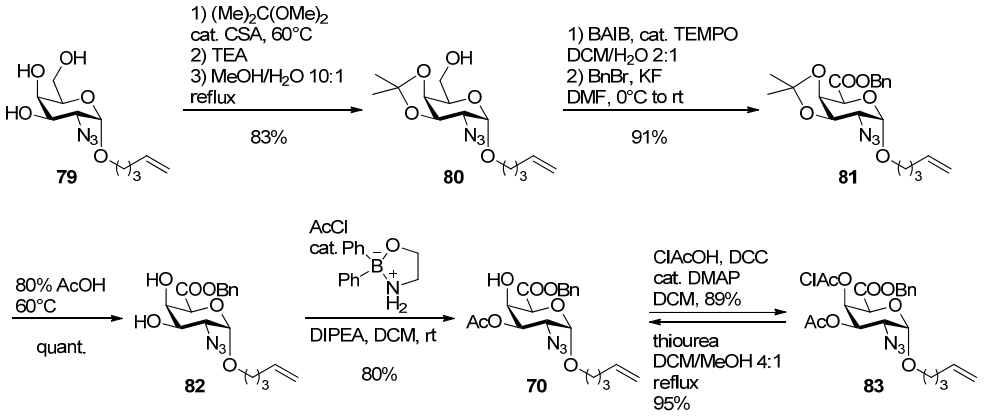
obtained by treatment with thiourea in refluxing DCM/MeOH, providing smoothly acceptor **70** in 95% yield as the sole product (Scheme 24).



Scheme 23: Lev removal.

Conditions	**Yield**	**70/78**
$NaBH_4$, H_2O, pH 5-8	/	
$NaHSO_3$, THF	n.r.	
$H_2NNH_2 \cdot AcOH$	80%	2:1
$H_2NNH_2 \cdot H_2O$, Py/AcOH 4:1, 5% 	75%	1:1

Table 6: Lev removal conditions.



Scheme 24: ClAc removal attempt on galactoside **83**.

5.3 Perspectives

Future work will concern the optimization of the synthesis of the building blocks starting from glucosamine hydrochloride, using the chloroacetate ester to protect position 4 in the donor, and the optimization of the glycosylation reaction conditions with building blocks **69b** and **70**. We will also explore the "2n+1" and "2n+2" glycosylation approaches employing the uronate disaccharide donor **85**, which could be obtained from disaccharide **84** by pentenyl hydrolysis (Figure 26).

Another important aspect we will also focus on is the elaboration of the linker moiety in order to allow protein or multivalent scaffold conjugation. In fact, the target oligomers obtained by hydrogenolysis contain a pentyl arm at the reducing end, and they are therefore unsuitable for conjugation. To this end, various options will be investigated. For example, the terminal C-C double bond of the pentenyl linker can be converted into an aldehyde by ozonolysis prior to global deprotection. Another option is to perform a chemoselective thiol-ene "click" reaction using a bifunctional organic thiol (Figure 25).

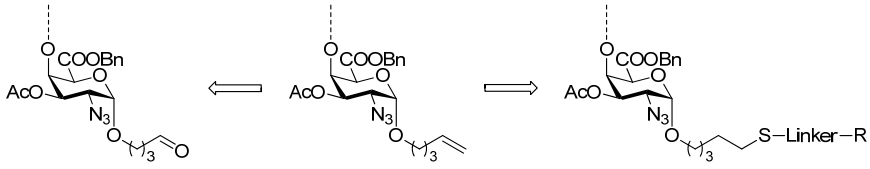


Figure 25: manipulation of the linker moiety.

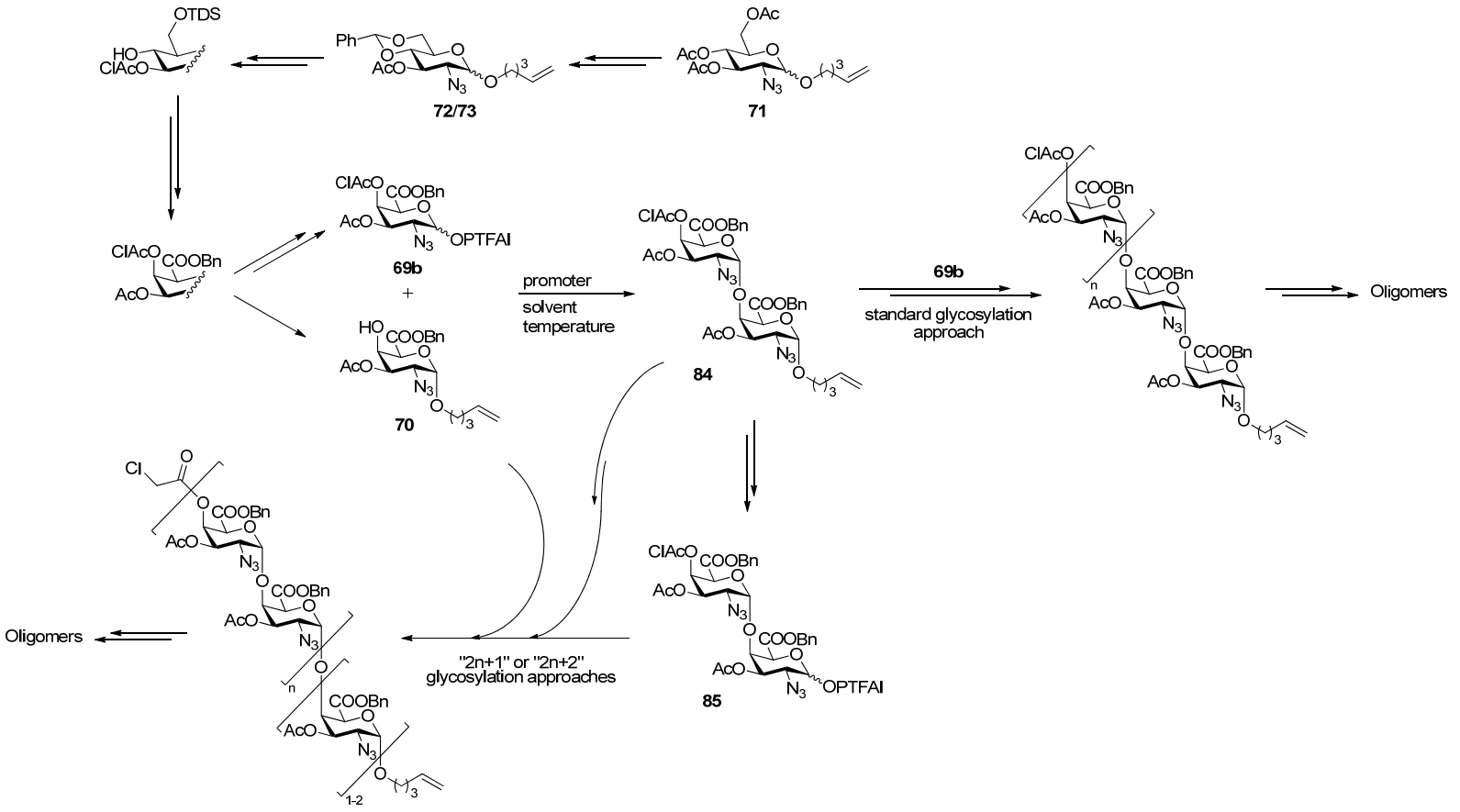


Figure 26: global synthetic strategy.

6

Experimental section

General experimental methods

Thin Layer Chromatography

Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) were performed on Merck precoated $60F_{254}$ 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 $(NH_4)_4Mo_4O_{24}$, 1 g of $Ce(SO_4)_2$, 31 mL of H_2SO_4 98%, 970 mL H_2O)
- sulphuric acid (50 mL of H_2SO_4 98%, 450 mL of MeOH, 450 mL H_2O)
- ninhydrin (2.7 g of 2,2-dihydroxyindane-1,3-dione, 27 mL of AcOH, 900 mL of EtOH)

with detection by charring at 196°C.

Flash chromatography

According to Still procedure,[105] 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) from Sigma-Aldrich. In some cases it was also used a flash purification system, Biotage SP1. Normal- and reverse phase Biotage SNAP cartridges (sizes from 10 g to 340 g, standard 50 μm silica) were used to purify the compounds.

NMR analysis

NMR spectra were recorded on a Bruker AMX 400 instrument (400 and 100.6 MHz for ^{1}H and ^{13}C, respectively), and were all run at room temperature (298K), unless otherwise noted. The samples were prepared using deuterated solvents ($CDCl_3$, D_2O and CD_3OD from Sigma-Aldrich). Chemical shifts (δ) are reported in ppm and the coupling constants (*J*) in Hz. Chemical shifts were referenced to the residual proton in the solvent (e.g. the $CHCl_3$, 0.01 % in 99.99 % $CDCl_3$), according to Gottlieb and

Nudelman.[106] Multiplicities are abbreviated as: br (broad), s (singlet), d (doublet), t (triplet), hept (heptet), m (multiplet) or combinations thereof. ^{1}H-NMR spectra were recorded for all the synthesized products. In the case of unknown structure, the characterization is reported by ^{1}H-NMR and ^{13}C-NMR. Bidimensional experiments (COSY, TOCSY, HSQC and HMBC) were used to better assign peaks to the structure. Carbon atoms and protons on each sugar residue were identified by numbers in accordance to Figure 27. C-6 methylene protons were labeled with letters “a” and “b”.

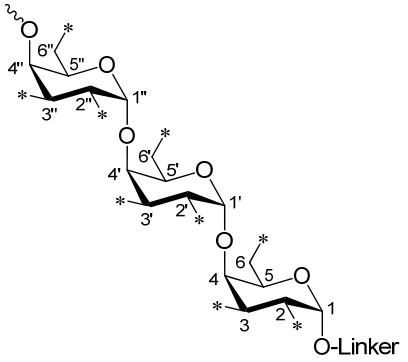


Figure 27

Mass analysis

Low resolution mass analyses were recorded in negative or positive mode on a Thermo Finnigan LCQ Advantage equipped with an ESI source. High resolution mass analyses were recorded on a Waters Micromass Q-Tof micro equipped with a LockSpray ESI source or on a Bruker Daltonics ICR-FTMS APEX II at C.I.G.A, University of Milan.

Optical rotatory power

Optical rotations were measured at r.t. with a Pelkin-Elmer 241 polarimeter (589 nm, D line from sodium lamp); the polarimeter tube was 100 mm thick and 1 ml of capacity. Optical rotatory powers were measured following the equation: $[\alpha]_D^{20} = (10000 \cdot \alpha)/(l \cdot c)$, where α is the observed rotation (on the average of 10

experimental values), *l* is the length of the observed layer in mm, *c* is the number of g of substance contained in 100 ml of solution. The optical rotation (α) can be expressed in angular degrees: values are given in 10^{-1} deg cm^2 g^{-1} unit.

Anhydrous environment

Unless otherwise stated, all the reaction were conducted under nitrogen atmosphere. When stirred overnight, the reaction were put under anhydrous atmosphere by an Argon-balloon.

Solvents

Unless otherwise stated, all the reactions were performed using dry solvents. DCM, Et_2O, MeOH, pyridine, toluene, DMF, CH_3CN and THF over molecular sieves were purchased from Sigma-Aldrich and used without further purifications.

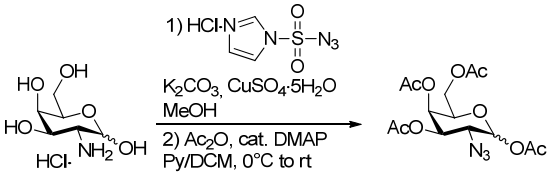
Competitive ELISA assay

96-Well flat-bottomed plates were incubated overnight at 4-8°C with a mixture of *S. thypi* Vi CPS (kindly provide by Sanofi Pasteur, Marcy L'Etoile, France) (1 mg/mL) and methylated human serum albumin (1 mg/mL). A solution of foetal calf serum (5%) in phosphate-buffered saline supplemented with Brij-35 (0.1%) and sodium azide (0.05%) was applied to the plates for blocking of nonspecific binding sites. The plates were incubated overnight at 4-8°C with a solution (1:200) of horse anti-Vi, used as reference serum (Remel, Lenexa, USA). When Vi analogs were tested, they were added to each well immediately before the addition of the reference serum. The plates were then incubated with alkaline phosphatase conjugate rabbit anti-horse IgG (Sigma-Aldrich, Milan, Italy), stained with *p*-nitrophenylphosphate, and the absorbance was measured at 405 nm with an Ultramark microplate reader (Bio-Rad Laboratories S.r.l., Milan, Italy).

6.1 3-*non*-*O*-Ac (3-OH) oligomers

6.1.1 First route – propargyl linker

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-galactopyranoside (8)

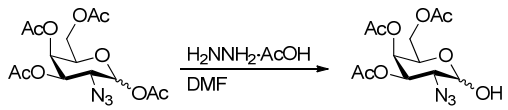


8

Anhydrous K_2CO_3 (19.8 g, 143 mmol) was added to a suspension of D-galactosamine hydrochloride (10.29 g, 47.7 mmol) in MeOH (250 mL) at 0°C and the mixture is left under vigorous stirring for 15 min. Imidazole-1-sulfonyl azide hydrochloride (11.99 g, 57.24 mmol) was then slowly added, followed by $CuSO_4 \cdot 5H_2O$ (120 mg, 0.48 mmol). The reaction was monitored by RPTLC (CH_3CN/MeOH/H_2O 10:5:1). After 2 h the solvent was removed, then the crude was coevaporated three times with toluene and kept under vacuum overnight. The crude was suspended in pyridine (64.4 mL, 763 mmol) and DCM (150 mL), catalytic DMAP was added. Ac_2O (36 mL, 382 mmol) was slowly added to the suspension at 0°C, then the reaction was warmed to rt and stirred for 3 h until completion (TCL, eluent hexane/AcOEt 6:4). The mixture was quenched with crushed ice and the solvent evaporated. The crude was diluted with DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash chromatography (hexane/AcOEt 6:4) afforded compound **8** (11.7118 g, 66%) as a white foam.

Spectroscopic data were in agreement with those reported in the literature.[3]

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-D-galactopyranose (9)

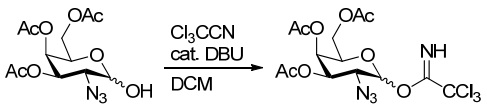


8 **9**

A solution of hydrazinium acetate was prepared by slow addition of acetic acid (1.67 mL, 29.3 mmol) to a stirred solution of hydrazine hydrate (1.85 mL, 38.1 mmol) in MeOH (19 mL) at 0°C. After 15 min, the solution was slowly added to compound **8** (10.9239 g, 29.3 mmol) dissolved in DMF (60 mL), and stirred for 1.5 h (TLC hexane/AcOEt 6:4). The solvent was evaporated, the crude was taken up with AcOEt and washed with brine. The organic phase was dried with Na_2SO_4, filtered and the solvent removed. Compound **9** was obtained as a light yellow foam (9.693 g, quant.) and used in the following step without further purification.

Spectroscopic data were in agreement with those reported in the literature.[4]

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-D-galactopyranosyl trichloroacetimidate (10)

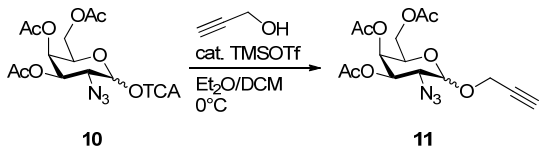


9 **10**

Compound **9** (10.48 g, 31.6 mmol) was dissolved in DCM (50 mL). Trichloroacetonitrile (31.7 mL, 316 mmol) and catalytic DBU were added and the reaction mixture was stirred for 1.5 h (TLC, hexane/AcOEt 6:4). The solvent was removed and the crude was purified by flash chromatography (hexane/AcOEt 6:4 + 2% TEA), affording compound **10** (12.94 g, 86%) as a white foam.

Spectroscopic data were in agreement with those reported in the literature.[5]

Propargyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-galactopyranoside (11)

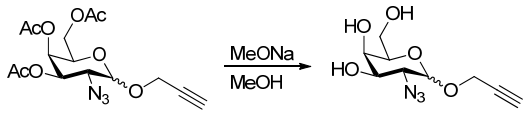


10 **11**

Compound **10** (12.94 g, 27.2 mmol) was dissolved in Et_2O (20 mL) and DCM (5 mL) under Argon atmosphere at 0°C, and propargyl alcohol (1.9 mL, 32.6 mmol) was added. Then a solution of TMSOTf (0.11 M in DCM, 12.7 mL, 1.4 mmol) was slowly added. The reaction was monitored by HPTLC (hexane/AcOEt 6:4). After 2 h the mixture was neutralized with TEA, filtered on a Celite pad and the solvent was removed. Flash chromatography (hexane/AcOEt 7:3 to 6:4) afforded pure **11** (8.84 g, 88%, α/β 3:1) as a white foam.

Spectroscopic data were in agreement with those reported in the literature.[6]

Propargyl 2-azido-2-deoxy-D-galactopyranoside (12)

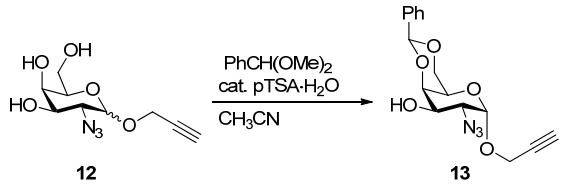


11 **12**

A solution of MeONa in MeOH (0.1 M, 2 mL, 5 mmol) was added to compound **11** (4.78 g, 12.95 mmol) dissolved in MeOH (40 mL) and the mixture was stirred for 2 h (TLC, DCM/MeOH 9:1). The reaction is neutralized with Amberlite IR-120, filtered and the solvent was removed *in vacuo*. The product was used in the following step without further purification (quant.).

Spectroscopic data were in agreement with those reported in the literature.[7]

Propargyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (13)



Compound **12** (3.15 g, 12.95 mmol) was dissolved in CH_3CN (30 mL), benzaldehyde dimethyl acetal (5.2 mL, 34.7 mmol) and *p*TSA·H_2O (246 mg, 1.3 mmol) were added. The reaction was stirred overnight (TLC, hexane/AcOEt 6:4), then quenched with TEA and the solvent was evaporated. Flash chromatography (hexane/AcOEt 8:2 to 6:4) afforded compound **13** (2.22 g, 52%) as a white solid.

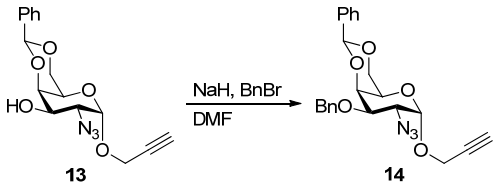
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.52 – 7.35 (m, 5H, arom.), 5.56 (s, 1H, PhC*H*), 5.19 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.30 – 4.23 (m, 4H, OC*H*$_2$CCH, H-6a, H-4), 4.15 (dd, $J_{2,3}$ = 10.6, $J_{3,4}$ = 3.7 Hz, 1H, H-3), 4.08 (dd, $J_{6a,6b}$ = 12.7, $J_{5,6b}$ = 1.7 Hz, 1H, H-6b), 3.78 (br d, *J* = 1.3 Hz, 1H, H-5), 3.65 (dd, $J_{2,3}$ = 10.6, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 2.48 (t, *J* = 2.4 Hz, 1H, OCH$_2$CC*H*), 2.47 (br s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 101.36 (Ph*C*H), 97.64 (C-1), 75.45 (C-4), 75.22 (OCH$_2$C*C*H), 69.07 (C-6), 67.66 (C-3), 63.36 (C-5), 60.60 (C-2), 55.56 (O*C*H$_2$CCH) ppm.

ESI HR-MS (MeOH) for $[C_{16}H_{17}N_3O_5Na]$+ calcd: 354.10604, found: 354.10591

$[\alpha]_D^{20}$ = 169.34 (*c* = 1 in $CHCl_3$)

Propargyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (14)



Compound **13** (1.2992 g, 3.92 mmol) and BnBr (930 µL, 7.84 mmol) were dissolved in DMF (20 mL), then NaH was slowly added portionwise over 2 h. The reaction was stirred for a further 1 h (TLC, hexane/AcOEt 6:4), then carefully quenched with MeOH and neutralized with Amberlite IR-120. The mixture is filtered and the solvent evaporated. The crude is taken up with AcOEt and washed with water and brine. The organic phase was dried with Na_2SO_4, filtered and evaporated *in vacuo*. Flash chromatography (hexane/AcOEt 8:2) furnished compound **14** (1.2224 g, 74%) as a white foam.

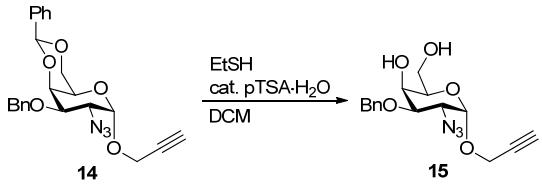
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.55 – 7.28 (m, 10H, arom.), 5.47 (s, 1H, PhC*H*), 5.19 (d, $J_{1,2}$ = 2.3 Hz, 1H, H-1), 4.74 (m, 2H, OCH_2Ph), 4.29 (m, 2H, OCH_2CCH), 4.24 (m, 1H, H-6a), 4.21 (m, 1H, H-4), 4.01 (m, 3H, H-6b, H-2, H-3), 3.69 (m, 1H, H-5), 2.46 (t, *J* = 2.4 Hz, 1H, OCH_2CCH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 101.16 (Ph*C*H), 97.75 (C-1), 76.21 (OCH_2C*C*H), 74.83 (C-3), 73.26 (C-4), 71.59 (O*C*H_2Ph), 69.40 (C-6), 63.54(C-5), 58.84 (C-2), 55.60 (O*C*H_2CCH) ppm.

ESI HR-MS (MeOH) for $[C_{23}H_{23}N_3O_5Na]^+$ 444.15299, found: 444.15277

$[\alpha]_D^{20}$ = 152.9 (*c* = 1 in $CHCl_3$)

Propargyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (15)



Compound **14** (1.3346 g, 3.17 mmol) was dissolved in DCM (20 mL). EtSH (1.4 mL, 19 mmol) and then *p*TSA·H_2O (120 mg, 0.63 mmol) were added. The reaction was stirred for 1 h (TLC, hexane/AcOEt 6:4), then quenched with TEA and the solvent was removed. Flash chromatography (hexane/AcOEt 5:5) afforded compound **15** (1.0346 g, 98%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45 – 7.28 (m, 5H, arom.), 5.15 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.71 (m, 2H, OC*H*$_2$Ph), 4.28 (m, 2H, OC*H*$_2$CCH), 4.12 (br d, *J* = 2.7 Hz, 1H, H-4), 3.95 – 3.87 (m, 2H, H-6a, H-5), 3.86 – 3.78 (m, 3H, H-3, H6b, H-2), 2.47 (t, *J* = 2.4 Hz, 1H, OCH$_2$CC*H*) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 96.94 (C-1), 76.14 (C-3), 75.40 (OCH$_2$C*C*H), 72.28 (O*C*H$_2$Ph), 70.10 (C-5), 67.69 (C-4), 62.96 (C-6), 58.98 (C-2), 55.28 (O*C*H$_2$CCH) ppm.

Propargyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (7a)

Compound **15** (1.0346 g, 3.11 mmol) and *sym*-collidine (1.6 mL, 12.4 mmol) were dissolved in DCM at -40°C. AcCl (331 µL, 4.66 mmol) was slowly added and the reaction mixture was stirred for 3 h (TLC, hexane/AcOEt 5:5). The reaction was quenched with MeOH, diluted with DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. The organic phase was dried with Na_2SO_4, filtered and evaporated *in vacuo*. Flash chromatography (hexane/AcOEt 6:4) afforded compound **7a** (894 mg, 77%) as a white solid.

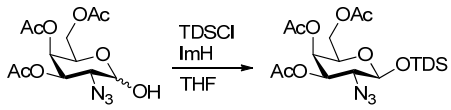
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.44 – 7.30 (m, 5H, arom.), 5.12 (d, $J_{1,2}$ = 3.3 Hz, 1H, H-1), 4.71 (m, 2H, OC*H*$_2$Ph), 4.37 – 4.21 (m, 4H, H-6a, H-6b, OC*H*$_2$CCH), 4.01 (br d, *J* = 1.0 Hz, 1H, H-4), 3.96 (br t, *J* = 6.1 Hz, 1H, H-5), 3.90 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 2.9 Hz, 1H, H-3), 3.78 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 3.3 Hz, 1H, H-2), 2.48 (t, *J* = 2.3 Hz, 1H, OCH$_2$CC*H*), 2.07 (s, 3H, OCOCH$_3$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 170.71 (*C*OCH$_3$), 96.64 (C-1), 76.12 (C-3), 74.70 (OCH$_2$C*C*H), 72.33 (O*C*H$_2$Ph), 68.46 (C-5), 66.36 (C-4), 63.29 (C-6), 58.88 (C-2), 55.04 (O*C*H$_2$CCH), 20,92 (*C*OCH$_3$) ppm.

ESI HR-MS (MeOH) for $[C_{18}H_{21}N_3O_6Na]$+ calcd: 398.13226, found: 398.13184

$[\alpha]_D^{20}$ = 98.7 (*c* = 1 in $CHCl_3$)

Thexyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (16)

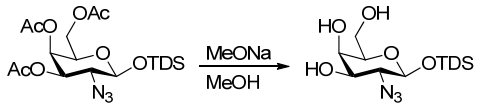


9 **16**

Compound **9** (7.38 g, 22 mmol) and imidazole (4.5 g, 66 mmol) were dissolved in DMF (20 mL). TDSCl (8.5 mL, 44 mmol) was slowly added at 0°C. After 15 min the reaction was warmed to rt and stirred overnight (TCL, hexane/AcOEt 6:4). The solvent was removed, the crude was taken up with AcOEt and washed with water and brine. The organic phase was dried with Na_2SO_4, filtered and evaporated *in vacuo*. Flash chromatography (hexane/AcOEt 8:2) afforded compound **16** (8.4088 g, 81%) as a white solid.

Spectroscopic data were in agreement with those reported in the literature.[8]

Thexyldimethylsilyl 2-azido-2-deoxy-β-D-galactopyranoside (17)

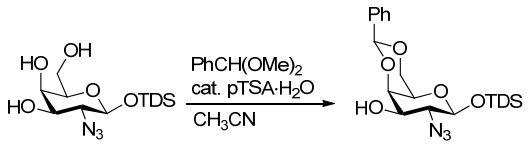


16 **17**

A solution of MeONa in MeOH (0.4 M, 11 mL, 4.5 mmol) was added to compound **16** (10.8 g, 22.8 mmol) dissolved in MeOH (80 mL). The reaction was followed by TLC (hexane/AcOEt 6:4). After 15 min the reaction was neutralized with Amberlite IR-120, filtered and the solvent was removed *in vacuo*, affording compound **17** (7.6744 g, 96%) as a white foam. The product was used in the following step without further purification.

Spectroscopic data were in agreement with those reported in the literature.[8]

Thexyldimethylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (18)

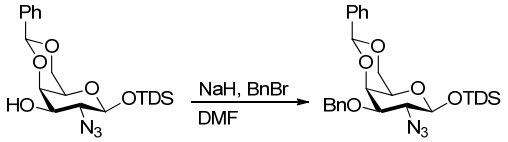


17 **18**

Compound **17** (7.6744 g, 22 mmol) was dissolved in CH_3CN (60 mL), then benzaldehyde dimethyl acetal (4.9 mL, 33 mmol) and *p*TSA·H_2O (418 mg, 2.2 mmol) were added. The reaction was monitored by TLC (hexane/AcOEt 7:3). After 2 h the mixture was quenched with TEA and the solvent was evaporated. Flash chromatography (hexane/AcOEt 8:2 + 1% TEA) afforded compound **18** (8.6797 g, 90%) as a white solid.

Spectroscopic data were in agreement with those reported in the literature.[8]

Thexyldimethylsilyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (19)



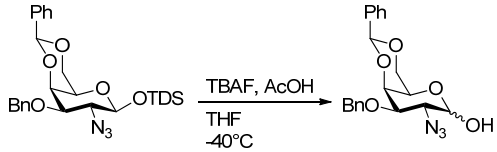
18 **19**

Compound **18** (8.6797 g, 19.9 mmol) was dissolved in DMF (70 mL), then BnBr (4.7 mL, 39.9 mmol) and TBAI (735 mg, 2 mmol) were added. NaH (799 mg, 29.9 mmol) was added portionwise. The reaction was monitored by TLC (hexane/AcOEt 7:3). After 15 min the mixture was quenched with MeOH and the solvent was removed. The crude was taken up in DCM and washed with water and brine. The organic phase was dried with Na_2SO_4, filtered and evaporated *in vacuo*. Flash chromatography (hexane/AcOEt 95:5 to 9:1) afforded compound **19** (9.834 g, 94%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.46 – 7.28 (m, 10H arom.), 5.49 (s, 1H, PhC*H*), 4.75 (s, 2H, OCH_2Ph), 4.53 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1), 4.25 (dd, $J_{6a,6b}$ = 12.3, $J_{5,6a}$ = 1.6 Hz, 1H, H-6a), 4.08 (br d, J = 3.5 Hz, 1H, H-4), 4.01 (dd, $J_{6a,6b}$ = 12.3, $J_{5,6b}$ = 1.9 Hz, 1H, H-6b), 3.78 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 7.6 Hz, 1H, H-2), 3.34 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 3.29 (m, 1H, H-5), 1.72 (m, 1H, OSiC$(CH_3)_2$C*H*$(CH_3)_2$), 0.94 (m, 12H, OSiC$(CH_3)_2$CH$(CH_3)_2$), 0.24 (m, 6H, OSi$(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{28}H_{39}N_3O_5SiNa]$+ calcd: 548.26, found: 548.5

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-galactopyranose (20)

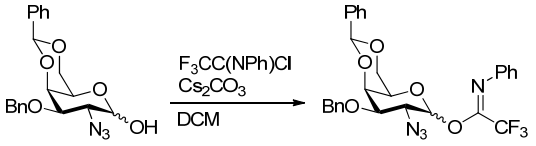


19 **20**

Compound **19** (2.6829 g, 5 mmol) was dissolved in THF (20 mL) at -40°C. AcOH (875 µL, 15.3 mmol) and TBAF (1.0 M in THF, 15 mL, 15 mmol) were slowly added. The reaction was monitored by TLC (hexane/AcOEt 5:5). After 1 h the solvent was concentrated, the mixture was diluted with AcOEt and washed with satd. NH_4Cl. The organic phase was dried with Na_2SO_4, filtered and evaporated *in vacuo*. Flash chromatography (hexane/AcOEt 5:5) afforded compound **20** (1.9239 g, quant.) as a white foam.

Spectroscopic data were in agreement with those reported in the literature.[9]

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl *N*-phenyltrifluoroacetimidate (6)



20 **6**

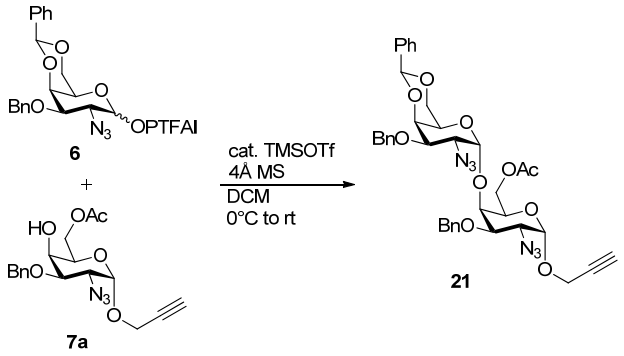
Compound **20** (709.5 mg, 1.85 mmol) and *N*-phenyltrifluoroacetimidoyl chloride (600 μL, 3.7 mmol) were dissolved in DCM (10 mL), then Cs_2CO_3 (912 mg, 2.8 mmol) was added portionwise. The reaction was monitored by TLC (hexane/AcOEt 5:5). After 3 h the reaction mixture was filtered on a Celite pad and the solvent was evaporated. Flash chromatography (hexane/AcOEt 7:3 + 0.1% TEA) afforded compound **6** (0.9732 g, 95%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 55°C): δ = 7.56 – 7.30 (m, 12H, arom.), 7.15 (m, 1H, arom.), 6.88 (m, 2H, arom.), 6.49 (br s, 1H, H-1), 5.50 (s, 1H, PhC*H*), 4.80 (m, 2H, OCH_2Ph), 4.30 (m, 2H, H-4, H-6a), 4.20 (dd, $J_{2,3}$ = 10.5, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 4.05 (m, 2H, H-3, H-6b), 3.76 (br s, 1H, H-5) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 55°C): δ = 101.00 (Ph*C*H), 95.11 (C-1), 74.85 (C-3), 72.68 (C-4), 71.59 (O*C*H_2Ph), 68.96 (C-6), 65.22 (C-5), 58.36 (C-2) ppm.

ESI HR-MS (MeOH) for $[C_{28}H_{25}F_3N_4O_5Na]$+ calcd: 577.1669, found: 577.1615

(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→4)-(propargyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (21)



Donor **6** (1.1655 g, 2.1 mmol) and acceptor **7a** (526.9 mg, 1.4 mmol) were coevaporated together three times with toluene and kept under vacuum overnight. They were dissolved in DCM (8 mL) under Argon atmosphere. Freshly activated 4Å molecular sieves (1.71 g) were added and the mixture was stirred at rt for 45 min. Then a solution of trimethylsilyl triflate (0.055 M in DCM, 2.5 mL, 0.14 mmol) was slowly added over 1 h at 0°C using a syringe pump. The reaction mixture was then warmed to rt and monitored by HPTLC (hexane/AcOEt 6:4). After 2 h the reaction was neutralized with TEA, filtered on a Celite pad and the solvent was evaporated. Flash chromatography (toluene/AcOEt 100:0 to 95:5) afforded compound **21** (735 mg, 71%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.36 (m, 15H, arom.), 5.32 (s, 1H, PhC*H*), 5.22 (d, $J_{1',2'}$ = 3.6 Hz, 1H, H-1'), 5.08 (d, $J_{1,2}$ = 2.7 Hz, 1H, H-1), 4.74 (m, 4H, 2 x OCH_2Ph), 4.54 (dd, $J_{6a,6b}$ = 11.1, $J_{5,6a}$ = 6.6 Hz, 1H, H-6a), 4.37 (dd, $J_{6a,6b}$ = 11.1, $J_{5,6b}$ = 7.9 Hz, 1H, H-6b), 4.29 (m, 2H, OCH_2CCH), 4.24 (d, J = 2.7 Hz, 1H, H-4'), 4.15 (d, J = 1.1 Hz, 1H, H-4), 4.03 (s, 1H, H-5), 4.00 (m, 3H, H-2; H-3; H-5'), 3.92 (dd, $J_{2',3'}$ = 10.8, $J_{3',4'}$ = 2.7 Hz, 1H, H-3'), 3.76 (dd, $J_{2',3'}$ = 10.8, $J_{1',2'}$ = 3.6 Hz, 1H, H-2'), 3.58 (dd, $J_{6'a,6'b}$ = 12.8, $J_{5',6'a}$ = 1.1 Hz, 1H, H-6'a), 3.34 (dd, $J_{6'a,6'b}$ = 12.8,

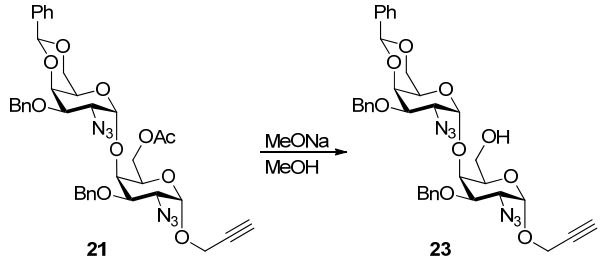
$J_{5',6'b}$ = 1.4 Hz, 1H, H-6'b), 2.50 (t, J = 2.3 Hz, 1H, OCH_2CCH), 2.11 (s, 3H, $COCH_3$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 172.31 ($COCH_3$), 100.89 (Ph*C*H), 99.81 (C-1'), 96.90 (C-1), 76.05 (C-3'), 75.39 (OCH_2CCH), 74.90 (C-5' or C-3), 73.00 (C-4'), 72.85 (C-4), 72.2 (OCH_2Ph), 71.33 (OCH_2Ph), 69.12 (C-6'), 69.00 (C-5' or C-3), 63.27 (C-5), 61.32 (C-6), 59.65 (C-2'), 59.28 (C-2), 55.40 (OCH_2CCH), 21.11 ($COCH_3$) ppm.

ESI HR-MS (MeOH) for $[C_{38}H_{40}N_6O_{10}Na]+$ calcd: 763.26981 found: 763.26939

$[\alpha]_D^{20}$ = 224.90 (*c* = 1 in $CHCl_3$)

(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→4)-(propargyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (23)



Compound **21** (735 mg, 0.99 mmol) was dissolved in MeOH (10 mL) and THF (2 mL), then a solution of sodium methoxide (0.4 M in MeOH, 250 μL, 0.1 mmol) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 2 h the mixture was neutralized with Amberlite IR-120, filtered and the solvent was removed *in vacuo*, affording compound **23** (684 mg, 99%) as a white foam.

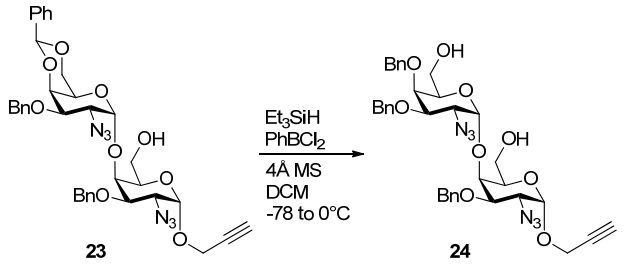
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.47 – 7.29 (m, 15H, arom.), 5.32 (s, 1H, PhC*H*), 5.22 (d, $J_{1',2'}$ = 3.6 Hz, 1H, H-1'), 5.13 (d, $J_{1,2}$ = 2.9 Hz, 1H, H-1), 4.81 – 4.65 (m, 4H, 2 x O*C*H_2Ph), 4.32 (br d, *J* = 2.7 Hz, 1H, H-4'), 4.30 (m, 2H, O*C*H_2CCH), 4.15 (br d, *J* = 1.2 Hz, 1H, H-4), 4.03 – 3.98 (m, 3H, H-2, H-3, H-5), 3.91 (m, 4H, H-5', H-6'a, H-6'b, H-3'), 3.75 (dd, $J_{2',3'}$ = 10.9, $J_{1',2'}$ = 3.6 Hz, 1H, H-2'), 3.66 (m, 1H, H-6a), 3.37 (m, 1H, H-6b), 2.48 (t, *J* = 2.3 Hz, 1H, OCH_2CC*H*) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 100.82 (Ph*C*H), 99.60 (C-1), 96.91 (C-1'), 76.14 (C-3'), 75.24 (OCH_2CC*C*H), 74.66 (C-2 or C-3 or C-5), 72.99 (C-4'), 72.82 (C-4), 72.08 (O*C*H_2Ph), 71.60 (C-5'), 71.20 (O*C*H_2Ph), 69.16 (C-6), 63.17 (C-2 or C-3 or C-5), 60.62 (C-6'), 59.77 (C-2'), 59.29 (C-2 or C-3 or C-5), 55.30 (O*C*H_2CCH) ppm.

ESI HR-MS (MeOH) for $[C_{36}H_38N6O9Na]+$ calcd: 721.25925, found: 721.25935

$[\alpha]_D^{20} = 166.04$ ($c = 1$ in $CHCl_3$)

(2-Azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(propargyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (24)



Compound **23** (242.5 mg, 0.35 mmol) was dissolved in DCM (10 mL). 3Å molecular sieves (1.25 g) were added and the mixture was stirred for 40 minutes. Triethylsilane (210 μL, 1.32 mmol) was added. $PhBCl_2$ (57 μL, 0.42 mmol) was added at -78°C. The reaction mixture was slowly warmed to 0°C over 4 hours. Further triethylsilane (100 μL, 0.32 mmol) and $PhBCl_2$ (26 μL 0.2, mmol) were added at -78°C, the reaction mixture was warmed to -30°C and stirred overnight. The reaction was monitored by TLC (hexane/AcOEt 6:4). The reaction was diluted with MeOH and neutralized with TEA, filtered on a Celite pad, diluted with DCM and washed with satd. $NaHCO_3$. The organic phase was dried with Na_2SO_4, filtered, and concentrated. Flash chromatography (hexane/AcOEt 6:4) afforded compound **24** (223 mg, 92%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.44 – 7.23 (m, 15H, arom.), 5.15 (d, $J_{1',2'}$ = 3.6 Hz, 1H, H-1'), 5.04 (d, $J_{1,2}$ = 2.7 Hz, 1H, H-1), 4.89 – 4.51 (m, 6H; 3 x OC*H*$_2$Ph), 4.27 (m, 2H, OC*H*$_2$CCH), 4.22 (br d, *J* = 1.9 Hz, 1H, H-4'), 4.03 – 3.94 (m, 4H, H-5', H-2, H-3, H-4), 3.92 – 3.86 (m, 2H, H-5, H-3'), 3.83 – 3.74 (m, 3H, H-6'a, H-6'b, H-2'), 3.43 (dd, $J_{6a,6b}$ = 11.2, $J_{5,6a}$ = 6.1 Hz, 1H, H-6a), 3.36 (dd, $J_{6a,6b}$ = 11.2, $J_{5,6b}$ = 5.7 Hz, 1H, H-6b), 2.48 (t, *J* = 2.2 Hz, 1H, OCH$_2$CC*H*) ppm.

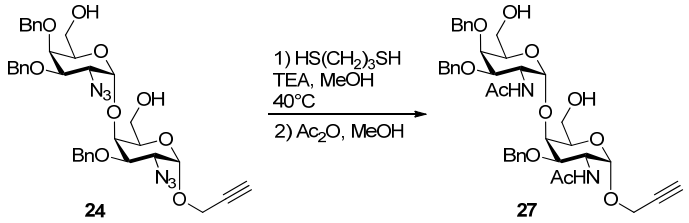
^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 99.34 (C-1), 96.87 (C-1'), 77.55 (C-3), 76.38 (C-3'), 75.35 (OCH$_2$C*C*H), 74.83 (O*C*H$_2$Ph), 74.09 (C-4'), 73.41 (C-4), 72.56

(O*C*H_2Ph), 72.30 (O*C*H_2Ph), 71.80 (C-5'), 71.27 (C-5), 62.13 (C-6), 60.89 (C-2), 60.48 (C-6'), 59.72 (C-2'), 55.28 (O*C*H_2CCH) ppm.

ESI HR-MS (MeOH) for $[C_{36}H_{40}N_6O_9Na]+$ calcd: 723.27490, found: 723.27436

$[\alpha]_D^{20}$ = 180.2 (*c* = 1 in $CHCl_3$)

(2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(propargyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (27)

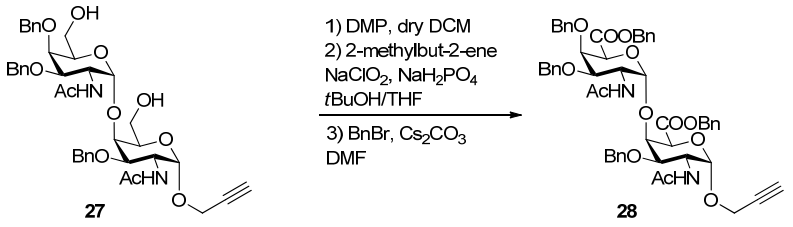


Compound **24** (172.5 mg, 0.25 mmol) was dissolved in MeOH (15 mL). Propanedithiol (500 μL, 5 mmol) and TEA (670 μL, 5.8 mmol) were added, and the mixture was heated at 40°C for 24 h. The reaction was monitored by TLC (hexane/AcOEt 5:5). Then the solvent was removed in vacuo, the crude was dissolved in MeOH (10 mL) and Ac_2O (472 μL, 5 mmol) was added. The reaction was monitored by TLC (AcOEt) and stirred overnight. Then the solvent was removed *in vacuo* and flash chromatography (DCM/MeOH 98:2) afforded compound **27** (93.4 mg, 51%) as a white foam.

The occurrence of the azide reduction was confirmed by the appearance of the acetamido signals in the ^{1}H NMR spectrum.

ESI-MS (MeOH) for $[C_{40}H_{48}N_2O_{11}Na]$+ calcd: 755.32, found: 755.40

(Benzyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyluronate)-(1→4)-(benzyl (propargyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosid)uronate) (28)



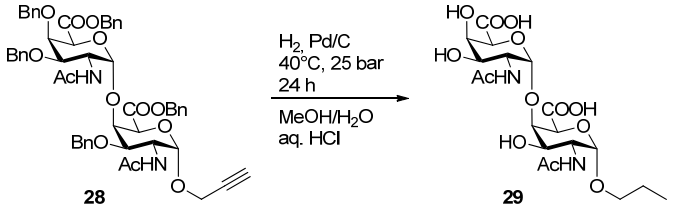
Compound **27** (93.4 mg, 0.13 mmol) was dissolved in DCM (5 mL) and Dess-Martin periodinane (165 mg, 0.39 mmol) was added. The reaction was monitored by TLC (hexane/AcOEt 5:5). After 2 h, the mixture was quenched with 10% aq. Na2S2O3. The organic phase was separated and washed with satd. NaHCO3. The combined aqueous phases were extracted with DCM. The combined organic phases were dried over Na2SO4, filtered and concentrated in vacuo. The residue was dissolved in THF (3 mL) and tBuOH (2 mL). 2-Methylbut-2-ene (1.4 mL, 13 mmol) and a solution of NaClO2 and NaH2PO4·2H2O (293.9 mg + 304.2 mg in 10 mL of water, 2.6 mmol + 1.95 mmol) were then added. The reaction was monitored by TLC (hexane/AcOEt 4:6 + 1% AcOH). After 2 h the mixture was concentrated to half volume, acidified with 5% aq. HCl to pH 2, then the solvents were evaporated and the mixture lyophilized. The crude was dissolved in DMF (10 mL), BnBr (309 μL, 2.6 mmol) and Cs_2CO_3 (423 mg, 1.3 mmol) were added. The reaction was monitored by TLC (hexane/AcOEt 7:3) and stirred overnight. Then the solvent was evaporated, the crude was taken up in DCM and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2 to 6:4) afforded compound **28** (61.1 mg, 50%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.55 – 7.11 (m, 25H, arom.), 5.39 (m, 2H, COOC*H*HPh, N*H*Ac), 5.15 (m, 2H, H-1, N*H*Ac), 5.06 – 4.95 (m, 3H, H-5',

COOCH*H*Ph, H-1'), 4.85 (d, *J* = 8.2 Hz, 2H, OC*H*HPh), 4.80 – 4.72 (m, 2H, OC*H*HPh, OC*H*HPh), 4.67 (d, *J* = 12.1 Hz, 2H, COOC*H*HPh), 4.61 – 4.49 (m, 6H, COOH*H*Ph, H-2', H-4, H-4', OCH*H*Ph, OC*H*HPh), 4.45 – 4.28 (m, 4H, H-2, H-5, OCH*H*Ph, OCH*H*Ph), 4.27 – 4.13 (m, 3H, OCH_2CCH, OCH*H*Ph), 3.75 (dd, $J_{3',4'}$ = 11.3, $J_{2',3'}$ = 2.7 Hz, 1H, H-3'), 3.55 (dd, $J_{3,4}$ = 11.3, $J_{2,3}$ = 2.7 Hz, 1H, H-3), 2.33 (t, *J* = 2.4 Hz, 1H, OCH$_2$CC*H*), 2.06 (s, 3H, HNCOCH_3), 1.95 (s, 3H, HNCOCH_3) ppm.

ESI-MS (MeOH) for $[C_{54}H_{56}N_2O_{13}Na]$+ calcd: 963.38, found: 963.51

(2-Acetamido-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(propyl 2-acetamido-2-deoxy-α-D-galactopyranosiduronic acid) (29)



Compound **28** (35.6 mg, 37.8 μmol) was dissolved in H_2O/MeOH/AcOEt 4:4:1 (9 mL + 5 drops of 5% aq. HCl). Pd/C was added and the mixture was stirred under hydrogen (30 bar) at 40°C for 24 h. The reaction mixture was then filtered on a Celite pad and lyophilized. Reverse phase flash chromatography (H_2O/CH_3CN 95:5) afforded compound **29** (17.3 mg, 93%)

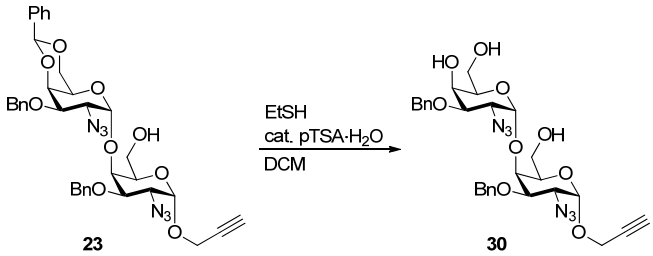
^{1}H NMR (400 MHz, D_2O, 25°C): δ = 5.09 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 5.03 (d, $J_{1',2'}$ = 3.5 Hz, 1H, H-1'), 4.48 (br d, J = 2.8 Hz, 1H, H-5'), 4.38 (m, 1H, H-5), 4.27 – 4.04 (m, 5H, H-4', H-2, H-2', H-3', H-3), 3.66 (m, 1H, OC*H*HCH_2CH_3), 3.50 (dt, J = 9.9, 6.3 Hz, 1H, OCH*H*CH_2CH_3), 2.07 (s, 3H, NHCOC*H*$_3$), 2.05 (s, 3H, NHCOC*H*$_3$), 1.60 (m, 2H, $OCH_2CH_2CH_3$), 0.91 (m, 3H, $OCH_2CH_2CH_3$).

^{13}C NMR (100.6 MHz, D_2O, 25°C): δ = 173.77 (*C*OOH or NH*C*OCH_3), 173.46 (*C*OOH or NH*C*OCH_3), 97.41 (C-1), 95.51 (C-1'), 76.82 (C-5'), 71.36 (C-5), 70.18 ($OCH_2CH_2CH_3$), 69.53 (C-4'), 68.93 (C-4), 66.83 (C-3), 65.93 (C-3'), 48.85 (C-2'), 48.21 (C-2), 21.29 (NHCOC*H*$_3$), 20.78 (NHCOC*H*$_3$), 20.53 ($OCH_2CH_2CH_3$), 8.13 ($OCH_2CH_2CH_3$) ppm.

ESI HR-MS (MeOH) for $[C_{19}H_{30}N_2O_{13}\text{-}H]^-$ calcd: 493.1675, found: 493.1627

$[\alpha]_D^{20}$ = 95.3 (*c* = 0.5 in H_2O)

(2-Azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(propargyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (30)

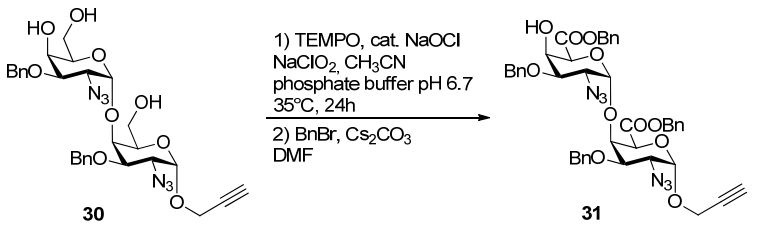


Compound **23** (244 mg, 0.35 mmol) was dissolved in DCM (10 mL). EtSH (155 μL, 2.1 mmol) and *p*TSA·H_2O (13 mg, 0.07 mmol) were added. The reaction was monitored by TLC (hexane/AcOEt 3:7). After 2 h, the mixture was neutralized with TEA and the solvent was evaporated. Flash chromatography (hexane/AcOEt 3:7) afforded compound **30** (203 mg, 95%) as a white foam.

The occurrence of the benzylidene cleavage was confirmed by the disappearance of the benzylidene signal in the ^{1}H NMR spectrum.

ESI-MS (MeOH) for $[C_{29}H_{34}N_6O_9Na]$+ calcd: 633.23, found: 633.1

(Benzyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyluronate)-(1→4)-(benzyl (propargyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosid)uronate) (31)



Compound **30** (48.8 mg, 80 μmol) and TEMPO (1.3 mg, 8 μmol) were dissolved in CH_3CN (1 mL) and sodium phosphate buffer (0.67 M, pH 6.7, 500 μL) and the mixture was heated to 35°C. Then $NaClO_2$ (28.9 mg, 320 μmol in 200 μL of water) and bleach (1.8 μL 13% NaOCl diluted in 200 μL of water, 3.2 μmol) were added. The mixture was stirred at 35°C for 24 h (TLC DCM/MeOH 7:3 + 0.1% AcOH), then cooled to room temperature. Water (5 mL) was added, and the pH was adjusted to 8.0 with 1 M NaOH. The reaction was quenched by pouring into cold (0°C) Na_2SO_3 solution, until the pH of the aqueous layer reached 8.5-9.0. After stirring for 0.5 h at room temperature, MTBE (5 mL) was added. The organic layer was separated and discarded. Then AcOEt was added, the aqueous layer was acidified with 5% aq. HCl to pH 3-4. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was dissolved in DMF (5 mL), BnBr (190 μL, 1.6 mmol) and KF (46.5 mg, 800 μmol) were added at 0°C. The reaction was monitored by TLC (hexane/AcOEt 6:4) and stirred for 3 h at rt. The solvent was then removed, the crude was taken up with DCM and washed with water. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2 to 6:4) afforded compound **31** (21.5 mg, 33%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.49 – 7.18 (m, 20H, arom.), 5.37 (d, *J* = 12.0 Hz, 1H, COOC*H*HPh), 5.30 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 5.24 (d, *J* = 12.0 Hz, 1H, COOCH*H*Ph), 5.09 (d, $J_{1',2'}$ = 3.7 Hz, 1H, H-1b), 5.04 (d, *J* = 12.3 Hz, 1H,

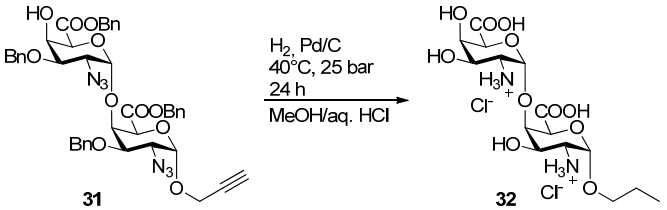
COO*H*HPh), 4.92 (br d, *J* = 1.7 Hz, 1H, H-5'), 4.80 (d, *J* = 12.3 Hz, 1H, COOH*H*Ph), 4.75 (d, *J* = 10.8 Hz, 1H, OC*H*HPh), 4.74 (d, *J* = 12.4 Hz, 1H, OC*H*HPh), 4.67 (d, *J* = 10.8 Hz, 1H, OCH*H*Ph), 4.61 (br d, *J* = 2.0 Hz, 1H, H-4), 4.50 (d, *J* = 12.4 Hz, 1H, OCH*H*Ph), 4.45 (br s, 1H, H-5), 4.41 (m, 1H, H-4'), 4.30 (ddd, *J* = 15.9, 12.8, 2.4 Hz, 2H, OCH_2CCH), 3.97 (dd, $J_{2',3'}$ = 10.4, $J_{3',4'}$ = 3.1 Hz, 1H, H-3'), 3.90 (dd, $J_{2,3}$ = 10.9, $J_{3,4}$ = 2.9 Hz, 1H, H-3), 3.74 (dd, $J_{2',3'}$ = 10.4, $J_{1',2'}$ = 3.7 Hz, 1H, H-2'), 3.69 (dd, $J_{2,3}$ = 10.9, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 2.37 (t, *J* = 2.4 Hz, 1H, OCH$_2$CC*H*), 2.30 (br s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 167.92 (*C*OOBn), 167.24 (*C*OOBn), 99.70 (C-1), 97.07 (C-1'), 78.08 (OCH$_2$*C*CH), 75.98 (C-3, OCH$_2$C*C*H), 75.41 (C-4'), 74.72 (C-3'), 72.29 (O*C*H$_2$Ph), 71.81 (O*C*H$_2$Ph), 70.52 (C-5), 70.39 (C-5'), 67.78 (COO*C*H$_2$Ph), 67.06 (C-4), 66.94 (COO*C*H$_2$Ph), 58.80 (C-2'), 58.63 (C-2), 55.90 (O*C*H$_2$CCH) ppm.

ESI-MS (MeOH) for $[C_{43}H_{42}N_6O_{11}Na]$+ calcd: 841.28, found: 841.1

$[\alpha]_D^{20}$ = 101.6 (*c* = 1 in $CHCl_3$)

(2-Amino-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(propyl 2-amino-2-deoxy-α-D-galactopyranosiduronic acid) dihydrochloride (32)



Compound **31** (35.6 mg, 43.9 μmol) was dissolved in H_2O/MeOH 1:1 (8 mL + 5 drops of 5% aq. HCl). Pd/C was added and the reaction was left under H_2 (30 bar) at 40°C for 24 h. The mixture was then filtered on a Celite pad and lyophilized, affording compound **32** (20 mg, 95%) as a white solid.

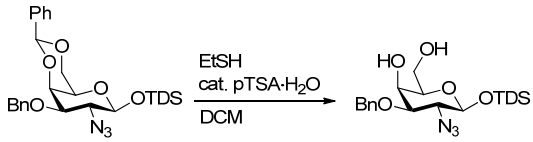
^{1}H NMR (400 MHz, D_2O, 25°C): δ = 5.22 (br s, 1H, H-1), 5.17 (br s, 1H, H-1'), 5.02 (br s, 1H, H-5), 4.39 (br s, 2H, H-5', H-4), 4.29 (br s, 1H, H-4), 4.19 (br d, *J* = 11.1 Hz, 1H, H-3'), 4.10 (br d, *J* = 10.8 Hz, 1H, H-3), 3.70 – 3.36 (m, 4H, OC*H*$_2$CH$_2$CH$_3$, H-2, H-2'), 1.54 (m, 2H, OCH$_2$C*H*$_2$CH$_3$), 0.82 (t, *J* = 7.4 Hz, 3H, OCH$_2$CH$_2$C*H*$_3$) ppm.

^{13}C NMR (100.6 MHz, D_2O, 25°C): δ = 96.16 (C-1), 95.17 (C-1'), 77.39 (C-4'), 71.54 (C-5), 70.69 (O*C*H$_2$CH$_2$CH$_3$), 70.15 (C-5'), 68.88 (C-4), 65.89 (C-3), 65.79 (C-3'), 50.54 (C-2, C-2'), 22.04 (OCH$_2$*C*H$_2$CH$_3$), 9.90 (OCH$_2$CH$_2$*C*H$_3$) ppm.

ESI HR-MS (MeOH) for $[C_{15}H_{26}N_2O_{11}\text{-H}]^-$ calcd: 409.1464, found: 409.1501

$[\alpha]_D^{20}$ = 127.1 (*c* = 0.5 in H_2O)

Thexyldimethylsilyl 2-azido-3-*O*-benzyl-2-deoxy-β-D-galactopyranoside (36)



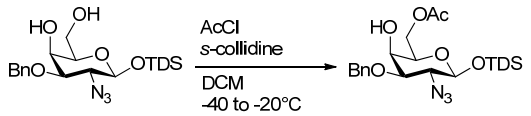
19 **36**

Compound **19** (2.5221 g, 4.8 mmol) was dissolved in DCM (30 mL). EtSH (2.13 mL, 28.8 mmol) and *p*TSA·H_2O (182 mg, 0.95 mmol) were added. After 10 min TLC (hexane/AcOEt 5:5) showed completion of the reaction, the mixture was neutralized with TEA and the solvent was removed. Flash chromatography (hexane/AcOEt 5:5) afforded compound **36** (2.055 g, 98%) as a light yellow oil.

The occurrence of the benzylidene cleavage was confirmed by the disappearance of the benzylidene signal in the ^{1}H NMR spectrum.

ESI-MS (MeOH) for $[C_{21}H_{35}N_3O_5SiNa]$+ calcd: 460.22, found: 460.3

Thexyldimethylsilyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-galactopyranoside (37)



36 **37**

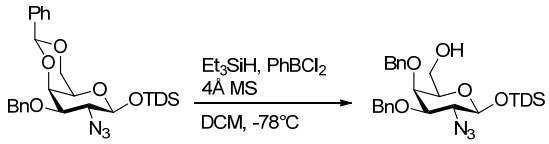
Compound **36** (3.254 g, 7.44 mmol) and *sym*-collidine (5 ml, 37.2 mmol) were dissolved in DCM (20 mL) at -40°C. AcCl (690 µL, 9.7 mmol) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 1 h the reaction was warmed to -20°C and stirred for a further 1 h. The mixture was quenched with MeOH, the solvent was evaporated. The crude is taken up with DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **37** (3.3163 g, 83%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.52 – 7.32 (m, 5H, arom.), 4.74 (m, 2H, OC*H*$_2$Ph), 4.43 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1), 4.38 – 4.27 (m, 2H, H-6a, H-6b), 3.88 (m, 1H, H-4), 3.65 – 3.51 (m, 2H, H-5, H-2), 3.28 (dd, $J_{2,3}$ = 10.1, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 2.40 (m, 1H, OH), 2.08 (s, 3H, $OCOCH_3$), 1.69 (hept, J = 6.9 Hz, 1H, $SiC(CH_3)_2CH(CH_3)_2$), 0.94 (m, 12H, $SiC(CH_3)_2CH(CH_3)_2$), 0.21 (br d, 6H, $Si(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 171.43 ($OCOCH_3$), 97.77 (C-1), 79.48 (C-3), 73.09 (OCH_2Ph), 72.77 (C-5), 66.39 (C-4), 65.81 (C-2), 63.89 (C-6), 34.59 ($SiC(CH_3)_2CH(CH_3)_2$, 21.46 ($OCOCH_3$), 20.64, 20.53 ($SiC(CH_3)_2CH(CH_3)_2$), 19.16, 19.05 ($SiC(CH_3)_2CH(CH_3)_2$), -2.02 ($Si(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{23}H_{37}N_3O_6SiNa]$+ calcd: 502.23, found: 502.4

Thexyldimethylsilyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (19a)



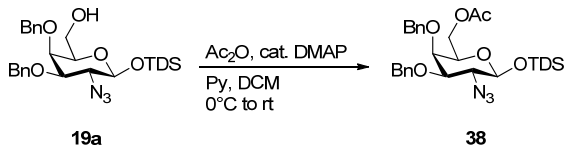
19 **19a**

Compound **19** (534.2 mg, 1.02 mmol) was dissolved in DCM (20 mL). 4Å molecular sieves (2.8 g) were added and the mixture was stirred for 45 min. Et_3SiH (623 μL, 3.9 mmol) was added and the mixture was cooled to -78°C. $PhBCl_2$ (160 μL, 1.22 mmol) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 1 h the mixture was neutralized with TEA, filtered on a Celite pad and the solvent was removed. The crude is taken up in DCM and washed with satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **19a** (514.4 mg, 95%) as a transparent oil.

The occurrence of the regioselective benzylidene ring opening was confirmed by the disappearance of the benzylidene signal in the ^1H NMR spectrum.

ESI-MS (MeOH) for $[C_{28}H_{41}N_3O_5SiNa]+$ calcd: 550.27, found: 550.5

Thexyldimethylsilyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (38)



19a **38**

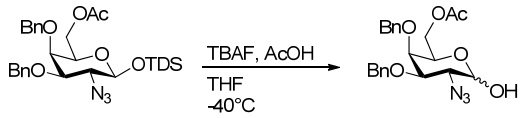
Compound **19a** (4.322 g, 8.19 mmol), pyridine (2.7 mL, 32.8 mmol) and catalytic DMAP were dissolved in DCM (20 mL) at 0°C. Acetic anhydride (1.5 mL, 16.4 mmol) was added dropwise, and the mixture was warmed to rt. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 1 h the reaction mixture was quenched with crushed ice, diluted with DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated, affording compound **38** (4.5044 g, 97%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.51 – 7.25 (m, 5H, arom.), 4.95 (d, J = 11.6 Hz, 1H, OC*H*HPh), 4.76 (m, 2H, OCH_2Ph), 4.64 (d, J = 11.6 Hz, 1H, OCH*H*Ph), 4.42 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1), 4.21 (dd, $J_{6a,6b}$ = 11.2, $J_{5,6a}$ = 7.3 Hz, 1H, H-6a), 4.04 (dd, $J_{6a,6b}$ = 11.2, $J_{5,6b}$ = 5.3 Hz, 1H, H-6b), 3.76 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 7.7 Hz, 1H, H-2), 3.74 (m, 1H, H-4), 3.50 (ddd, $J_{5,6a}$ = 7.3, $J_{5,6b}$ = 5.3, $J_{4,5}$ = 1.1 Hz, 1H, H-5), 3.30 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 2.9 Hz, 1H, H-3), 1.99 (s, 3H, $OCOCH_3$), 1.68 (hept, J = 6.9 Hz, 1H, $SiC(CH_3)_2CH(CH_3)_2$), 0.94 (m, 12H, $SiC(CH_3)_2CH(CH_3)_2$), 0.19 (s, 5H, $Si(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 170.56 ($OCOCH_3$), 97.40 (C-1), 80.71 (C-3), 74.39 (OCH_2Ph), 73.06 (OCH_2Ph), 72.45 (C-5), 72.17 (C-4), 65.74 (C-2), 63.31 (C-6), 33.92 ($SiC(CH_3)_2CH(CH_3)_2$, 20.74 ($OCOCH_3$), 20.00, 19.91 ($SiC(CH_3)_2CH(CH_3)_2$), 18.51, 18.42 ($SiC(CH_3)_2CH(CH_3)_2$), -1.96 ($Si(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{30}H_{43}N_3O_6SiNa]$+ calcd: 619.28, found: 619.1

6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-D-galactopyranose (38a)



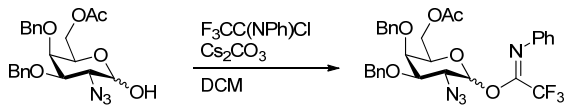
38 **38a**

Compound **38** (512.5 mg, 0.92 mmol) was dissolved in THF (15 mL) at -40°C. Acetic acid (158 µL, 2.76 mmol) and TBAF (1 M in THF, 2.8 ml, 2.8 mmol) were added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 45 min the solvent was removed, the crude was taken up with AcOEt and washed with satd. NH_4Cl. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 6:4) afforded compound **38a** (383.2 mg, 98%, mixture of anomers) as a light yellow oil.

The occurrence of the desilylation was confirmed by the disappearance of the TDS signals in the ^1H NMR spectrum.

ESI-MS (MeOH) for $[C_{22}H_{25}N_3O_6Na]+$ calcd: 450.16, found: 450.2

6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl *N*-phenyltrifluoroacetimidate (39)



38a **39**

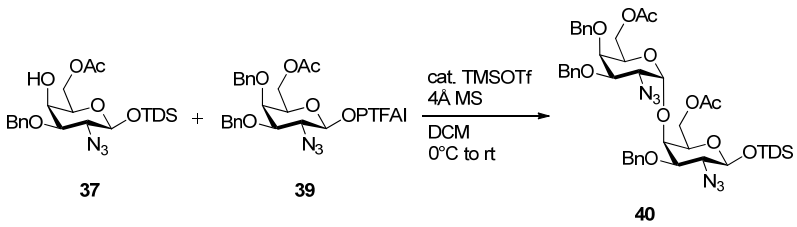
Compound **38a** (2.4967 g, 5.84 mmol) and *N*-phenyltrifluoroacetimidoyl chloride (1.89 mL, 11.7 mmol) were dissolved in DCM (20 mL), then Cs_2CO_3 (2.85 g, 8.76 mmol) was added. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 2 h the mixture was filtered on a Celite pad and the solvent removed. Flash chromatography (hexane/AcOEt 7:3 + 0.1% TEA) afforded compound **39** (3.5364 g, quant.) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 50°C): δ = 7.51 – 7.24 (m, 12H, arom.), 7.13 (m, 1H, arom.), 6.86 (m, 2H, arom.), 5.46 (br d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.97 (d, J = 11.4 Hz, 1H, OC*H*HPh), 4.80 (m, 2H, OCH_2Ph), 4.64 (d, J = 11.4 Hz, 1H, OCH*H*Ph), 4.24 (dd, $J_{6a,6b}$ = 11.2, $J_{5,6a}$ = 6.7 Hz, 1H, H-6a), 4.14 (dd, $J_{6a,6b}$ = 11.2, $J_{5,6b}$ = 5.8 Hz, 1H, H-6b), 4.09 (dd, $J_{2,3}$ = 10.5, $J_{1,2}$ = 7.8 Hz, 1H, H-2), 3.82 (m, 1H, H-4), 3.59 (m, 1H, H-5), 3.44 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 2.2 Hz, 1H, H-3), 1.98 (s, 3H, $OCOCH_3$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 50°C): δ = 96.01 (C-1), 80.91 (C-3), 74.71 (O*C*H_2Ph), 73.55 (C-5), 73.20 (O*C*H_2Ph), 72.04 (C-4), 62.68 (C-6), 62.21 (C-2), 20.53 ($OCO*C*H_3$) ppm.

ESI HR-MS (MeOH) for $[C_{30}H_{29}F_3N_4O_6Na]$+ calcd: 621.1931, found: 621.1991

(6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(thexyldimethylsilyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-galactopyranoside) (40)



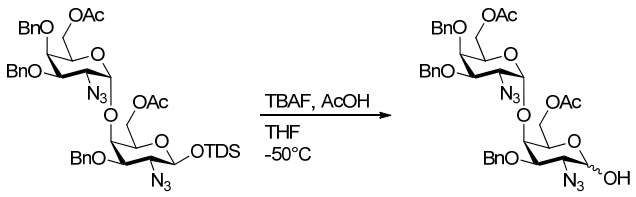
Donor **39** (2.0643 g, 3.45 mmol) and acceptor **37** (1.1034 g, 2.3 mmol) were coevaporated three times with toluene and left under vacuum overnight. They were dissolved in DCM (10 mL), freshly activated 4Å molecular sieves (3.5 g) were added and the mixture was stirred for 45 min under Argon atmosphere. A solution of TMSOTf (0.055 M in DCM, 4.6 mL, 0.23 mmol) was added dropwise *via* a syringe pump over 1 h. The reaction was warmed to rt and monitored by HPTLC (hexane/AcOEt 8:2). After 2h the mixture was quenched with TEA, filtered on a Celite pad and the solvent was removed. Flash chromatography (hexane/AcOEt 9:1 to 8:2) afforded compound **40** (1.5996 g, 78%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.52 – 7.25 (m, 15H, arom.), 4.97 (d, $J_{1',2'}$ = 3.5 Hz, 1H, H-1'), 4.93 (d, *J* = 11.2 Hz, 1H, OC*H*HPh), 4.86 (d, *J* = 11.1 Hz, 1H, OC*H*HPh), 4.81 (m, 2H, OCH*H*Ph, OC*H*HPh), 4.67 (d, *J* = 12.5 Hz, 1H, OCH*H*Ph), 4.56 (d, *J* = 11.2 Hz, 1H, OCH*H*Ph), 4.49 – 4.39 (m, 2H, H-1H-6a), 4.42 – 4.29 (m, 2H, H-5', H-6b), 4.13 – 4.04 (m, 2H, H-3', H-6'a), 4.03 – 3.90 (m, 4H, H-4', H-2', H-4, H-6'b), 3.58 – 3.48 (m, 2H, H-2, H-5), 3.18 (dd, $J_{2,3}$ = 10.6, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 2.08 (s, 3H, $OCOCH_3$), 1.86 (s, 3H, $OCOCH_3$), 1.70 (hept, *J* = 6.9 Hz, 1H, $SiC(CH_3)_2CH(CH_3)_2$), 0.92 (m, 12H, $SiC(CH_3)_2CH(CH_3)_2$), 0.20 (br d, 6H, $Si(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 99.50 (C-1'), 97.33 (C-1), 78.08 (C-3), 77.1 (C-3'), 74.70 (O*C*H_2Ph), 72.82 (C-4), 72.58 (C-4'), 72.38 (2 x O*C*H_2Ph), 72.26 (C-5), 69.01 (C-5'), 65.73 (C-2), 62.36 (C-6'), 62.19 (C-6), 60.17 (C-2'), 34.01 (SiC$(CH_3)_2$*C*H$(CH_3)_2$), 20.68 (2 x OCO*C*H_3), 19.96 (SiC$(CH_3)_2$CH(*C*$H_3)_2$), 18.46 (SiC(*C*$H_3)_2$CH$(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{45}H_{60}N_6O_{11}SiNa]$+ calcd: 911.40, found: 911.3

(6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-D-galactopyranose) (40a)



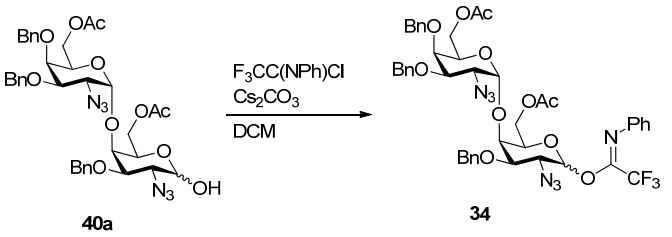
40 **40a**

Compound **40** (1.3192 g, 1.48 mmol) was dissolved in THF (20 mL) at -50°C. TBAF (1 M in THF, 1.78 mL, 1.78 mmol) was slowly added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 1 h acetic acid (17 μL, 0.3 mmol) was added, the mixture was diluted with water and extracted with DCM. The combined organic phases were dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 7:3) afforded compound **40a** (839.4 mg, 76%) as a light yellow oil.

The occurrence of the desilylation was confirmed by the disappearance of the TDS signals in the ^{1}H NMR spectrum.

ESI-MS (MeOH) for $[C_{37}H_{42}N_6O_{11}Na]$+ calcd: 769.28, found: 769.5

(6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-galactopyranoside) *N*-phenyltrifluoroacetimidate (34)



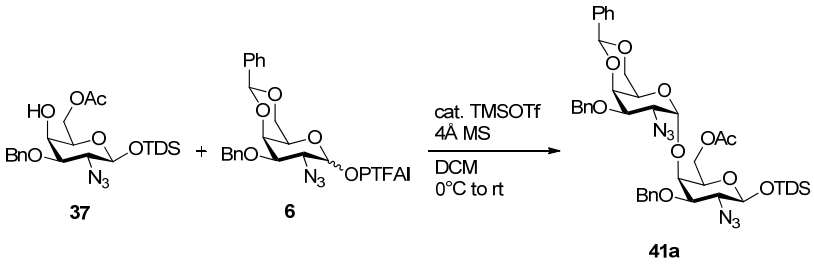
Compound **40a** (937.5 mg, 1.26 mmol) and *N*-phenyltrifluoroacetimidoyl chloride (405 μL, 2.5 mmol) were dissolved in DCM (10 mL), then Cs_2CO_3 (619 mg, 1.9 mmol) was added. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 1 h the mixture was filtered on a Celite pad and the solvent removed. Flash chromatography (hexane/AcOEt 8:2 + 0.1% TEA) afforded compound **34** (1.1363 g, 98%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 50°C): δ = 7.53 – 7.25 (m, 12H, arom.), 7.13 (m, 1H, arom.), 6.86 (m, 2H, arom.), 5.44 (br d, $J_{1,2}$ = 8.2 Hz, 1H, H-1), 5.02 (br s, 1H, H-1'), 4.94 (d, *J* = 11.2 Hz, 1H, OC*H*HPh), 4.84 (m, 3H, OCH_2Ph, OC*H*HPh), 4.71 (d, *J* = 12.4 Hz, 1H, OCH*H*Ph), 4.58 (d, *J* = 11.2 Hz, 1H, OCH*H*Ph), 4.42 (m, 2H, H-6a, H-6b or H-6'a, H-6'b), 4.33 (br t, $J_{5,6a} = J_{5,6b}$ = 6.6 Hz, 1H, H-5' or H-5), 4.20 – 3.97 (m, 6H, H-6'a, H-6'b or H-6a, H-6b, H-3', H-2', H-4, H-4'), 3.86 (dd, $J_{2,3}$ = 10.3, $J_{1,2}$ = 8.2 Hz, 1H, H-2), 3.58 (br t, *J* = 6.1 Hz, 1H, H-5 or H-5'), 3.33 (dd, $J_{2,3}$ = 10.3, $J_{3,4}$ = 2.8 Hz, 1H, H-3), 2.05 (s, 3H, $OCOCH_3$), 1.91 (s, 3H, $OCOCH_3$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 50°C): δ = 99.53 (C-1'), 95.92 (C-1), 78.58 (C-3), 77.25 (C-3'), 74.77 (O*C*H_2Ph), 73.42 (C-5 or C-5'), 73.02 (C-4, C-4'), 72.48 (2 x O*C*H_2Ph), 69.48 (C-5' or C-5), 62.53 (C-6' or C-6), 62.13 (C-2), 61.76 (C-6 or C-6'), 60.57 (C-2'), 20.52 (2 x OCO*C*H_3) ppm.

ESI HR-MS (MeOH) for $[C_{45}H_{46}F_3N_7O_{11}Na]$+ calcd: 940.3099, found: 940.3110

(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→4)-(thexyldimethylsilyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-galactopyranoside) (41a)



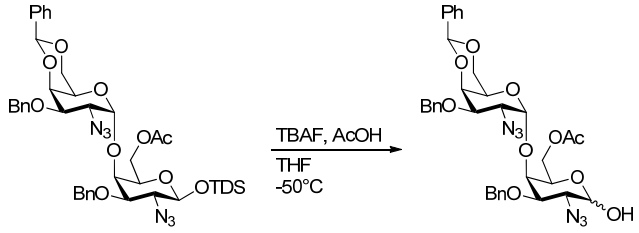
Donor **6** (319 mg, 0.57 mmol) and acceptor **37** (183 mg, 0.38 mmol) were coevaporated three times with toluene and left under vacuum overnight. They were dissolved in DCM (5 mL), freshly activated 4Å molecular sieves (0.5 g) were added and the mixture was stirred for 45 min under Argon atmosphere. A solution of TMSOTf (0.055 M in DCM, 690 μL, 38 μmol) was added dropwise *via* a syringe pump over 1 h. The reaction was warmed to rt and monitored by HPTLC (hexane/AcOEt 8:2). After 2h the mixture was quenched with TEA, filtered on a Celite pad and the solvent was removed. Flash chromatography (hexane/AcOEt 8:2 to 7:3) afforded compound **41a** (304.4 mg, 87%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.53 – 7.29 (m, 15H, arom.), 5.38 (s, 1H, PhC*H*), 5.05 (d, $J_{1',2'}$ = 3.4 Hz, 1H, H-1'), 4.84 – 4.73 (m, 3H, OCH_2Ph, OC*H*HPh), 4.69 (d, *J* = 11.9 Hz, 1H, OCH*H*Ph), 4.55 – 4.47 (m, 2H, H-6a, H-1), 4.38 (dd, $J_{6a,6b}$ = 11.1, $J_{5,6b}$ = 7.2 Hz, 1H, H-6b), 4.21 (br d, *J* = 3.3 Hz, 1H, H-4'), 4.13 – 4.04 (m, 3H, H-5', H-3', H-4), 4.00 (dd, $J_{2',3'}$ = 10.7, $J_{1',2'}$ = 3.4 Hz, 1H, H-2'), 3.71 (dd, $J_{6'a,6'b}$ = 12.7, $J_{5',6'a}$ = 1.5 Hz, 1H, H-6'a), 3.60 – 3.51 (m, 2H, H-5, H-2), 3.49 (dd, $J_{6'a,6'b}$ = 12.7, $J_{5',6'b}$ = 1.8 Hz, 1H, H-6'b), 3.24 (dd, $J_{2,3}$ = 10.6, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 2.11 (s, 3H, $OCOCH_3$), 1.72 (m, 1H, $SiC(CH_3)_2CH(CH_3)_2$), 0.94 (m, 12H, $SiC(CH_3)_2CH(CH_3)_2$), 0.23 (m, 6H, $Si(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 170.37 (O*C*OCH_3), 100.72 (Ph*C*H), 100.05 (C-1'), 97.32 (C-1), 78.88 (C-3), 74.19 (C-3'), 72.91 (C-4'), 72.56 (C-4), 72.42 (O*C*H_2Ph), 72.11 (C-5), 71.16 (O*C*H_2Ph), 69.09 (C-6'), 65.71 (C-2), 63.13 (C-5'), 61.69 (C-6), 59.00 (C-2'), 34.02 (SiC$(CH_3)_2$*C*H$(CH_3)_2$), 20.85 (OCO*C*H_3), 20.04, 19.97 (SiC$(CH_3)_2$CH(*C*$H_3)_2$), 18.54 (SiC(*C*$H_3)_2$CH$(CH_3)_2$), 18.47 (SiC(*C*$H_3)_2$CH$(CH_3)_2$), -1.98 (Si$(CH_3)_2$), -3.04 (Si$(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{43}H_{56}N_6O_{10}SiNa]$+ calcd: 867.37, found: 867.2

(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-D-galactopyranose) (41)



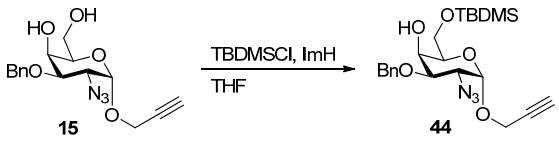
41a **41**

Compound **41a** (280 mg, 0.33 mmol) was dissolved in THF (5 mL) at -50°C. TBAF (1 M in THF, 400 µL, 0.4 mmol) was slowly added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 1 h acetic acid (4 µL, 0.07 mmol) was added, the mixture was diluted with water and extracted with DCM. The combined organic phases were dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 7:3) afforded compound **41a** (188 mg, 82%) as a light yellow oil.

The occurrence of the desilylation was confirmed by the disappearance of the TDS signals in the ^{1}H NMR spectrum.

ESI-MS (MeOH) for $[C_{35}H_{38}N_6O_{10}Na]$+ calcd: 725.25, found: 725.5

Propargyl 2-azido-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-2-deoxy-α-D-galactopyranoside (44)

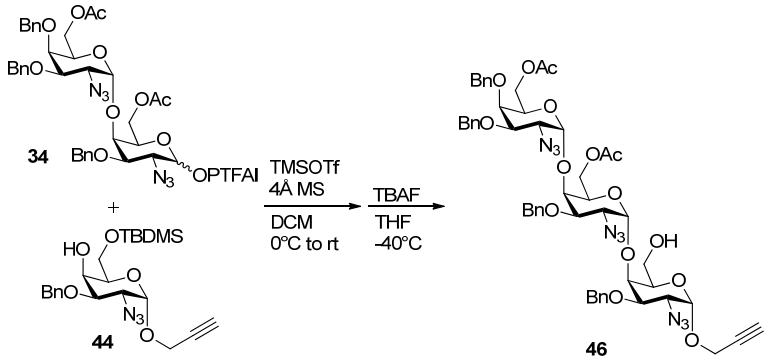


Compound **15** (167.6 mg, 0.50 mmol) was dissolved in THF (5 mL). Imidazole (136 mg, 2 mmol) was added and the mixture was cooled to 0°C. TBDMSCl (113 mg, 0.75 mmol) was added, the mixture was slowly warmed to rt and stirred overnight. The reaction was monitored by TLC (hexane/AcOEt 6:4). The solvent was removed, the crude was taken up in AcOEt and washed with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 85:15) afforded compound **44** (195.9 mg, 87%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.47 – 7.31 (m, 5H, arom.), 5.14 (d, $J_{1,2}$ = 3.0 Hz, 1H, H-1), 4.75 (m, 2H, OC*H*$_2$Ph), 4.29 (m, 2H, OC*H*$_2$CCH), 4.13 (br s, 1H, H-4), 3.95 – 3.75 (m, 5H, H-6a, H-6b, H-5, H-3, H-2), 2.48 (t, *J* = 2.3 Hz, 1H, OCH$_2$CC*H*), 0.92 (s, 9H, OSiC(C*H*$_3$)$_3$), 0.10 (s, 6H, OSi(C*H*$_3$)$_2$).

ESI-MS (MeOH) for $[C_{22}H_{33}N_3O_5SiNa]$+ calcd: 470.21, found: 470.3

(6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(propargyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (46)



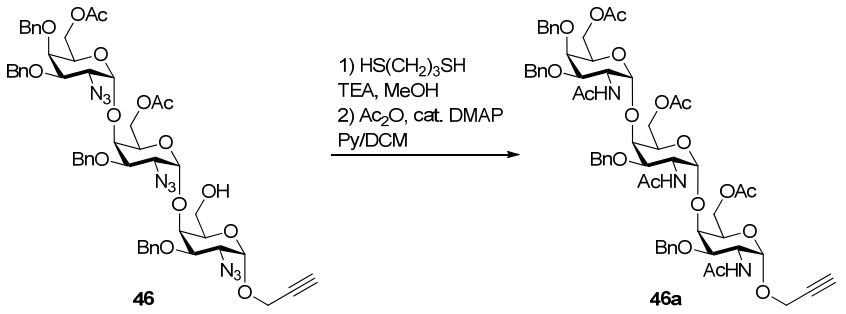
Donor **34** (304 mg, 331 μmol) and acceptor **44** (95.8 mg, 214 μmol) were coevaporated three times with toluene and left under vacuum overnight. They were dissolved in DCM (3 mL), freshly activated 4Å molecular sieves (400 mg) were added and the mixture was stirred for 45 min under Argon atmosphere. A solution of TMSOTf (0.055 M in DCM, 420 μL, 21 μmol) was added dropwise via a syringe pump over 1 h. The reaction was warmed to rt and monitored by HPTLC (hexane/AcOEt 8:2). After 3 h the mixture was quenched with TEA, filtered on a Celite pad and the solvent was removed. Flash chromatography (hexane/AcOEt 9:1 to 6:4) afforded crude compound 45 (193 mg) as a light yellow oil. It was dissolved in THF (5 mL) at -40°C. TBAF (1 M in THF, 321 μL, 321 μmol) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 2 h the mixture was allowed to warm to 0°C overnight. Then acetic acid (6 μL, 107 μmol) was added, then the mixture is diluted with AcOEt and washed with brine. Then organic phase was dried over Na2SO4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2 to 6:4) afforded compound 46 (133.7 mg, 59% over two steps) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.50 – 7.22 (m, 20H, arom.), 5.17 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.14 (d, $J_{1',2'}$ = 3.5 Hz, 1H, H-1'), 4.95 (d, $J_{1'',2''}$ = 2.2 Hz, 1H, H-1''), 4.91 – 4.86 (m, 2H, 2 x OC*H*HPh), 4.85 – 4.76 (m, 3H, OCH_2Ph, OC*H*HPh), 4.73 (d, *J* = 12.1 Hz, 1H, OCH*H*Ph), 4.66 (d, *J* = 12.0 Hz, 1H, OCH*H*Ph), 4.52 (d, *J* = 11.2 Hz, 1H, OCH*H*Ph), 4.34 – 4.22 (m, 6H, H-5'' or H-5', H-5' or H-5'', OCH_2CCH, H-5, H-6''a or H-6'a), 4.19 – 4.11 (m, 2H, H-4', H-6''b or H-6'b), 4.06 – 3.98 (m, 3H, H-6'a or H-6''a, H-3'', H-2''), 3.97 – 3.69 (m, 9H, H-4'', H-3', H-4, H-3, H-6a, H-6b, H-6'b or H-6''b, H-2', H-2), 2.49 (t, *J* = 2.4 Hz, 1H, OCH_2CCH), 1.97 (s, 3H, OCOCH_3), 1.82 (s, 3H, OCOCH_3) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 169.86 (2 x O*C*OCH_3), 99.10 (C-1''), 98.49 (C-1'), 96.78 (C-1), 78.43 (OCH_2CCH), 77.27 (C-3''), 75.52 (C-3), 75.18 (OCH_2CCH), 74.98 (C-3'), 74.67 (O*C*H_2Ph), 72.99 (C-5), 72.51 (C-4'), 72.38 (O*C*H_2Ph), 72.28 (C-4''), 72.17 (O*C*H_2Ph), 71.82 (O*C*H_2Ph), 71.28 (C-4), 68.89 (C-5' or C-5''), 68.82 (C-5'' or C-5'), 62.07 (C-6'' or C-6'), 61.33 (C-6' or C-6''), 60.59 (C-6), 60.45 (C-2'), 60.24 (C-2''), 59.64 (C-2), 55.12 (O*C*H_2CCH), 20.71 (OCO*C*H_3), 20.63 (OCO*C*H_3) ppm.

ESI-MS (MeOH) for $[C_{53}H_{59}N_9O_{15}Na]$+ calcd: 1084.40, found: 1084.5

(2-Acetamido-6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(2-acetamido-6-*O*-acetyl-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(propargyl 2-acetamido-6-*O*-acetyl-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (46a)



Compound **46** (68.1 mg, 64 μmol) was dissolved in MeOH (5 mL). Propanedithiol (193 μL, 1.9 mmol) and TEA (267 μL, 1.9 mmol) were added, and the mixture was heated at 40°C for 24 h. The reaction was monitored by TLC (hexane/AcOEt 6:4 and H_2O/MeOH 5:5 + 5 drops of 25% aq. NH_3). Then the solvent was removed *in vacuo*, the crude was dissolved in pyridine (5 mL) and cooled to 0°C. Catalytic DMAP and Ac_2O (180 μL, 1.9 mmol) were added and the mixture was warmed to rt. The reaction was monitored by TLC (AcOEt) and stirred overnight. Then the solvent was removed *in vacuo* and flash chromatography (hexane/AcOEt 1:9 to AcOEt/MeOH 9:1) afforded compound **46a** (38 mg, 52%) as a colorless oil.

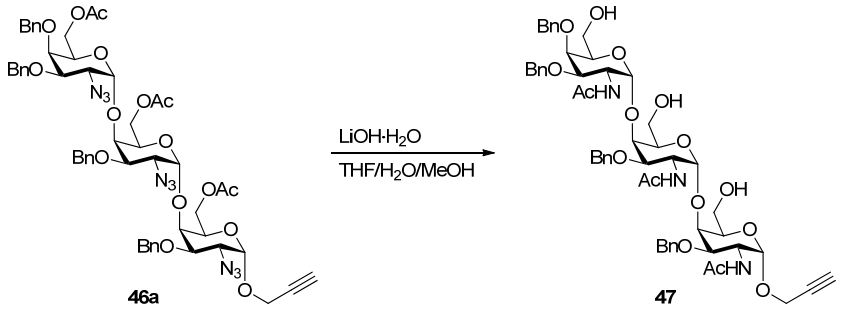
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45 – 7.22 (m, 20H, arom.), 5.35 (d, $J_{2',NH}$ = 8.9 Hz, 1H, N*H*Ac), 5.22 (d, $J_{2,NH}$ = 9.4 Hz, 1H, N*H*Ac), 5.17 (d, $J_{2'',NH}$ = 8.9 Hz, 1H, N*H*Ac), 5.00 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.98 – 4.72 (m, 7H, H-1', H-1'', 4 x OC*H*HPh, H-2'), 4.61 – 4.37 (m, 8H, 4 x OC*H*HPh, H-2, H-2', H-5, H-5' or H-5''), 4.29 – 4.18 (m, 4H, H-6''a or H-6a, H-4'', OCH_2CCH), 4.17 – 4.08 (m, 2H, H-4, H-6a or H-6''a), 4.07 – 3.80 (m, 7H, H-6'a, H-6'b, H-6''b or H-6b, H-4', H-5'' or H-5', H-3', H-3''), 3.66 (t, $J_{5,6a}$ = $J_{5,6b}$ = 10.1 Hz, 1H, H-6b or H-6''b), 3.53 (dd, $J_{2,3}$ = 11.1, $J_{3,4}$ = 2.6 Hz, 1H, H-3), 2.48 (t, J = 2.4 Hz, 1H, OCH$_2$CC*H*), 2.11 (s, 3H,

OCOC*H*$_3$), 1.95 (m, 6H, OCOC*H*$_3$, HNCOC*H*$_3$), 1.90 (m, 6H, 2 x HNCOC*H*$_3$), 1.85 (s, 3H, OCOC*H*$_3$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 170.41 – 169.6 (6 x *C*OCH$_3$), 98.71 (C-1' or C-1''), 98.46 (C-1'' or C-1'), 96.73 (C-1), 76.80 (C-3'), 75.19 (C-3), 75.10 (OCH$_2$*C*CH), 74.37 (C-3''), 74.21 (O*C*H$_2$Ph), 71.94 (O*C*H$_2$Ph), 71.87 (C-4'), 71.78 (O*C*H$_2$Ph), 71.54 (C-4), 71.33 (C-4'), 69.30 (C-5'' or C-5'), 69.04 (C-5), 68.92 (C-5' or C-5''), 62.06 (C-6'), 61.74 (C-6'' or C-6), 60.35 (C-6 or C-6''), 54.99 (O*C*H$_2$CCH), 49.22 (C-2'), 48.94 (C-2''), 48.33 (C-2), 23.38 (3 x HNCO*C*H$_3$), 20.72 (3 x OCO*C*H$_3$) ppm.

ESI-MS (MeOH) for $[C_{61}H_{73}N_3O_{19}Na]$+ calcd: 1174.47, found: 1174.2

(2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(propargyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (47)

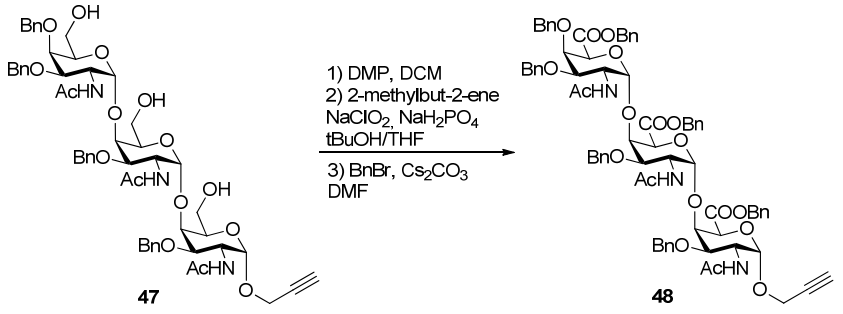


Compound **46a** (38 mg, 33 μmol) was dissolved in H_2O/MeOH/THF 1:2.5:0.5 (4 mL). LiOH·H_2O (4 mg, 100 μmol) was added and the mixture was stirred for 24 h. The reaction was monitored by TLC (AcOEt/MeOH 9:1). After completion, the mixture was quenched with Amberlite IR-120, filtered and the solvent was removed. Flash chromatography (AcOEt/MeOH 100:0 to 9:1) afforded compound **47** (27.6 mg, 82%).

The occurrence of complete deacetylation was confirmed by the disappearance of the acetyl signals in the ^{1}H NMR spectrum.

ESI-MS (MeOH) for $[C_{55}H_{67}N_3O_{16}Na]$+ calcd: 1048.44, found: 1048.3

(Benzyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyluronate)-(1→4)-(benzyl (propargyl 2-acetamido-3-*O*-benzyl-2-deoxy-galactopyranosid)uronate) (48)



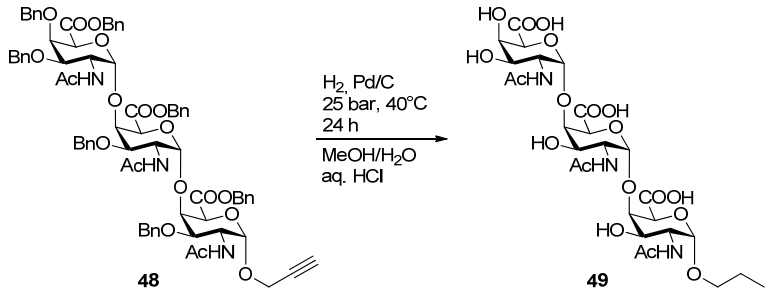
Compound **47** (27.6 mg, 27 μmol) was dissolved in DCM (2.5 mL) and Dess-Martin periodinane (51.7 mg, 122 μmol) was added. The reaction was monitored by HPTLC (AcOEt/MeOH 9:1). After 3 h, the mixture was quenched with 10% aq. $Na_2S_2O_3$. The organic phase was separated and washed with satd. $NaHCO_3$. The combined aqueous phases were extracted with DCM. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in THF (1 mL) and tBuOH (2 mL). 2-Methylbut-2-ene (424 μL) and a solution of $NaClO_2$ and $NaH_2PO_4 \cdot 2H_2O$ (73 mg + 95 mg in 1 mL of water, 810 μmol + 608 μmol) were then added. The reaction was monitored by TLC (DCM/MeOH 8:2 + 1% AcOH). After 1 h the mixture was concentrated to half volume, acidified with 5% aq. HCl to pH 2, then the solvents were evaporated *in vacuo*. The crude was dissolved in DMF (3 mL), BnBr (100 μL, 810 μmol) and Cs_2CO_3 (132 mg, 405 μmol) were added. The reaction was monitored by TLC (hexane/AcOEt 2:8) and stirred overnight. Then the solvent was evaporated, the crude was taken up in DCM and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography hexane/AcOEt 3:7 to 2:8) afforded compound **48** (17.3 mg, 34%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.49 – 7.11 (m, 35H, arom.), 5.39 (m, 3H, 2 x N*H*Ac, COOC*H*HPh), 5.11 (m, 2H, H-1, N*H*Ac), 5.04 – 4.94 (m, 5H, H-5', H-5'', H-1', H-1'', COOCH*H*Ph), 4.89 – 4.71 (m, 6H, COOC*H*$_2$Ph, 3 x OC*H*HPh, H-2'), 4.66 (d, *J* = 12.2 Hz, 1H, COOC*H*HPh), 4.59 – 4.48 (m, 6H, COOCH*H*Ph, H-4, H-4'', H-2'', OCH*H*Ph, OC*H*HPh), 4.45 – 4.33 (m, 4H, H-2, H-5, OCH*H*Ph, H-4'), 4.30 (d, *J* = 12.8 Hz, 1H, OCH*H*Ph), 4.26 – 4.13 (m, 3H, OC*H*$_2$CCH, OCH*H*Ph), 3.82 (dd, $J_{3',4'}$ = 11.1, $J_{2',3'}$ = 2.5 Hz, 1H, H-3'), 3.73 (dd, $J_{3'',4''}$ = 11.4, $J_{2'',3''}$ = 2.6 Hz, 1H, H-3''), 3.52 (dd, $J_{3,4}$ = 11.2, $J_{2,3}$ = 2.8 Hz, 1H, H-3), 2.33 (t, *J* = 2.4 Hz, 1H, OCH_2CCH), 2.06 (s, 3H, HNCOC*H*$_3$), 2.05 (s, 3H, HNCOC*H*$_3$), 1.92 (s, 3H, HNCOC*H*$_3$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 170.64, 170.31, 169.81 (3 x NH*C*OCH_3), 168.54, 168.44, 167.41 (3 x *C*OOBn), 100.11 (C-1'), 99.57 (C-1''), 97.20 (C-1), 77.02 (C-3'), 75.15 (OCH_2*C*CH), 75.08 (C-3''), 74.80 (C-3 or C-4' or C-4), 74.75 (C-4' or C-4 or C-3), 74.68 (C-4 or C-4' or C-3), 74.22 (O*C*H_2Ph), 74.13 (C-4''), 72.07 (O*C*H_2Ph), 71.85 (C-5''), 71.59 (O*C*H_2Ph), 71.29 (O*C*H_2Ph), 71.22 (C-5'), 70.43 (C-5), 67.65 (COO*C*H_2Ph), 67.42 (COO*C*H_2Ph), 66.43 (COO*C*H_2Ph), 55.96 (O*C*H_2CCH), 48.32 (C-2'), 47.92 (C-2 or C-2''), 47.87 (C-2'' or C-2), 23.62, 23.54, 23.29 (3 x HNCO*C*H_3) ppm.

ESI HR-MS (MeOH) for $[C_{76}H_{79}N_3O_{19}Na]$+ calcd: 1360.5205, found: 1360.6083

(2-Acetamido-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(2-acetamido-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(propyl 2-acetamido-2-deoxy-α-D-galactopyranosiduronic acid) (49)



Compound **48** (13 mg, 6.9 μmol) was dissolved in H_2O/MeOH/AcOEt 3:3:1 (7 mL + 5 drops of 5% aq. HCl). Pd/C was added and the mixture was stirred under hydrogen (25 bar) at 40°C overnight. The reaction mixture was then filtered on a Celite pad and lyophilized, affording compound **49** (8.7 mg, quant.).

^{1}H NMR (400 MHz, D_2O, 25°C): δ = 5.01 (m, 5H, H-1, H-1', H-1'', H-5, H-5'), 4.60 (br s, 1H, H-5''), 4.42 (m, 2H, H-4, H-4'), 4.30 (br s, 1H, H-4''), 4.18 – 3.95 (m, 6H, H-3, H-3', H-3'', H-2, H-2', H-2''), 3.59 (m, 1H, OC*H*HCH$_2$CH$_3$), 3.42 (m, 1H, OCH*H*CH$_2$CH$_3$), 1.98 (m, 9H, 3 x HNCOC*H$_3$*), 1.52 (m, 2H, OCH$_2$C*H*$_2$CH$_3$), 0.83 (t, *J* = 7.1 Hz, 3H, OCH$_2$CH$_2$C*H$_3$*) ppm.

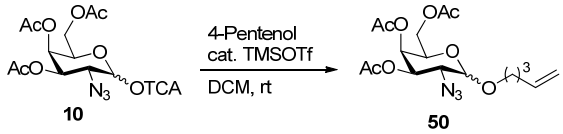
^{13}C NMR (100.6 MHz, D_2O, 25°C): δ = 174.73, 172.64, 171.68 (3 x *C*OOH, 3 x NH*C*OCH$_3$), 98.50, 98.42, 96.99 (C-1, C-1', C-1''), 77.51 (C-4 or C-4'), 77.46 (C-4' or C-4), 71.33 (C-5 or C-5'), 71.05 (O*C*H$_2$CH$_2$CH$_3$), 70.65 (C-5' or C-5), 69.55 (C-5''), 69.17 (C-4''), 66.72, 66.22, 65.90 (C-3, C-3', C-3''), 49.60, 49.30 (C-2, C-2', C-2''), 22.16 (2 x NHCO*C*H$_3$), 21.98 (OCH$_2$*C*H$_2$CH$_3$), 21.90 (NHCO*C*H$_3$), 9.80 (OCH$_2$CH$_2$*C*H$_3$) ppm.

ESI HR-MS (MeOH) for $[C_{27}H_{41}N_3O_{19}\text{-}H]^-$ calcd: 710.2261, found: 710.2290

$[\alpha]_D^{20}$ = 63.8 (*c* = 0.26 in H_2O)

6.1.2 Second route – pentenyl linker

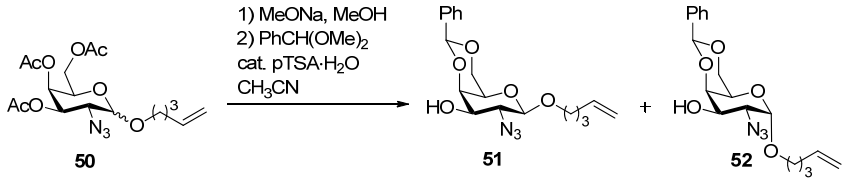
4-Pentenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-galactopyranoside (50)



Compound **10** (9.4214 g, 19.8 mmol) and 4-pentenol (2.45 mL, 23.8 mmol) were dissolved in DCM (5 mL) and Et_2O (10 mL). TMSOTf (2 M in DCM, 1.9 mmol) was then added dropwise at rt. The reaction was monitored by HPTLC (hexane/AcOEt 6:4). After 2 h the mixture was neutralized with TEA and the solvent evaporated. Flash chromatography (hexane/AcOEt 7:3) afforded compound **50** (7.908 g, quant., α/β = 1:1).

Spectroscopic data were in agreement with those reported in the literature.[10]

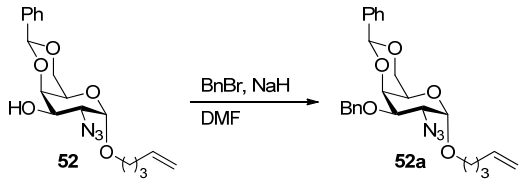
4-Pentenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-D-galactopyranoside (51 and 52)



Crude **50** (19.8 mmol) was dissolved in MeOH (25 mL), then a solution of sodium methoxide (0.4 M in MeOH, 5 mL, 2 mmol) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt 5:5). After 1 h the mixture was neutralized with Amberlite IR-120, filtered and the solvent was removed *in vacuo*. The residue was dissolved in CH_3CN (20 mL), benzaldehyde dimethyl acetal (6 mL, 40 mmol) and *p*TSA·H_2O (380 mg, 2 mmol) were added. The reaction was stirred overnight (TLC, hexane/AcOEt 7:3), then quenched with TEA and the solvent was evaporated. Flash chromatography (hexane/AcOEt 8:2 to 6:4 + 0.1% TEA) afforded compound **51** (3.22 g, 45%) and **52** (2.862 g, 40%) as white foams.

Spectroscopic data were in agreement with those reported in the literature.[11]

4-Pentenyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (52a)



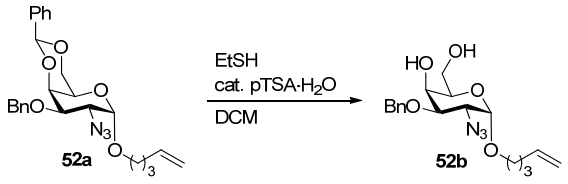
Compound **52** (2.862 g, 7.9 mmol) and BnBr (1.9 mL, 16 mmol) were dissolved in DMF (15 mL). NaH (240 mg, 10 mmol) was added portionwise. The reaction was monitored by TLC (hexane/AcOEt 7:3). After 2h the mixture was quenched with MeOH and the solvent was removed. The crude was taken up in DCM and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **52a** (3.3885 g, 95%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.59 – 7.30 (m, 10H, arom.), 5.84 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.50 (s, 1H, PhC*H*–), 5.13 – 4.98 (m, 3H, $O(CH_2)_3CHCH_2$, H-1), 4.77 (m, 2H, OCH_2Ph), 4.30 – 4.23 (m, 2H, H-6a, H-4), 4.09 – 4.02 (m, 2H, H-3, H-6b), 3.91 (dd, $J_{2,3}$ = 10.7, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 3.74 (dt, *J* = 9.8, 6.6 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.66 (br d, *J* = 1.2 Hz, 1H, H-5), 3.55 (dt, *J* = 9.8, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.18 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.76 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 138.07 ($O(CH_2)_3CHCH_2$), 115.20 ($O(CH_2)_3CHCH_2$), 101.10 (PhCH–), 98.83 (C-1), 74.54 (C-3), 73.35 (C-4), 71.43 (OCH_2Ph), 69.57 (C-6), 68.07 ($OCH_2(CH_2)_2CHCH_2$), 63.03 (C-5), 58.93 (C-2), 30.38 ($O(CH_2)_3CH_2CHCH_2$), 28.76 ($OCH_2CH_2CH_2CHCH_2$) ppm.

ESI HR-MS (MeOH) for $[C_{25}H_{29}N_3O_5Na]$+ calcd: 474.2005, found: 473.9853

4-Pentenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (52b)



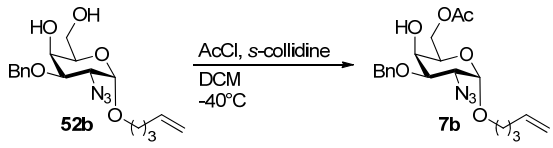
Compound **52a** (1.7992 g, 4.0 mmol) was dissolved in DCM (10 mL). EtSH (1.8 mL, 24 mmol) and then *p*TSA·H_2O (152 mg, 0.8 mmol) were added. The reaction was stirred for 1 h (TLC hexane/AcOEt 6:4), then quenched with TEA and the solvent was removed. Flash chromatography (hexane/AcOEt 5:5) afforded compound **52b** (1.38 g, 95%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.46 – 7.34 (m, 5H, arom.), 5.84 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.11 – 4.98 (m, 2H, $O(CH_2)_3CHCH_2$), 4.97 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.85 – 4.66 (m, 2H, OCH_2Ph), 4.16 (br dd, *J* = 3.2, 1.0 Hz, 1H, H-4), 4.00 – 3.92 (m, 2H, H-6a, H-3), 3.87 – 3.81 (m, 2H, H-5, H-6b), 3.74 (dt, *J* = 9.8, 6.6 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.69 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 3.51 (dt, *J* = 9.7, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.17 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.85 (br s, 1H), 1.76 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 138.01 ($O(CH_2)_3CHCH_2$), 115.23 ($O(CH_2)_3CHCH_2$), 98.27 (C-1), 75.98 (C-3), 72.28 (OCH_2Ph), 96.56 (C-5), 67.92 ($OCH_2(CH_2)_2CHCH_2$), 67.83 (C-4), 63.15 (C-6), 59.21 (C-2), 30.36 ($O(CH_2)_3CH_2CHCH_2$), 28.71 ($OCH_2CH_2CH_2CHCH_2$) ppm.

ESI-MS (MeOH) for $[C_{18}H_{25}N_3O_5Na]$+ calcd: 386.17, found: 386.2

4-Pentenyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (7b)



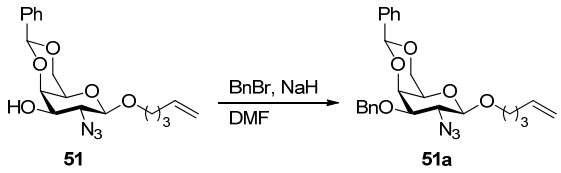
Compound **52b** (1.2781 g, 3.52 mmol) was dissolved in DCM (5 mL). *Sym*-collidine (2.3 mL, 17.6 mmol) was added and the mixture was cooled to -40°C. AcCl (326 µL, 4,6 mmol) was added dropwise. The reaction was followed by TLC (hexane/AcOEt 6:4). After 2 h, the mixture was quenched with MeOH, diluted with DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. Then organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 6:4) afforded compound **7b** (1.3838 g, 97%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.47 – 7.33 (m, 5H, arom.), 5.84 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.10 – 4.98 (m, 2H, $O(CH_2)_3CHCH_2$), 4.94 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.75 (m, 2H, OCH_2Ph), 4.36 (dd, $J_{6a,6b}$ = 11.6, $J_{5,6a}$ = 5.0 Hz, 1H, H-6a), 4.28 (dd, $J_{6a,6b}$ = 11.6, $J_{5,6b}$ = 7.3 Hz, 1H, H-6b), 4.06 (br dd, $J_{3,4}$ = 3.2, $J_{4,5}$ = 1.4 Hz, 1H, H-4), 4.01 – 3.96 (m, 1H), 3.94 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 3.71 (dt, J = 9.5, 6.5 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.67 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 3.53 (dt, J = 9.8, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.17 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 2.10 (s, 3H, $OCOCH_3$), 1.76 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 137.98 ($O(CH_2)_3CHCH_2$), 115.24 ($O(CH_2)_3CHCH_2$), 98.21 (C-1), 76.00 (C-3), 72.35 (OCH_2Ph), 68.02 ($OCH_2(CH_2)_2CHCH_2$), 67.99 (C-5), 66.57 (C-4), 63.63 (C-6), 59.16 (C-2), 30.38 ($O(CH_2)_3CH_2CHCH_2$), 28.71 ($OCOCH_3$), 20.96 ($OCH_2CH_2CH_2CHCH_2$) ppm.

ESI HR-MS (MeOH) for $[C_{20}H_{27}N_3O_6Na]$+ calcd: 428.1792, found: 428.1750

4-Pentenyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (51a)

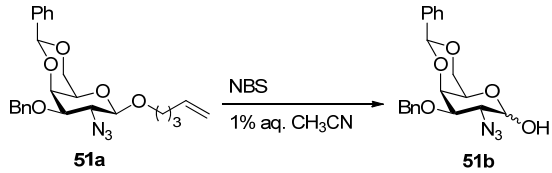


51 **51a**

Compound **51** (419 mg, 1.16 mmol) and BnBr (207 µL, mmol) were dissolved in DMF (20 mL). NaH (33.6 mg, 1.4 mmol) was added portionwise and the mixture was stirred for 2h (TLC hexane/AcOEt 6:4). Then the solvent was removed, the crude was taken up in DCM and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 7:3) afforded compound **51a** (494 mg, 1.1 mmol, 95%) as a white foam.

ESI-MS (MeOH) for $[C_{25}H_{29}N_3O_5Na]+$ calcd: 474.20, found: 474.1

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-galactopyranose (51b)

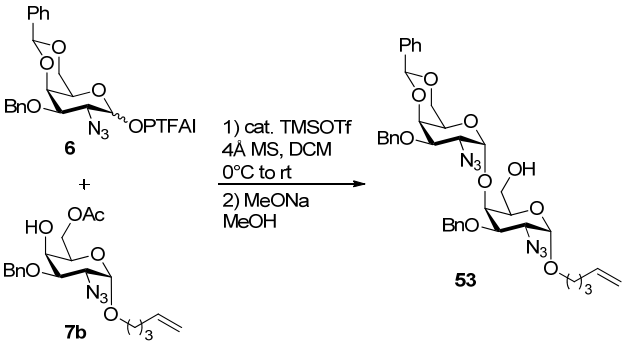


51a **51b**

Compound **51a** (494 mg, 1.1 mmol) was dissolved in 1% aq. CH_3CN (10 mL), freshly recrystallized NBS (587 mg, 3.3 mmol) was added and the mixture was stirred for 2 h in the dark (TLC hexane/AcOEt 1:1). Then the reaction mixture was diluted with Et_2O and washed with 10% aq. $Na_2S_2O_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 1:1) afforded compound **51b** (413 mg, 98%, mixture of anomers) as a white foam.

Spectroscopic data were in agreement with those reported in the literature.[9]

(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→4)-(4-pentenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (53)



Donor **6** (300 mg, 0.54 mmol) and acceptor **7b** (145.9 mg, 0.36 mmol) were coevaporated together three times with toluene and kept under vacuum overnight. They were dissolved in DCM (4 mL) under Argon atmosphere. Freshly activated 4Å molecular sieves (450 mg) were added and the mixture was stirred at rt for 45 min. Then a solution of trimethylsilyl triflate (0.055 M in DCM, 654 μL, 36 μmol) was slowly added over 1 h at 0°C using a syringe pump. The reaction mixture was then warmed to rt and monitored by HPTLC (hexane/AcOEt 7:3). After 2 h the reaction was neutralized with TEA, filtered on a Celite pad and the solvent was evaporated. Flash chromatography (hexane/AcOEt 7:3) afforded the crude disaccharide. It was then dissolved in MeOH (2 mL) and THF (2 mL) and treated with a solution of MeONa (0.4 M in MeOH, 1.1 mL, 0.4 mmol). The reaction was monitored by TLC (hexane/AcOEt 6:4). After 5 h the mixture was neutralized with Amberlite IR-120, filtered and the concentrated. Flash chromatography (hexane/AcOEt 7:3) afforded compound **53** (184.5 mg, 70%) as a white foam.

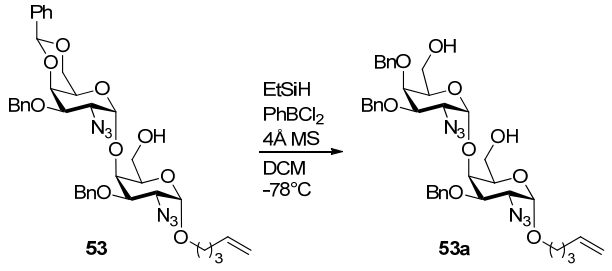
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.51 – 7.30 (m, 15H, arom.), 5.84 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.34 (s, 1H, PhC*H*–), 5.15 (d, $J_{1,2}$ = 2.8 Hz, 1H, H-1), 5.11 – 4.99 (m, 3H, $O(CH_2)_3CHCH_2$, H-1'), 4.83 (d, *J* = 11.7 Hz, 1H, OC*H*HPh), 4.75 (br s, 2H, OCH_2Ph), 4.71 (d, *J* = 11.7 Hz, 1H, OCH*H*Ph), 4.34 (br d, *J* = 2.8 Hz, 1H, H-4), 4.17 (m, 1H, H-4'), 4.07 – 3.89 (m, 7H, H-5, H-3, H-2, H-

3', H-6'a, H-6'b, H-5'), 3.75 (dt, *J* = 9.7, 6.6 Hz, 1H, OC*H*H$(CH_2)_2CHCH_2$), 3.71 – 3.63 (m, 2H, H-6a, H-2'), 3.53 (dt, *J* = 9.7, 6.4 Hz, 1H, OCH*H*$(CH_2)_2CHCH_2$), 3.40 (dd, $J_{6a,6b}$ = 12.8, $J_{5,6b}$ = 1.8 Hz, 1H, H-6b), 2.18 (m, 2H, $O(CH_2)_2C$*H*$_2CHCH_2$), 1.76 (m, 2H, OCH_2C*H*$_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 138.03 ($O(CH_2)_3$*C*HCH_2), 115.24 ($O(CH_2)_3CH$*C*H_2), 100.82 (Ph*C*H–), 99.63 (C-1), 98.29 (C-1'), 75.91 (C-3), 74.75 (C-3'), 73.18 (C-4'), 72.84 (C-4), 71.95 (O*C*H_2Ph), 71.23 (O*C*H_2Ph), 71.09 (C-5'), 69.19 (C-6), 67.96 (O*C*$H_2(CH_2)_2CHCH_2$), 63.14 (C-5), 60.81 (C-6'), 59.85 (C-2'), 59.32 (C-2), 30.40 ($O(CH_2)_3$*C*H_2CHCH_2), 28.75 (OCH_2*C*$H_2CH_2CHCH_2$) ppm.

ESI-MS (MeOH) for $[C_{38}H_{44}N_6O_9Na]$+ calcd: 751.31, found: 751.2

(2-Azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(4-pentenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (53a)



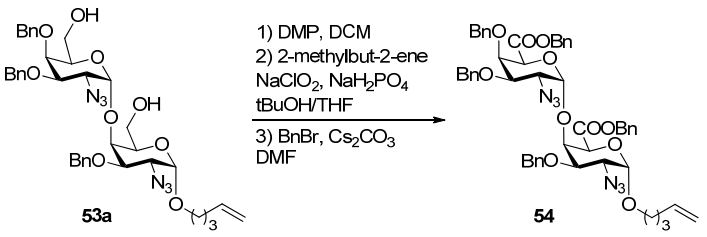
Compound **53** (320.7 mg, 0.44 mmol) was dissolved in DCM (10 mL). 4Å molecular sieves (1.5 g) were added and the mixture was stirred for 45 min at rt. Et_3SiH (267 µL, 1.67 mmol) was added and the mixture was cooled to -78°C. $PhBCl_2$ (69 µL, 0.53 mmol) was added dropwise and the mixture was slowly warmed to 0°C. The reaction was monitored by TLC (hexane/AcOEt 5:5). After 1 h the mixture was quenched with TEA at -78°C, filtered and concentrated. The crude was taken up in DCM, washed with satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 7:3) afforded compound **53a** (300.8 mg, 93%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.51 – 7.26 (m, 15H, arom.), 5.84 (ddt, *J* = 16.9, 10.1, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.05 (m, 3H, $O(CH_2)_3CHCH_2$, H-1'), 4.96 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.89 (d, *J* = 11.3 Hz, 1H, OC*H*HPh), 4.82 (d, *J* = 11.0 Hz, 1H, OC*H*HPh), 4.79 (d, *J* = 11.3 Hz, 1H, OC*H*HPh), 4.73 (d, *J* = 11.3 Hz, 1H, OCH*H*Ph), 4.70 (d, *J* = 11. Hz, 1H, OCH*H*Ph), 4.57 (d, *J* = 11.3 Hz, 1H, OCH*H*Ph), 4.25 (br d,*J* = 2.7 Hz, 1H, H-4), 4.08 – 3.76 (m, 8H, H-5', H-2', H-3', H-4', H-3, H-5, H-6a, H-6b), 3.76 – 3.64 (m, 2H, OC*H*H$(CH_2)_2CHCH_2$, H-2), 3.51 (dt, *J* = 9.7, 6.4 Hz, 1H, OCH*H*$(CH_2)_2CHCH_2$), 3.45 (dd, $J_{6'a,6'b}$ = 11.3, $J_{5',6'a}$ = 6.0 Hz, 1H, H-6'a), 3.39 (dd, $J_{6'a,6'b}$ = 11.3, $J_{5',6'b}$ = 5.8 Hz, 1H, H-6'b), 2.37 (br s, 1H, OH), 2.17 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.76 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 137.89 ($O(CH_2)_3\mathit{C}HCH_2$), 115.08 ($O(CH_2)_3CH\mathit{C}H_2$), 99.25 (C-1'), 98.13 (C-1), 77.53 (C-3'), 76.03 (C-3), 74.72 ($O\mathit{C}H_2Ph$), 74.19 (C-4), 73.40 (C-4'), 72.35 ($O\mathit{C}H_2Ph$), 72.21 ($O\mathit{C}H_2Ph$), 71.69 (C-5'), 70.68 (C-5), 67.82 ($O\mathit{C}H_2(CH_2)_2CHCH_2$), 62.02 (C-6'), 60.83 (C-2'), 60.59 (C-6), 59.77 (C-2), 30.24 ($O(CH_2)_3\mathit{C}H_2CHCH_2$), 28.61 ($OCH_2\mathit{C}H_2CH_2CHCH_2$) ppm.

ESI-MS (MeOH) for $[C_{38}H_{46}N_6O_9Na]$+ calcd: 753.32, found: 753.1

(Benzyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyluronate)-(1→4)-(benzyl (4-pentenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosid)uronate) (54)



Compound **53a** (84.8 mg, 0.12 mmol) was dissolved in DCM (5 mL) and Dess-Martin periodinane (153 mg, 0.36 mmol) was added. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 1 h, the mixture was quenched with 10% aq. $Na_2S_2O_3$. The organic phase was separated and washed with satd. $NaHCO_3$. The combined aqueous phases were extracted with DCM. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in THF (3 mL) and tBuOH (6 mL). 2-Methylbut-2-ene (1.3 mL) and a solution of $NaClO_2$ and $NaH_2PO_4 \cdot 2H_2O$ (271 mg + 280 mg in 7 mL of water, 2.4 mmol + 1.8 mmol) were then added. The reaction was monitored by TLC (DCM/MeOH 9:1 + 1% AcOH). After 1 h the mixture was concentrated to half volume, acidified with 5% aq. HCl to pH 2, then the solvents were evaporated and the mixture lyophilized. The crude was dissolved in DMF (5 mL), BnBr (285 µL, 2.4 mmol) and Cs_2CO_3 (782 mg, 2.4 mmol) were added. The reaction was monitored by TLC (hexane/AcOEt 7:3) and stirred overnight. Then the solvent was evaporated, the crude was taken up in DCM and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (toluene/acetone 98:2 to 95:5) afforded compound **54** (70.7 mg, 63%) as a white foam.

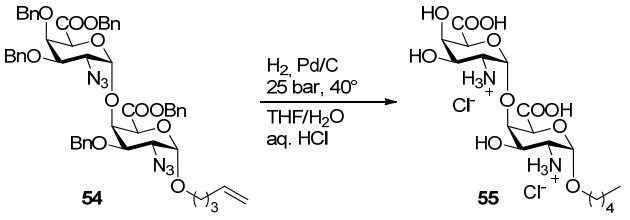
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.50 – 7.08 (m, 25H, arom.), 5.78 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.30 (m, 2H, $COOCH_2Ph$), 5.15 (d, $J_{1',2'}$ =

3.6 Hz, 1H, H-1'), 5.12 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.99 (m, 2H, $O(CH_2)_3CHC\mathit{H}_2$), 4.90 (d, J = 1.5 Hz, 1H, H-5'), 4.85 – 4.71 (m, 5H, $OC\mathit{H}_2Ph$, 2 x $OC\mathit{H}HPh$, $COO\mathit{H}HPh$), 4.66 (d, J = 12.1 Hz, 1H, $COOH\mathit{H}Ph$), 4.63 (br d, J = 1.9 Hz, 1H, H-4), 4.50 (d, J = 12.4 Hz, 1H, $OCH\mathit{H}Ph$), 4.41 (br s, 1H, H-5), 4.34 (m, 2H, $OCH\mathit{H}Ph$, H-4'), 4.02 (dd, $J_{2',3'}$ = 10.7, $J_{3',4'}$ = 2.6 Hz, 1H, H-3'), 3.94 (m, 2H, H-2', H-3), 3.74 (dt, J = 9.9, 6.6 Hz, 1H, $OC\mathit{H}H(CH_2)_2CHCH_2$), 3.58 (m, 2H, H-2, $OCH\mathit{H}(CH_2)_2CHCH_2$), 2.12 (m, 2H, $O(CH_2)_2C\mathit{H}_2CHCH_2$), 1.72 (m, 2H, $OCH_2C\mathit{H}_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 168.22 ($\mathit{C}OOBn$), 167.78 ($\mathit{C}OOBn$), 137.92 ($O(CH_2)_3\mathit{C}HCH_2$), 115.32 ($O(CH_2)_3CH\mathit{C}H_2$), 100.13 (C-1'), 98.34 (C-1), 77.23 (C-3'), 75.53 (C-4), 74.97 (C-4'), 74.81 (C-3), 74.69 ($O\mathit{C}H_2Ph$), 72.54 ($O\mathit{C}H_2Ph$), 71.85 ($O\mathit{C}H_2Ph$), 71.57 (C-5'), 70.34 (C-5), 68.59 ($O\mathit{C}H_2(CH_2)_2CHCH_2$), 67.98 ($COO\mathit{C}H_2Ph$), 67.06 ($COO\mathit{C}H_2Ph$), 59.42 (C-2'), 59.13 (C-2), 30.31 ($O(CH_2)_3\mathit{C}H_2CHCH_2$), 28.30 ($OCH_2\mathit{C}H_2CH_2CHCH_2$) ppm.

ESI HR-MS (MeOH) for $[C_{52}H_{54}N_6O_{11}Na]+$ calcd: 961.3743, found: 961.3825

(2-Amino-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(pentyl 2-amino-2-deoxy-α-D-galactopyranosiduronic acid) dihydrochloride (55)



Compound **54** (31.5 mg, 33.5 μmol) was dissolved in H_2O/THF 3:1 (8 mL + 5 drops of 5% aq. HCl). Pd/C was added and the mixture was left under hydrogen (30 bar) at 30°C for 24 h. Then the mixture was filtered and lyophilized. Reversed-phase flash chromatography (H_2O/CH_3CN 95:5) afforded compound **55** (15.4 mg, 90%) as a white solid.

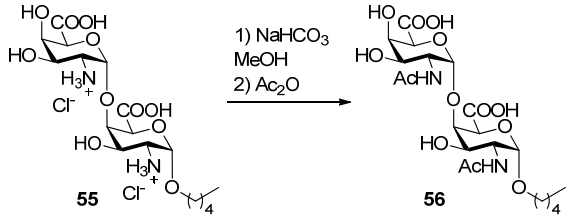
^{1}H NMR (400 MHz, D_2O, 25°C): δ = 5.24 (d, $J_{1',2'}$ = 3.7 Hz, 1H, H-1'), 5.19 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.78 (br s, 1H, H-5'), 4.42 (d, J = 3.1 Hz, 1H, H-4), 4.32 (br s, 1H, H-5), 4.30 (br d, J = 2.9 Hz, 1H, H-4'), 4.22 (dd, $J_{2,3}$ = 11.1, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 4.12 (dd, $J_{2',3'}$ = 11.1, $J_{3',4'}$ = 3.2 Hz, 1H, H-3'), 3.74 (dt, J = 9.7, 6.7 Hz, 1H, OC*H*H$(CH_2)_3CH_3$), 3.55 (m, 3H, OCH*H*$(CH_2)_3CH_3$, H-2, H-2'), 1.61 (m, 2H, $OCH_2CH_2(CH_2)_2CH_3$), 1.31 (m, 4H, $O(CH_2)_2CH_2CH_2CH_3$), 0.86 (m, 3H, $O(CH_2)_4CH_3$) ppm.

^{13}C NMR (100.6 MHz, D_2O, 25°C): δ = 174.83 (2 x *C*OOH), 96.32 (C-1'), 95.42 (C-1), 77.55 (C-4), 72.59 (C-5'), 70.75 (C-5), 69.62 (C-4'), 69.05 ($OCH_2(CH_2)_3CH_3$), 66.83 (C-3'), 66.29 (C-3), 50.87 (C-2 or C-2'), 50.80 (C-2' or C-2), 28.41 ($OCH_2CH_2(CH_2)_2CH_3$), 27.69 ($O(CH_2)_2CH_2CH_2CH_3$), 21.74 ($O(CH_2)_2CH_2CH_2CH_3$), 13.37 ($O(CH_2)_4CH_3$) ppm.

ESI HR-MS (MeOH) for $[C_{17}H_{30}N_2O_{11}\text{-}H]^-$ calcd: 437.1777, found: 437.1820

$[\alpha]_D^{20}$ = 139.9 (*c* = 0.39 in H_2O)

(2-Acetamido-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(pentyl 2-acetamido-2-deoxy-α-D-galactopyranosiduronic acid) (56)



Compound **55** (5.9 mg, 11.5 μmol) was dissolved in MeOH (2 mL). $NaHCO_3$ (3.9 mg, 31.2 μmol) was added and the mixture was stirred for 15 min. Then Ac_2O (30 μL, 300 μmol) was added and the mixture was stirred overnight. The reaction was monitored by RPTLC (H_2O/CH_3CN 8:2 + 1% AcOH). The mixture was quenched with water and neutralized with Amberlite IR-120, filtered and concentrated *in vacuo*. Reverse phase flash chromatography (H_2O/CH_3CN 95:5 to 7:3) afforded compound **56** (6 mg, quant.).

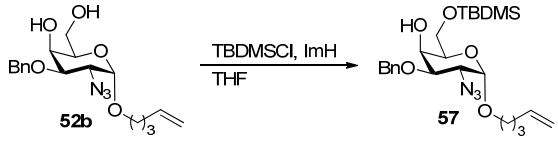
^{1}H NMR (400 MHz, D_2O, 25°C): δ = 4.98 (br s, 1H, H-1), 4.91 (d, $J_{1',2'}$ = 3.5 Hz, 1H, H-1'), 4.68 (br s, 1H, H-5), 4.35 (br s, 1H, H-5'), 4.25 (br d, J = 3.1 Hz, 1H , H-4), 4.20 (m, 2H, H-4', H-2), 4.13 – 3.94 (m, 3H, H-2', H-3', H-3), 3.65 (dt, J = 10.0, 6.5 Hz, 1H, OC*H*H$(CH_2)_3CH_3$), 3.46 (dt, J = 10.0, 6.0 Hz, 1H, OCH*H*$(CH_2)_3CH_3$), 2.04 (s, 3H, HNCOC*H*$_3$), 1.98 (s, 3H, HNCOC*H*$_3$), 1.53 (m, 2H, $OCH_2CH_2(CH_2)_2CH_3$), 1.27 (m, 4H, $O(CH_2)_2CH_2CH_2CH_3$), 0.82 (m, 3H, $O(CH_2)_4CH_3$) ppm.

^{13}C NMR (100.6 MHz, D_2O, 25°C): δ = 174.96 (*C*OOH or NH*C*OCH_3), 174.62 (*C*OOH or NH*C*OCH_3), 98.56 (C-1), 96.67 (C-1'), 77.97 (C-5'), 72.52 (C-5), 70.68 (C-4'), 70.08 (C-4), 68.50 ($OCH_2(CH_2)_3CH_3$), 67.98 (C-3), 67.08 (C-3'), 50.00 (C-2'), 49.36 (C-2), 28.22 ($OCH_2CH_2(CH_2)_2CH_3$), 27.62 ($O(CH_2)_2CH_2CH_2CH_3$), 22.43 (NHCO*C*H_3), 21.92 (NHCO*C*H_3), 21.68 ($O(CH_2)_2CH_2CH_2CH_3$), 13.36 ($O(CH_2)_4CH_3$) ppm.

ESI HR-MS (MeOH) for $[C_{21}H_{34}N_2O_{13}\text{-}H]^-$ calcd: 521.1988, found: 521.1942

$[\alpha]_D^{20} = 82.1$ ($c = 0.4$ in H_2O)

4-Pentenyl 2-azido-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-2-deoxy-α-D-galactopyranoside (57)



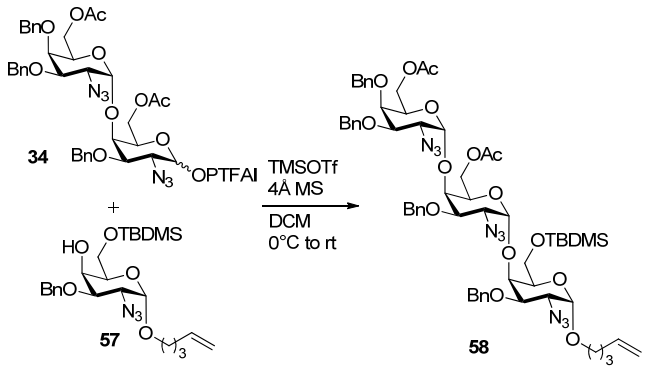
Compound **52b** (130 mg, 0.37 mmol) was dissolved in THF (5 mL). Imidazole (100.6 mg, 1.48 mmol) was added and the mixture was cooled to 0°C. TBDMSCl (83 mg, 0.55 mmol) was added, the mixture was slowly warmed to rt and stirred overnight. The reaction was monitored by TLC (hexane/AcOEt 6:4). The solvent was removed, the crude was taken up in AcOEt and washed with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **57** (148.5 mg, 84%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.47 – 7.32 (m, 5H, arom.), 5.84 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.10 – 4.97 (m, 2H, $O(CH_2)_3CHCH_2$), 4.92 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.76 (m, 2H, OCH_2Ph), 4.14 (br d, *J* = 2.6 Hz, 1H, H-4), 3.92 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 3.89 (dd, $J_{6a,6b}$ = 9.4 Hz, $J_{5,6a}$ = 5.2 Hz, 1H, H-6a), 3.84 – 3.67 (m, 4H, H-6b, H-5, H-2, $OCHH(CH_2)_2CHCH_2$), 3.49 (dt, *J* = 9.7, 6.5 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.57 (br s, 1H, OH), 2.17 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.75 (m, 2H, $OCH_2CH_2CH_2CHCH_2$), 0.93 (s, 9H, $SiC(CH_3)_3$), 0.10 (s, 3H, $Si(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 137.96 ($O(CH_2)_3CHCH_2$), 115.02 ($O(CH_2)_3CHCH_2$), 98.05 (C-1), 76.23 (C-3), 71.92 (OCH_2Ph), 70.03 (C-5), 67.56 ($OCH_2(CH_2)_2CHCH_2$), 66.45 (C-4), 62.67 (C-6), 59.20 (C-2), 30.28 ($O(CH_2)_3CH_2CHCH_2$), 28.60 ($OCH_2CH_2CH_2CHCH_2$), 25.86 ($SiC(CH_3)_3$), 18.29 ($SiC(CH_3)_3$), -5.41 ($Si(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{24}H_{39}N_3O_5Na]$+ calcd: 500.26, found: 500.3

(6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(4-pentenyl 2-azido-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-2-deoxy-α-D-galactopyranoside) (58)



Donor **34** (405 mg, 0.44 mmol) and acceptor **57** (137 mg, 0.29 mmol) were coevaporated together three times with toluene and kept under vacuum overnight. They were dissolved in DCM (4 mL) under Argon atmosphere. Freshly activated 4Å molecular sieves (545 mg) were added and the mixture was stirred at rt for 45 min. Then a solution of trimethylsilyl triflate (0.055 M in DCM, 527 μL, 0.03 mmol) was slowly added over 1 h at 0°C using a syringe pump. The reaction mixture was then warmed to rt and monitored by TLC (hexane/AcOEt 7:3). After 3 h the reaction was neutralized with TEA, filtered on a Celite pad and the solvent was evaporated. Flash chromatography (hexane/AcOEt 9:1) afforded compound **58** (303.2 mg, 86%) as a white foam.

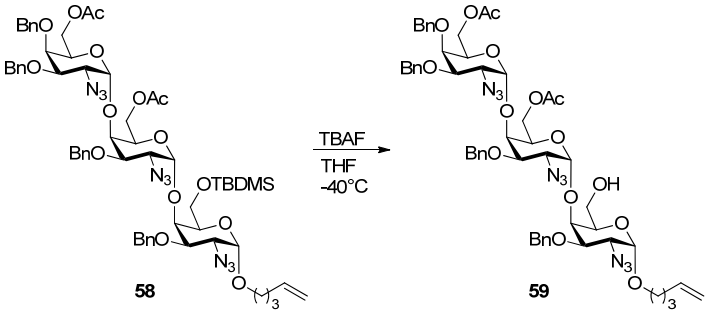
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.50 – 7.22 (m, 20H, arom.), 5.81 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.21 (d, $J_{1',2'}$ = 3.6 Hz, 1H, H-1'), 5.08 – 4.96 (m, 2H, $O(CH_2)_3CHCH_2$), 4.94 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.92 (d, $J_{1'',2''}$ = 3.6 Hz, 1H, H-1''), 4.90 – 4.85 (m, 3H, 3 x OC*H*HPh), 4.82 (d, *J* = 11.2 Hz, 1H, OCH*H*Ph), 4.76 (d, *J* = 11.2 Hz, 1H, OCH*H*Ph), 4.73 (d, *J* = 12.2 Hz, 1H, OCH*H*Ph), 4.67 (d, *J* = 11.9 Hz, 1H, OCH*H*Ph), 4.50 (d, *J* = 11.2 Hz, 1H,

OCH*H*Ph), 4.38 (br dd, *J* = 9.8, 5.3 Hz, 1H, H-5'), 4.31 (br d, *J* = 2.8 Hz, 1H, H-4), 4.30 – 4.22 (m, 2H, H-5'', H-6'a), 4.20 (br d, *J* = 5.6 Hz, 1H, H-4'), 4.07 – 3.92 (m, 7H, H-6'b, H-3'', H-6''a, H-3'', H-6b, H-4'', H-2''), 3.89 (dd, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 3.79 – 3.65 (m, 5H, H-6b, H-5, H-6''b, H-2', OC*H*H$(CH_2)_2$CHCH_2), 3.57 (dd, $J_{2,3}$ = 10.8, $J_{1,2}$ = 3.6 Hz, 1H, H-2), 3.49 (dt, *J* = 9.8, 6.3 Hz, 1H, OCH*H*$(CH_2)_2$CHCH_2), 2.14 (m, 2H, O$(CH_2)_2$C*H*$_2$CHCH_2), 1.93 (s, 3H, OCOCH_3), 1.77 (s, 3H, OCOCH_3), 1.73 (m, 2H, OCH_2C*H*$_2$$CH_2CHCH_2$), 0.94 (s, 9H, SiC$(CH_3)_3$), 0.12 (s, 3H, Si$(CH_3)_2$), 0.11 (s, 3H, Si$(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 169.81 (O*C*OCH_3), 169.61 (O*C*OCH_3), 137.92 (O$(CH_2)_3$*C*HCH_2), 115.03 (O$(CH_2)_3$CH*C*H$_2$), 99.11 (C-1'), 98.46 (C-1), 98.16 (C-1''), 76.86 (C-3''), 75.01 (C-3', C-3), 74.65 (O*C*H$_2$Ph), 72.28 (O*C*H$_2$Ph), 72.20 (C-4', C-4''), 71.93 (2 x O*C*H$_2$Ph), 71.74 (C-4), 70.93 (C-5), 68.76 (C-5''), 68.27 (C-5'), 67.66 (O*C*H$_2$$(CH_2)_2CHCH_2$), 62.01 (C-6''), 61.07 (C-6'), 60.41 (C-6), 60.07, 59.96, 59.83 (C-2'', C-2', C-2), 30.27 (O$(CH_2)_3$*C*H$_2$CHCH_2), 28.60 (OCH_2*C*H$_2$$CH_2CHCH_2$), 25.85 (SiC(*C*H$_3)_3$), 20.71 (OCO*C*H$_3$), 20.61 (OCO*C*H$_3$), 18.19 (Si*C*$(CH_3)_3$), -5.37 (Si$CH_3$), -5.49 (Si$CH_3$) ppm.

ESI HR-MS (MeOH) for $[C_{61}H_{79}N_9O_{15}SiNa]$+ calcd: 1228.5363, found: 1228.5380

(6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(4-pentenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (59)



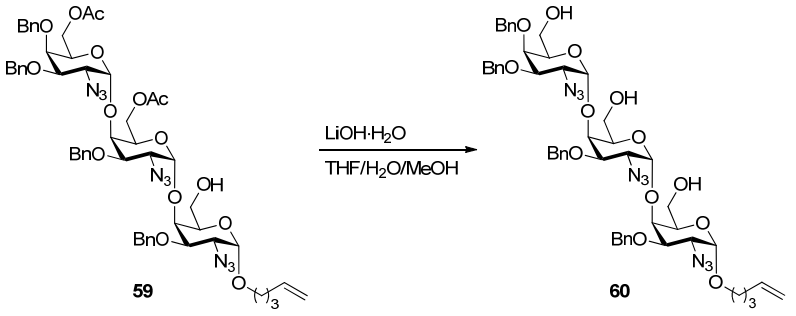
Compound **58** (303 mg, 0.25 mmol) was dissolved in THF (5 mL) at -40°C. TBAF (1.0 M in THF, 325 µL, 0.33 mmol) was slowly added. The reaction was monitored by TLC (hexane/AcOEt 6:4) and slowly warmed to 0°C overnight. Then the solvent was concentrated, the mixture was diluted with AcOEt and washed with satd. NH_4Cl. The organic phase was dried with Na_2SO_4, filtered and evaporated *in vacuo*. Flash chromatography (hexane/AcOEt 7:3) afforded compound **59** (260.8 mg, 96%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.49 – 7.23 (m, 20H, arom.), 5.82 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.13 (d, $J_{1',2'}$ = 3.5 Hz, 1H, H-1'), 5.08 – 4.97 (m, 2H, $O(CH_2)_3CHCH_2$), 4.94 (m, 2H, H-1, H-1''), 4.90 – 4.80 (m, 4H, 4 x OCHPh), 4.77 (d, *J* = 11.2 Hz, 1H, OCHPh), 4.72 (d, *J* = 12.0 Hz, 1H, OCHPh), 4.65 (d, *J* = 12.0 Hz, 1H, OCHPh), 4.52 (d, *J* = 11.2 Hz, 1H, OCHPh), 4.34 – 4.23 (m, 4H, H-5'', H-5', H-6'a, H-4), 4.17 (br d, *J* = 2.7 Hz, 1H, H-4'), 4.12 (br dd, *J* = 10.8 Hz, 5.8 Hz, 1H, H-6'b), 4.05 – 3.98 (m, 3H, H-6''a, H-2'', H-3''), 3.97 – 3.66 (m, 9H, H-4'', H-3', H-3, H-5, H-6a, H-6''b, H-2', H-6b, $OCHH(CH_2)_2CHCH_2$), 3.59 (dd, $J_{2,3}$ = 10.8, $J_{1,2}$ = 3.6 Hz, 1H, H-2), 3.49 (dt, *J* = 9.7, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.15 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.96 (s, 3H, $OCOCH_3$), 1.80 (s, 3H, $OCOCH_3$), 1.73 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 169.87 (2 x O*C*OCH$_3$), 137.91 (O(CH$_2$)$_3$*C*HCH$_2$), 115.07 (O(CH$_2$)$_3$CH*C*H$_2$), 99.09 (C-1'), 98.50 (C-1), 98.16 (C-1''), 77.33 (C-3''), 75.28 (C-3 or C-3'), 75.08 (C-3' or C-3), 74.67 (O*C*H$_2$Ph), 73.08 (C-4), 72.49 (C-4'), 72.38 (O*C*H$_2$Ph), 72.22 (C-4''), 72.07 (O*C*H$_2$Ph), 71.84 (O*C*H$_2$Ph), 70.77 (C-5), 68.86 (C-5'' or C-5'), 68.78 (C-5' or C-5''), 67.76 (O*C*H$_2$(CH$_2$)$_2$CHCH$_2$), 62.05 (C-6'' or C-6'), 61.32 (C-6' or C-6''), 60.73 (C-6), 60.45 (C-2'), 60.22 (C-2''), 59.73 (C-2), 30.24 (O(CH$_2$)$_3$*C*H$_2$CHCH$_2$), 28.57 (OCH$_2$*C*H$_2$CH$_2$CHCH$_2$), 20.72 (OCO*C*H$_3$), 20.46 (OCO*C*H$_3$) ppm.

ESI HR-MS (MeOH) for [$C_{55}H_{65}N_9O_{15}Na$]+ calcd: 1114.4498, found: 1114.4502

(2-Azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(4-pentenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside) (60)



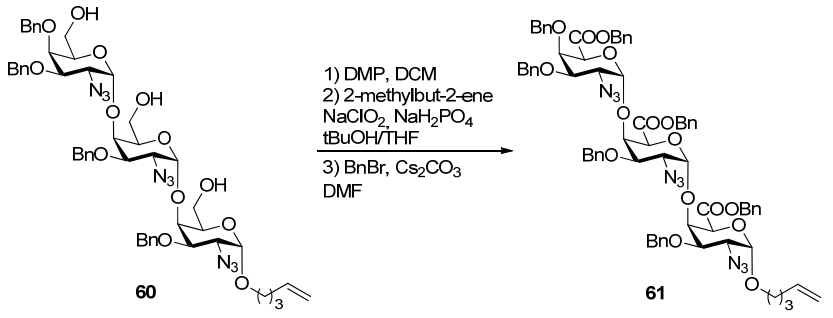
Compound **59** (260 mg, 0.24 mmol) was dissolved in THF/MeOH/H_2O 1:5:1 (7 mL) and LiOH·H_2O (20 mg, 0.48 mmol) was added. The reaction was monitored by TLC (hexane/AcOEt 4:6). After 24 h the mixture was neutralized with Amberlite IR-120, filtered and the solvent was evaporated *in vacuo*, affording compound **60** (245 mg, quant.) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.51 – 7.23 (m, 20H, arom.), 5.84 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.12 – 4.98 (m, 4H, H-1', H-1'', $O(CH_2)_3CHCH_2$), 4.96 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.87 (d, *J* = 11.3 Hz, 1H, OC*H*HPh), 4.85 (d, *J* = 11.7 Hz, 1H, OC*H*HPh), 4.83 (d, *J* = 11.5 Hz, 1H, OC*H*HPh), 4.78 (d, *J* = 11.3 Hz, 1H, OC*H*HPh), 4.74 (d, *J* = 11.6 Hz, OCH*H*Ph), 4.71 (d, *J* = 11.3 Hz, OCH*H*Ph), 4.67 (d, *J* = 11.7 Hz, OCH*H*Ph), 4.56 (d, *J* = 11.3 Hz, 1H, OCH*H*Ph), 4.28 (br d, *J* = 2.8 Hz, 1H, H-4), 4.24 (br d, *J* = 2.2 Hz, 1H, H-4'), 4.07 (br t, *J* = 7.0 Hz, 1H, H-5'), 4.04 – 3.56 (m, 13H, H-5'', H-4'', H-3', H-3'', H-3, H-2'', H-5, H-6a, H-6b, H-2', $OCHH(CH_2)_2CHCH_2$, H-2, H-6'a), 3.51 (dt, *J* = 9.7, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.44 – 3.31 (m, 3H, H-6'b, H-6''a, H-6''b), 2.32 (br s, 1H, OH), 2.17 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.75 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 137.89 ($O(CH_2)_3CHCH_2$), 115.10 ($O(CH_2)_3CHCH_2$), 98.92 (C-1'), 98.82 (C-1''), 98.10 (C-1), 77.13 (C-3' or C-3''), 76.01 (C-3'' or C-3'), 75.88 (C-3), 74.72 (OCH_2Ph), 73.57 (C-4), 73.33 (C-4''), 72.56 (C-3'), 72.35 (OCH_2Ph), 72.14 (OCH_2Ph), 71.80 (OCH_2Ph), 71.34 (C-5'), 71.22 (C-5''), 70.72 (C-5), 67.82 ($OCH_2(CH_2)_2CHCH_2$), 61.80 (C-6''), 60.68 (C-6), 60.54 (C-2''), 60.37 (C-2'), 60.37 (C-6'), 59.81 (C-2), 30.24 ($O(CH_2)_3CH_2CHCH_2$), 28.59 ($OCH_2CH_2CH_2CHCH_2$) ppm.

ESI HR-MS (MeOH) for $[C_{51}H_{61}N_9O_{13}Na]$+ calcd: 1030.4287, found: 1030.4807

(Benzyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyluronate)-(1→4)-(benzyl (4-pentenyl 2-azido-3-*O*-benzyl-2-deoxy-galactopyranosid)uronate) (61)



Compound **60** (242 mg, 0.24 mmol) was dissolved in DCM (10 mL) and Dess-Martin periodinane (467 mg, 1.1 mmol) was added. The reaction was monitored by TLC (tol/AcOEt 7:3). After 8 h, the mixture was quenched with 10% aq. $Na_2S_2O_3$. The organic phase was separated and washed with satd. $NaHCO_3$. The combined aqueous phases were extracted with DCM. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in THF (5 mL) and tBuOH (10 mL). 2-Methylbut-2-ene (3.8 mL) and a solution of $NaClO_2$ and $NaH_2PO_4 \cdot 2H_2O$ (814 mg + 842 mg in 20 mL of water, 7.2 mmol + 5.4 mmol) were then added. The reaction was monitored by TLC (DCM/MeOH 8:2 + 1% AcOH). After 1 h the mixture was concentrated to half volume, acidified with 5% aq. HCl to pH 2, then the solvents were evaporated and the mixture lyophilized. The crude was dissolved in DMF (20 mL), BnBr (856 μL, 7.2 mmol) and K_2CO_3 (1.0 g, 7.2 mmol) were added. The reaction was monitored by TLC (hexane/AcOEt 6:4) and stirred overnight. Then the solvent was evaporated, the crude was taken up in DCM and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (toluene/acetone 98:2 to 95:5) afforded compound **61** (130 mg, 41%) as a white foam.

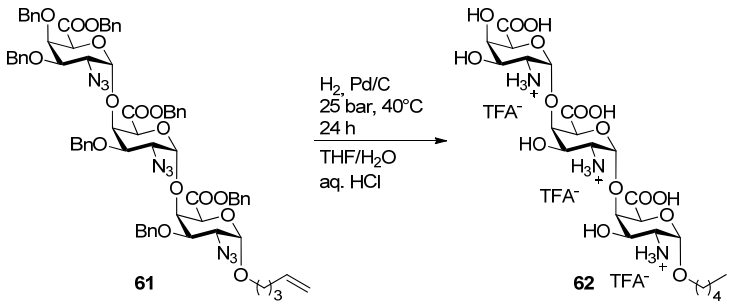
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.52 – 7.04 (m, 35H, arom.), 5.75 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.32 (s, 2H, $COOCH_2Ph$), 5.23 (d, $J_{1',2'}$ = 3.7 Hz, 1H, H-1'), 5.10 – 4.91 (m, 4H, H-1, $COOCHHPh$, H-1'', $O(CH_2)_3CHCH_2$), 4.91 – 4.66 (m, 10H, H-5', $OCHHPh$, H-5'', $COOCHHPh$, $OCHHPh$, $OCHHPh$, OCH_2Ph, $COOCHHPh$, H-4), 4.62 – 4.44 (m, 4H, H-4', $COOCHHPh$, $OCHHPh$, $OCHHPh$), 4.42 – 4.26 (m, 3H, H-5, $OCHHPh$, H-4), 4.03 – 3.85 (m, 4H, H-3'', H-3', H-2'', H-3), 3.71 (dt, *J* = 10.4, 6.5 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.65 – 3.45 (m, 3H, H-2', $OCHH(CH_2)_2CHCH_2$, H-2), 2.08 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.96 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 167.83 (*C*OOBn), 167.69 (*C*OOBn), 167.27 (*C*OOBn), 137.72 ($O(CH_2)_3CHCH_2$), 115.12 ($O(CH_2)_3CHCH_2$), 99.86 (C-1''), 99.27 (C-1'), 98.15 (C-1), 77.05 (C-3''), 75.34 (C-4'), 75.01 (C-3'), 74.79 (C-4''), 74.66 (C-4), 74.49 (OCH_2Ph), 74.28 (C-3), 72.37 (OCH_2Ph), 71.84 (OCH_2Ph), 71.57 (OCH_2Ph), 71.40 (C-5''), 70.79 (C-5'), 70.04 (C-5), 68.41 ($OCH_2(CH_2)_2CHCH_2$), 67.74 (*C*OOBn), 67.65 (*C*OOBn), 66.85 (*C*OOBn), 59.20 (C-2''), 59.07 (C-2'), 58.93 (C-2), 30.13 ($O(CH_2)_3CH_2CHCH_2$), 28.45 ($OCH_2CH_2CH_2CHCH_2$) ppm.

ESI HR-MS (MeOH) for $[C_{72}H_{73}N_9O_{16}Na]$+ calcd: 1342.5067, found: 1342.4950

$[\alpha]_D^{20}$ = 143.6 (*c* = 1 in $CHCl_3$)

(2-Amino-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(2-amino-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(pentyl 2-amino-2-deoxy-α-D-galactopyranosiduronic acid) *tris*-trifluoroacetate salt (62)



Compound **61** (23 mg, 17.4 μmol) was dissolved in H_2O/THF 3:1 (8 mL + 5 drops of 5% aq. HCl). Pd/C was added and the mixture was stirred under hydrogen (35 bar) at 35°C for 24 h. The reaction mixture was filtered on a Celite pad and lyophilized. Reverse phase flash chromatography (H_2O/CH_3CN + 0.1% TFA 95:5 to 9:1) afforded compound **62** (15 mg, 90%) as a white solid.

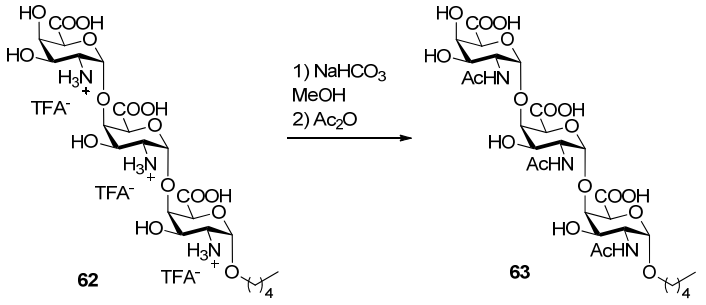
^{1}H NMR (400 MHz, D_2O, 25°C): δ = 5.24 (d, $J_{1'',2''}$ = 3.8 Hz, 1H, H-1''), 5.20 (d, $J_{1',2'}$ = 3.8 Hz, 1H, H-1'), 5.16 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.98 (s, 1H, H-5'), 4.85 (s, 1H, H-5''), 4.39 (m, 2H, H-4, H-4''), 4.34 (s, 1H, H-5), 4.30 (m, 1H, H-4'), 4.26 – 4.17 (m, 2H, H-3'', H-3), 4.11 (dd, $J_{2',3'}$ = 11.1, $J_{3',4'}$ = 3.2 Hz, 1H, H-3'), 3.70 (dt, J = 9.7, 6.7 Hz, 1H, OC*H*HCH$_2$CH$_2$CH$_2$CH$_3$), 3.59 – 3.45 (m, 4H, H-2'', OCH*H*CH$_2$CH$_2$CH$_2$CH$_3$, H-2, H-2'), 1.56 (m, 2H, OCH$_2$C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.26 (m, 4H, OCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_3$), 0.81 (m, 3H, OCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_3$) ppm.

^{13}C NMR (400 MHz, D_2O, 25°C): δ = 174.53 (*C*OOH'), 174.20 (*C*OOH''), 173.08 (*C*OOH), 95.92 (C-1'', C-1'), 95.21 (C-1), 77.29 (C-4 or C-4''), 77.15 (C-4'' or C-4), 71.53 (C-5'), 71.05 (C-5''), 70.34 (C-5), 69.02 (O*C*H$_2$(CH$_2$)$_3$CH$_3$), 68.95 (C-4'), 66.03 (C-3'), 65.79 (C-3), 65.44 (C-3''), 50.51 (C-2, C-2', C-2''), 28.30 (OCH$_2$*C*H$_2$(CH$_2$)$_2$CH$_3$), 27.57 (O(CH$_2$)$_2$*C*H$_2$CH$_2$CH$_3$), 21.66 (O(CH$_2$)$_2$CH$_2$*C*H$_2$CH$_3$), 13.28 (O(CH$_2$)$_4$*C*H$_3$) ppm.

ESI HR-MS (MeOH) for $[C_{23}H_{39}N_3O_{16}\text{-}H]^-$ calcd: 612.2257, found: 612.2310

$[\alpha]_D^{20} = 143.6$ ($c = 1$ in H_2O)

(2-Acetamido-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(2-acetamido-2-deoxy-α-D-galactopyranosyluronic acid)-(1→4)-(pentyl 2-acetamido-2-deoxy-α-D-galactopyranosiduronic acid) (63)



Compound **62** (5 mg, 5.2 μmol) was dissolved in MeOH (2 mL). $NaHCO_3$ (2.6 mg, 31.2 μmol) was added and the mixture was stirred for 15 min. Then Ac_2O (30 μL, 300 μmol) was added and the mixture was stirred overnight. The reaction was monitored by RPTLC (H_2O/CH_3CN 7:3). The mixture was quenched with water and neutralized with Amberlite IR-120, filtered and concentrated *in vacuo*. HPLC purification (H_2O/CH_3CN + 0.1% TFA 95:5 to 7:3) afforded compound **63** (3.7 mg, 97%).

^{1}H NMR (400 MHz, D_2O, 25°C): δ = 5.15 – 4.88 (m, 5H, H-1, H-1', H-1'', H-5, H-5'), 4.50 – 4.40 (m, 3H, H-5'', H-4, H-4'), 4.31 (br s, 1H, H-4''), 4.23 – 3.99 (m, 6H, H-2, H-2', H-2'', H-3, H-3', H-3''), 3.67 (m, 1H, OC*H*H$(CH_2)_3CH_3$), 3.49 (m, 1H, OCH*H*$(CH_2)_3CH_3$), 1.98 (m, 9H, 3 x NHCOC*H*$_3$), 1.54 (m, 2H, $OCH_2CH_2CH_2CH_2CH_3$), 1.26 (m, 4H, $OCH_2CH_2CH_2CH_2CH_3$), 0.83 (m, 3H, $OCH_2CH_2CH_2CH_2CH_3$) ppm.

^{13}C NMR (100.6 MHz, D_2O, 25°C): δ = 174.66, 172.56 (3 x *C*OOH, 3 x NH*C*OCH_3), 98.57, 98.46, 96.88 (C-1, C-1', C-1''), 77.71 (C-4 or C-4'), 77.64 (C-4' or C-4), 71.38 (C-5 or C-5'), 71.24 (C-5' or C-5), 70.25 (C-5''), 69.38 (C-4'') , 68.77 (O*C*H$_2(CH_2)_3CH_3$), 67.05, 66.50, 66.27 (C-3, C-3', C-3''), 49.74, 49.36 (C-2,

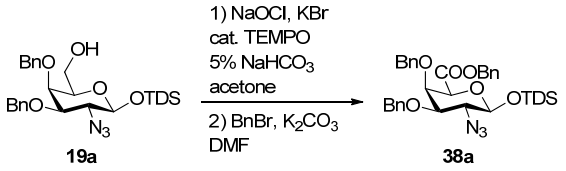
C-2' C-2''), 28.20 ($OCH_2CH_2(CH_2)_2CH_3$), 27.57 ($O(CH_2)_2CH_2CH_2CH_3$), 22.23 (3 x $NHCOCH_3$), 21.68 ($O(CH_2)_2CH_2CH_2CH_3$), 13.33 ($O(CH_2)_4CH_3$) ppm.

ESI HR-MS (MeOH) for $[C_{29}H_{45}N_3O_{19}\text{-}H]^-$ calcd: 738.2574, found: 738.2632

$[\alpha]_D^{20} = 52.3$ ($c = 0.26$ in H_2O)

6.2 3-*O*-Ac oligomers

Benzyl (thexyldimethylsilyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-galactopyranosiduronate) (38a)



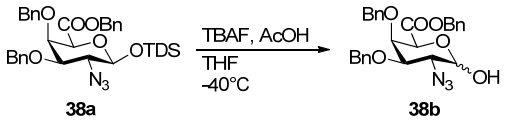
Compound **19a** (216 mg, 0.41 mmol) was dissolved in acetone (20 mL) and 5% aq. $NaHCO_3$ (10 mL), and the mixture was cooled to 0°C. KBr (97 mg, 0.82 mmol), TEMPO (90 mg, 0.57 mmol) and NaOCl 13% (1.7 mL) were added sequentially. After 30 min TLC analysis (DCM/MeOH 8:2 + 1% AcOH) showed complete consumption of the starting material. The solvent was removed *in vacuo* and co-evaporated three times with toluene. The crude was left under vacuum overnight, then it was dissolved in DMF (10 mL). BnBr (240 μL, 2.1 mmol) and K_2CO_3 (280 mg, 2.1 mmol) were added. The reaction was monitored by TLC (hexane/AcOEt 7:3). After 5 h the solvent was evaporated, the crude was taken up in AcOEt and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **38a** (217.4 mg, 84%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45 – 7.22 (m, 15H, arom.), 5.14 (m, 2H, COOCH_2Ph), 4.84 (d, J = 11.7 Hz, 1H, OC*H*HPh), 4.72 (s, 2H, OCH_2Ph), 4.57 (d, J = 11.7 Hz, 1H, OCH*H*Ph), 4.46 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1), 4.24 (dd, $J_{3,4}$ = 3.0, $J_{4,5}$ = 1.3 Hz, 1H, H-4), 4.00 (d, $J_{4,5}$ = 1.3 Hz, 1H, H-5), 3.80 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 7.7 Hz, 1H, H-2), 3.34 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 1.70 (hept, J = 6.8 Hz, 1H, SiC$(CH_3)_2$C*H*$(CH_3)_2$), 0.92 (m, 12H, SiC$(CH_3)_2$CH$(CH_3)_2$), 0.24 (s, 3H, Si$(CH_3)_2$), 0.21 (s, 3H, Si$(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 167.68 (*C*OOBn), 97.22 (C-1), 79.83 (C-3), 74.53 (O*C*H_2Ph), 74.11 (C-5), 74.06 (C-4), 72.69 (O*C*H_2Ph), 67.07 (COO*C*H_2Ph), 65.24 (C-2), 33.94 (SiC$(CH_3)_2$*C*H$(CH_3)_2$), 20.00 (SiC$(CH_3)_2$CH(*C*$H_3)_2$), 19.94 (SiC$(CH_3)_2$CH(*C*$H_3)_2$), 18.50 (SiC(*C*$H_3)_2$CH$(CH_3)_2$), 18.44 (SiC(*C*$H_3)_2$CH$(CH_3)_2$), -1.91 (Si$(CH_3)_2$), -3.22 (Si$(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{35}H_{45}N_3O_6SiNa]$+ calcd: 654.30, found: 654.2

Benzyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-D-galactopyranuronate (38b)



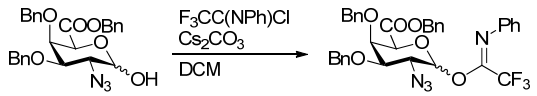
38a **38b**

Compound **38a** (217 mg, 0.34 mmol) was dissolved in THF (5 mL) at -40°C. AcOH (26 μL, 0.44 mmol) and TBAF (1.0 M in THF, 440 μL, 0.44 mmol) were added dropwise. The reaction was monitored by TLC (hexane/AcOEt 7:3). After 3 h the solvent was concentrated, the mixture was diluted with AcOEt and washed with satd. NH_4Cl. The organic phase was dried with Na_2SO_4, filtered and evaporated *in vacuo*. Flash chromatography (hexane/AcOEt 7:3) afforded compound **38b** (140 mg, 85%, mixture of anomers) as a pale yellow oil.

The occurrence of the desilylation was confirmed by the disappearance of the TDS signals in the ^1H NMR spectrum.

ESI-MS (MeOH) for $[C_{27}H_{27}N_3O_6Na]+$ calcd: 512.17, found: 512.2

(Benzyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-D-galactopyranuronate) *N*-phenyltrifluoroacetimidate (67)



38b **67**

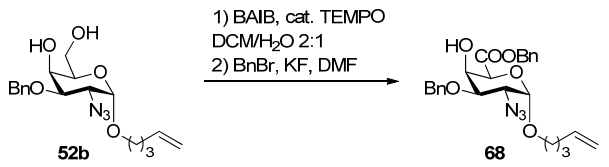
Compound **38b** (140 mg, 0.29 mmol) and *N*-phenyltrifluoroacetimidoyl chloride (92 μL, 0.57 mmol) were dissolved in DCM (5 mL), then Cs_2CO_3 (140 mg, 0.43 mmol) was added portionwise. The reaction was monitored by TLC (hexane/ethyl actetate 6:4) and stirred overnight. Then the mixture was filtered on a Celite pad and the solvent was evaporated. Flash chromatography (hexane/AcOEt 8:2 + 0.1% TEA) afforded compound **67** (187.5 mg, 97%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.44 – 7.19 (m, 12H, arom.), 7.09 (m, 1H, arom.), 6.83 (m, 2H, arom.), 5.43 (s, 1H, H-1), 5.15 (m, 2H, COOC*H*$_2$Ph), 4.82 (d, *J* = 11.5 Hz, 1H, OC*H*HPh), 4.74 (s, 2H, OC*H*$_2$Ph), 4.53 (d, *J* = 11.5 Hz, 1H, OCH*H*Ph), 4.25 (br s, 1H, H-4), 4.12 (m, 1H, H-2), 3.93 (br s, 1H, H-5), 3.45 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 2.8 Hz, 1H, H-3) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 95.81 (C-1), 80.30 (C-3), 74.88 (O*C*H$_2$Ph), 74.79 (C-5), 73.89 (C-4), 73.08 (O*C*H$_2$Ph), 67.39 (COO*C*H$_2$Ph), 61.94 (C-2) ppm.

ESI-MS (MeOH) for $[C_{35}H_{31}F_3N_4O_6Na]$+ calcd: 683.21, found: 683.1

Benzyl (4-pentenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate) (68)



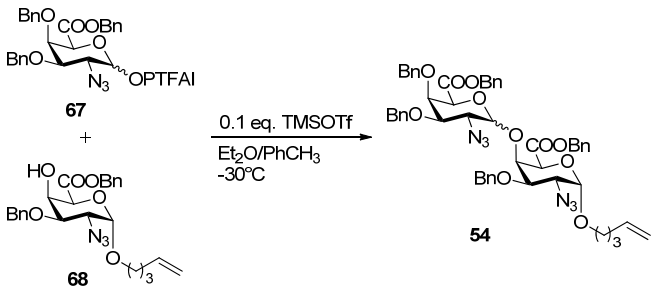
Compound **52b** (109.6 mg, 0.31 mmol) was dissolved in DCM/H_2O 2:1 (10 mL), then BAIB (300 mg, 0.93 mmol) and TEMPO (15 mg, 0.1 mmol) were added. The reaction was monitored by TLC (DCM/MeOH 8:2 + 1% AcOH). After 3 h the mixture was quenched with 5% aq. $Na_2S_2O_3$, diluted with water and extracted with AcOEt. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was dissolved in DMF (7 mL) at 0°C. KF (180 mg, 3.1 mmol) and BnBr (184 μL, 1.6 mmol) were added, and the mixture was stirred overnight at rt (TLC hexane/AcOEt 6:4). Then the solvent was removed, the crude was taken up in DCM and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 7:3) afforded compound **68** (121.5 mg 82%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45 – 7.33 (m, 10H, arom.), 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.30 (s, 2H, $COOCH_2Ph$), 5.08 – 4.95 (m, 3H, H-1, $O(CH_2)_3CHCH_2$), 4.75 (m, 2H, OCH_2Ph), 4.45 (m, 2H, H-4, H-5), 4.00 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.1 Hz, 1H, H-3), 3.74 (m, 2H, $OCHH(CH_2)_2CHCH_2$, H-2), 3.55 (dt, *J* = 9.8, 6.5 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.45 (br s, 1H, OH), 2.15 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.74 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 168.28 (*C*OOBn), 137.87 ($O(CH_2)_3CHCH_2$), 115.31 ($O(CH_2)_3CHCH_2$), 98.39 (C-1), 75.52 (C-3), 72.38 (OCH_2Ph), 70.04 (C-4), 68.54 ($OCH_2(CH_2)_2CHCH_2$), 67.53 (C-5), 67.38 ($COOCH_2Ph$), 58.70 (C-2), 30.27 ($O(CH_2)_3CH_2CHCH_2$), 28.63 ($OCH_2CH_2CH_2CHCH_2$) ppm.

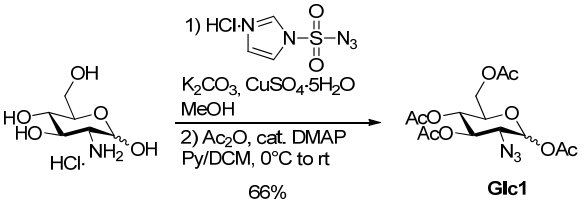
ESI-MS (MeOH) for $[C_{25}H_{29}N_3O_6Na]$+ calcd: 490.19, found: 490.3

Glycosylation with uronate building blocks 67 and 68



Donor **67** (60.4 mg, 91.5 µmol) and acceptor **68** (25 mg, 61 µmol) were coevaporated three times with toluene and kept under vacuum overnight. They were dissolved in toluene/Et_2O 1:2 (750 µL) under Argon atmosphere. Freshly activated 4Å molecular sieves (85 mg) were added and the mixture was stirred at rt for 45 min. Then a solution of trimethylsilyl triflate (0.1 M in toluene, 120 µL, 12 µmol) was slowly added over 1 h at -30°C. The reaction was monitored by HPTLC (hexane/AcOEt 7:3). After 2 h the reaction was neutralized with TEA, filtered on a Celite pad and the solvent was evaporated. Flash chromatography (hexane/AcOEt 85:25 to 75:25) afforded compound **54** (38.6 mg, 68%, mixture of anomers) as a white foam.

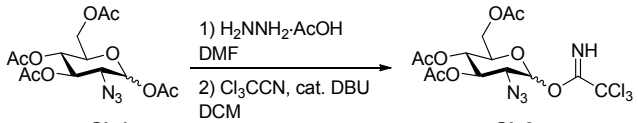
1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranoside (Glc1)



Anhydrous K_2CO_3 (29.65 g, 214.5 mmol) was added to a suspension of D-glucosamine hydrochloride (15.42 g, 71.5 mmol) in MeOH (250 mL) at 0°C and the mixture is left under vigorous stirring for 15 min. Imidazole-1-sulfonyl azide hydrochloride (17.90 g, 85.8 mmol) was then slowly added, followed by $CuSO_4 \cdot 5H_2O$ (179 mg, 0.715 mmol). The reaction was monitored by TLC (DCM/MeOH 7:3). After 4 h the solvent was removed, then the crude was coevaporated with toluene and kept under vacuum overnight. The crude was suspended in pyridine (96.5 mL, 1.144 mol), catalytic DMAP was added. Ac_2O (54 mL, 0.572 mol) was slowly added to the suspension at 0°C, then the reaction was warmed to rt and stirred overnight until completion (TCL, eluent hexane/AcOEt 6:4). The mixture was quenched with crushed ice and concentrated *in vacuo*, then the residue was taken up with DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash chromatography (hexane/AcOEt 7:3) afforded compound **Glc1** (16.62 g, 62%) as a white foam.

Spectroscopic data were in agreement with those reported in the literature.[12]

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-D-glucopyranosyl trichloroacetimidate (Glc2)

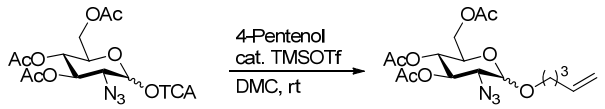


Glc1 **Glc2**

A solution of hydrazinium acetate was prepared by slow addition of acetic acid (6.6 mL, 78 mmol) to a stirred solution of hydrazine hydrate (2.9 mL, 60 mmol) in MeOH (30 mL) at 0°C. After 15 min, the solution was slowly added to compound **Glc1** (16.62 g, 44.2 mmol) dissolved in DMF (200 mL), and stirred for 2 h (TLC hexane/AcOEt 6:4). The solvent was evaporated, the crude was taken up with DCM and washed with brine. The organic phase was dried with Na_2SO_4, filtered and the solvent removed. The residue was dissolved in DCM (200 mL), trichloroacetonitrile (26.6 mL, 265.2 mmol) and catalytic DBU were added and the reaction mixture was stirred overnight (TLC, hexane/AcOEt 6:4). The solvent was removed and the crude was purified by flash chromatography (hexane/AcOEt 6:4 + 1% TEA), affording compound **Glc2** (16.44 g, 78% over two steps) as a white foam.

Spectroscopic data were in agreement with those reported in the literature.[12]

4-Pentenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-glucopyranoside (71)

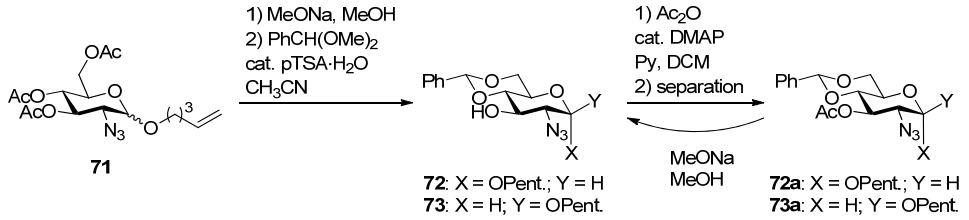


Glc2 **71**

Compound **Glc2** (16.44 g, 34.6 mmol) was dissolved in DCM (150 mL) under Argon atmosphere and 4-pentenol (3.57 mL, 34.6 mmol) was added. Then a solution of TMSOTf (0.5 M in DCM, 6.92 mL, 3.46 mmol) was slowly added. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 1 h the mixture was neutralized with TEA, filtered on a Celite pad and the solvent was removed. Flash chromatography (hexane/AcOEt 8:2) afforded pure **71** (13.819 g, quant., α/β 1:1) as a white foam.

Spectroscopic data were in agreement with those reported in the literature.[12]

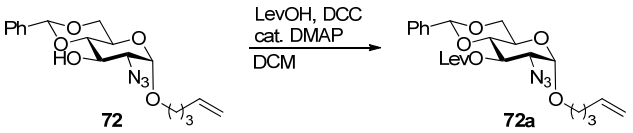
4-Pentenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranoside (72 and 73)



A solution of MeONa in MeOH (0.4 M, 7.8 mL, 3.12 mmol) was added to compound **71** (8.33 g, 20.8 mmol) dissolved in MeOH (100 mL) and the mixture was stirred for 15 min (TLC, hexane/AcOEt 6:4). The reaction is neutralized with Amberlite IR-120, filtered and the solvent was removed *in vacuo*. The product (5.68 g, 20.8 mmol) was dissolved in CH_3CN (200 mL), benzaldehyde dimethyl acetal (6.2 mL, 41.6 mmol) and *p*TSA·H_2O (400 mg, 2.08 mmol) were added. The reaction was stirred for 4 h (TLC, hexane/AcOEt 6:4), then quenched with TEA and the solvent was evaporated. The residue was dissolved in DCM (100 mL), pyridine (6.3 mL, 77.6 mmol), catalytic DMAP and Ac_2O (3.7 mL, 38.8 mmol) were sequentially added to the solution at 0°C. The reaction was warmed to rt and stirred for 3 h (TLC hexane/AcOEt 8:2). The mixture was quenched with water and the solvent was removed. The crude was taken up in DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Pure compound **72a** was obtained by crystallization (hexane/AcOEt). Pure compound **73a** was obtained after flash chromatography (hexane/AcOEt 9:1) of the concentrated mother liquor. The two compounds were independently subjected to Zémplen deactylation, affording compound **72** (3.83 g, 52%) and **73** (2.023 g, 27%) as white foams.

Spectroscopic data were in agreement with those reported in the literature.[12]

4-Pentenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-α-D-glucopyranoside (72a)



A solution of MeONa in MeOH (0.4 M, 3.55 mL, 1.42 mmol) was added to compound **72** (1.912 g, 4.74 mmol) dissolved in MeOH (45 mL). The reaction was followed by TLC (hexane/AcOEt 8:2). After 3 h the reaction was neutralized with Amberlite IR-120, filtered and the solvent was removed *in vacuo*. The crude was dissolved in DCM (90 mL), levulinic acid (0.73 mL, 7.11 mmol), DCC (1.66 g, 8.1 mmol), and DMAP (116 mg, 0.95 mmol) were sequentially added and the mixture was stirred for 3 h at rt (TLC hexane/AcOEt 7:3). The reaction mixture was filtered on a Celite pad and the solvent evaporated. Flash chromatography (hexane/AcOEt 8:2 to 7:3) afforded compound **72a** (1.045 g, 48%) as a white foam.

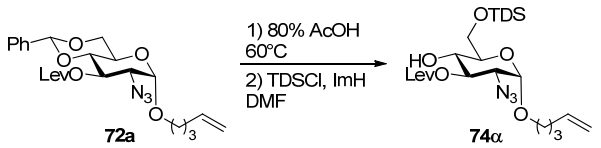
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.53 – 7.34 (m, 5H, arom.), 5.84 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.62 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.54 (s, 1H, PhC*H*), 5.17 – 4.94 (m, 3H, $O(CH_2)_3CHCH_2$, H-1), 4.31 (dd, $J_{5,6a}$ = 10.3, $J_{6a,6b}$ = 4.9 Hz, 1H, H-6a), 3.98 (m, 1H, H-5), 3.85 – 3.73 (m, 2H, H-6b, $OCHH(CH_2)_2CHCH_2$), 3.66 (t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1H, H-4), 3.54 (dt, J = 9.6, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.22 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 3.6 Hz, 1H, H-2), 2.81 (m, 2H, $H_3CCOCH_2CH_2COO$), 2.69 (m, 2H, $H_3CCOCH_2CH_2COO$), 2.20 (m, 5H, $O(CH_2)_2CH_2CHCH_2$, $H_3CCOCH_2CH_2COO$), 1.80 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 171.90 ($OCOCH_2CH_2COCH_3$), 137.85 ($O(CH_2)_3CHCH_2$), 115.42 ($O(CH_2)_3CHCH_2$), 101.75 (PhCH), 99.14 (C-1), 79.68 (C-4), 69.36 (C-3), 68.96 (C-6), 68.23 ($OCH_2(CH_2)_2CHCH_2$), 62.94 (C-5), 61.74 (C-2), 38.18 ($H_3CCOCH_2CH_2COO$), 30.32 ($O(CH_2)_3CH_2CHCH_2$), 29.89

(H_3*C*$COCH_2CH_2COO$), 28.96 (OCH_2*C*$H_2CH_2CHCH_2$), 28.14 (H_3CCOCH_2*C*H_2COO) ppm.

ESI-MS (MeOH) for $[C_{23}H_{29}N_3O_7Na]$+ calcd: 482.19, found: 482.1

4-Pentenyl 2-azido-2-deoxy-3-*O*-levulinoyl-6-*O*-thexyldimethylsilyl-α-D-glucopyranoside (74α)



Compound **72a** (1.045 g, 2.27 mmol) was dissolved in 80% aq. AcOH (25 mL) and the mixture was stirred at 60°C (TLC hexane/AcOEt 7:3). After 1 h, the reaction mixture was neutralized with solid K_2CO_3, diluted with water and extracted with DCM. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in DMF (20 mL). Imidazole (0.464 g, 6.81 mmol) was added and the mixture was cooled to 0°C. TDSCl (0.67 mL, 3.41 mmol) was added, and the mixture was warmed to rt. The reaction was monitored by TLC (hexane/AcOEt 3:7). After 2 h the solvent was removed, the crude was taken up in DCM and washed with water. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **74α** (1.062 g, 91%) as a light yellow oil.

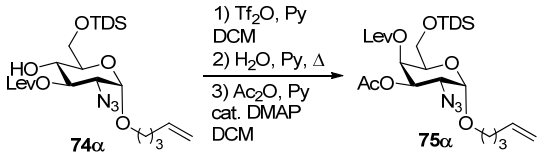
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.83 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.41 (dd, $J_{2,3}$ = 10.7, $J_{3,4}$ = 8.6 Hz, 1H, H-3), 5.11 – 4.97 (m, 2H, $O(CH_2)_3CHCH_2$), 4.93 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 3.86 (br d, J = 4.2 Hz, 2H, H-6a, H-6b), 3.82 – 3.64 (m, 3H, $OCHH(CH_2)_2CHCH_2$, H-5, H-4), 3.49 (dt, J = 9.6, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.14 (dd, $J_{2,3}$ = 10.7, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 3.00 – 2.77 (m, 2H, $H_3CCOCH_2CH_2COO$), 2.75 – 2.57 (m, 2H, $H_3CCOCH_2CH_2COO$), 2.25 – 2.13 (m, 5H, $H_3CCOCH_2CH_2COO$, $O(CH_2)_2CH_2CHCH_2$), 1.76 (m, 2H, $OCH_2CH_2CH_2CHCH_2$), 1.64 (hept, 1H, $OSiC(CH_3)_2CH(CH_3)_2$), 0.89 (m, 12H, $OSiC(CH_3)_2CH(CH_3)_2$), 0.14 (s, 6H, $OSi(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 207.54 ($OCOCH_2CH_2COCH_3$), 173.02 ($OCOCH_2CH_2COCH_3$), 138.00 ($O(CH_2)_3CHCH_2$), 115.22 ($O(CH_2)_3CHCH_2$), 98.06

(C-1), 73.80 (C-3), 71.64 (C-5), 70.82 (C-4), 67.72 ($OCH_2(CH_2)_2CHCH_2$), 63.21 (C-6), 60.86 (C-2), 38.56 ($H_3CCOCH_2CH_2COO$), 34.28 ($OSiC(CH_3)_2CH(CH_3)_2$), 30.37 ($O(CH_2)_3CH_2CHCH_2$), 29.88 ($H_3CCOCH_2CH_2COO$), 28.71 ($OCH_2CH_2CH_2CHCH_2$), 28.39 ($H_3CCOCH_2CH_2COO$), 20.45 ($OSiC(CH_3)_2CH(CH_3)_2$), 18.65 ($OSiC(CH_3)_2CH(CH_3)_2$), -3.30 ($OSi(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{24}H_{43}N_3O_7SiNa]+$ calcd: 536.28, found: 536.2

4-Pentenyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-levulinoyl-6-*O*-thexyldimethylsilyl-α-D-galactopyranoside (75α)



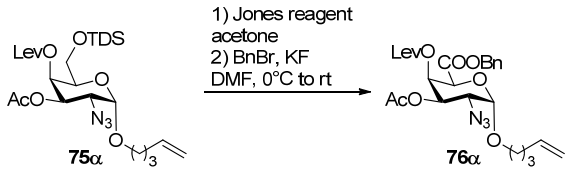
Compound **74α** (100 mg, 0.195 mmol) was dissolved in DCM (2 mL) and the mixture was cooled to 0°C. Pyridine (32 µL, 0.39 mmol) and Tf_2O (39 µL, 0.234 mmol) were added dropwise. The mixture was monitored by TLC (hexane/AcOEt 8:2). After 30 min, more pyridine (1 mL) and water (193 µL, 10.7 mmol) were added, and the mixture was stirred overnight at 40°C. The reaction mixture was then diluted with DCM and washed with 5% aq. HCl. The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude was dissolved in DCM (2 mL), pyridine (63 µL, 0.78 mmol), catalytic DMAP and Ac_2O (37 µL, 0.39 mmol) were sequentially added at rt. After 1 h the mixture was quenched with water, diluted with DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 9:1) afforded compound **75α** (65 mg, 60%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42 – 7.32 (m, 5H, arom.), 5.80 (m, 2H, $O(CH_2)_3CHCH_2$, H-4), 5.41 (dd, $J_{2,3}$ = 11.1, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 5.27 (d, J = 11.9 Hz, 1H, $COOCHPh$), 5.17 – 5.12 (m, 2H, $COOCHPh$), 5.08 – 4.97 (m, 2H, $O(CH_2)_3CHCH_2$), 4.65 (br d, J = 1.6 Hz, 1H, H-5), 3.79 (dt, J = 9.7, 6.5 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.66 (dd, $J_{2,3}$ = 11.1, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 3.58 (dt, J = 9.7, 6.5 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.68 – 2.47 (m, 3H, $H_3CCOCH_2CH_2COO$, H_3CCOCH_2CHCOO), 2.33 – 2.24 (m, 1H, H_3CCOCH_2CHCOO), 2.22 – 2.13 (m, 5H, $H_3CCOCH_2CH_2COO$, $O(CH_2)_2CH_2CHCH_2$), 2.06 (s, 3H, $OCOCH_3$), 1.76 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 205.99 ($OCOCH_2CH_2\mathit{C}OCH_3$), 171.45 ($O\mathit{C}OCH_2CH_2COCH_3$), 170.07 ($\mathit{C}OOBn$ or $O\mathit{C}OCH_3$), 166.78 ($O\mathit{C}OCH_3$ or $\mathit{C}OOBn$), 137.73 ($O(CH_2)_3\mathit{C}HCH_2$), 115.44 ($O(CH_2)_3CH\mathit{C}H_2$), 98.47 (C-1), 68.91 (C-5), 68.87 ($O\mathit{C}H_2(CH_2)_2CHCH_2$), 68.81(C-4), 67.82 (C-3), 67.74 ($COO\mathit{C}H_2Ph$), 57.1 (C-2), 37.75 ($H_3CCO\mathit{C}H_2CH_2COO$), 30.21 ($O(CH_2)_3\mathit{C}H_2CHCH_2$), 29.86 ($H_3\mathit{C}COCH_2CH_2COO$), 28.58 ($OCH_2\mathit{C}H_2CH_2CHCH_2$), 27.66 ($H_3CCOCH_2\mathit{C}H_2COO$), 20.69 ($OCO\mathit{C}H_3$) ppm.

ESI-MS (MeOH) for $[C_{26}H_{45}N_3O_8SiNa]+$ calcd: 578.29, found: 578.3

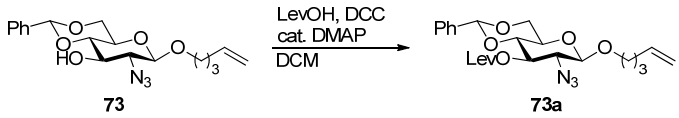
Benzyl (4-pentenyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-levulinoyl-α-D-galactopyranosiduronate) (76α)



Compound **75α** (300 mg, 0.54 mmol) was dissolved in acetone (4 mL) at -15°C. Jones reagent (3 mL) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 20 min the mixture was quenched carefully with EtOH, neutralized with satd. $NaHCO_3$ and washed with chloroform. The aqueous phase was acidified to pH 2 with 5% aq. HCl and extracted with chloroform. The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude was dissolved in DMF (5 mL), KF (0.29 g, 5 mmol) and BnBr (0.3 mL, 2.5 mmol) were added at 0°C. The mixture was warmed to rt and stirred overnight (TLC hexane/AcOEt 8:2). The solvent was evaporated, the crude was taken up in DCM and washed with water. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **76α** (177 mg, 54%) as a pale yellow oil.

ESI-MS (MeOH) for $[C_{25}H_{31}N_3O_9Na]+$ calcd: 540.20, found: 540.1

4-Pentenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-D-glucopyranoside (73a)



73 **73a**

A solution of MeONa in MeOH (0.4 M, 2.75 mL, 1.1 mmol) was added to compound **73** (1.481 g, 3.67 mmol) dissolved in MeOH (35 mL). The reaction was followed by TLC (hexane/AcOEt 8:2). After 2 h the reaction was neutralized with Amberlite IR-120, filtered and the solvent was removed *in vacuo*. The crude was dissolved in DCM (35 mL), levulinic acid (0.56 mL, 5.51 mmol), DCC (1.287 g, 6.24 mmol), and DMAP (90 mg, 0.73 mmol) were sequentially added and the mixture was stirred for 3 h at rt (TLC hexane/AcOEt 8:2). The reaction mixture was filtered on a Celite pad and the solvent evaporated. Flash chromatography (hexane/AcOEt 8:2 to 7:3) afforded compound **73a** (1.96 g, quant.) as a white foam.

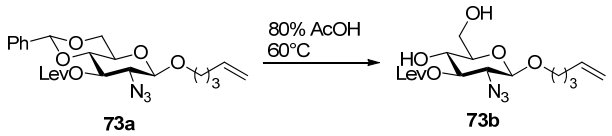
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.49 – 7.34 (m, 5H, arom.), 5.84 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.52 (s, 1H, PhC*H*), 5.18 – 4.99 (m, 3H, H-3, $O(CH_2)_3CHCH_2$), 4.50 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.37 (dd, $J_{6a,6b}$ = 10.5, $J_{5,6a}$ = 5.0 Hz, 1H, H-6a), 3.97 (dt, J = 9.5, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.81 (t, $J_{5,6b}$ = $J_{6a,6b}$ = 10.3 Hz, 1H, H-6b), 3.74 – 3.60 (m, 2H, H-4, $OCHH(CH_2)_2CHCH_2$), 3.55 – 3.43 (m, 2H, H-2, H-5), 2.79 (m, 2H, $H_3CCOCH_2CH_2COO$), 2.69 (m, 2H, $H_3CCOCH_2CH_2COO$), 2.27 – 2.15 (m, 5H, $O(CH_2)_2CH_2CHCH_2$, $H_3CCOCH_2CH_2COO$), 1.78 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 206.10 ($OCOCH_2CH_2COCH_3$), 171.79 ($OCOCH_2CH_2COCH_3$), 137.86 ($O(CH_2)_3CHCH_2$), 115.33 ($O(CH_2)_3CHCH_2$), 102.85 (PhCH), 101.57 (C-1), 78.81 (C-4), 71.54 (C-3), 70.71 ($OCH_2(CH_2)_2CHCH_2$), 68.63 (C-6), 66.55 (C-5), 64.99 (C-2), 38.13

($H_3CCOCH_2CH_2COO$), 30.09 ($O(CH_2)_3CH_2CHCH_2$), 29.89 ($H_3CCOCH_2CH_2COO$), 28.88 ($OCH_2CH_2CH_2CHCH_2$), 28.12 ($H_3CCOCH_2CH_2COO$) ppm.

ESI-MS (MeOH) for $[C_{23}H_{29}N_3O_7Na]+$ calcd: 482.19, found: 482.3

4-Pentenyl 2-azido-2-deoxy-3-*O*-levulinoyl-β-D-glucopyranoside (73b)



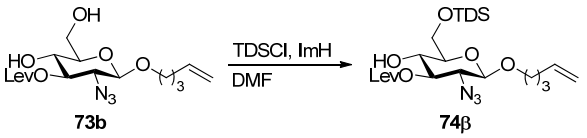
73a **73b**

Compound **73a** (0.1.686 g, 3.67 mmol) was dissolved in 80% aq. AcOH (30 mL) and the mixture was stirred at 60°C (TLC hexane/AcOEt 7:3). After 1 h, the reaction mixture was neutralized with solid K_2CO_3, diluted with water and extracted with DCM. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 3:7) afforded compound **73b** (1.295 g, 95%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.67 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 4.95 – 4.82 (m, 2H, $O(CH_2)_3CHCH_2$), 4.73 (dd, $J_{3,4}$ = 10.4, $J_{2,3}$ = 9.1 Hz, 1H, H-3), 4.27 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 3.80 (m, 2H, $OCHH(CH_2)_2CHCH_2$, H-6a), 3.69 (dd, *J* = 11.9, 4.6 Hz, 1H, H-6b), 3.56 (t, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, 1H, H-4), 3.45 (dt, *J* = 9.5, 6.7 Hz, 1H, $OCH(CH_2)_2CHCH_2$), 3.27 (m, 2H, H-2, H-5), 2.82 (ddd, *J* = 18.6, 9.1, 5.0 Hz, 1H, $H_3CCOCHHCH_2COO$), 2.65 (ddd, *J* = 18.6, 6.2, 4.6 Hz, 1H, $H_3CCOCHHCH_2COO$), 2.52 (ddd, *J* = 16.6, 9.1, 4.5 Hz, 1H, , $H_3CCOCH_2CHHCOO$), 2.42 (ddd, *J* = 16.6, 6.2, 5.0 Hz, 1H, $H_3CCOCH_2CHHCOO$), 2.09 – 1.98 (m, 5H, $H_3CCOCH_2CH_2COO$, $O(CH_2)_2CH_2CHCH_2$), 1.61 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

ESI-MS (MeOH) for $[C_{16}H_{25}N_3O_7Na]$+ calcd: 394.16, found: 394.1

4-Pentenyl 2-azido-2-deoxy-3-*O*-levulinoyl-6-*O*-thexyldimethylsilyl-β-D-glucopyranoside (74β)



73b **74β**

Compound **73b** (1.363 g, 3.67 mmol) was dissolved in DMF (35 mL). Imidazole (0.75 g, 11 mmol) was added and the mixture was cooled to 0°C. TDSCl (1.08 mL, 5.51 mmol) was added, and the mixture was warmed to rt. The reaction was monitored by TLC (hexane/AcOEt 3:7). After 2 h the solvent was removed, the crude was taken up in DCM and washed with water. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **74β** (1.603 g, 85%) as a light yellow oil.

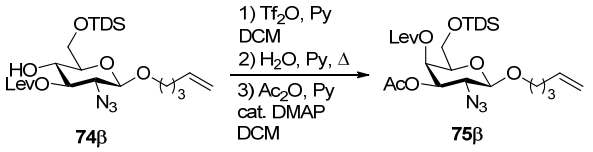
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 5.83 (ddt, J = 16.9, 10.1, 6.7 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.13 – 4.95 (m, 2H, $O(CH_2)_3CHCH_2$)), 4.88 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 9.1 Hz, 1H, H-3), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 3.98 – 3.86 (m, 3H, $OCHH(CH_2)_2CHCH_2$, H-6a, H-6b), 3.65 (t, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, 1H, H-4), 3.58 (dt, J = 9.6, 6.6 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.43 – 3.34 (m, 2H, H-2, H-5), 2.96 – 2.77 (m, 2H, $H_3CCOCH_2CH_2COO$), 2.74 – 2.55 (m, 2H, $H_3CCOCH_2CH_2COO$), 2.25 – 2.13 (m, 5H, $H_3CCOCH_2CH_2COO$, $O(CH_2)_2CH_2CHCH_2$), 1.76 (m, 2H, $OCH_2CH_2CH_2CHCH_2$), 1.64 (m, 1H, $OSiC(CH_3)_2CH(CH_3)_2$), 0.89 (m, 12H, $OSiC(CH_3)_2CH(CH_3)_2$), 0.14 (m, 6H, $OSi(CH_3)_2$) ppm.

^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 207.49 ($OCOCH_2CH_2COCH_3$), 172.86 ($OCOCH_2CH_2COCH_3$), 138.01 ($O(CH_2)_3CHCH_2$), 115.17 ($O(CH_2)_3CHCH_2$), 101.98 (C-1), 75.92 (C-3), 75.28 (C-5), 70.88 (C-4), 69.54 ($OCH_2(CH_2)_2CHCH_2$), 63.84 (C-2), 63.57 (C-6), 38.51 ($H_3CCOCH_2CH_2COO$), 34.30 ($OSiC(CH_3)_2CH(CH_3)_2$), 30.17 ($O(CH_2)_3CH_2CHCH_2$), 29.90 ($H_3CCOCH_2CH_2COO$), 28.90 ($OCH_2CH_2CH_2CHCH_2$), 28.36

($H_3CCOCH_2CH_2COO$), 20.47 ($OSiC(CH_3)_2CH(CH_3)_2$), 18.65 ($OSiC(CH_3)_2CH(CH_3)_2$), -3.31 ($OSi(CH_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{24}H_{43}N_3O_7SiNa]+$ calcd: 536.28, found: 536.3

4-Pentenyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-levulinoyl-6-*O*-thexyldimethylsilyl-β-D-galactopyranoside (75β)



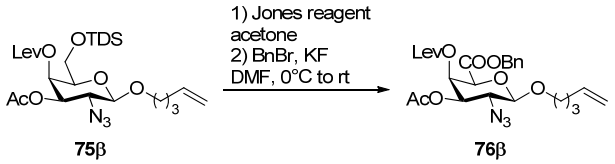
Compound **74β** (200 mg, 0.39 mmol) was dissolved in DCM (4 mL) and the mixture was cooled to 0°C. Pyridine (63 µL, 0.78 mmol) and Tf_2O (79 µL, 0. 47 mmol) were added dropwise. The mixture was monitored by TLC (hexane/AcOEt 8:2). After 30 min, more pyridine (2 mL) and water (700 µL, 39 mmol) were added, and the mixture was stirred overnight at 40°C. The reaction mixture was then diluted with DCM and washed with 5% aq. HCl. The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude was dissolved in DCM (4 mL), pyridine (315 µL, 3.9 mmol), catalytic DMAP and Ac_2O (185 µL, 1.95 mmol) were sequentially added at rt. After 1 h the mixture was quenched with water, diluted with DCM and washed with 5% aq. HCl, satd. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 9:1) afforded compound **75β** (173.4 mg, 80%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42 – 7.31 (m, 5H, arom.), 5.84 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.68 (dd, $J_{3,4}$ = 3.5, $J_{4,5}$ = 1.4 Hz, 1H, H-4), 5.27 (d, *J* = 11.9 Hz, 1H, $COOCHHPh$), 5.13 (d, *J* = 11.9 Hz, 1H, $COOCHHPh$), 5.11 – 4.97 (m, 2H, $O(CH_2)_3CHCH_2$), 4.79 (dd, $J_{2,3}$ = 10.9, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.27 (d, $J_{4,5}$ = 1.4 Hz, 1H, H-5), 4.08 (dt, *J* = 9.4, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.71 (dd, $J_{2,3}$ = 10.9, $J_{1,2}$ = 8.0 Hz, 1H, H-2), 3.60 (dt, *J* = 9.5, 6.8 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.72 – 2.50 (m, 3H, $H_3CCOCH_2CH_2COO$, $H_3CCOCH_2CHHCOO$), 2.37 – 2.25 (m, 1H, $H_3CCOCH_2CHHCOO$), 2.25 – 2.15 (m, 5H, $H_3CCOCH_2CH_2COO$, $O(CH_2)_2CH_2CHCH_2$), 2.06 (s, 3H, $OCOCH_3$), 1.80 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 205.96 ($OCOCH_2CH_2\mathit{C}OCH_3$), 171.53 ($O\mathit{C}OCH_2CH_2COCH_3$), 170.08 ($\mathit{C}OOBn$ or $O\mathit{C}OCH_3$), 165.81 ($O\mathit{C}OCH_3$ or $\mathit{C}OOBn$), 137.97 ($O(CH_2)_3\mathit{C}HCH_2$), 115.26 ($O(CH_2)_3CH\mathit{C}H_2$), 102.32 (C-1), 72.36 (C-5), 70.66 (C-3), 70.14 ($O\mathit{C}H_2(CH_2)_2CHCH_2$), 67.75 (C-4), 67.73 ($COO\mathit{C}H_2Ph$), 60.64 (C-2), 37.72 ($H_3CCO\mathit{C}H_2CH_2COO$), 30.10 ($O(CH_2)_3\mathit{C}H_2CHCH_2$), 29.91 ($H_3\mathit{C}COCH_2CH_2COO$), 28.77 ($OCH_2\mathit{C}H_2CH_2CHCH_2$), 27.67 ($H_3CCOCH_2\mathit{C}H_2COO$), 20.64 ($OCO\mathit{C}H_3$) ppm.

ESI-MS (MeOH) for $[C_{26}H_{45}N_3O_8SiNa]+$ calcd: 578.29, found: 578.1

Benzyl (4-pentenyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-levulinoyl-β-D-galactopyranosiduronate) (76β)



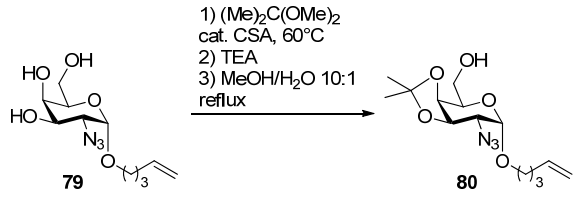
75β **76β**

Compound **75β** (100 mg, 0.17 mmol) was dissolved in acetone (2 mL) at -15°C. Jones reagent (1 mL) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 15 min the mixture was quenched carefully with EtOH, neutralized with satd. $NaHCO_3$ and washed with chloroform. The aqueous phase was acidified to pH 2 with 5% aq. HCl and extracted with chloroform. The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude was dissolved in DMF (2 mL), KF (87 mg, 1.5 mmol) and BnBr (90 µL, 0.75 mmol) were added at 0°C. The mixture was warmed to rt and stirred overnight (TLC hexane/AcOEt 8:2). The solvent was evaporated, the crude was taken up in DCM and washed with water. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 8:2) afforded compound **76β** (50 mg, 57%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.40 – 7.32 (m, 5H, arom.), 5.83 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.68 (dd, $J_{3,4}$ = 3.5, $J_{4,5}$ = 1.5 Hz, 1H, H-4), 5.26 (d, J = 11.9 Hz, 1H, $COOCHHPh$), 5.13 (d, J = 11.9 Hz, 1H, $COOHHPh$), 5.10 – 4.96 (m, 2H, $O(CH_2)_3CHCH_2$), 4.79 (dd, $J_{2,3}$ = 10.9, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 4.37 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1), 4.27 (d, $J_{4,5}$ = 1.5 Hz, 1H, H-5), 4.08 (dt, J = 9.4, 6.3 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.70 (dd, $J_{2,3}$ = 10.9, $J_{1,2}$ = 8.0 Hz, 1H, H-2), 3.60 (dt, J = 9.5, 6.8 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.70 – 2.49 (m, 3H, $H_3CCOCH_2CH_2COO$, $H_3CCOCH_2CHHCOO$), 2.35 – 2.25 (m, 1H, $H_3CCOCH_2CHHCOO$), 2.25 – 2.13 (m, 5H, $O(CH_2)_2CH_2CHCH_2$, $H_3CCO(CH_2)_2COO$), 1.79 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

ESI-MS (MeOH) for $[C_{25}H_{31}N_3O_9Na]$+ calcd: 540.20, found: 540.2

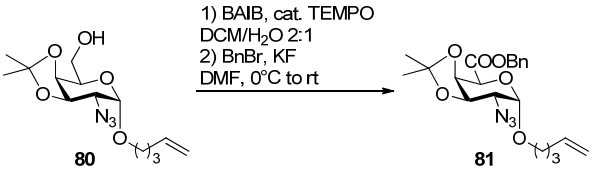
4-Pentenyl 2-azido-2-deoxy-3,4-di-*O*-isopropylidene-α-D-galactopyranoside (80)



Compound **79** (200 mg, 0.73 mmol) was dissolved in 2,2-dimethoxypropane (7 mL). CSA (17 mg, 0.073 mmol) was added and the mixture was refluxed for 3 h (TLC hexane/AcOEt 6:4). Then the reaction mixture was quenched with TEA, stirred of 15 min, the solvent was evaporated and the crude was coevaporated with toluene. The residue was dissolved in MeOH/H_2O 10:1 (11 mL) and the mixture was refluxed for 2.5 h. Then the solvent was evaporated *in vacuo* and the residue was purified by flash chromatography (hexane/AcOEt 7:3 + 0.1% TEA), yielding compound **80** (190.9 mg, 83%) as a pale yellow oil.

ESI-MS (MeOH) for $[C_{14}H_{23}N_3O_5Na]$+ calcd: 336.15, found: 336.1

Benzyl (4-pentenyl 2-azido-2-deoxy-3,4-di-*O*-isopropylidene-α-D-galactopyranosiduronate) (81)



Compound **80** (0.91 g, 2.7 mmol) was dissolved in DCM/H_2O 2:1 (15 mL), then BAIB (1.305 g, 4.05 mmol) and TEMPO (42 mg, 0.27 mmol) were added. The reaction was monitored by TLC (DCM/MeOH 7:3). After 3 h the mixture was quenched with ethanol, the solvent was evaporated and the residue was coevaporated with toluene. The crude was dissolved in DMF (30 mL) at 0°C. KF (1.74 g, 3 mmol) and BnBr (1.78 mL, 1.5 mmol) were added, and the mixture was stirred overnight at rt (TLC hexane/AcOEt 7:3). Then the solvent was removed, the crude was taken up in DCM and washed with water. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 9:1) afforded compound **81** (1.0288 g 91%) as a pale yellow oil.

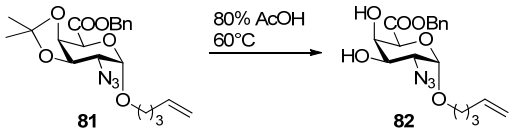
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.44 – 7.32 (m, 5H, arom.), 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.42 (d, *J* = 12.3 Hz, 1H, $COOCHHPh$), 5.22 (d, *J* = 12.3 Hz, 1H, $COOCHHPh$), 5.10 – 4.96 (m, 3H, $O(CH_2)_3CHCH_2$, H-1), 4.70 (d, $J_{4,5}$ = 2.9 Hz, 1H, H-5), 4.57 (dd, $J_{3,4}$ = 5.4, $J_{4,5}$ = 2.9 Hz, 1H, H-4), 4.45 (dd, $J_{2,3}$ = 8.4, $J_{3,4}$ = 5.4 Hz, 1H, H-3), 3.77 (dt, *J*=9.8, 6.6 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.53 (dt, *J*=9.8, 6.4 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.43 (dd, $J_{2,3}$ = 8.4, $J_{1,2}$ = 3.3 Hz, 1H, H-2), 2.15 (m, 2H, $O(CH_2)_2CH_2CHCH_2$), 1.73 (m, 2H, $OCH_2CH_2CH_2CHCH_2$), 1.53 (s, 3H, $C(CH_3)_2$), 1.35 (s, 3H, $C(CH_3)_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 167.49 (*C*OOBn), 137.68 ($O(CH_2)_3CHCH_2$), 115.25 ($O(CH_2)_3CHCH_2$), 110.20 ($C(CH_3)_2$), 98.16 (C-1), 73.31 (C-3), 73.17 (C-4), 68.51 ($OCH_2(CH_2)_2CHCH_2$), 67.67 (C-5), 67.06 ($COOCH_2Ph$),

60.54 (C-2), 30.09 ($O(CH_2)_3\mathit{C}H_2CHCH_2$), 28.46 ($OCH_2\mathit{C}H_2CH_2CHCH_2$), 28.12 ($C(\mathit{C}H_3)_2$), 26.14 ($C(\mathit{C}H_3)_2$) ppm.

ESI-MS (MeOH) for $[C_{21}H_{27}N_3O_6Na]+$ calcd: 440.18, found: 440.2

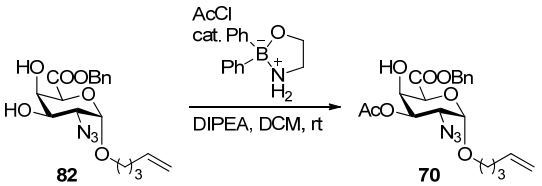
Benzyl (4-pentenyl 2-azido-2-deoxy-α-D-galactopyranosiduronate) (82)



Compound **81** (1.0 g, 2.4 mmol) was dissolved in 80% aq. AcOH (15 mL) and heated at 70°C. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 4 h the mixture was cooled to rt, neutralized with K_2CO_3, diluted with water and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered and concentrated, affording compound **82** (905.7 mg, quant.) as a pale yellow oil.

ESI-MS (MeOH) for $[C_{18}H_{23}N_3O_6Na]+$ calcd: 400.15, found: 400.2

Benzyl (4-pentenyl 3-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosiduronate) (70)



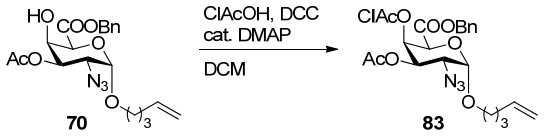
Compound **82** (127 mg, 0.34 mmol) was dissolved in CH_3CN (5 mL), 2-aminoethyldiphenilborinate (8 mg, 34 μmol), DIPEA (118 μL, 0.68 mmol) and AcCl (36 μL, 0.51 mmol) were sequentially added. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 2 h the mixture was diluted with water and extracted with DCM. The organic phase was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (hexane/AcOEt 7:3) afforded compound **70** (111.9 mg, 80%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.44 – 7.34 (m, 5H, arom.), 5.81 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H, $O(CH_2)_3CHCH_2$), 5.37 (dd, $J_{2,3}$ = 11.1, $J_{3,4}$ = 3.1 Hz, 1H, H-3), 5.34 – 5.23 (m, 2H, $COOCH_2Ph$), 5.13 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 5.09 – 4.96 (m, 2H, $O(CH_2)_3CHCH_2$), 4.57 (br d, $J_{4,5}$ = 1.5 Hz, 1H, H-5), 4.53 (dd, $J_{3,4}$ = 3.1, $J_{4,5}$ = 1.5 Hz, 1H, H-4), 3.80 (dt, J = 9.7, 6.6 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 3.74 (dd, $J_{2,3}$ = 11.0, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 3.58 (dt, J = 9.7, 6.5 Hz, 1H, $OCHH(CH_2)_2CHCH_2$), 2.23 – 2.13 (m, 5H, $OCOCH_3$), 1.77 (m, 2H, $OCH_2CH_2CH_2CHCH_2$) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$, 25°C): δ = 115.27 ($O(CH_2)_3CHCH_2$), 98.42 (C-1), 69.96 (C-4), 69.82 (C-3), 68.60 ($OCH_2(CH_2)_2CHCH_2$), 68.39 (C-5), 67.43 ($COOCH_2Ph$), 56.83 (C-2), 30.11 ($O(CH_2)_3CH_2CHCH_2$), 28.46 ($OCH_2CH_2CH_2CHCH_2$), 20.93 ($OCOCH_3$) ppm.

ESI HR-MS (MeOH) for $[C_{20}H_{25}N_3O_7Na]$+ calcd: 442.1585, found: 442.1602

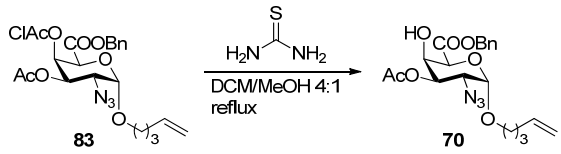
Benzyl (4-pentenyl 3-*O*-acetyl-2-azido-4-*O*-chloroacetyl-2-deoxy-α-D-galactopyranosiduronate) (83)



Compound **70** (110 mg, 0.27 mmol) was dissolved in DCM (3 mL), chloroacetic acid (38 mg, 0.41 mmol), DCC (95 mg, 0.46 mmol) and DMAP (6 mg, 5 μmol) were sequentially added. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 30 min the mixture was filtered on a Celite pad and the solvent was evaporated. Flash chromatography (hexane/AcOEt 9:1) afforded compound **83** (119 mg, 89%) as a pale yellow oil.

ESI-MS (MeOH) for $[C_{22}H_{26}ClN_3O_8Na]+$ calcd: 518.13, found: 518.2

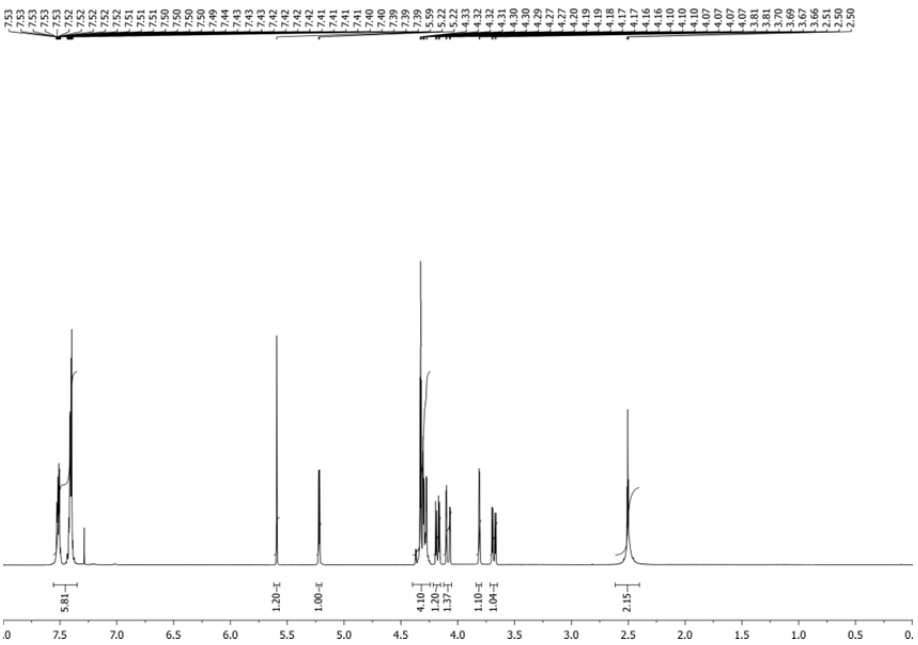
ClAc removal on compound 83



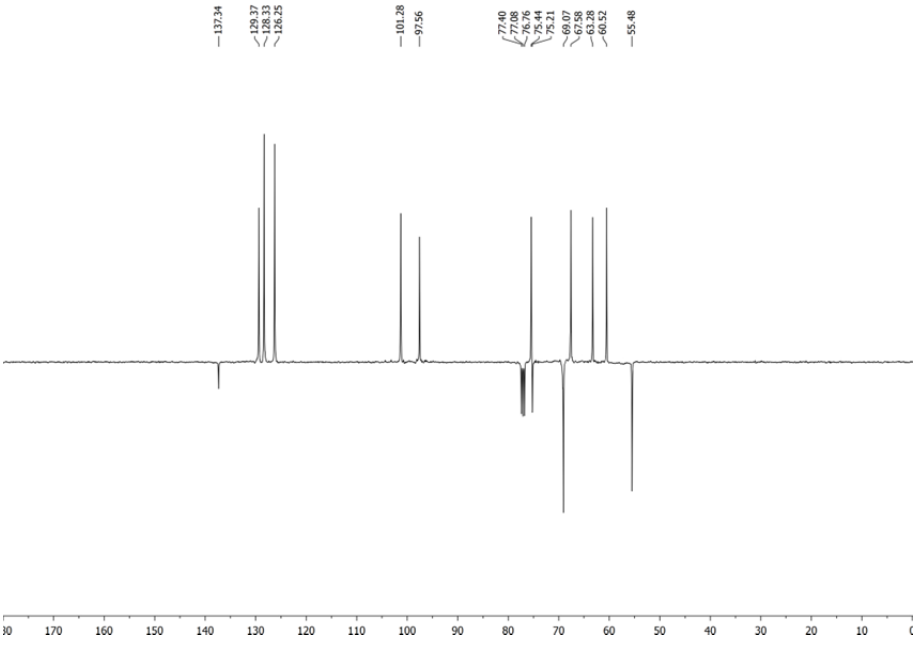
Compound **83** (30 mg, 60 μmol) was dissolved in DCM/MeOH 4:1 (5 mL), thiourea (23 mg, 300 μmol) was added and the mixture was refluxed overnight (TLC hexane/AcOEt 6:4). Then the solvent was concentrated and the crude was purified by flash chromatography (hexane/AcOEt 7:3), affording compound **70** (23.9 mg, 95%) as a pale yellow oil.

6.3 NMR spectra

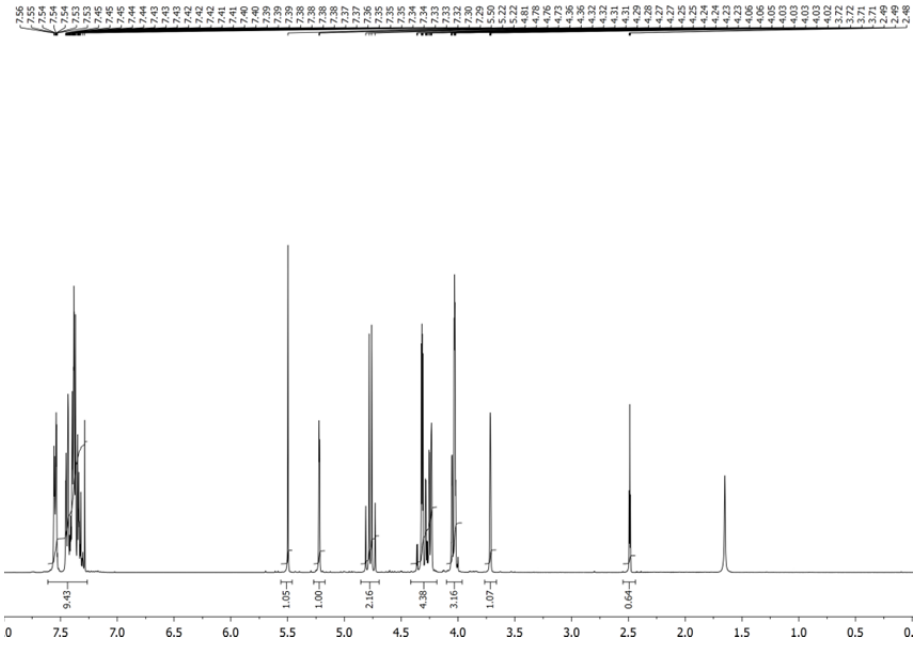
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **13**



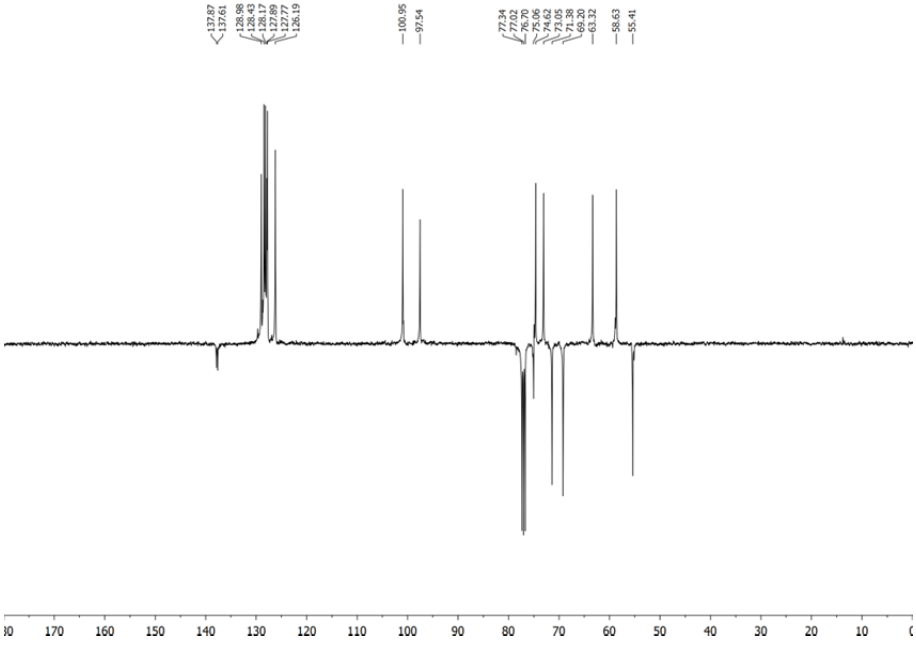
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



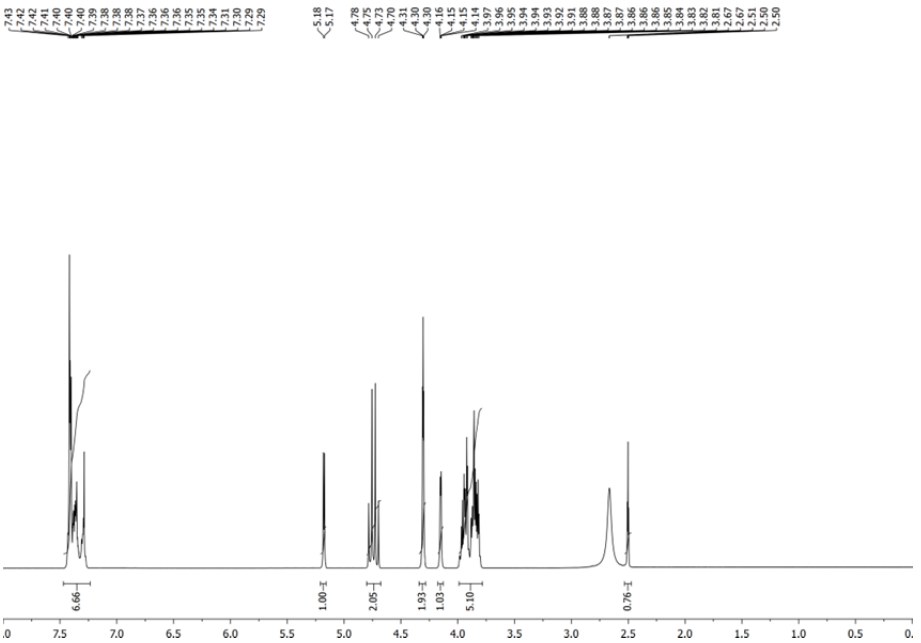
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **14**



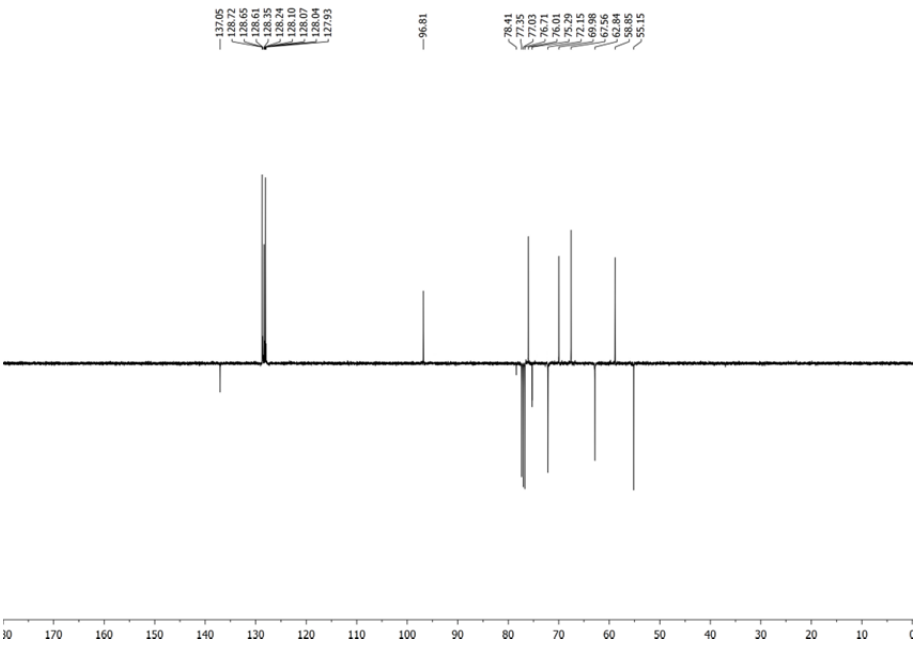
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



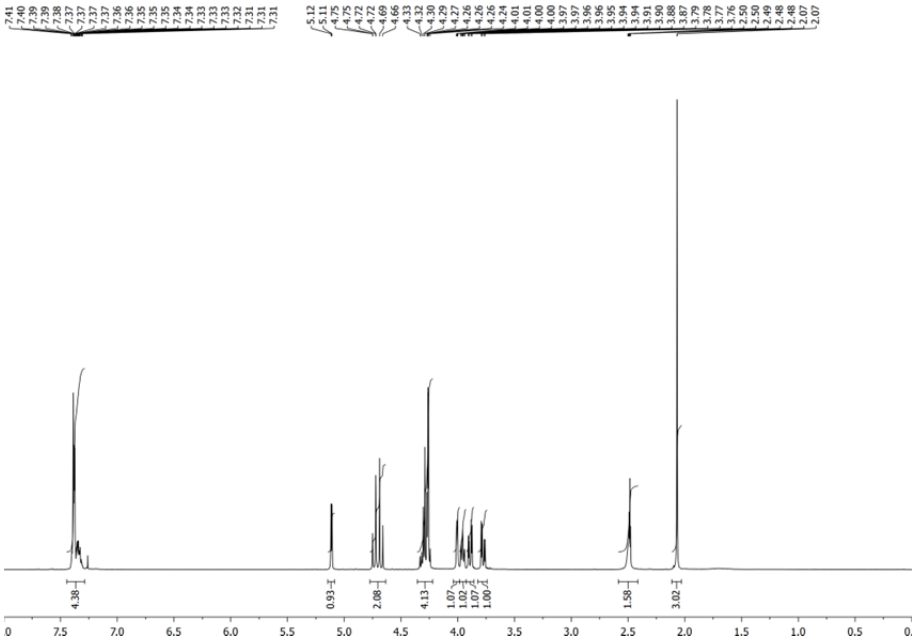
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **15**



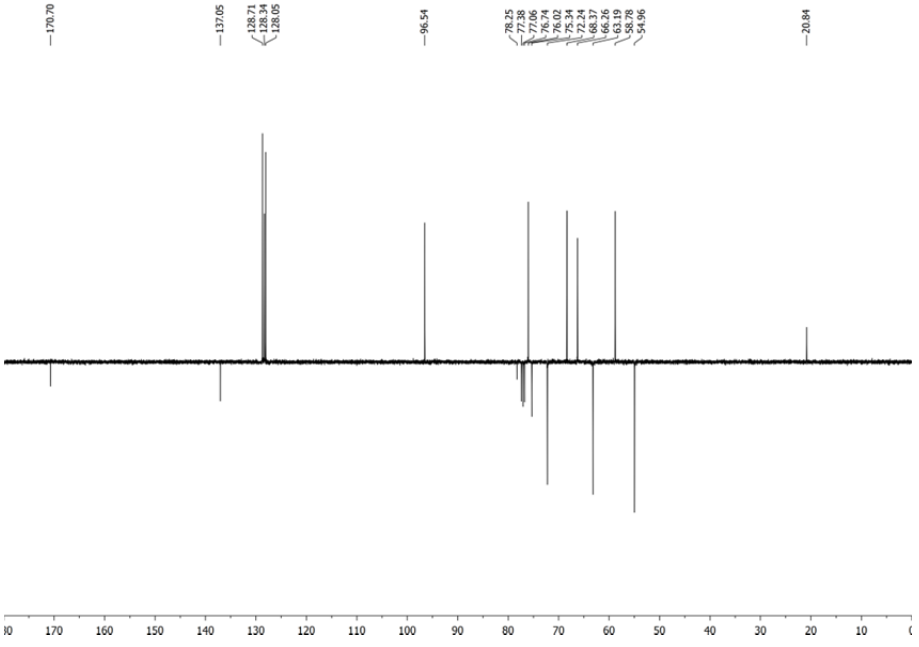
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



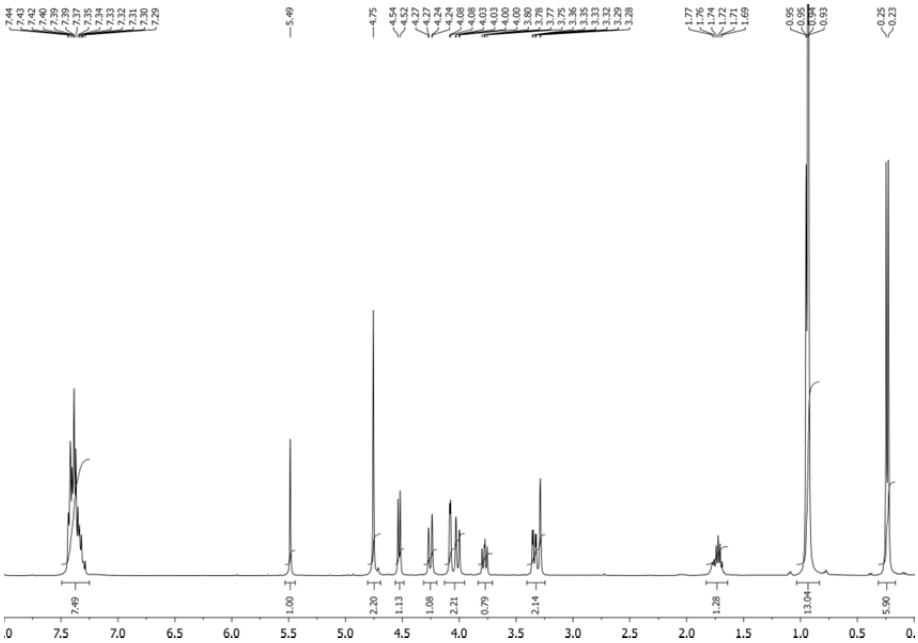
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **7a**



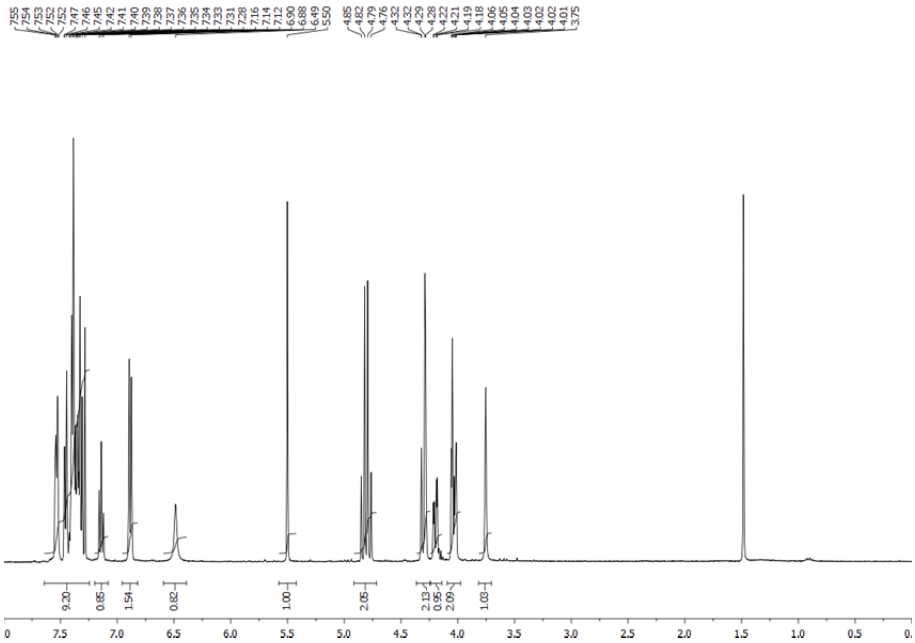
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



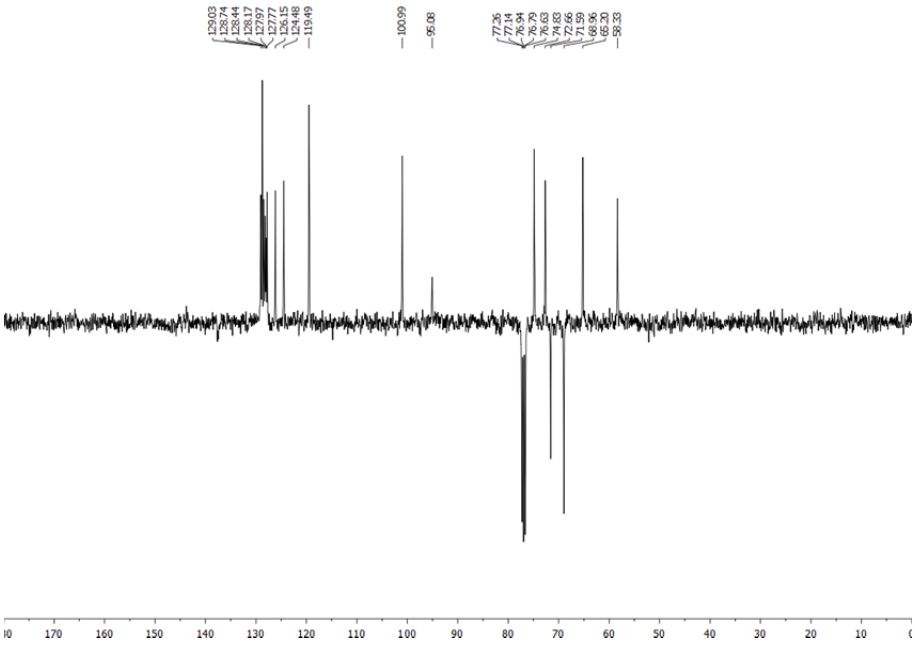
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **19**



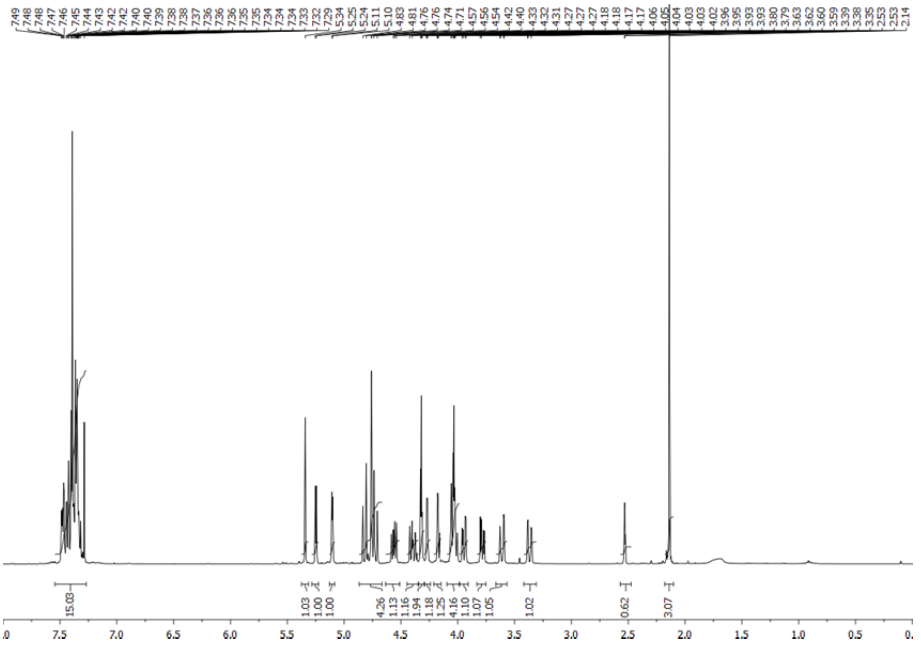
^{1}H NMR spectrum (400 Mhz, $CDCl_3$, 55°C) of compound **6α**



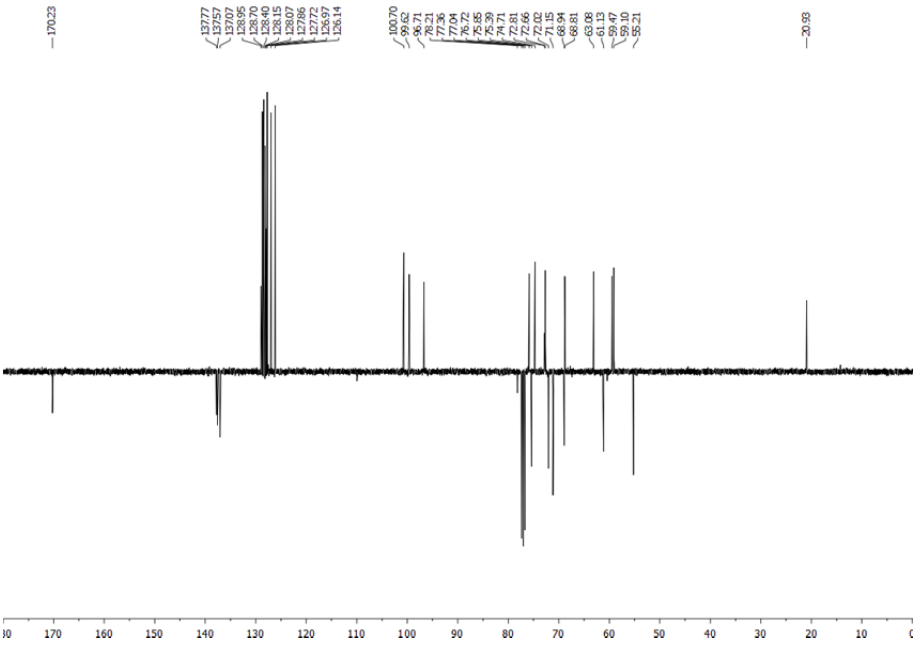
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$, 55°C)



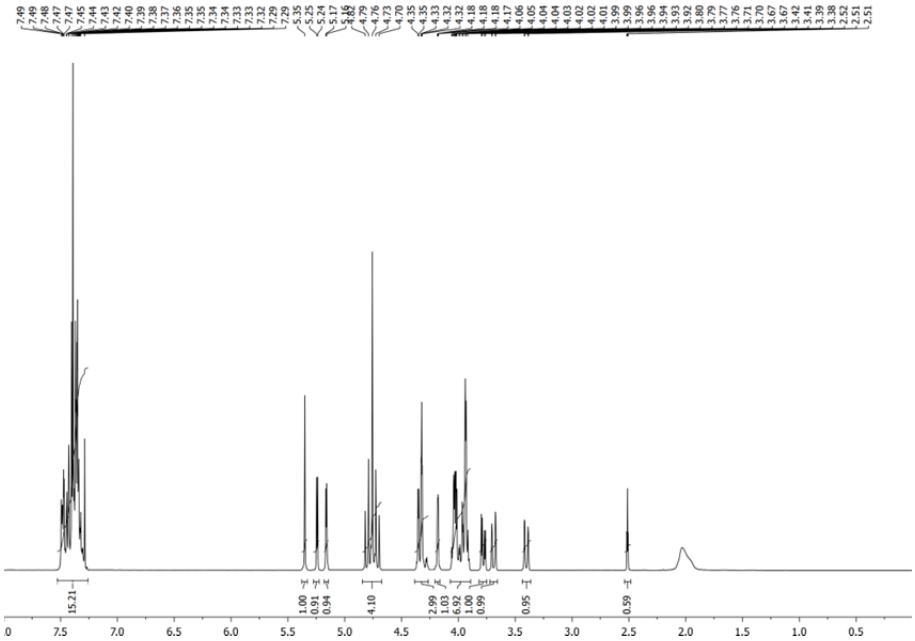
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **21**



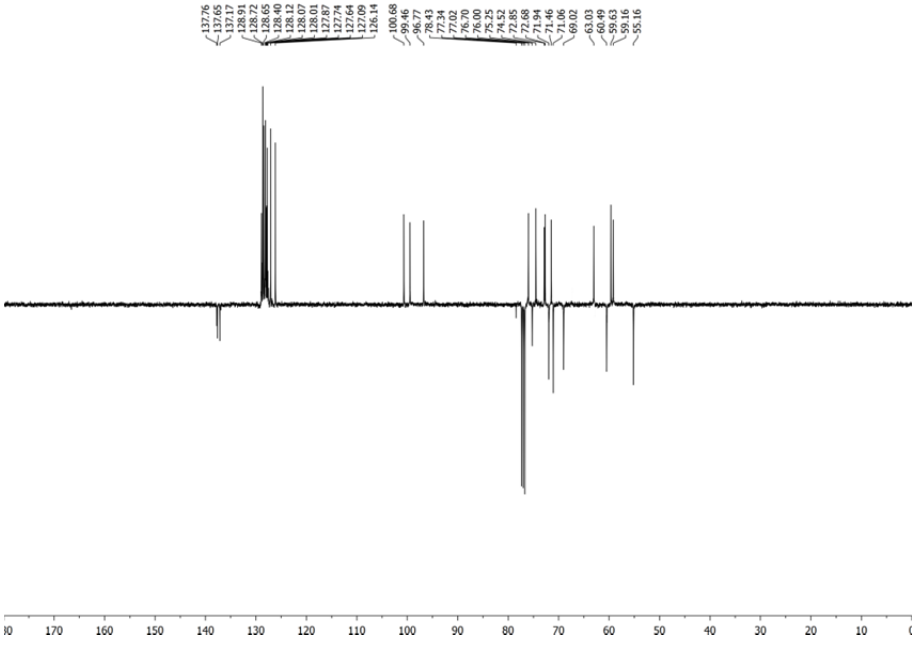
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



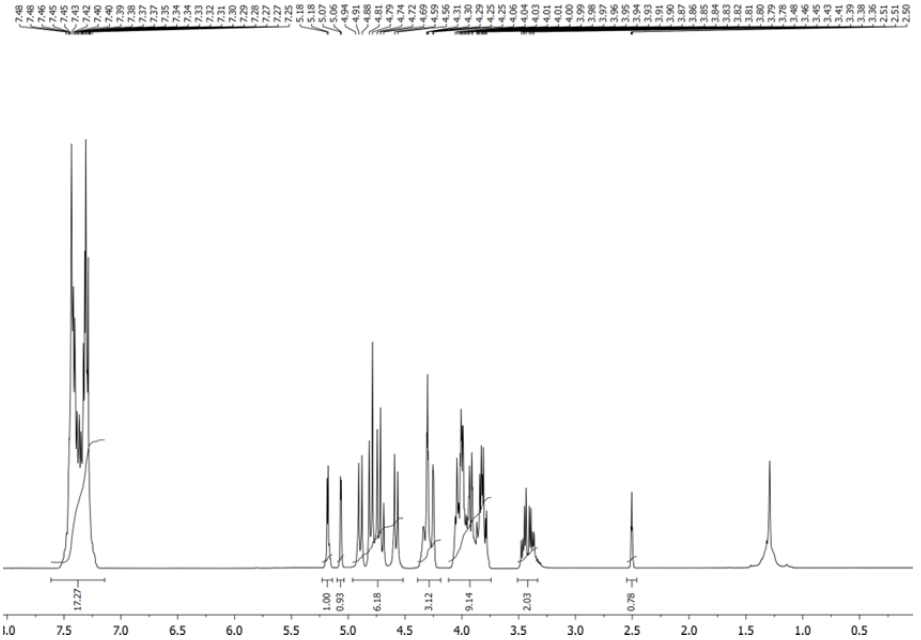
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **23**



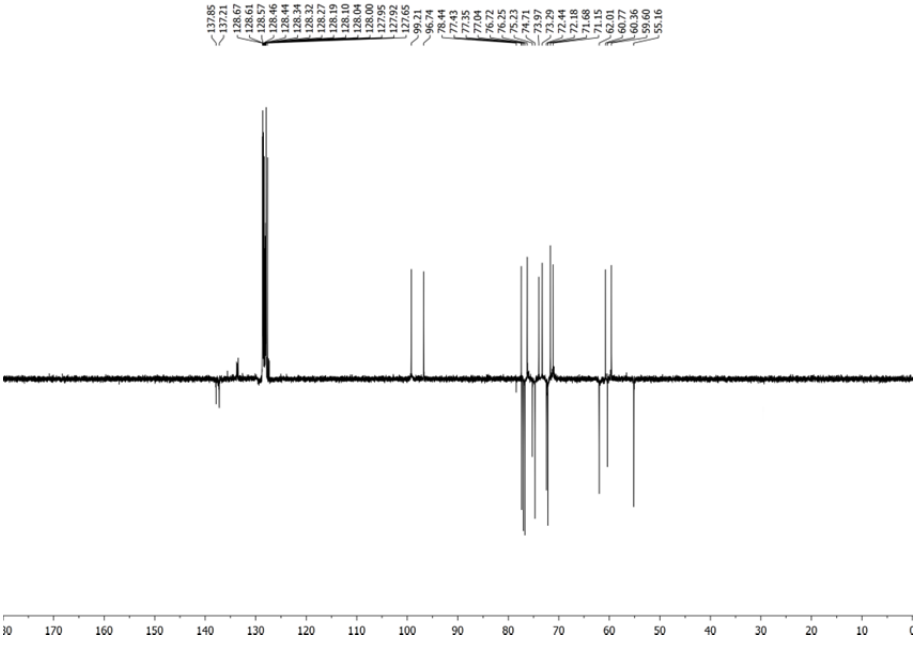
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



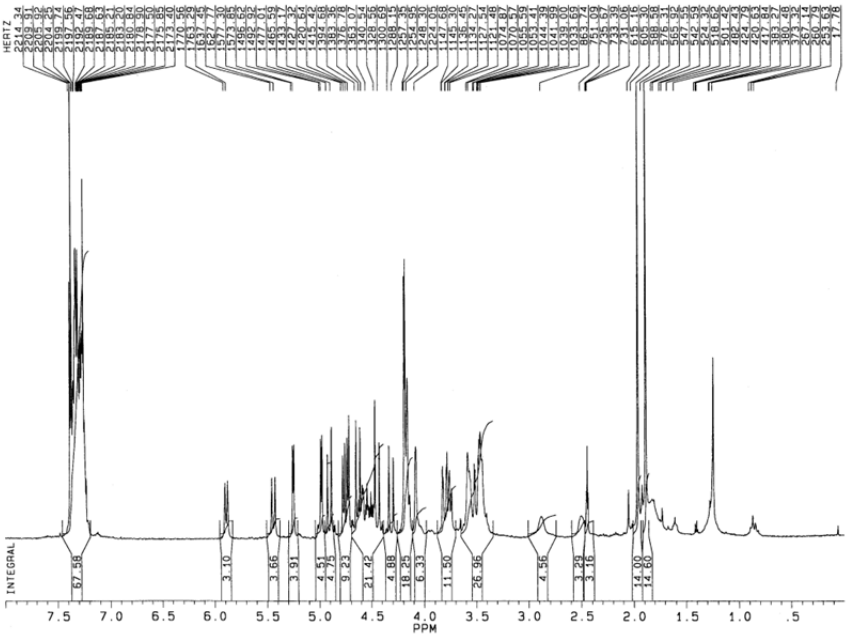
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **24**



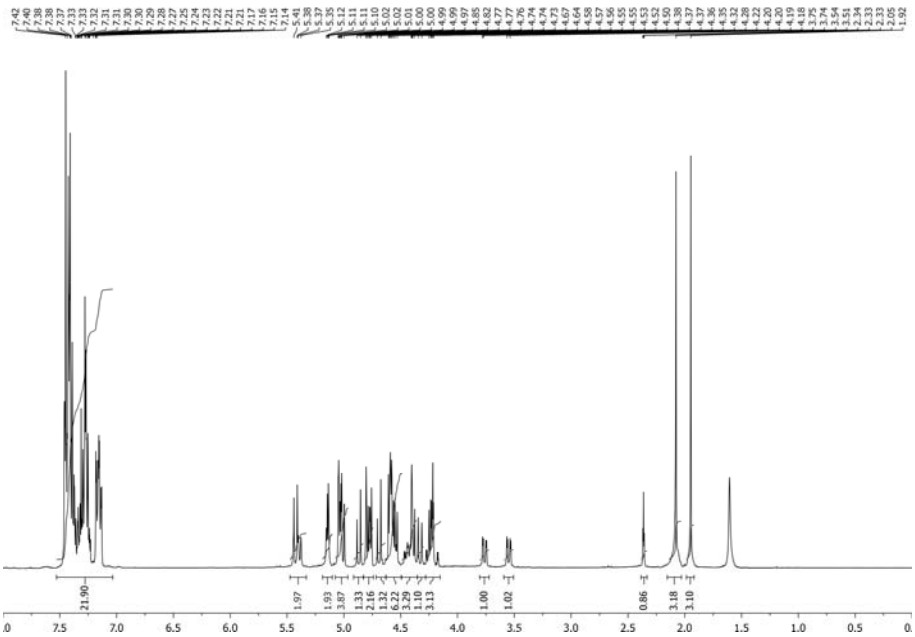
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



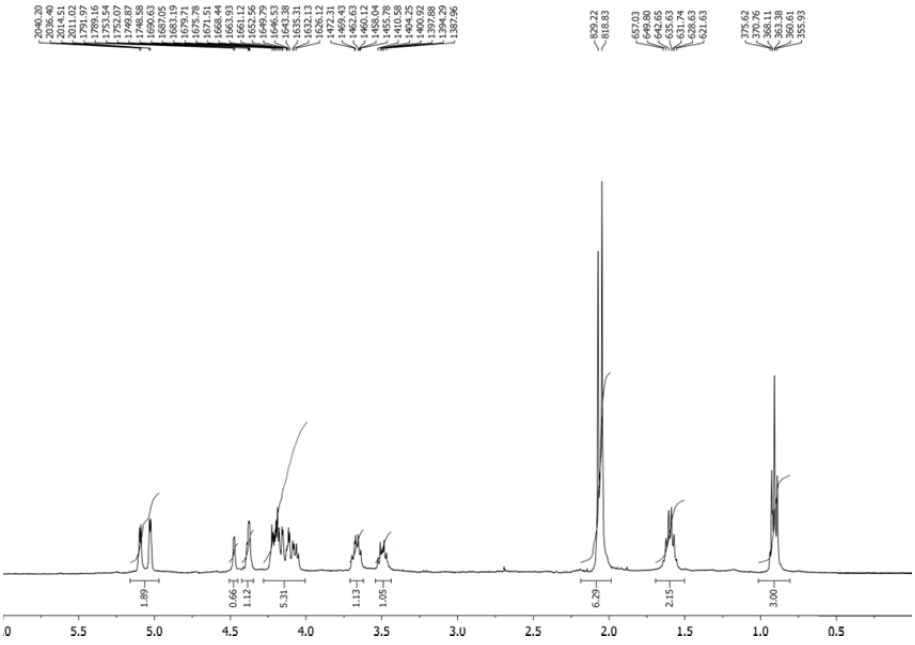
^{1}H NMR spectrum (300 Mhz, $CDCl_3$) of compound **27**



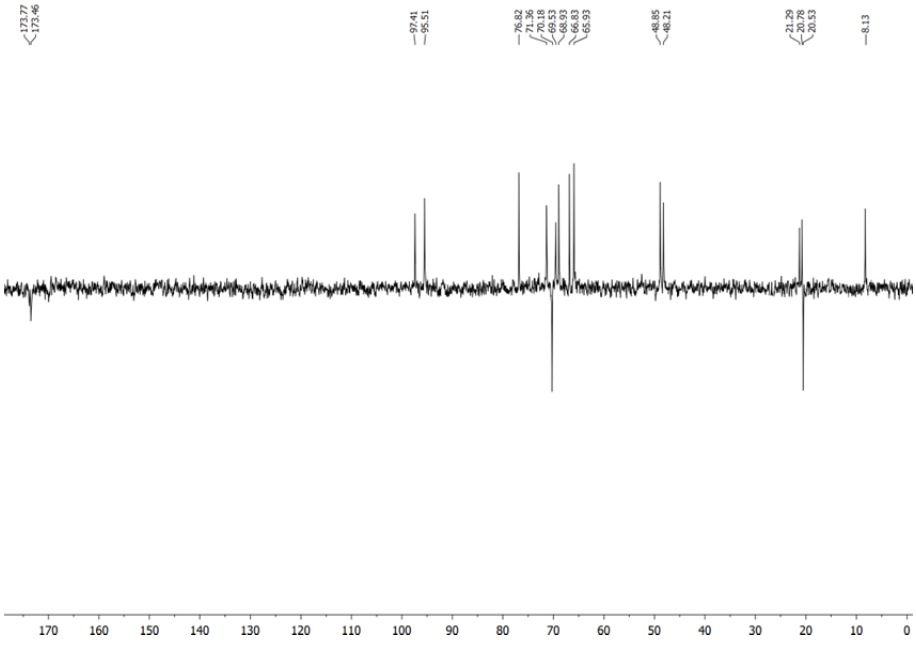
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **28**



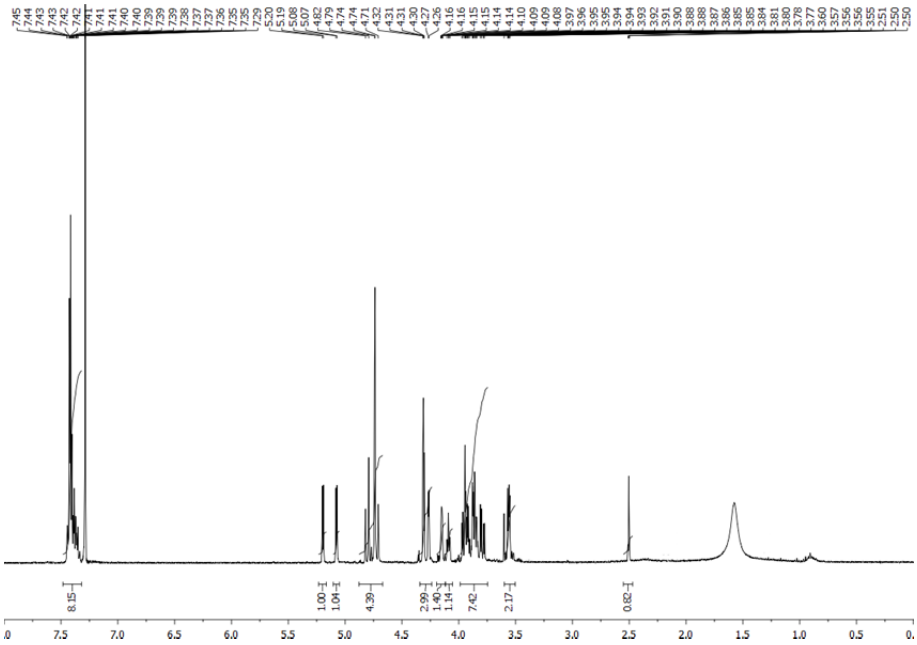
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **29**



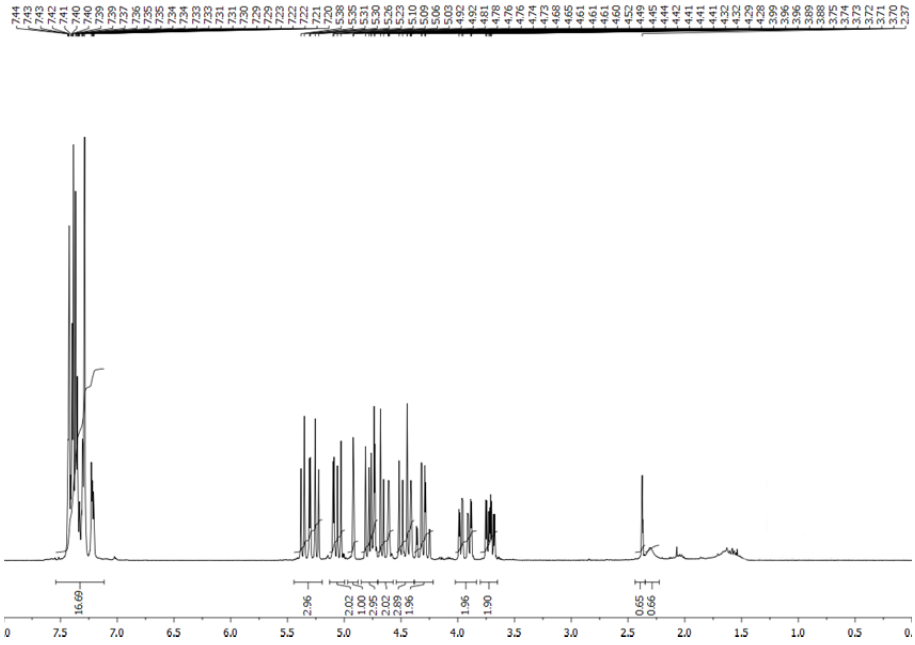
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



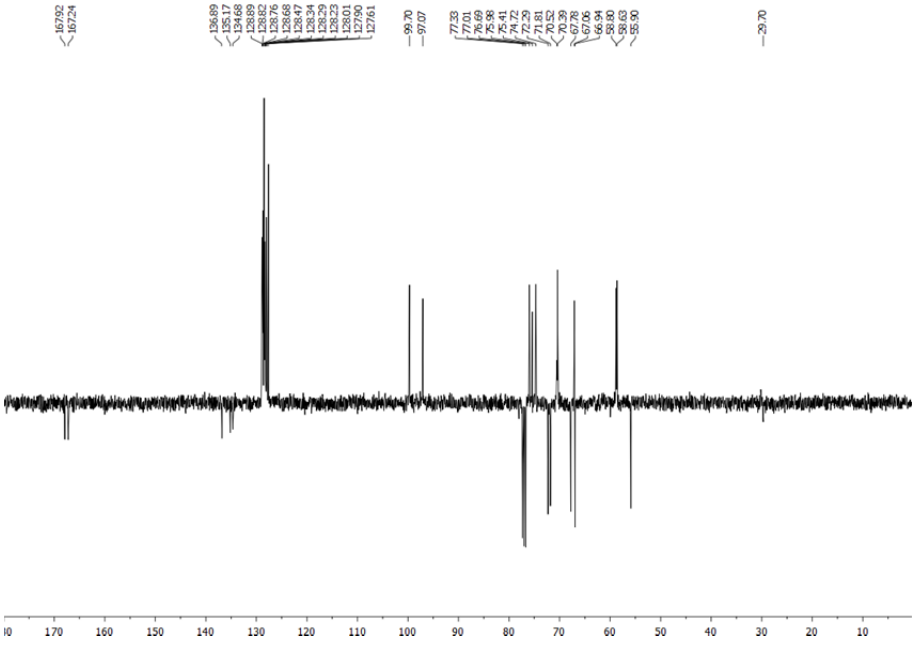
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **30**



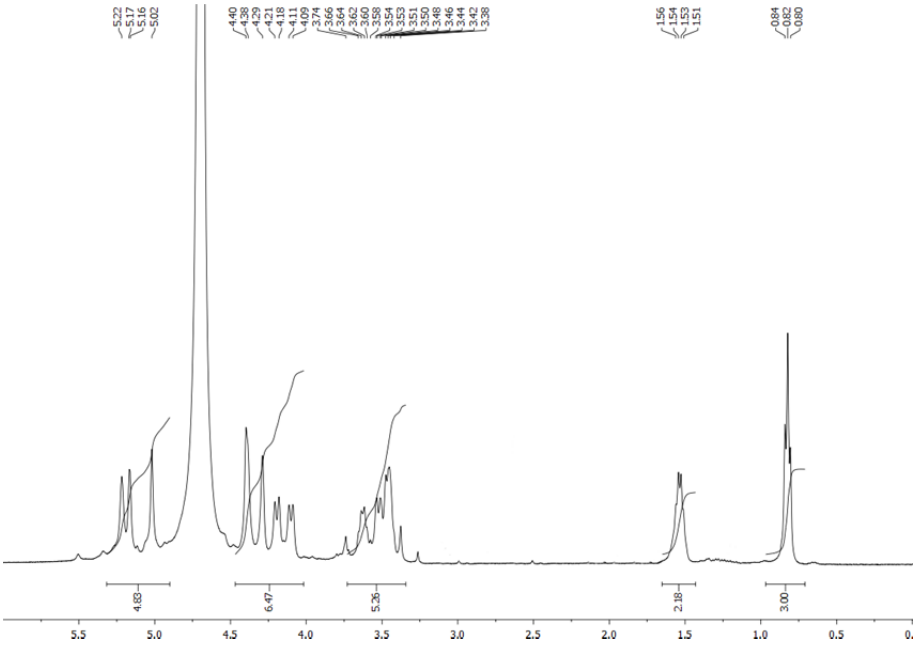
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **31**



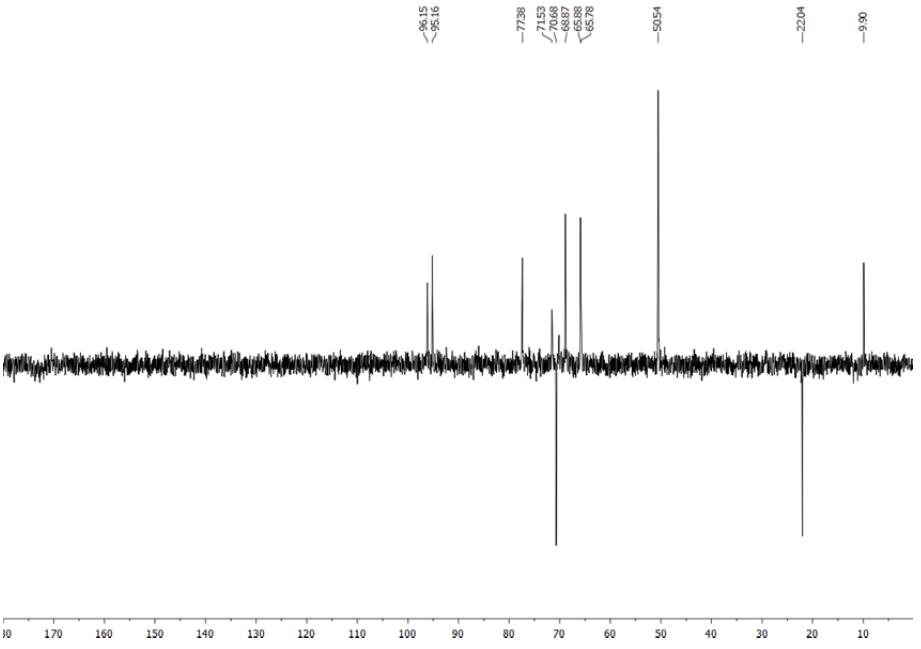
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



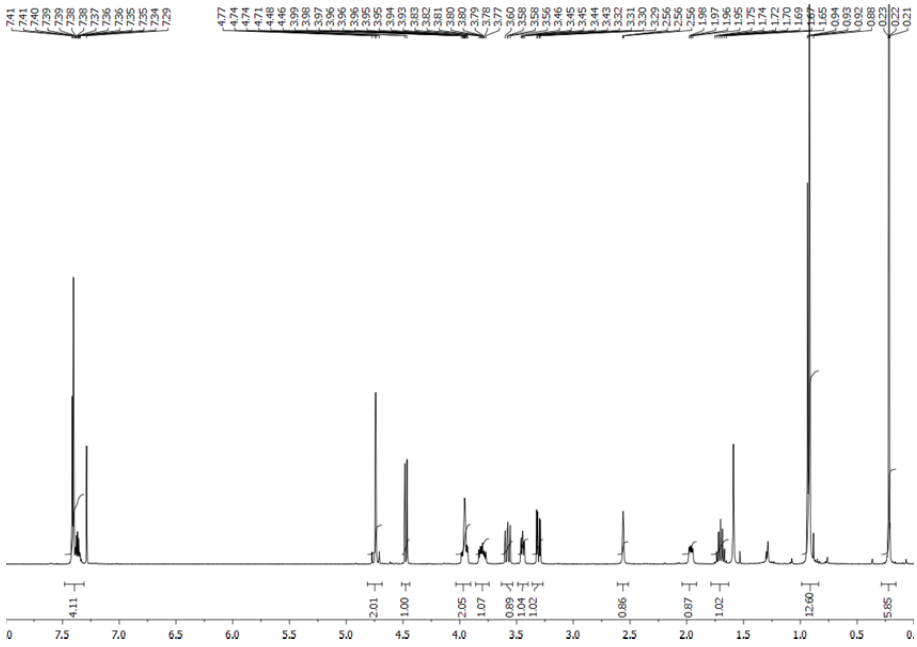
^{1}H NMR spectrum (400 Mhz, D_2O) of compound **32**



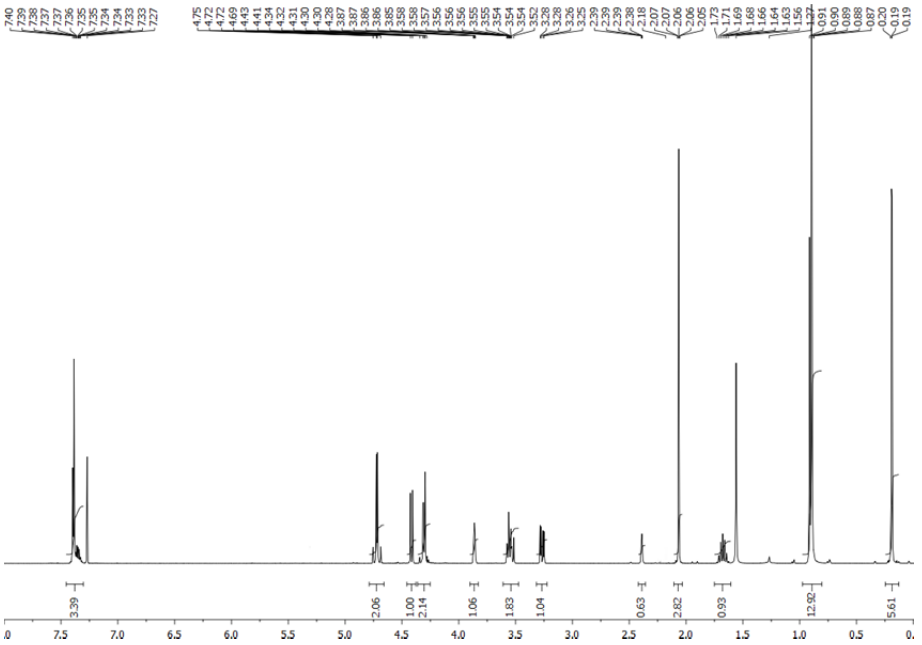
^{13}C NMR spectrum (100.6 MHz, D_2O)



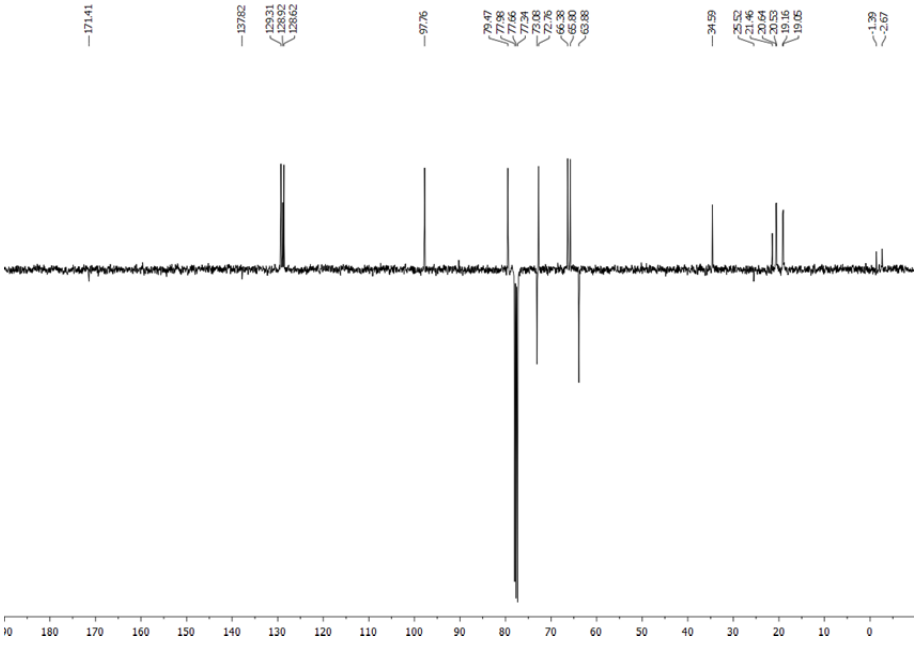
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **36**



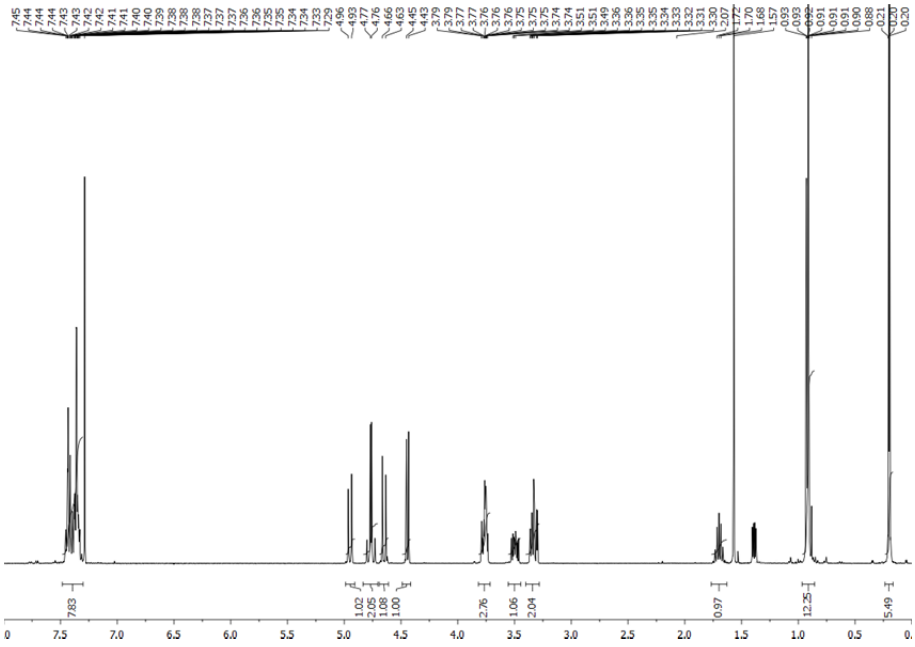
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **37**



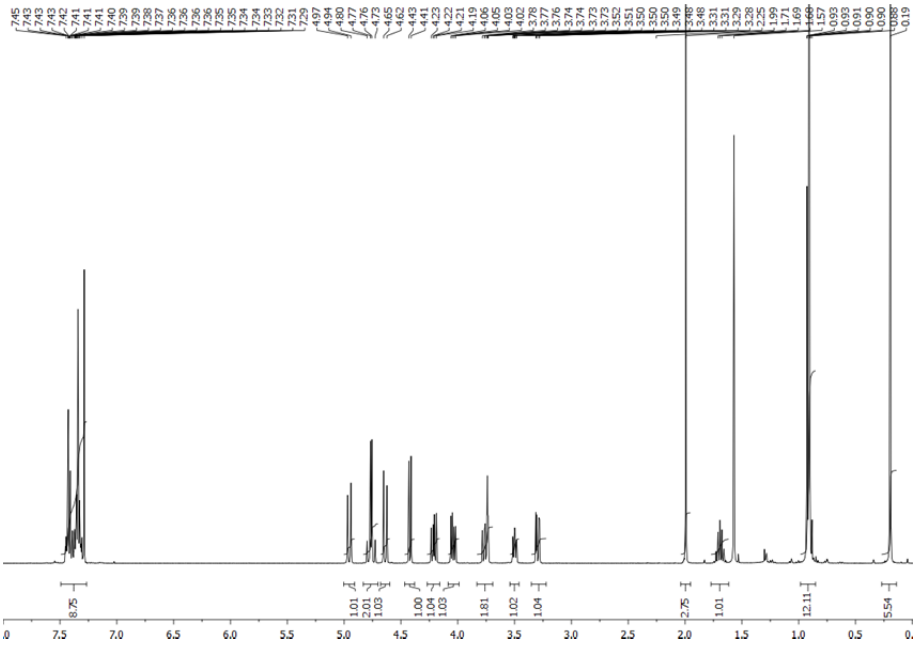
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



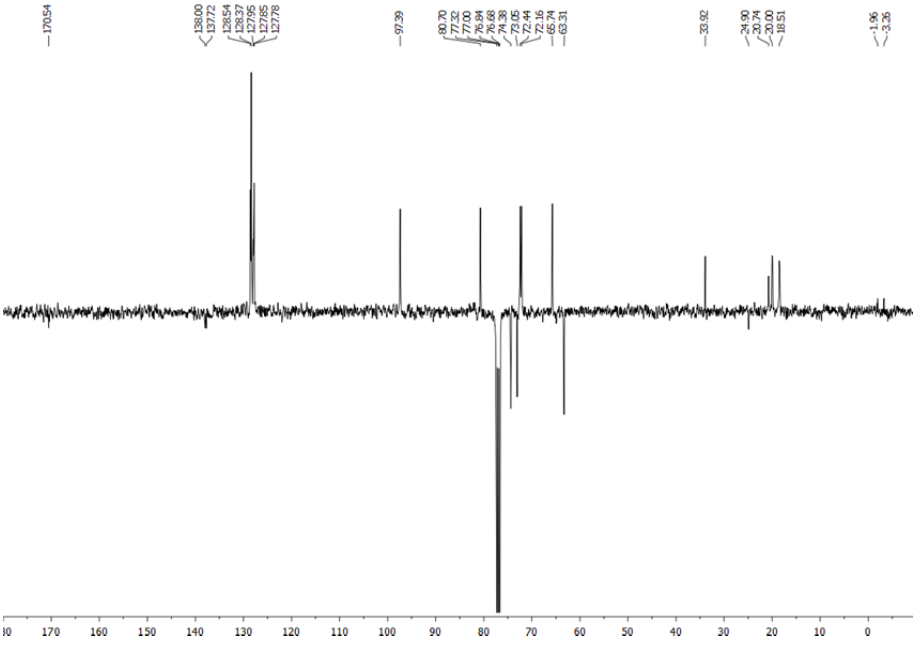
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **19a**



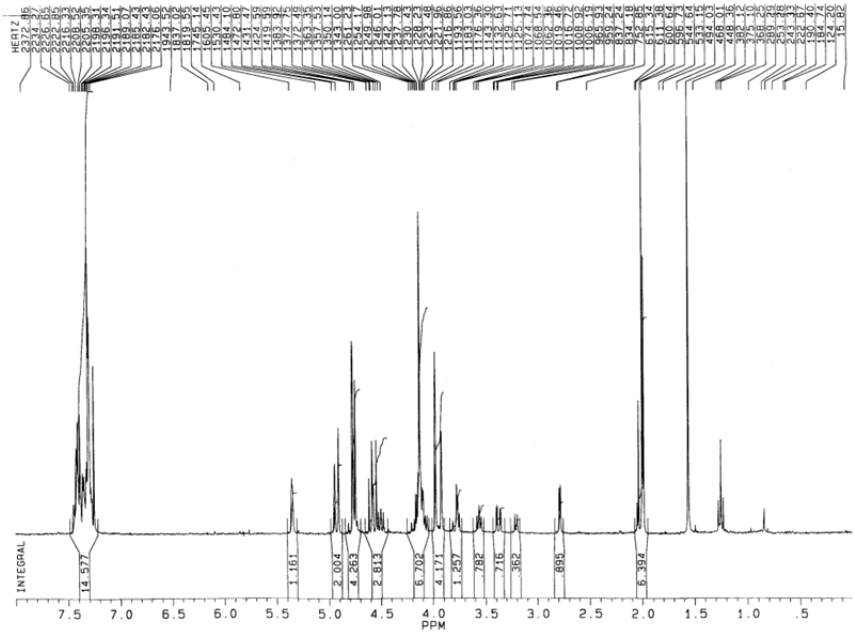
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **38**



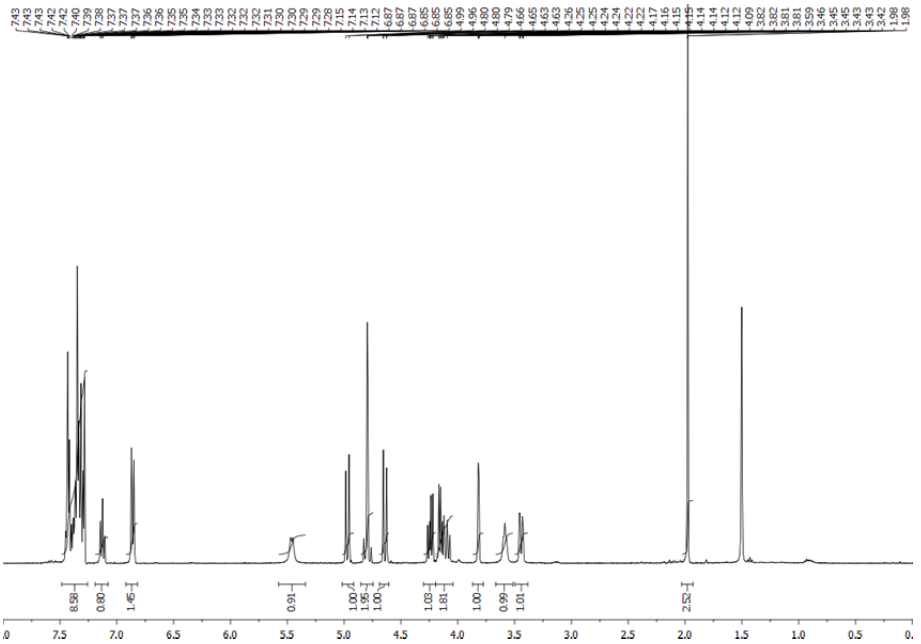
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



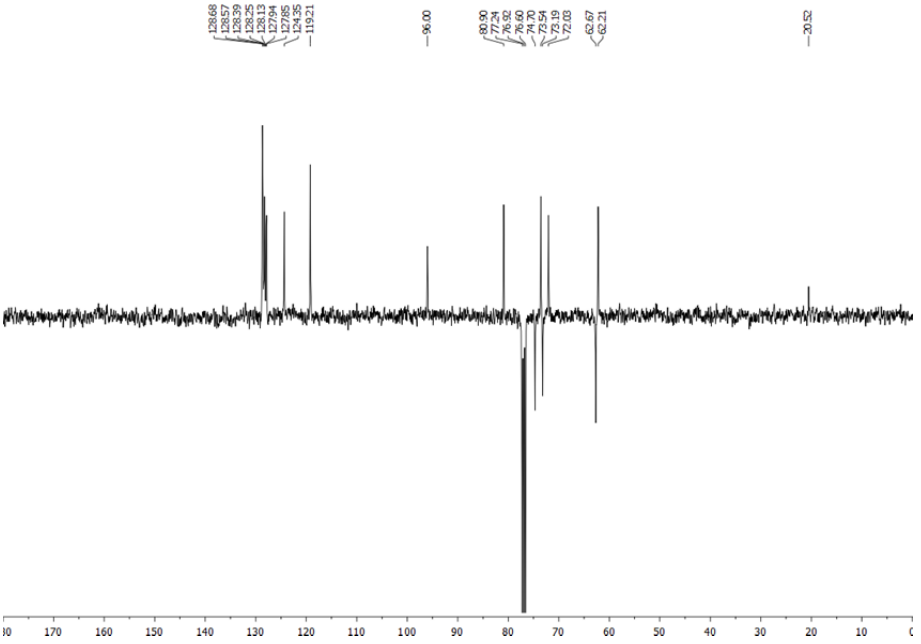
^{1}H NMR spectrum (300 Mhz, $CDCl_3$) of compound **38a**



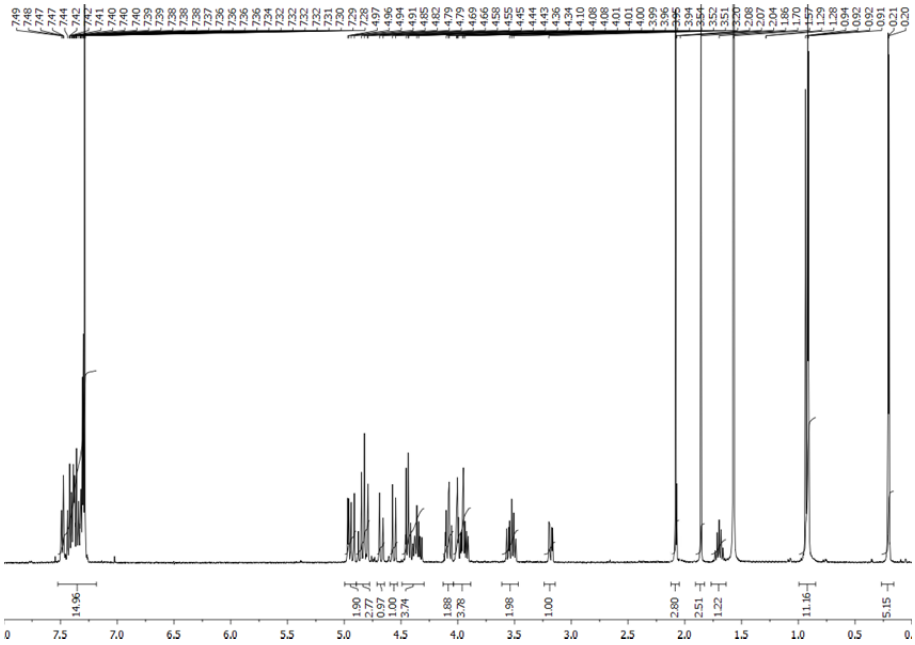
^{1}H NMR spectrum (400 Mhz, $CDCl_3$, 50°C) of compound **39β**



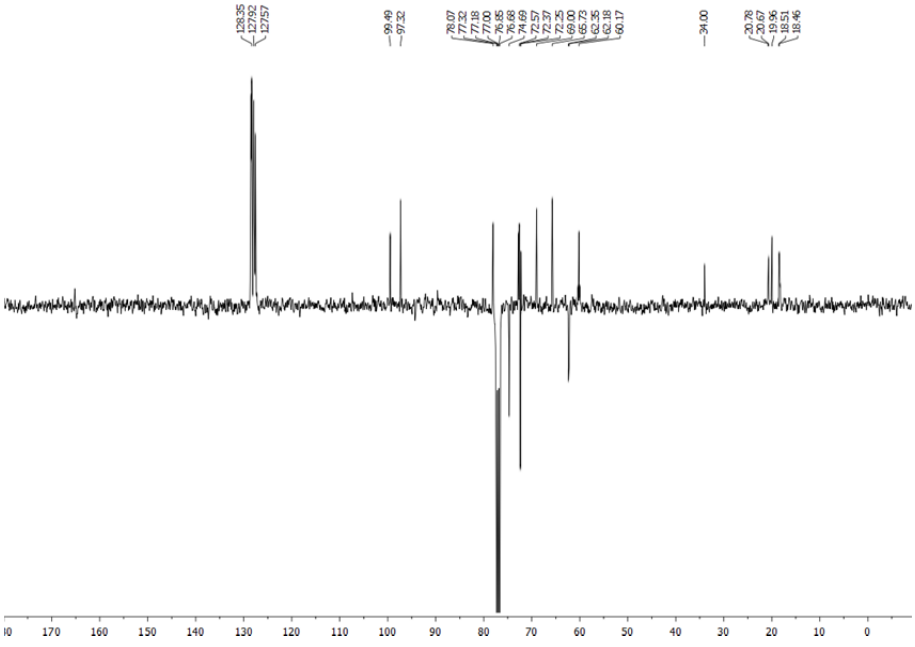
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$, 50°C)



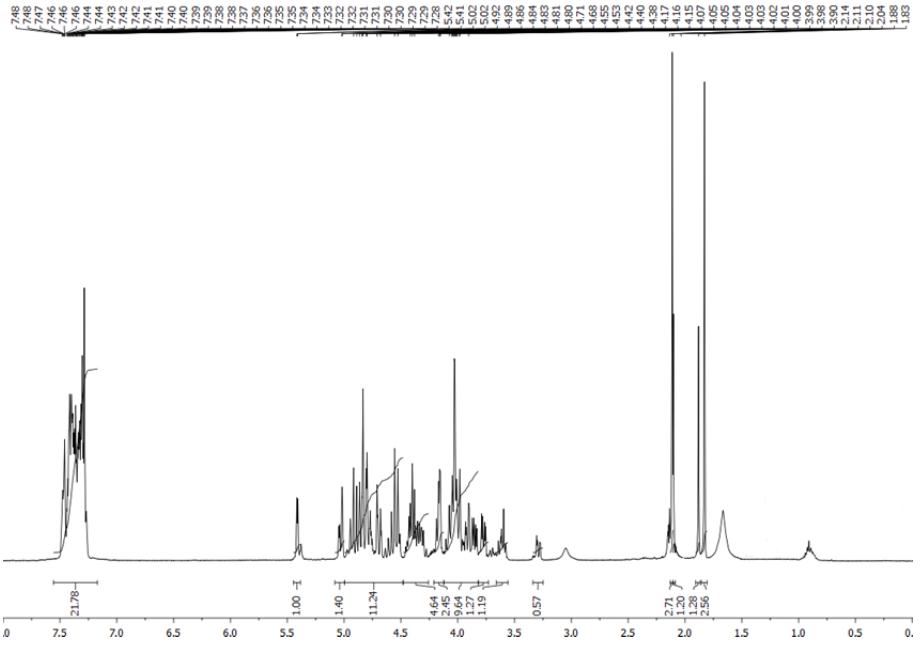
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **40**



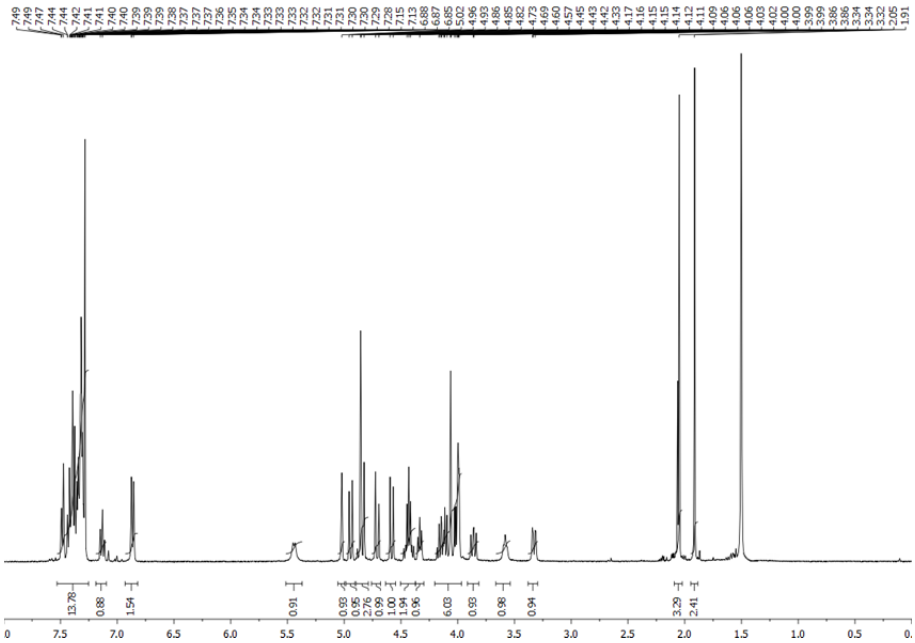
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



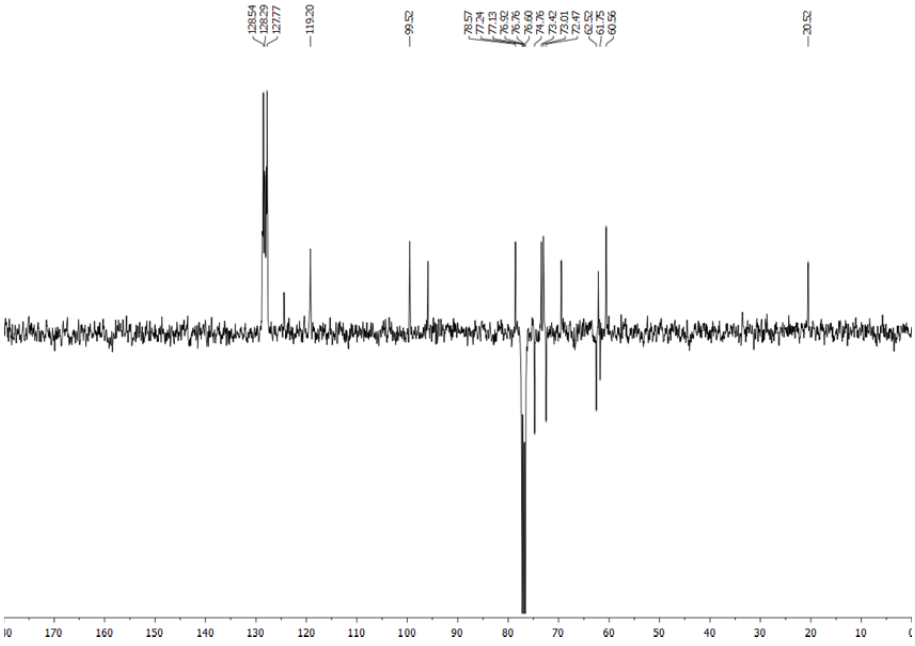
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **40a**



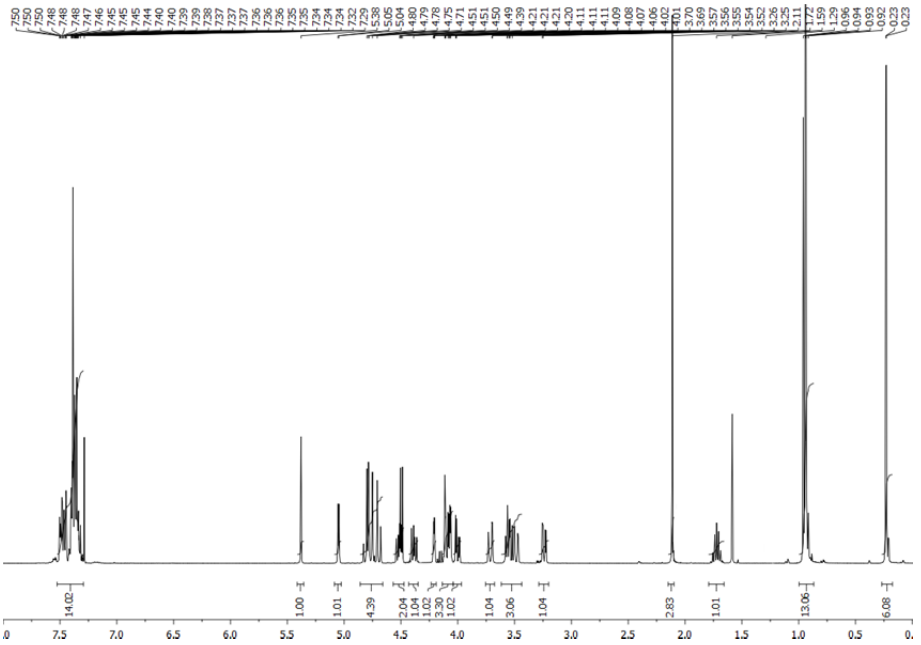
^{1}H NMR spectrum (400 Mhz, $CDCl_3$, 50°C) of compound **34β**



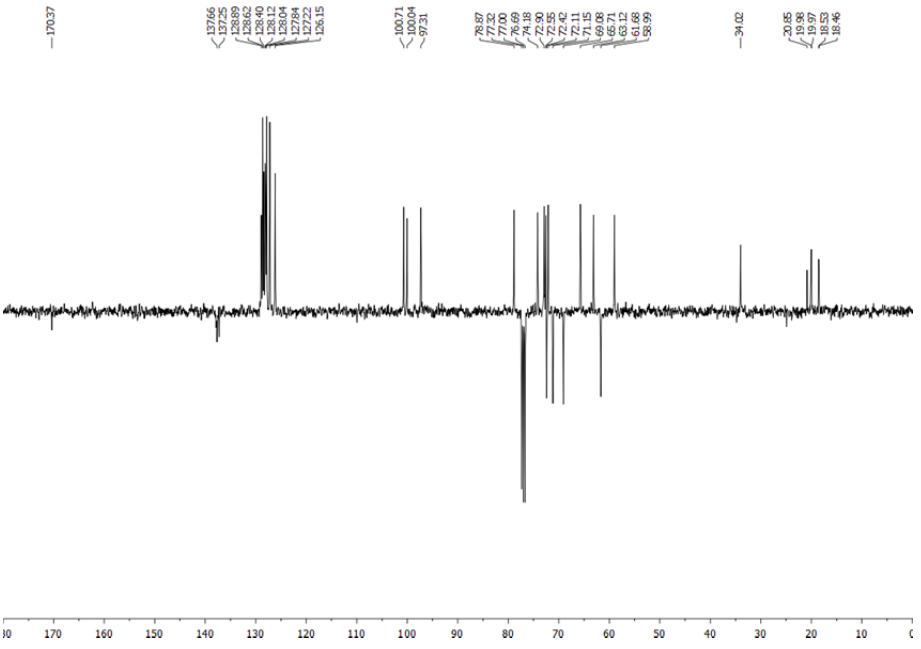
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$, 50°C)



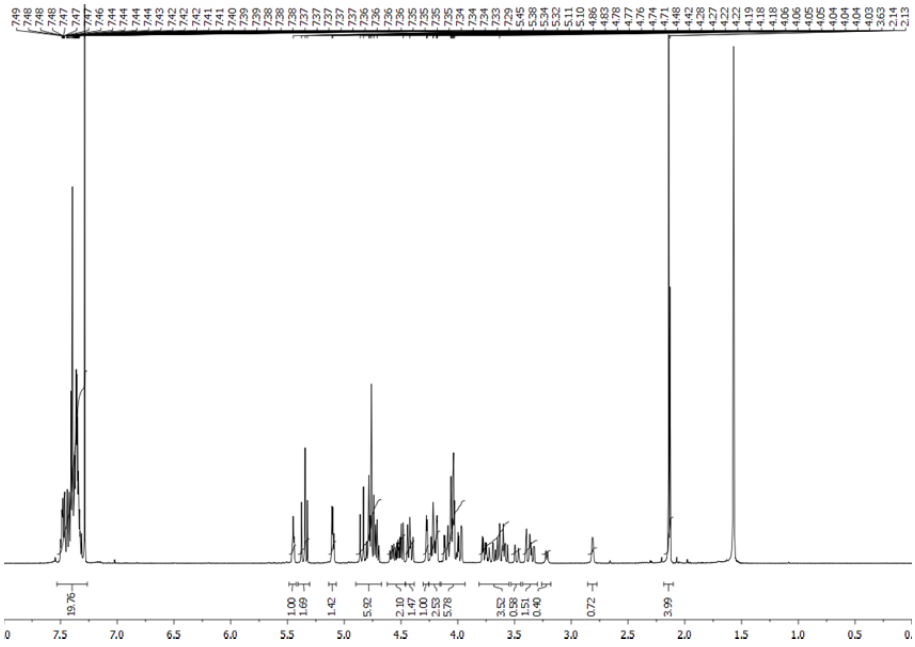
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **41a**



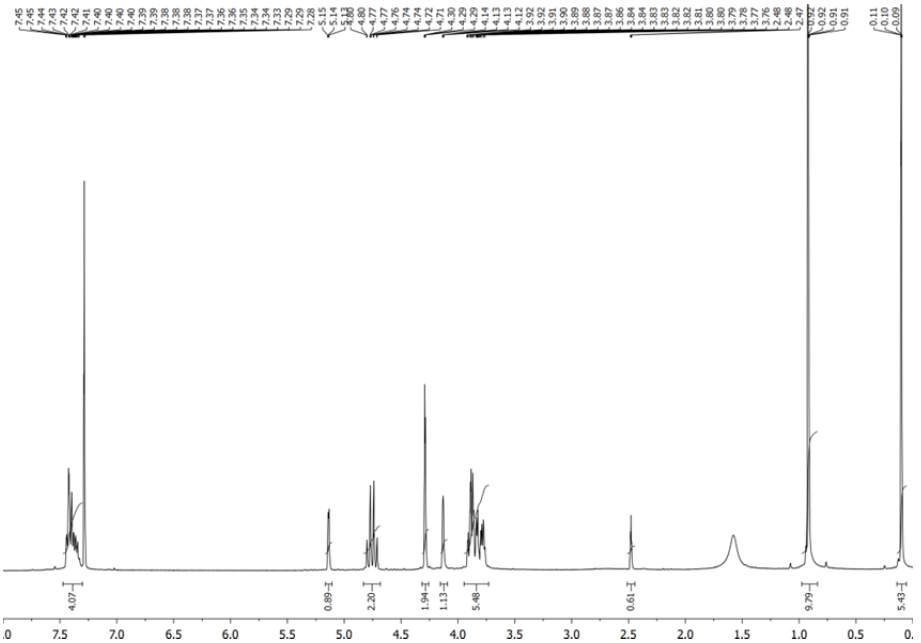
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



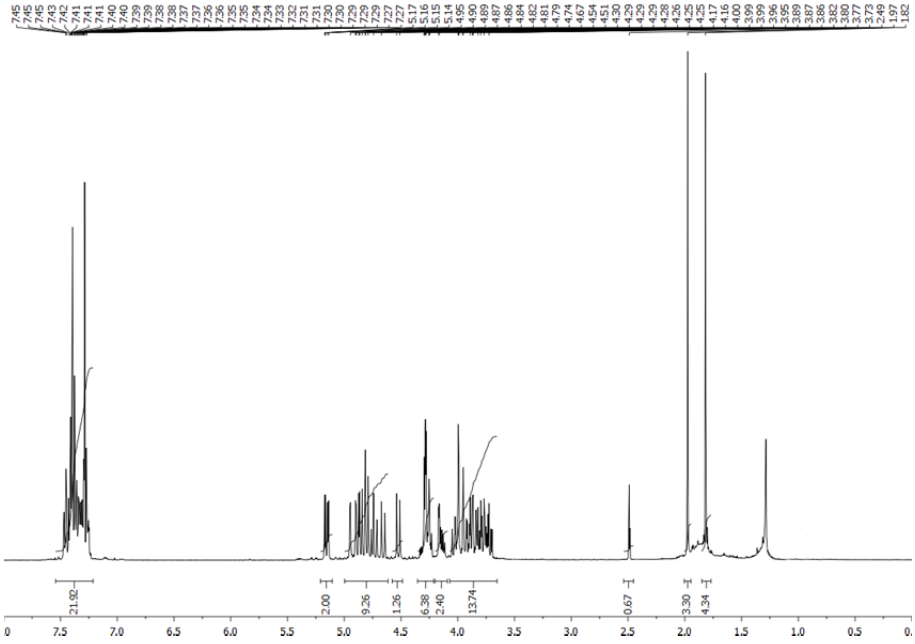
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **41**



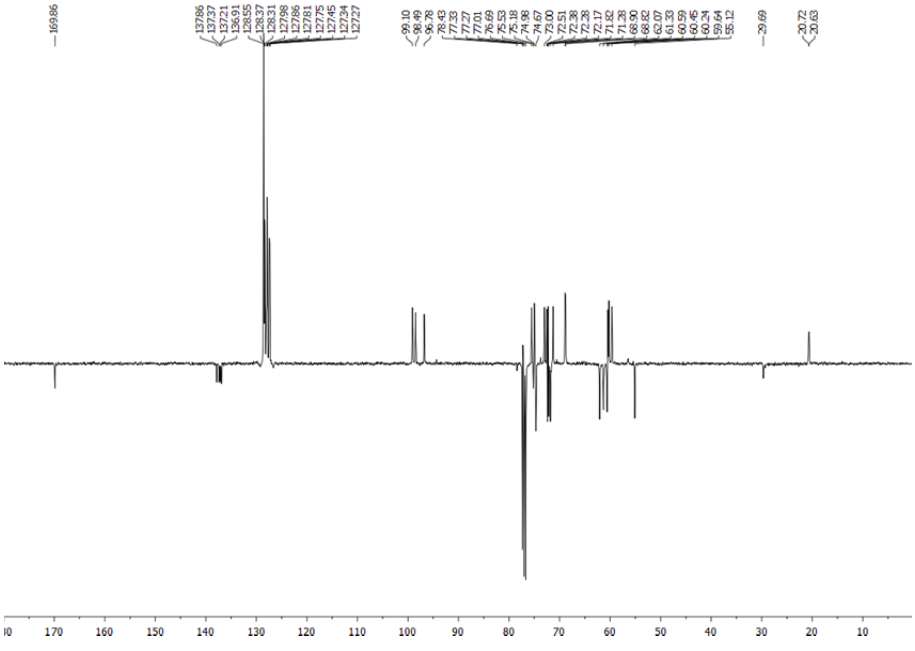
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **44**



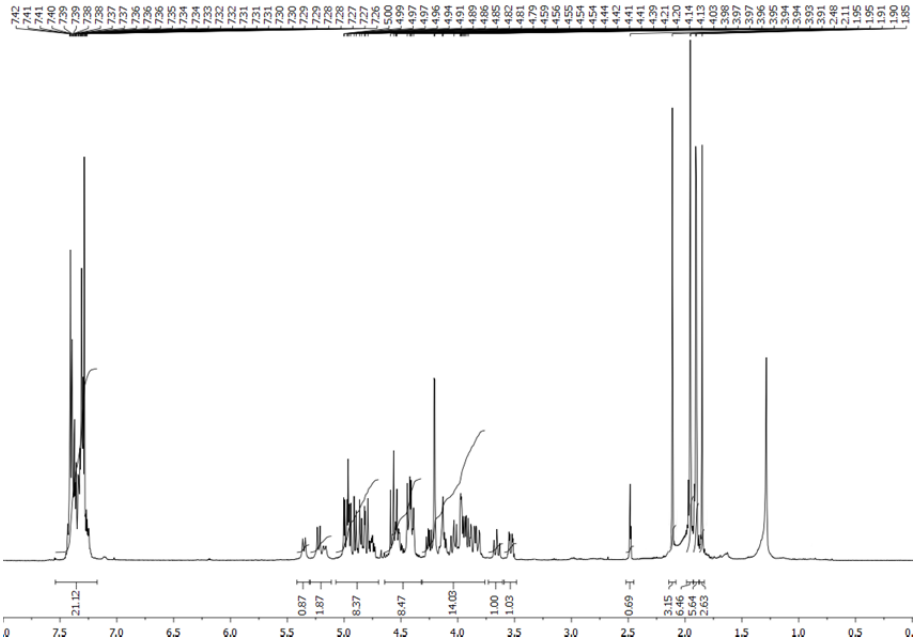
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **46**



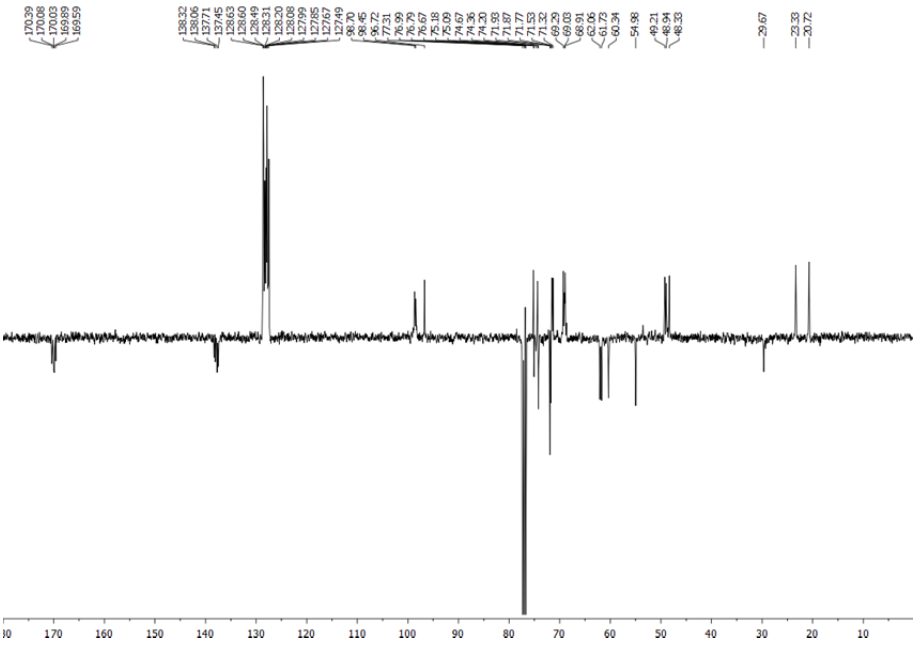
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



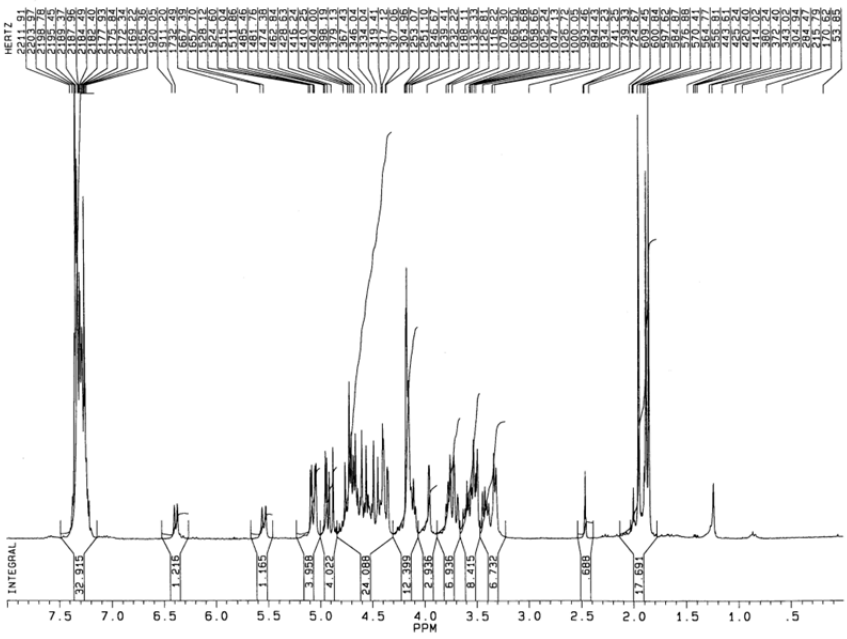
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **46a**



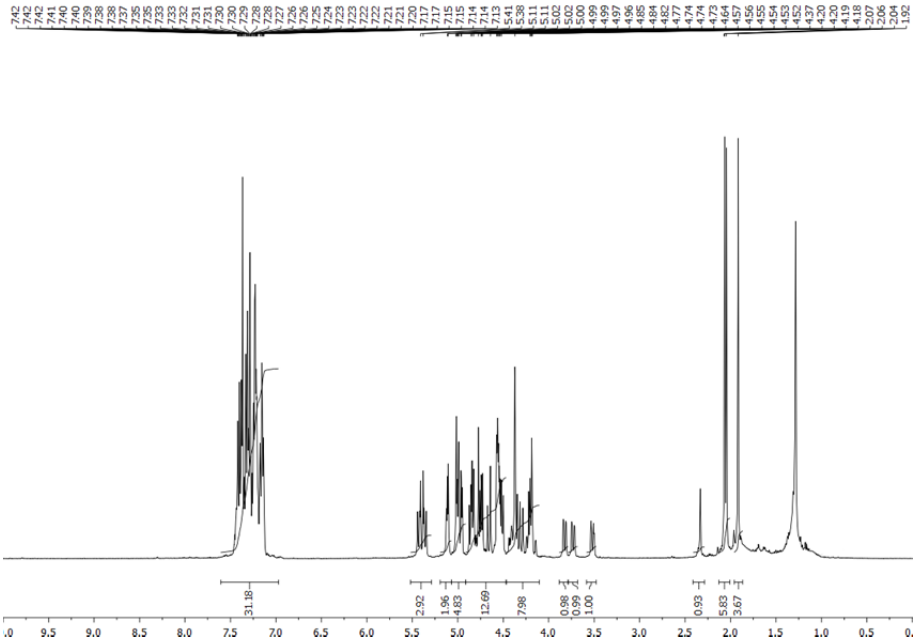
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



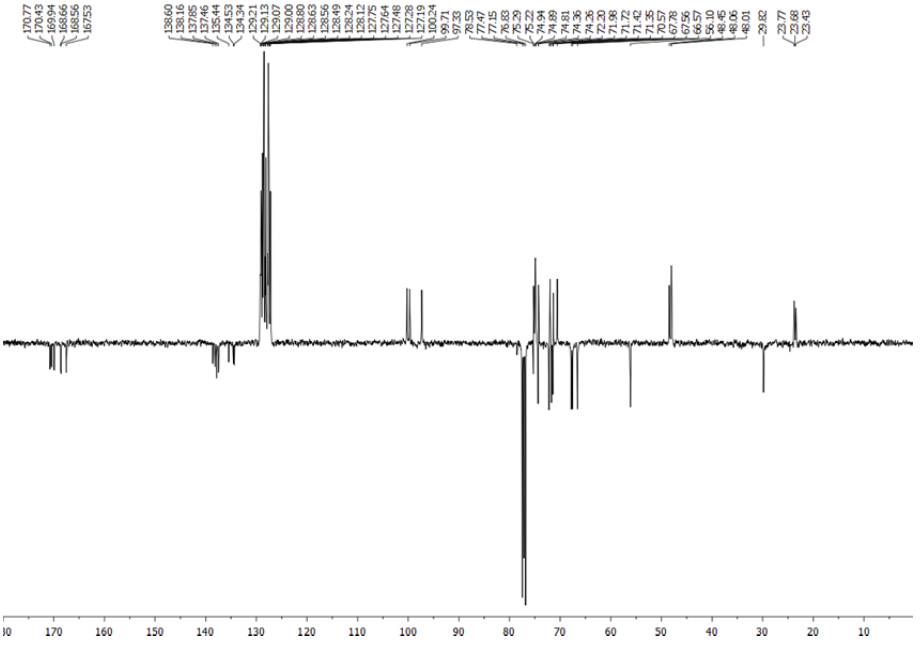
^{1}H NMR spectrum (300 Mhz, $CDCl_3$) of compound **47**



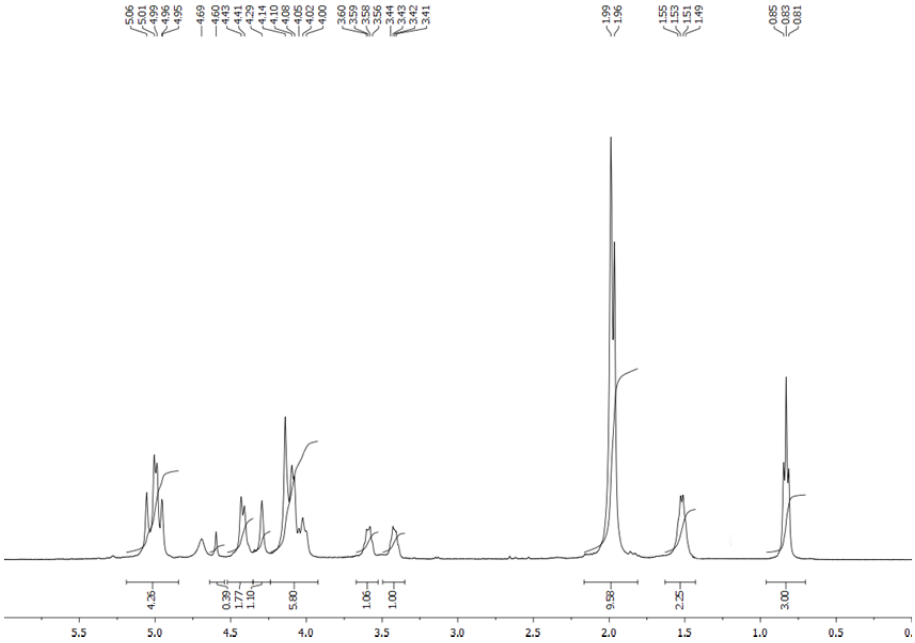
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **48**



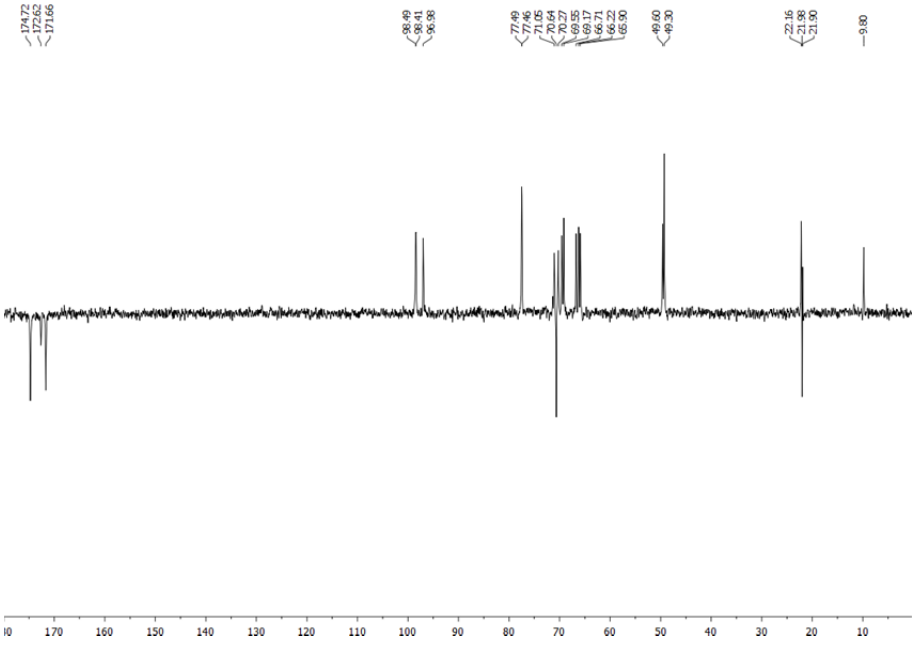
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



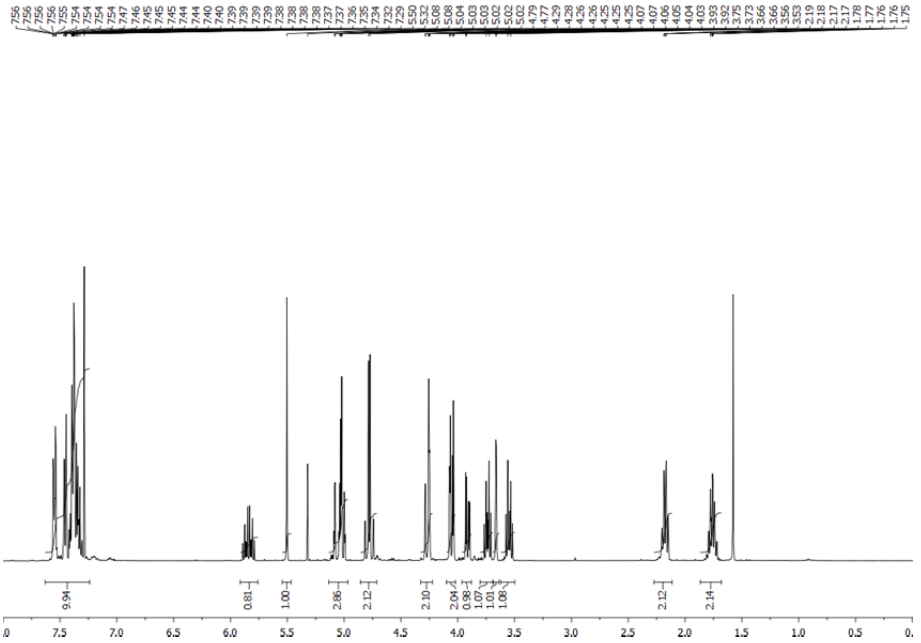
^{1}H NMR spectrum (400 Mhz, D_2O) of compound **49**



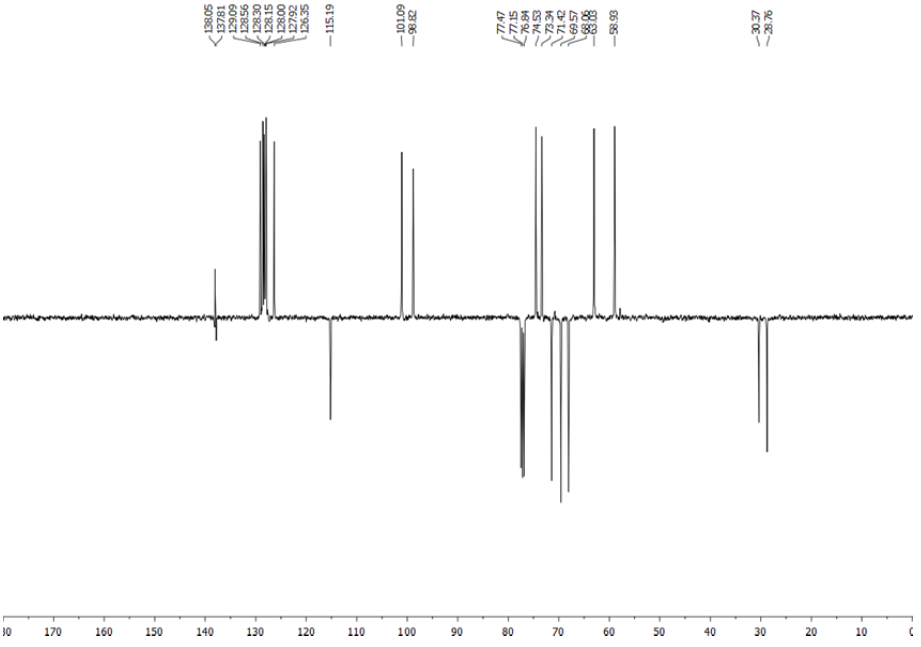
^{13}C NMR spectrum (100.6 MHz, D_2O)



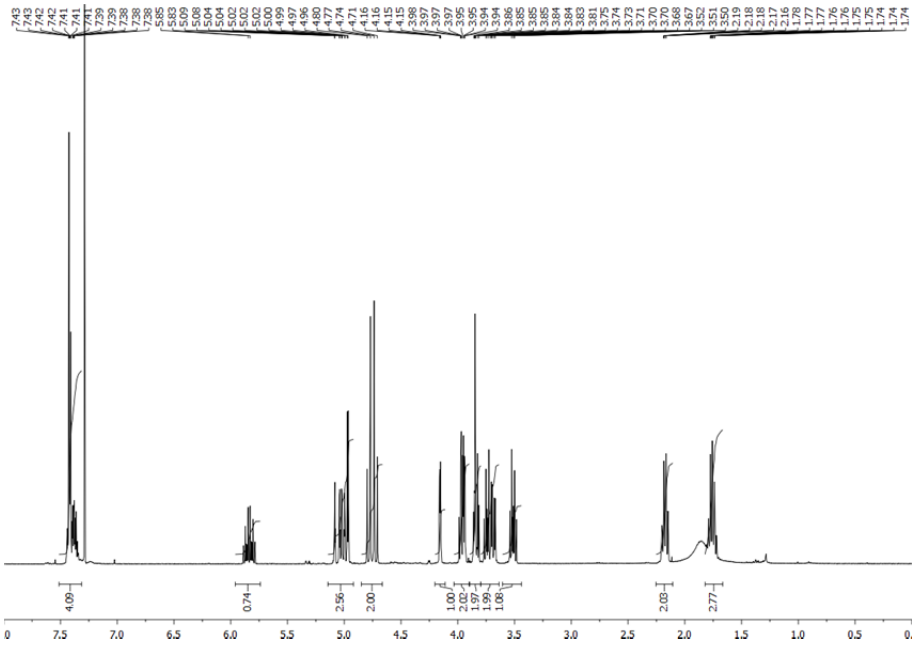
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **52a**



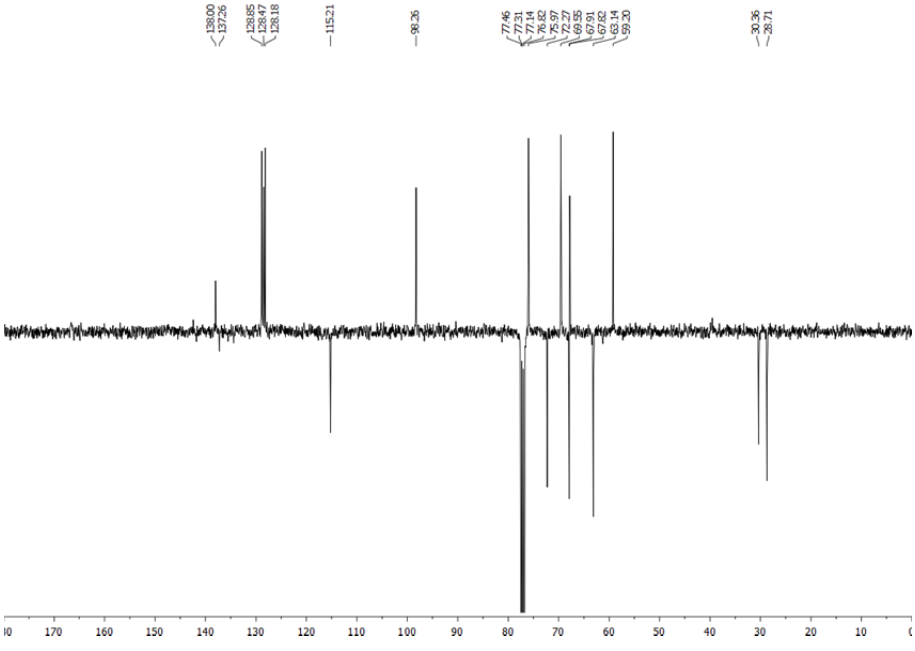
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



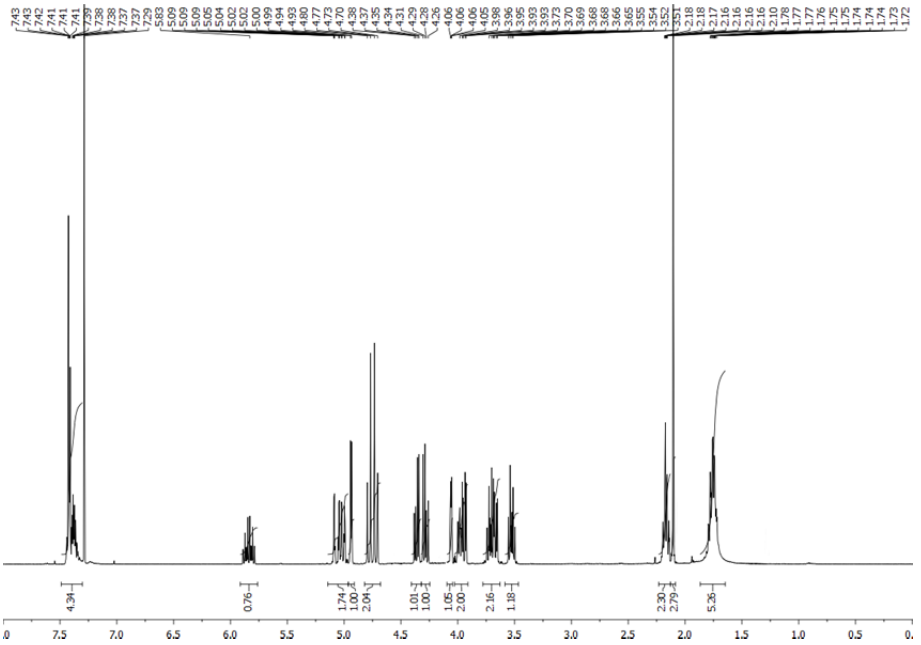
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **52b**



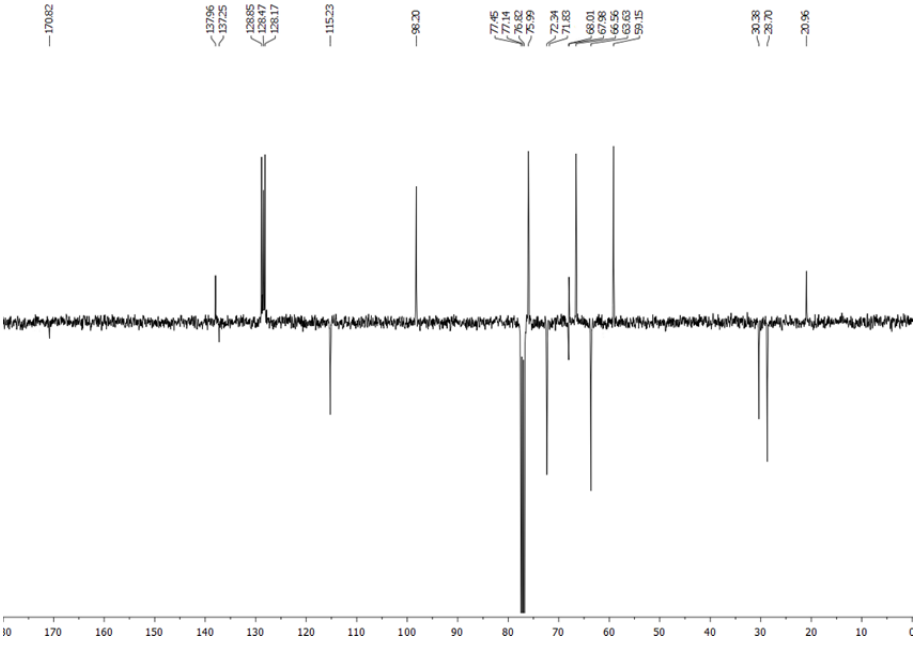
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



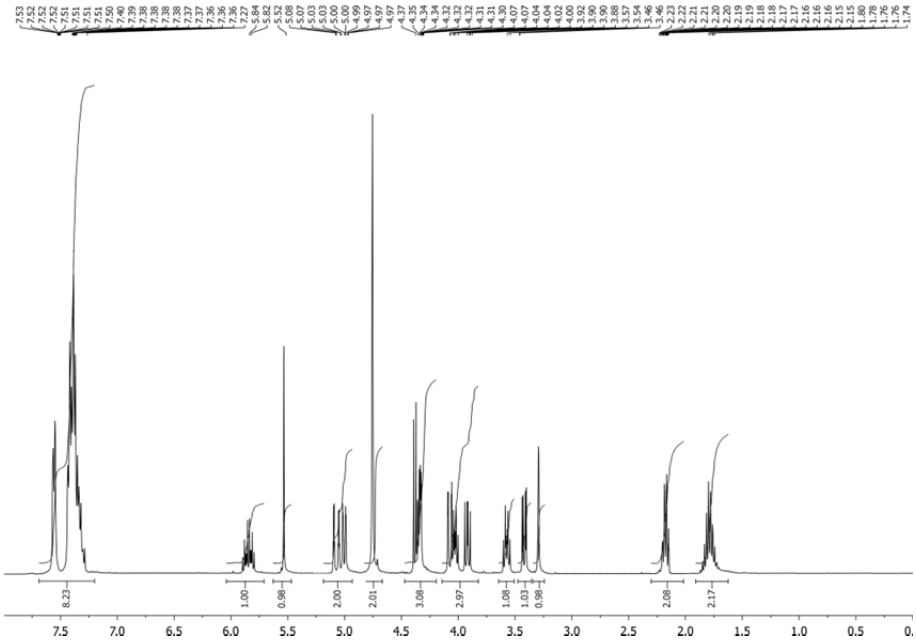
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **7b**



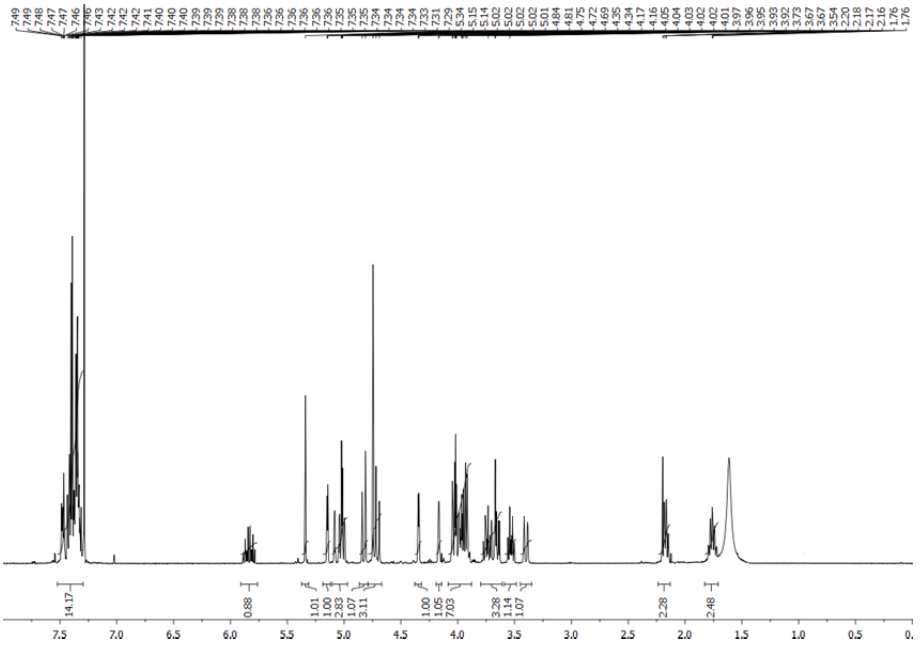
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



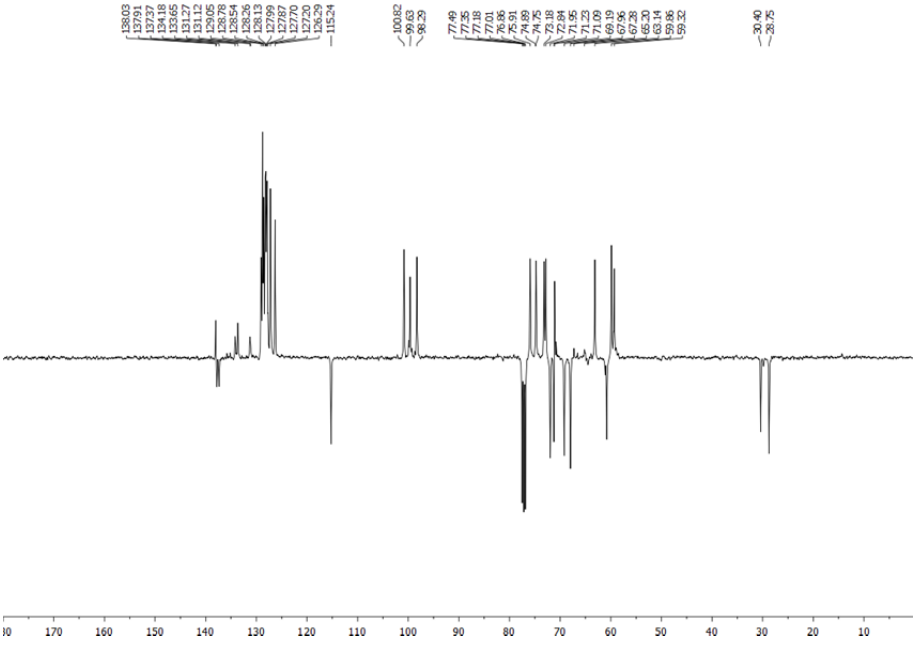
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **51a**



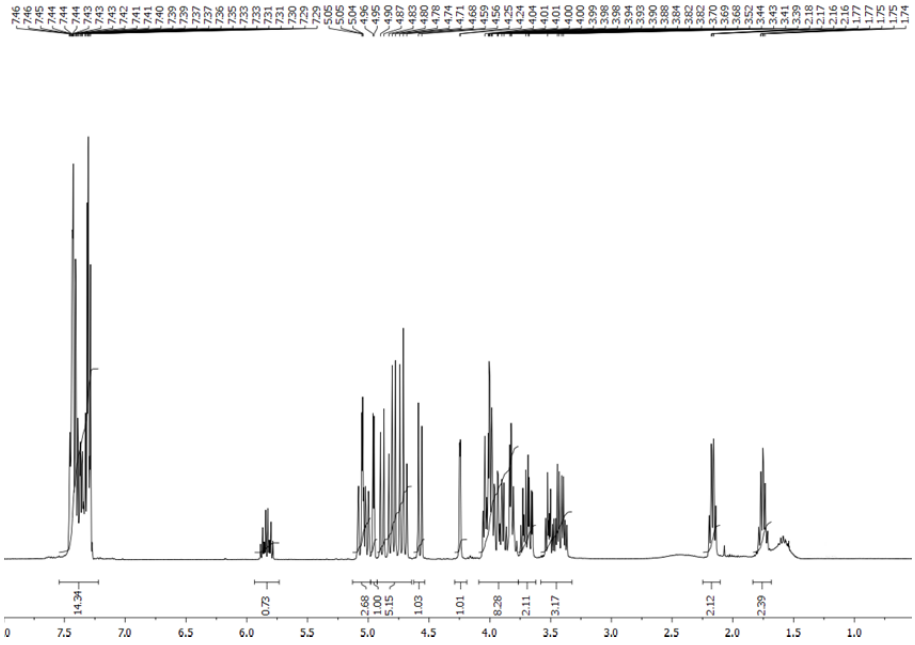
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **53**



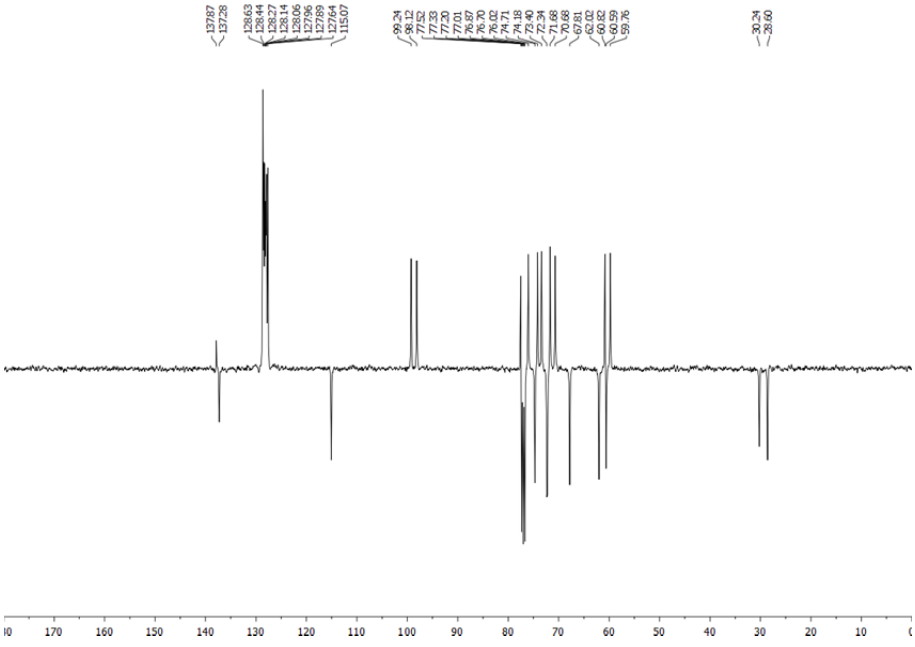
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



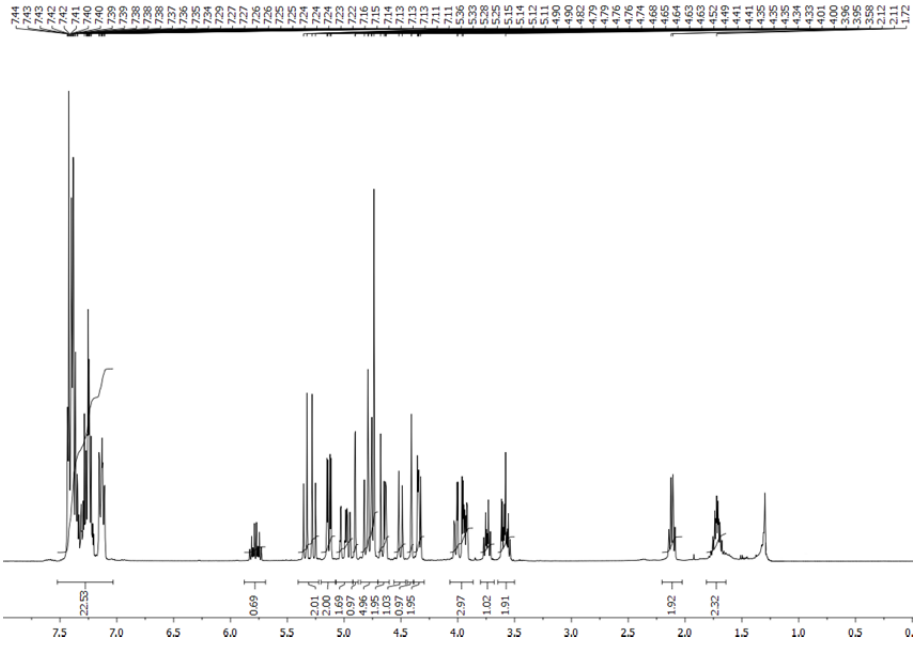
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **53a**



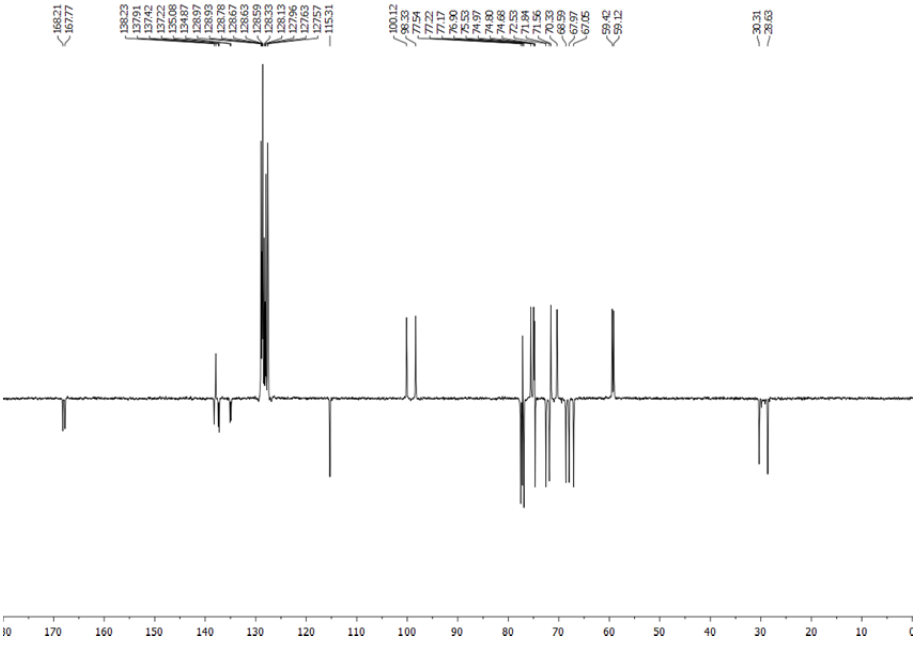
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



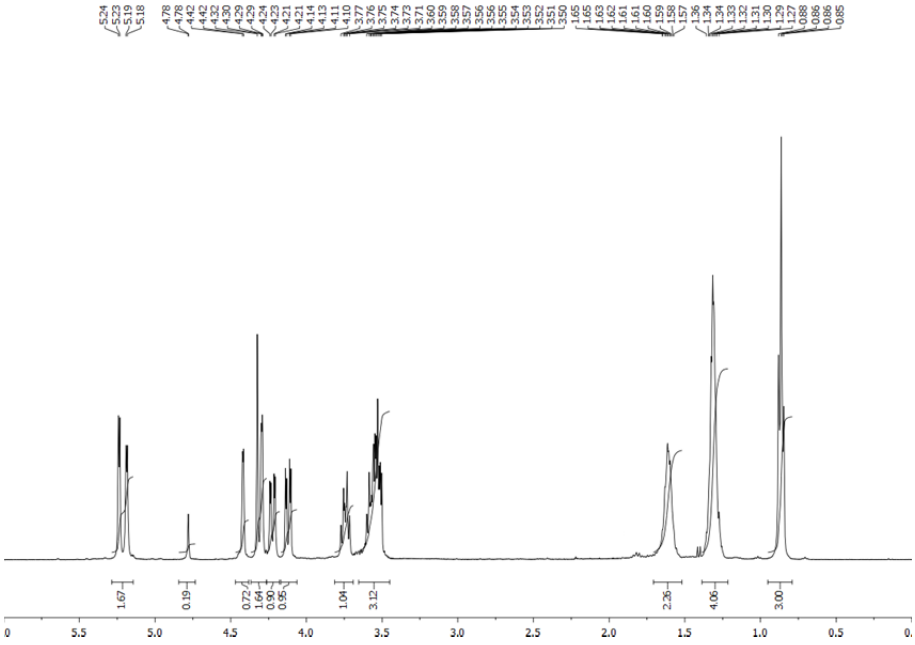
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **54**



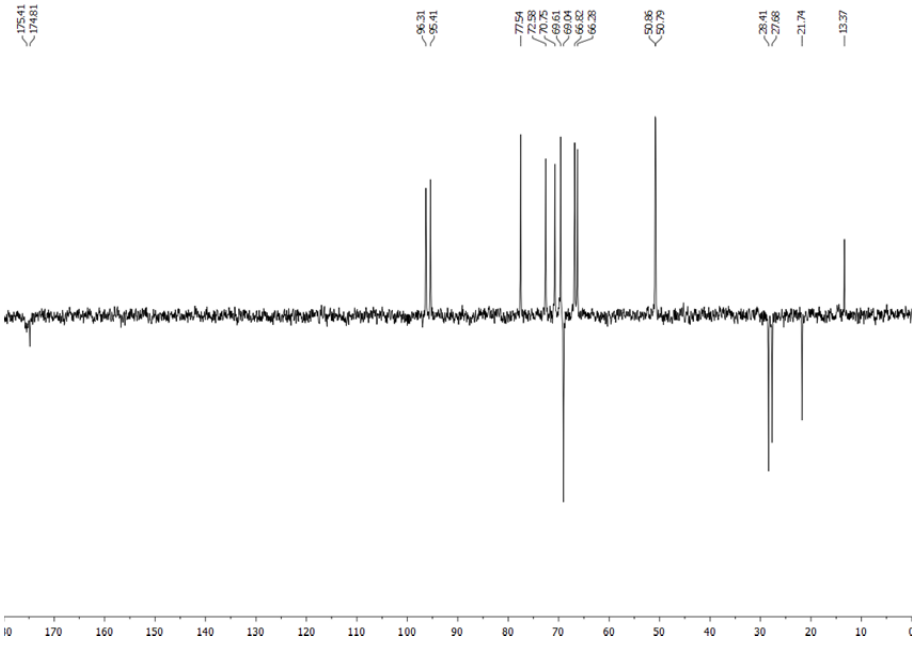
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



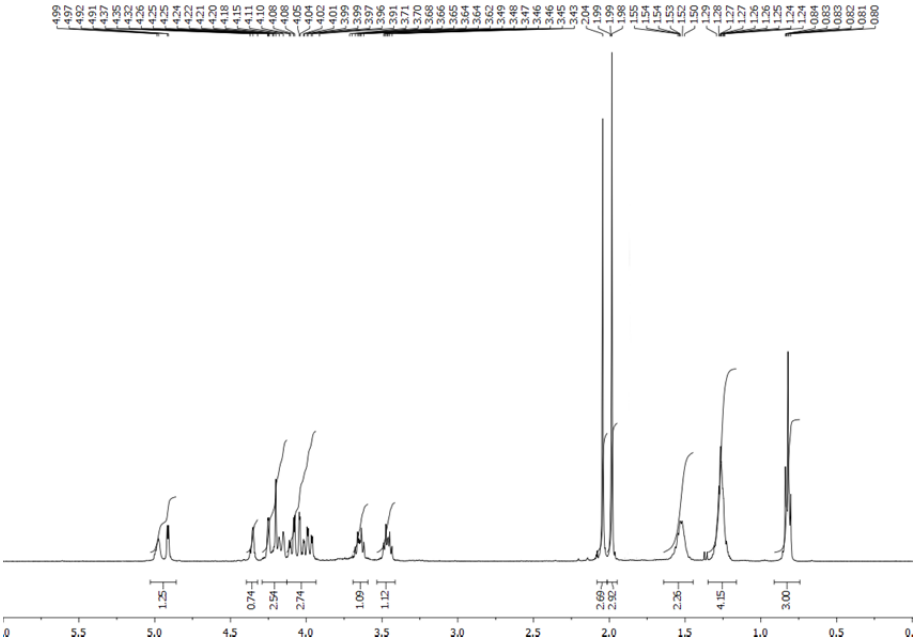
^{1}H NMR spectrum (400 Mhz, D_2O) of compound **55**



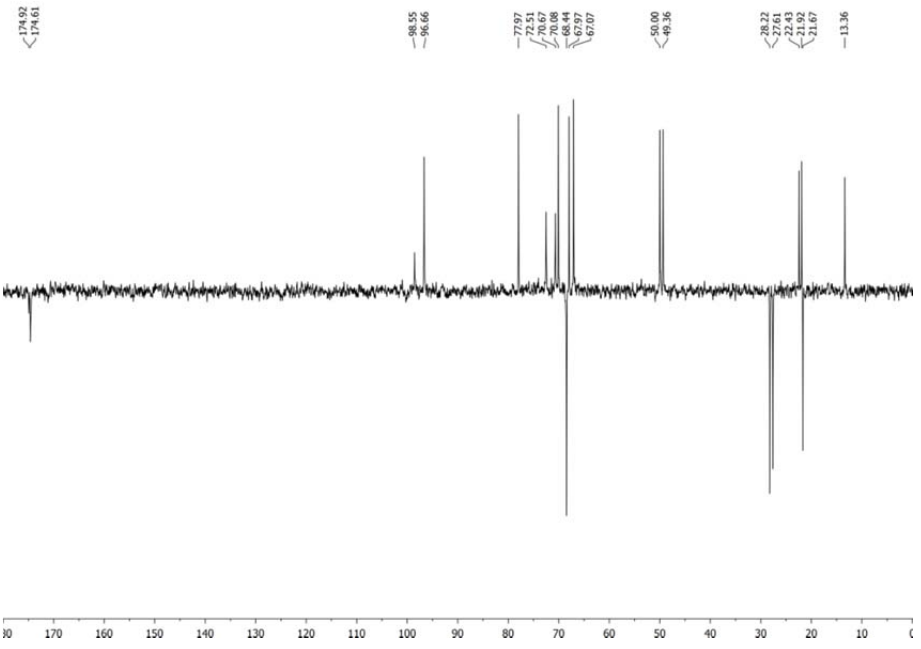
^{13}C NMR spectrum (100.6 MHz, D_2O)



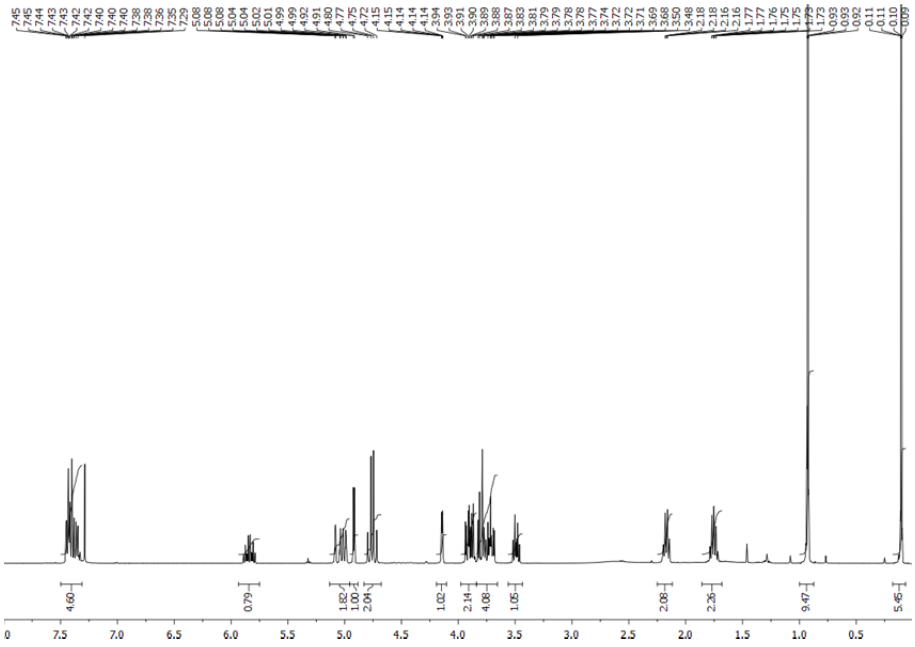
^{1}H NMR spectrum (400 Mhz, D_2O) of compound **56**



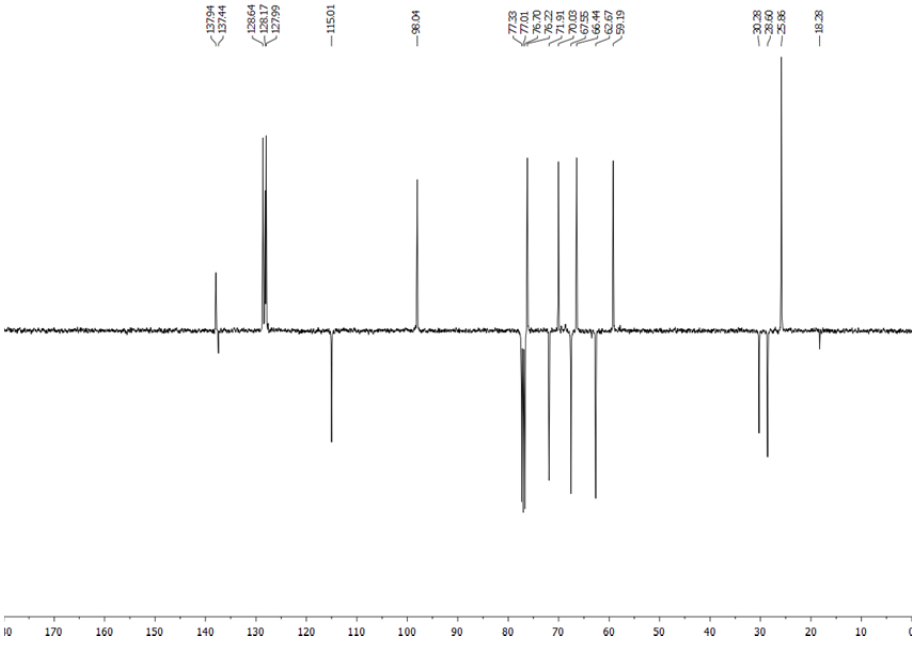
^{13}C NMR spectrum (100.6 MHz, D_2O)



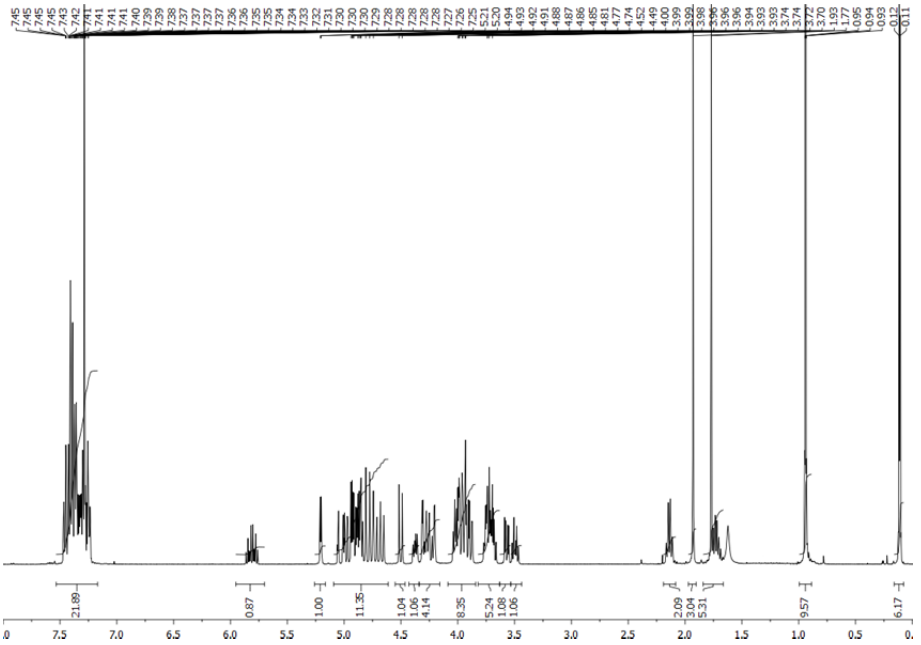
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **57**



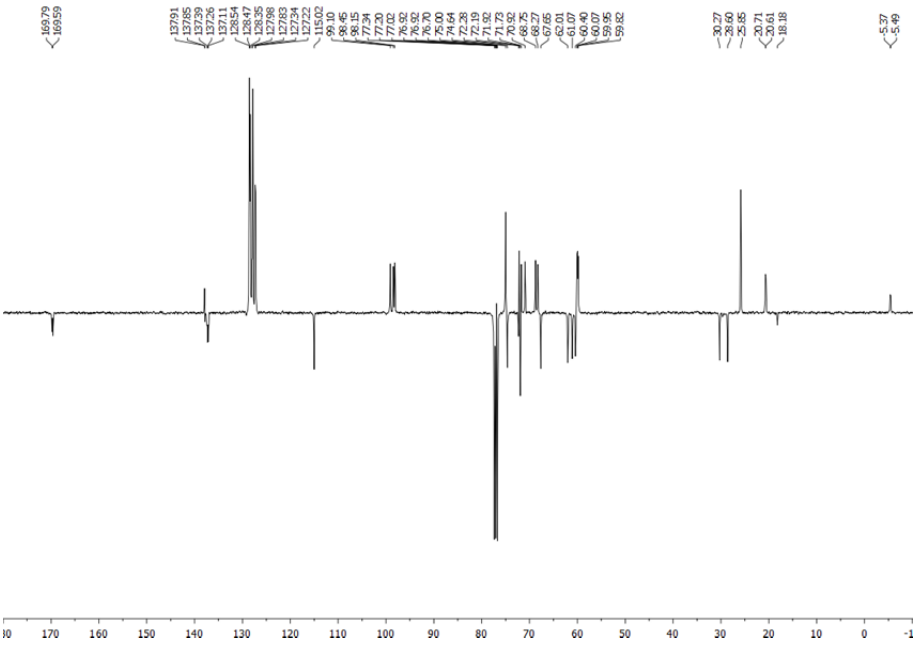
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



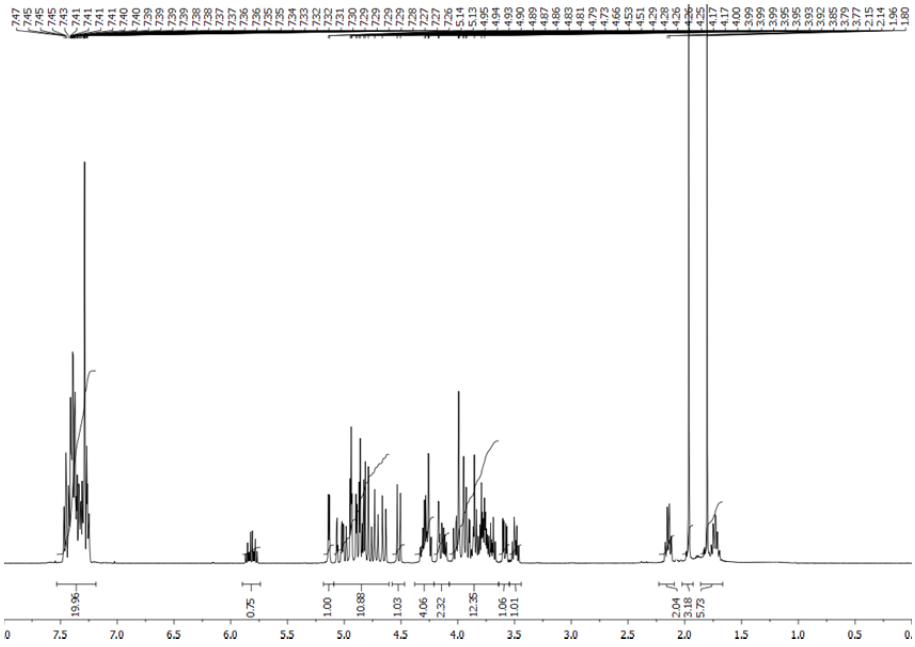
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **58**



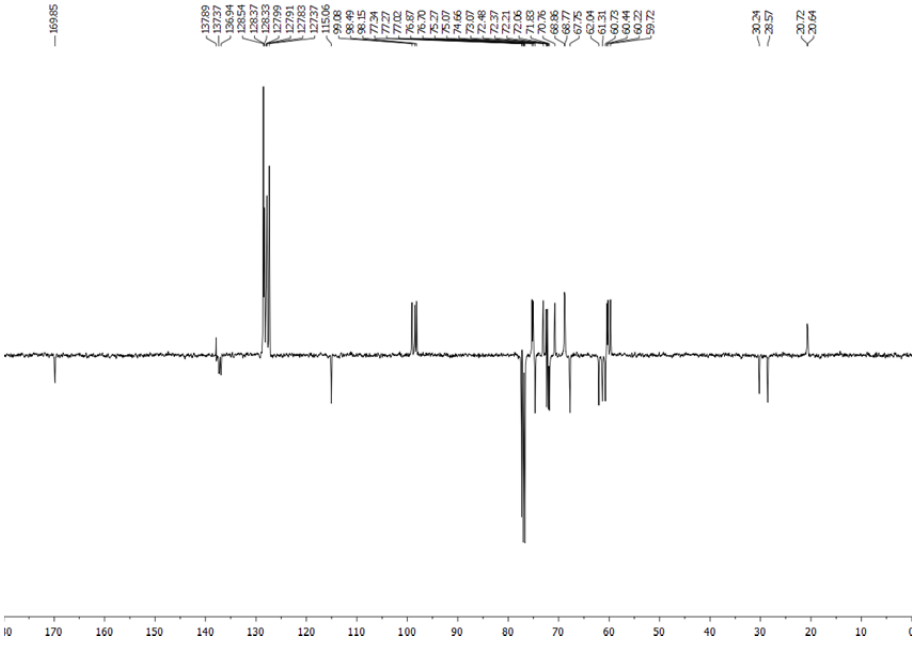
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



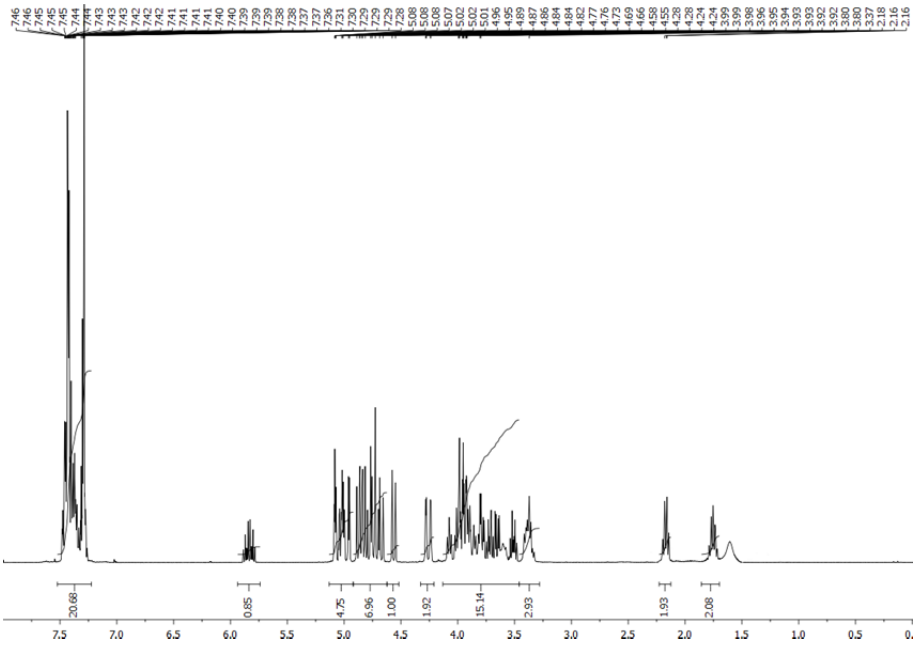
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **59**



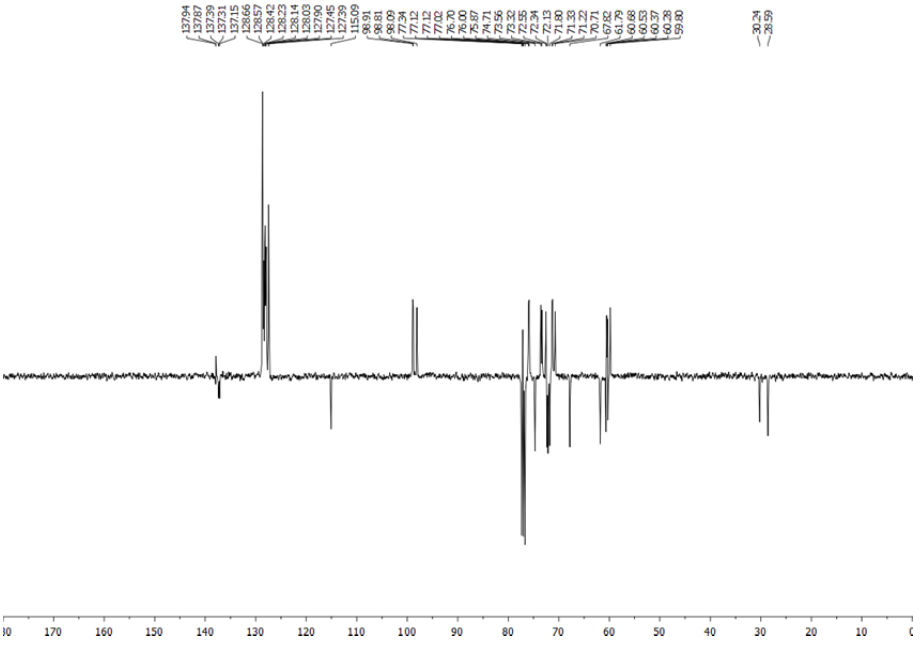
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



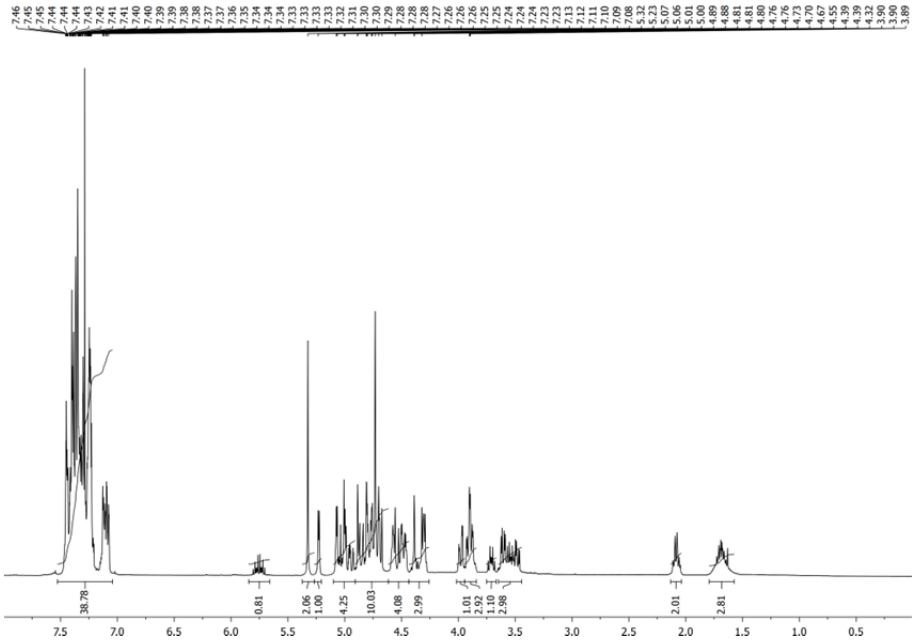
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **60**



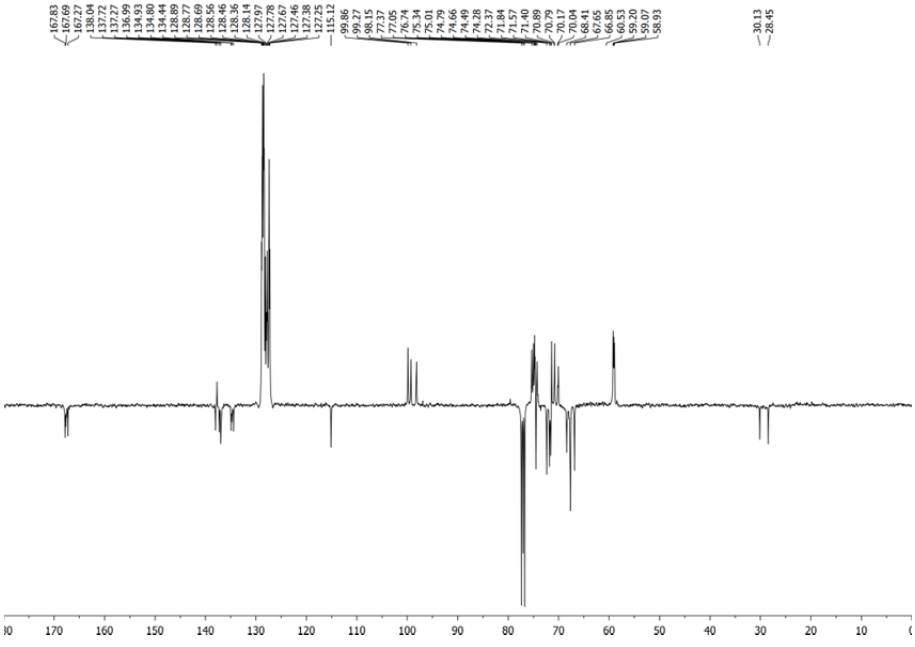
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



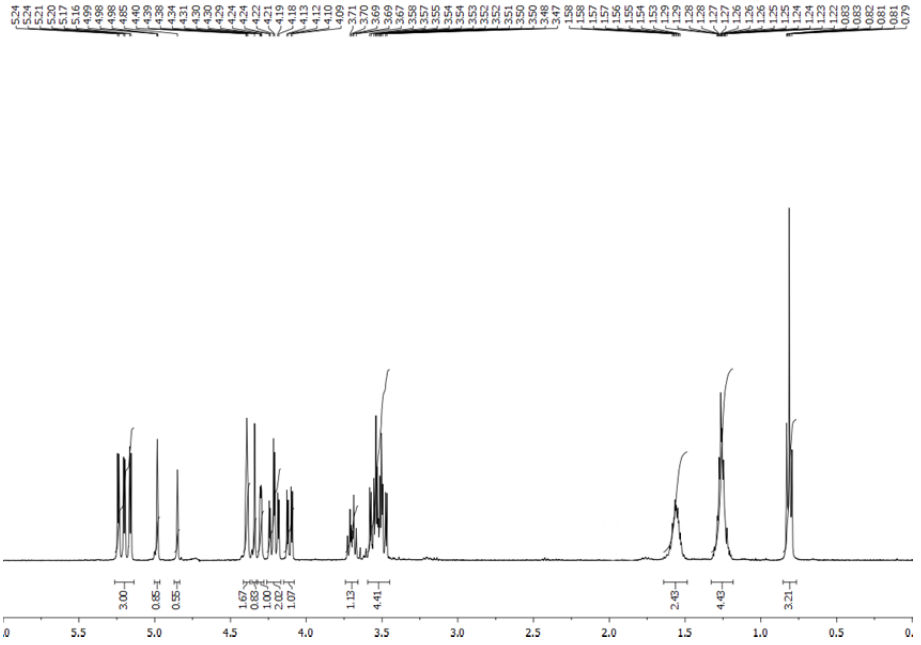
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **61**



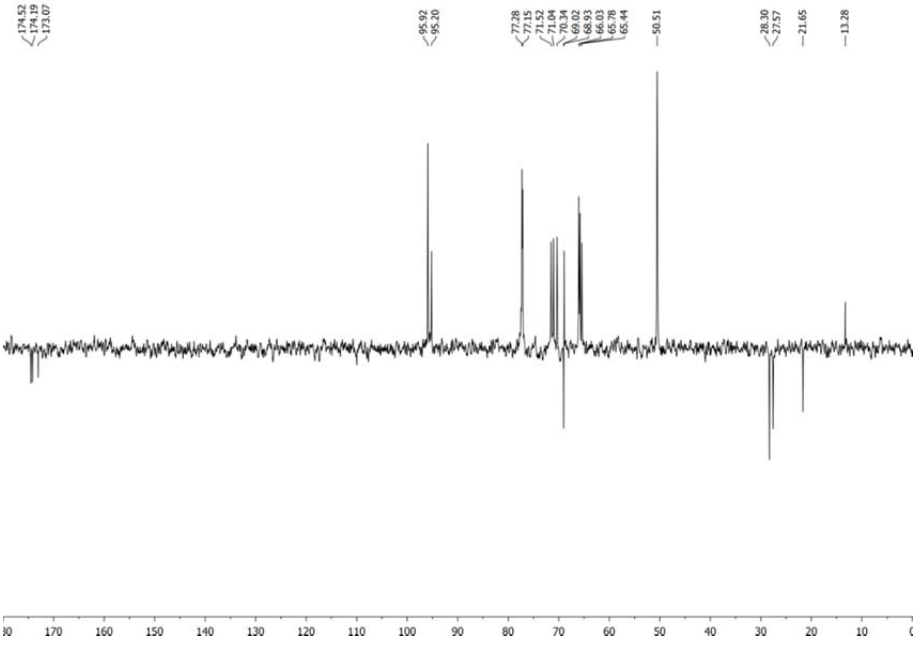
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



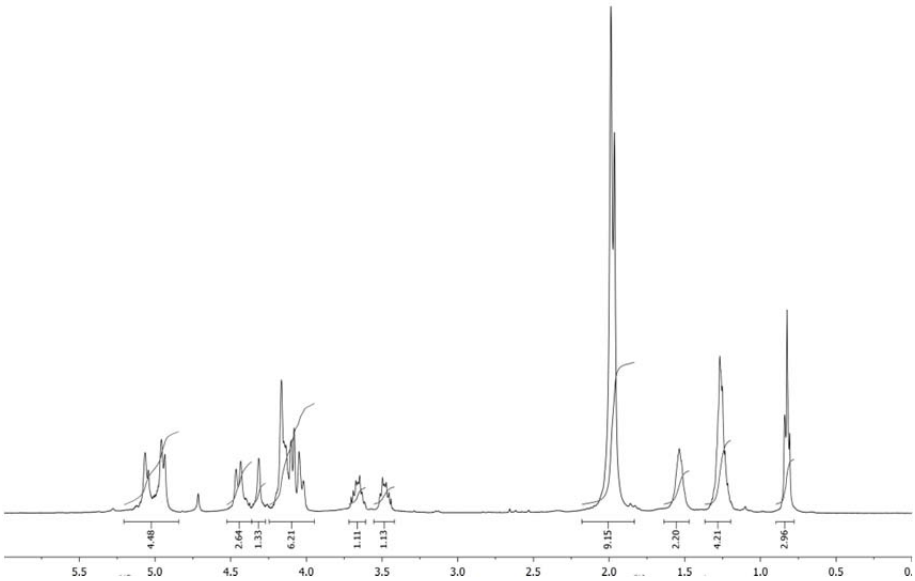
^{1}H NMR spectrum (400 Mhz, D_2O) of compound **62**



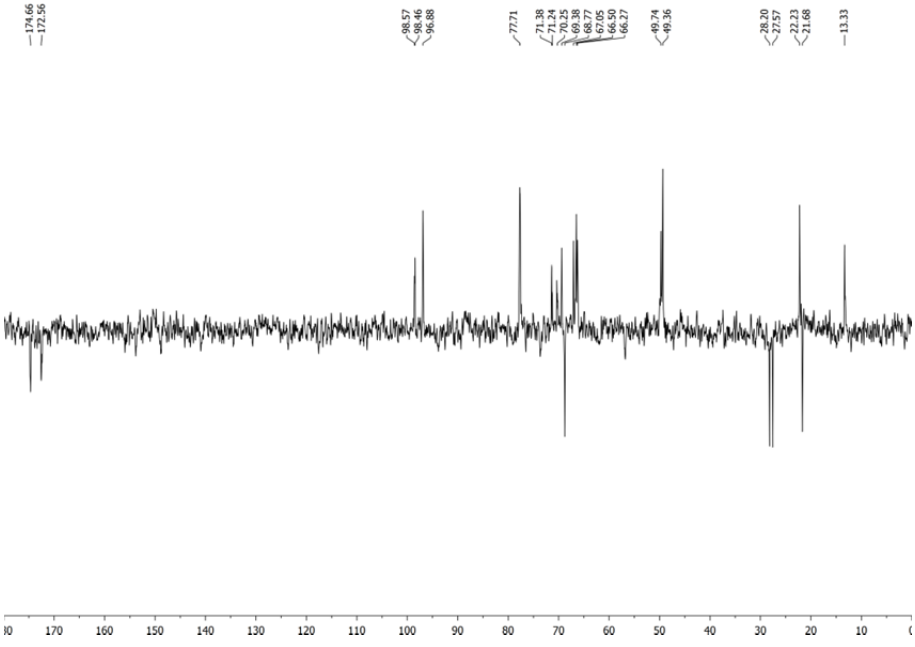
^{13}C NMR spectrum (100.6 MHz, D_2O)



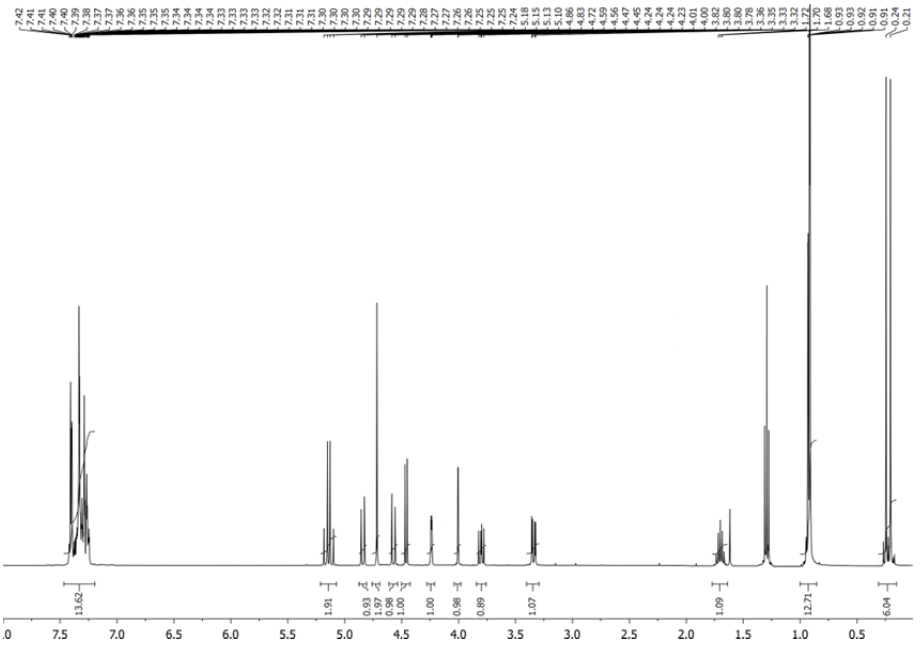
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **63**



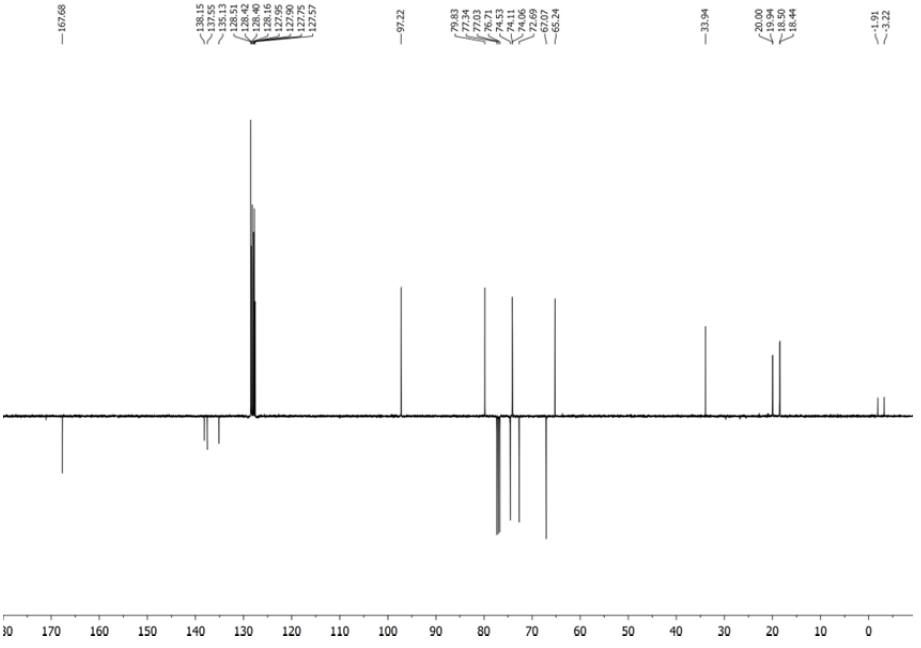
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



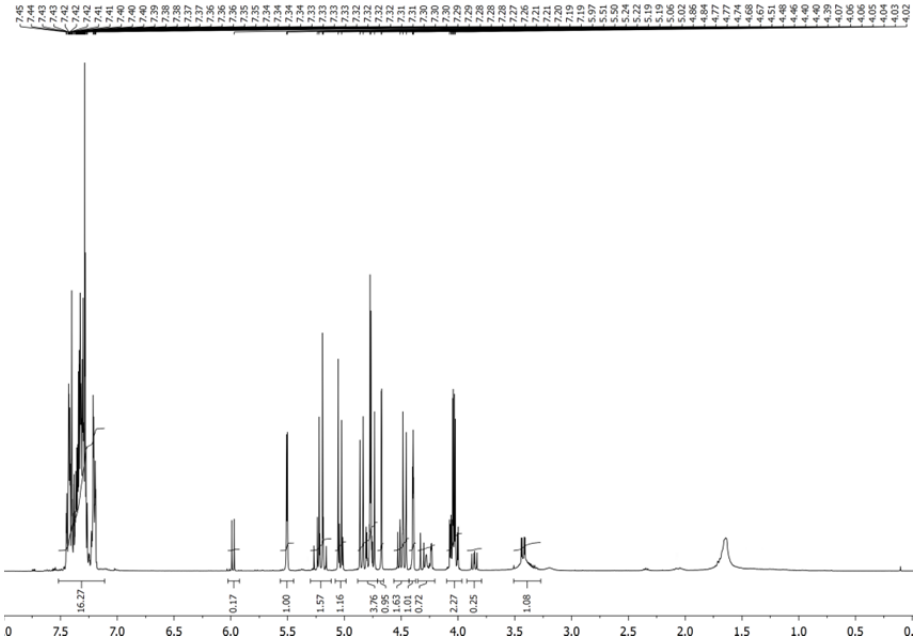
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **38a**



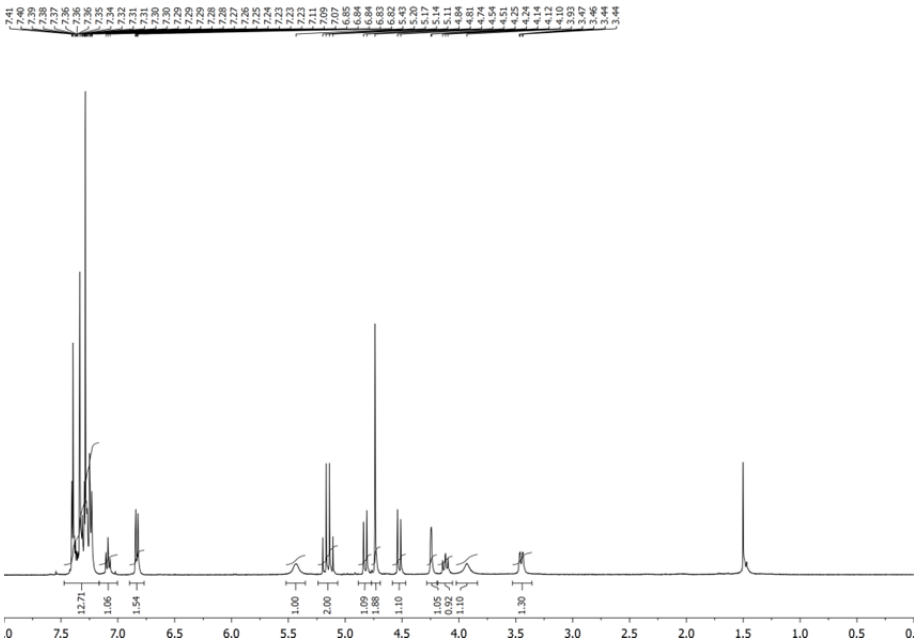
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



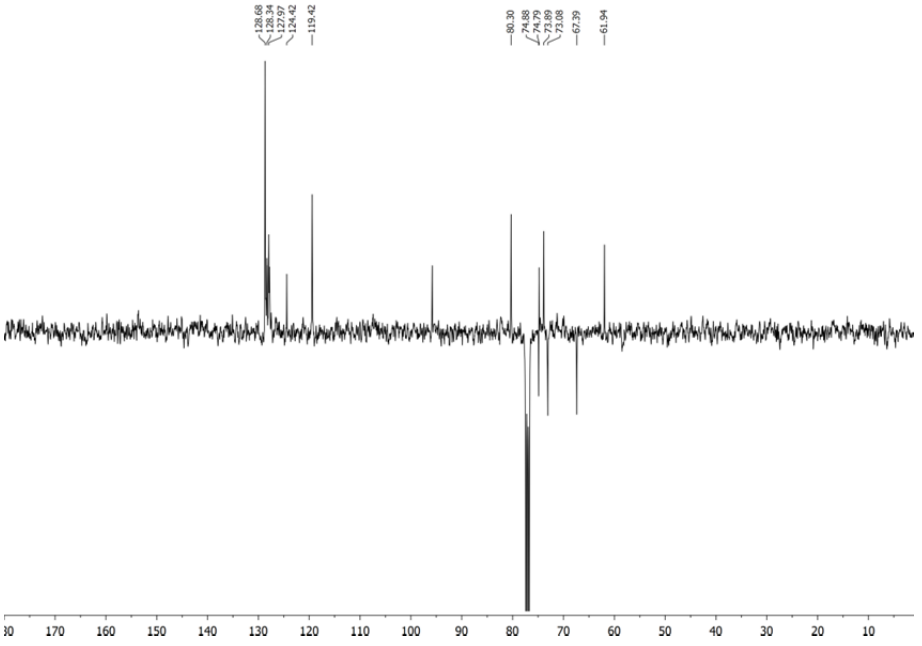
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **38b**



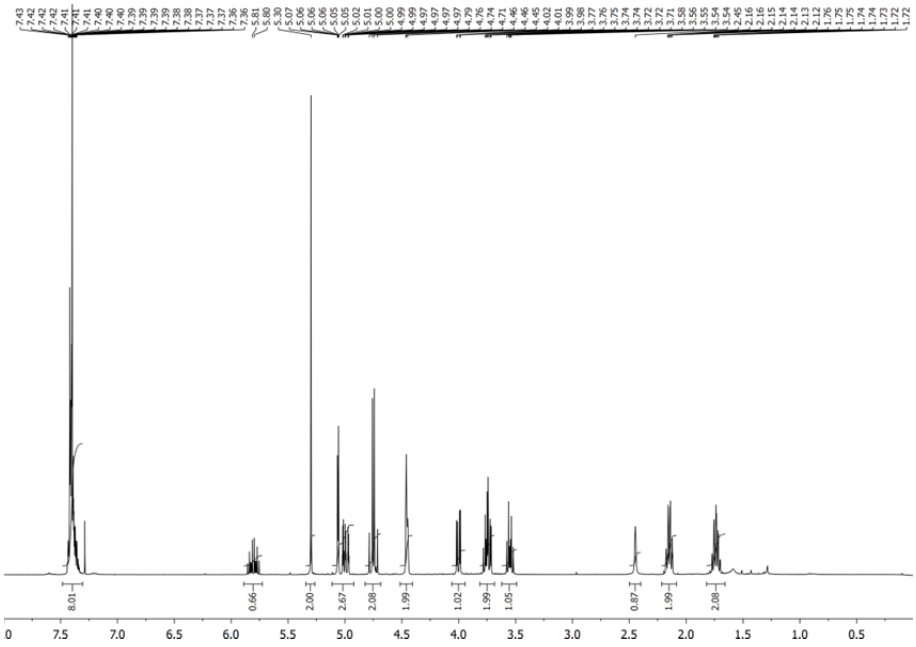
^{1}H NMR spectrum (400 Mhz, $CDCl_3$, 50°C) of compound **67β**



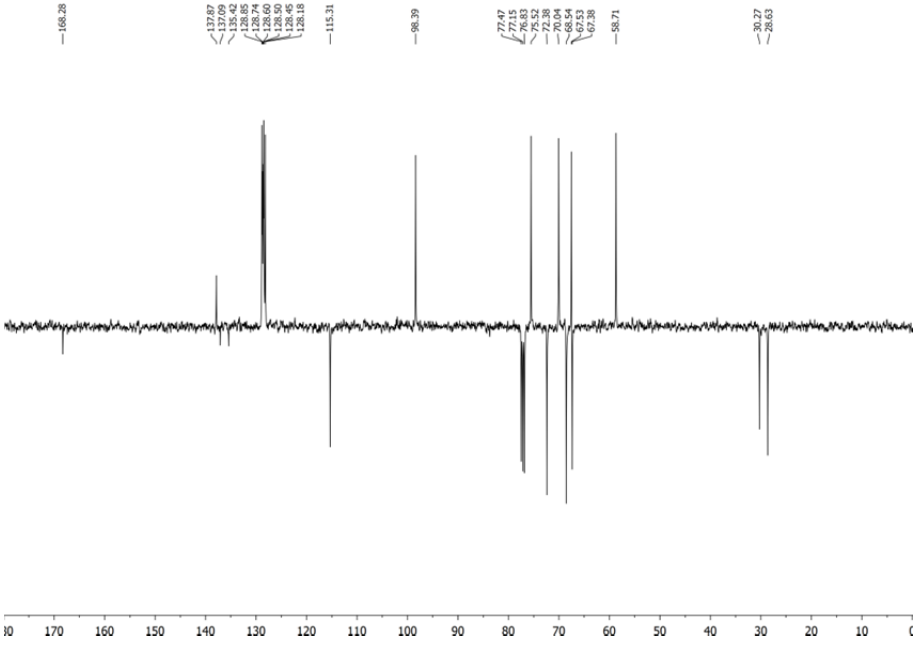
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$, 50°C)



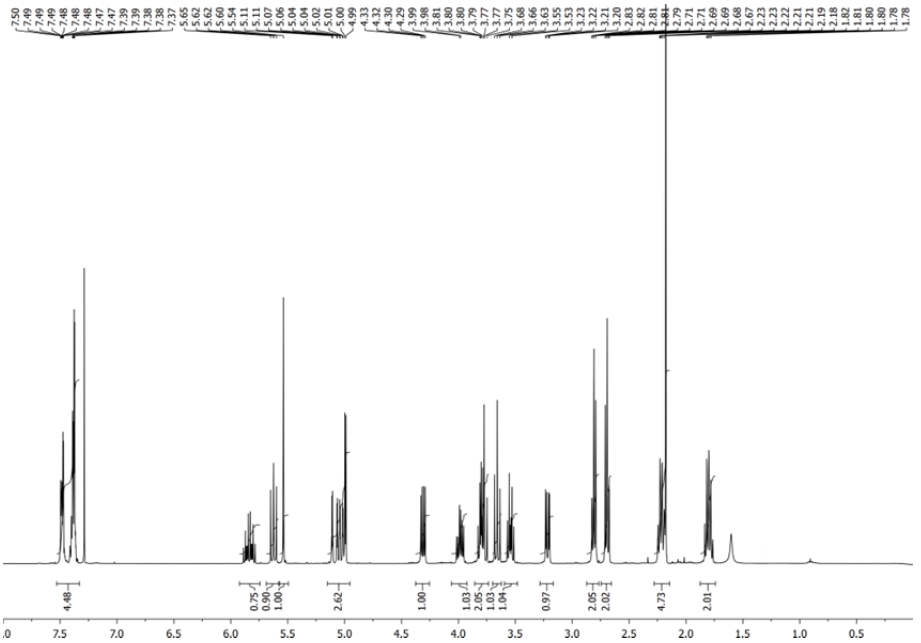
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **68**



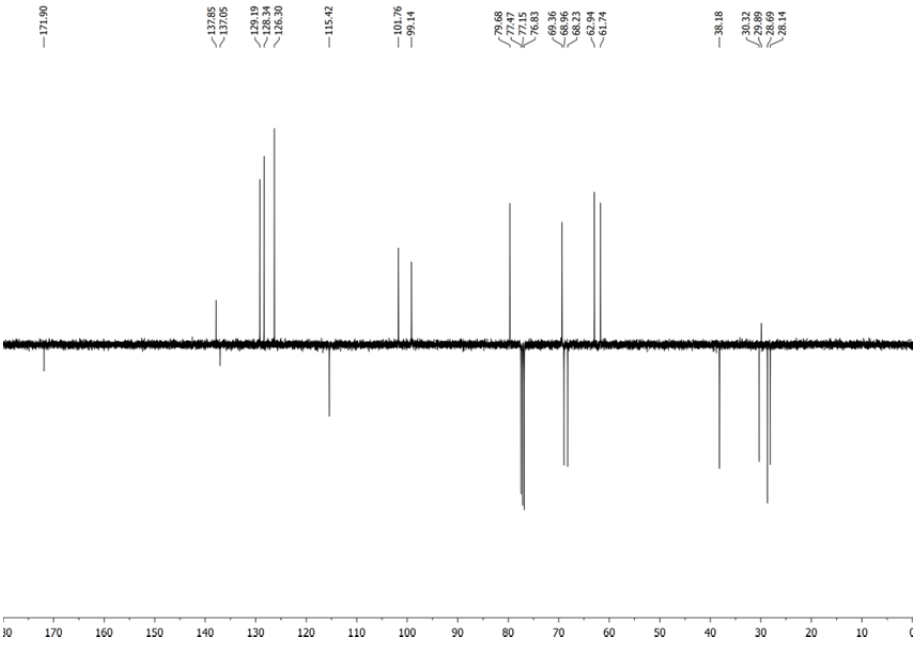
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



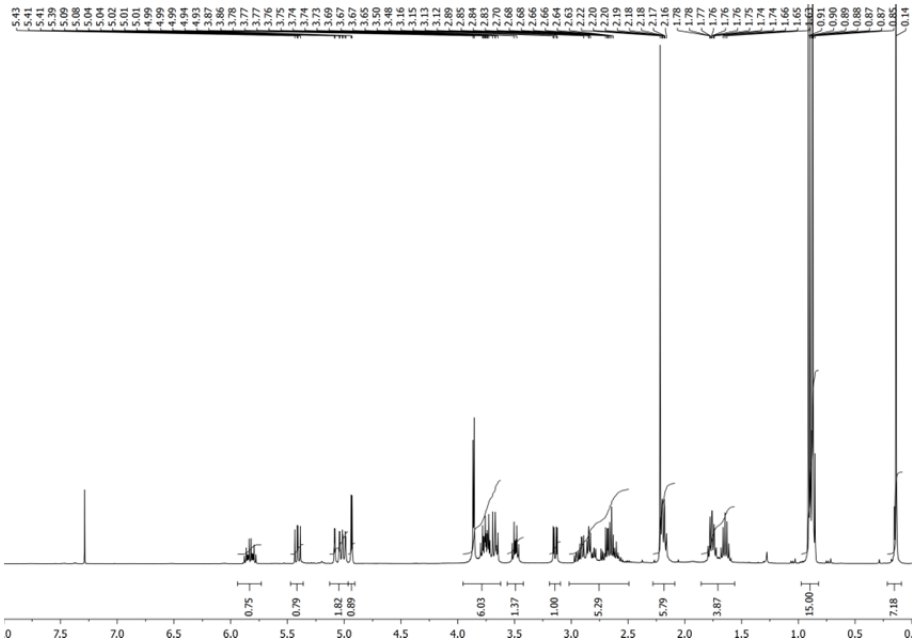
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **72a**



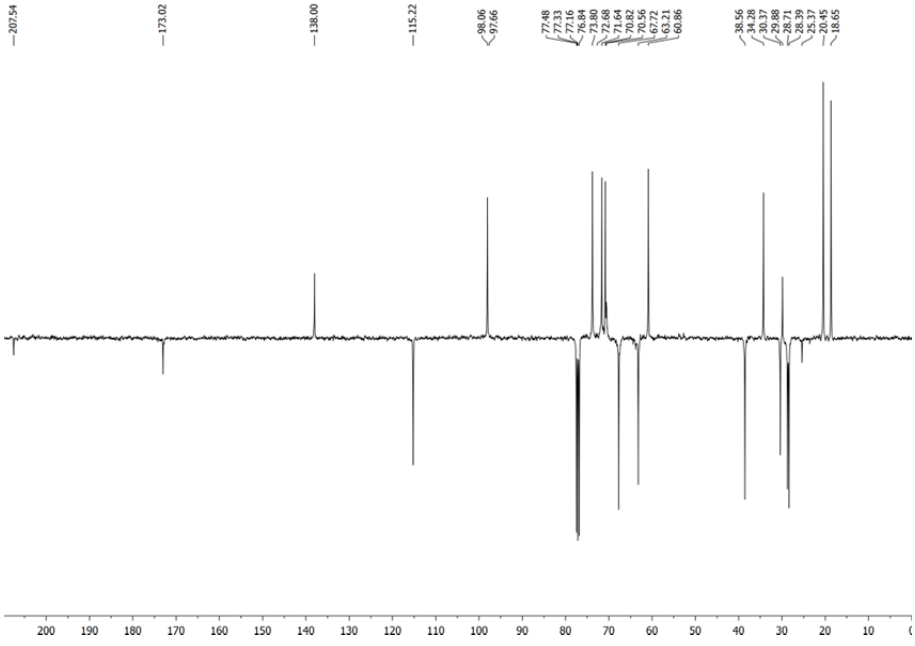
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



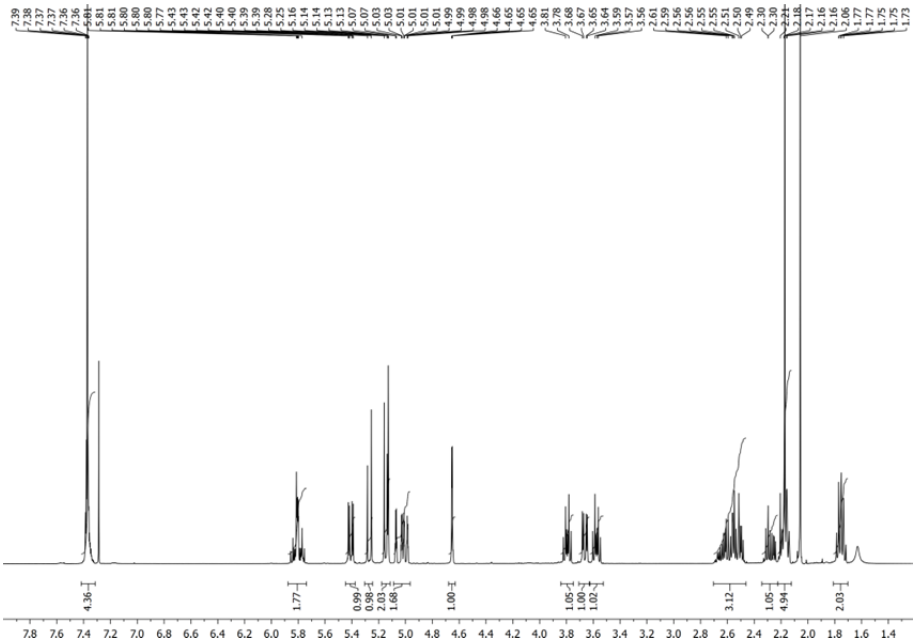
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **74α**



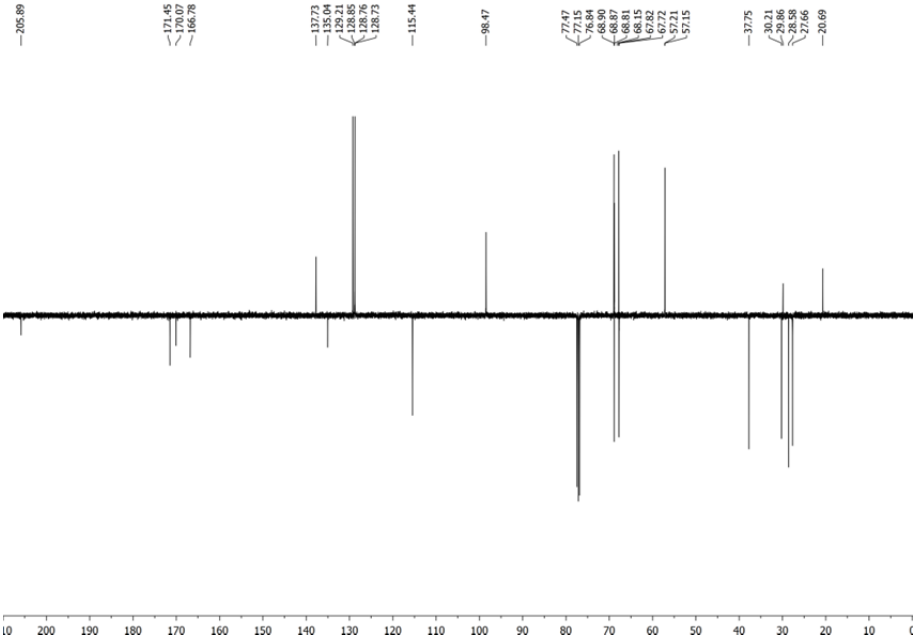
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



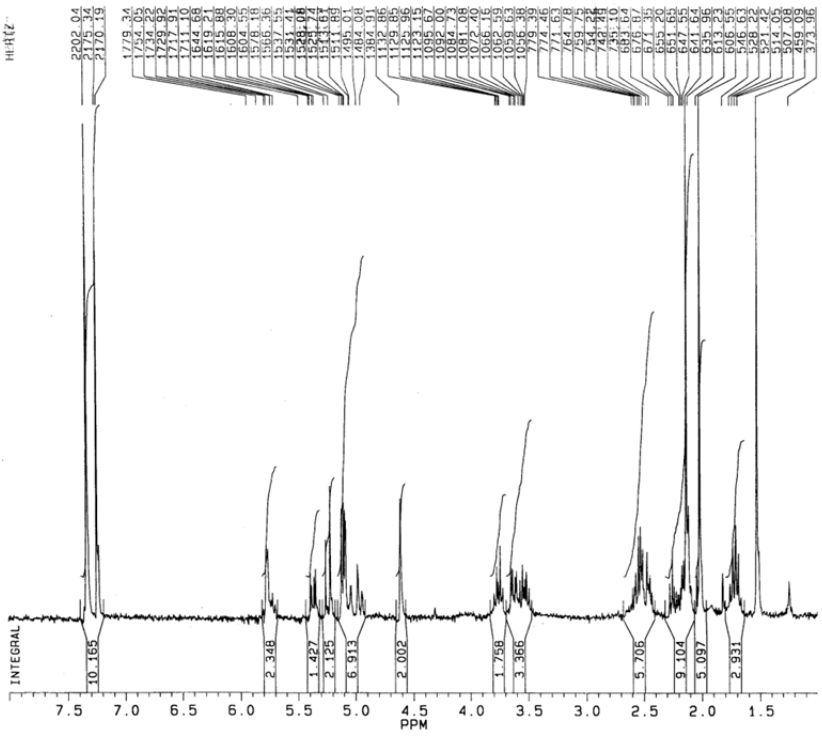
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **75α**



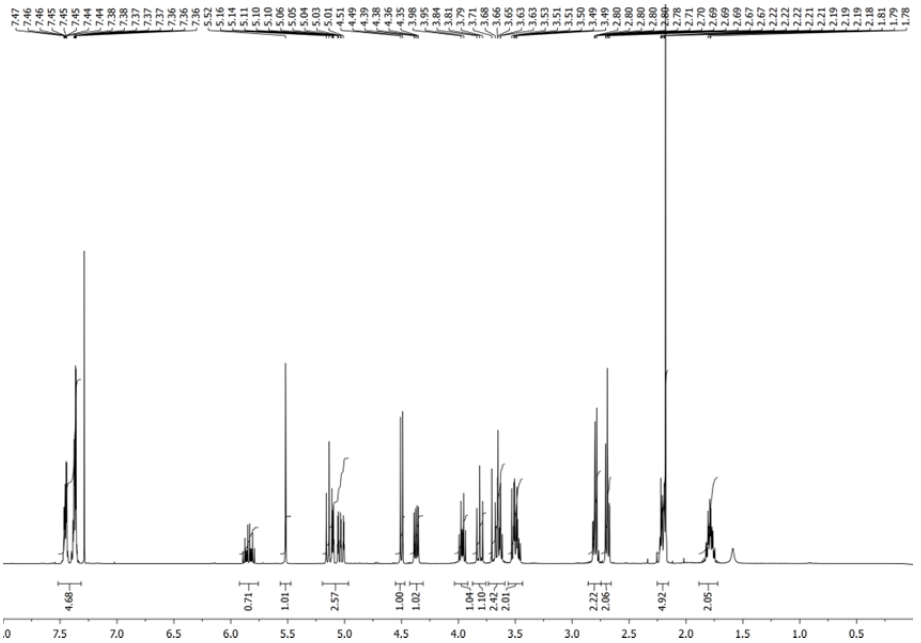
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



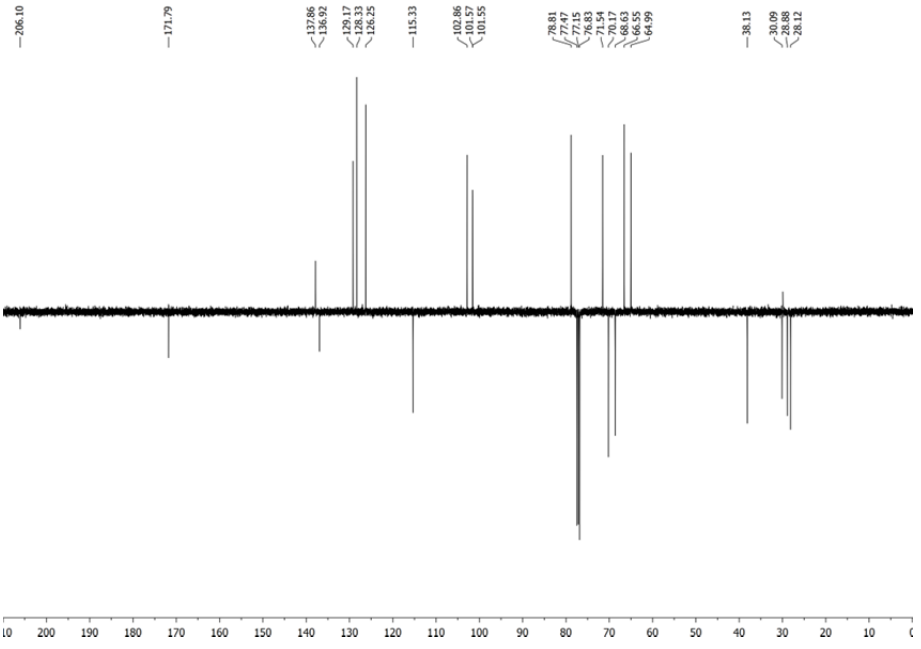
^{1}H NMR spectrum (300 Mhz, $CDCl_3$) of compound **76α**



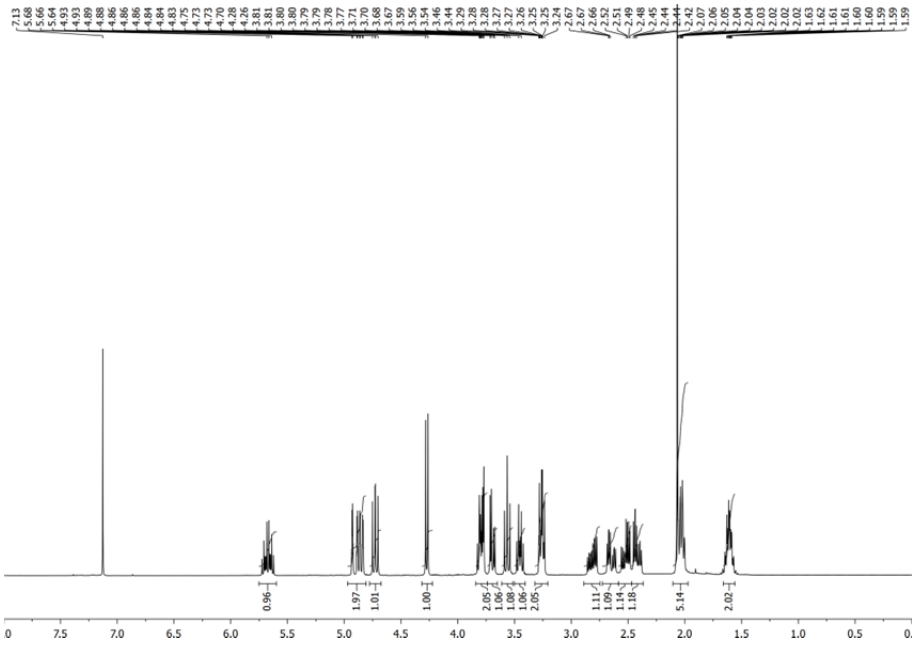
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **73a**



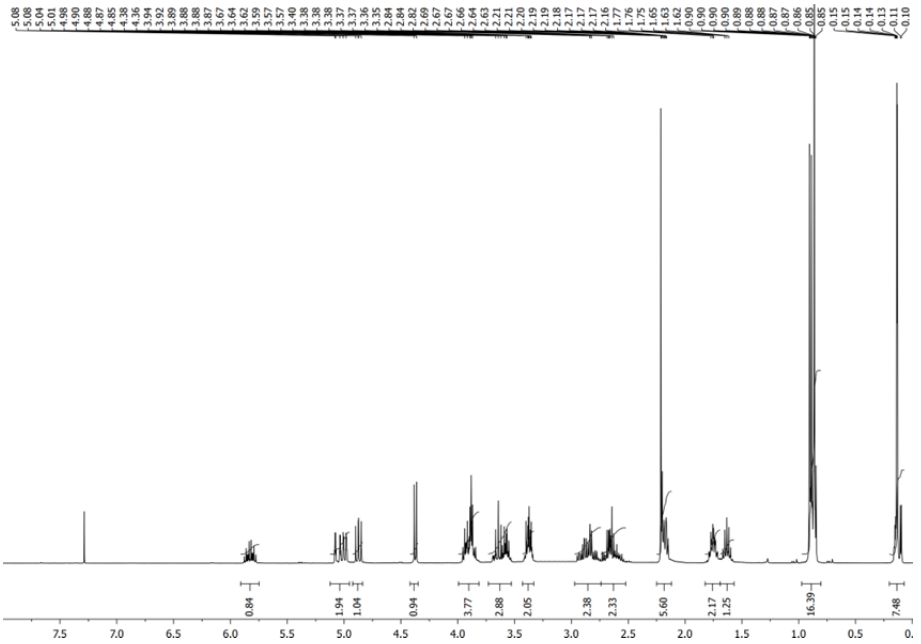
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



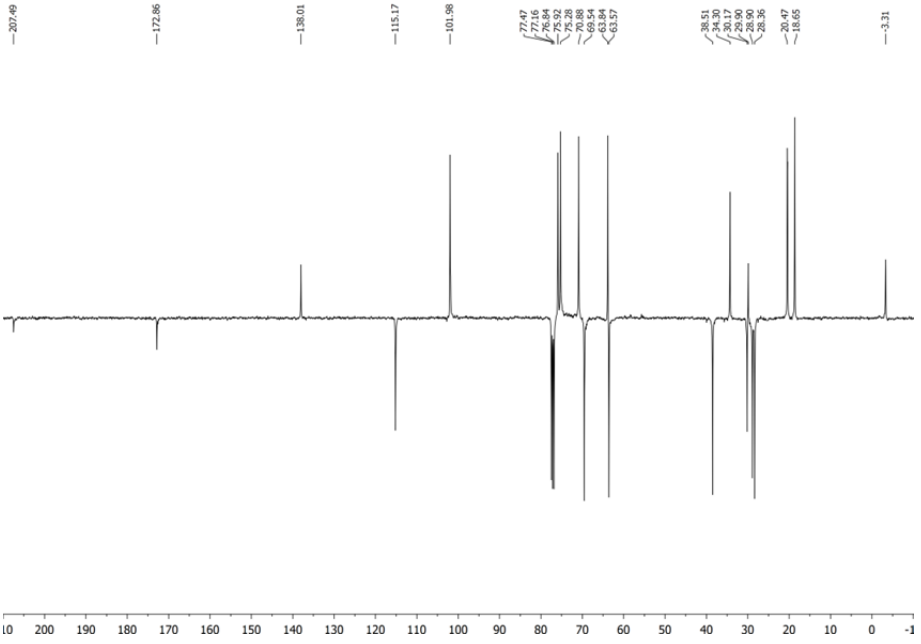
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **73b**



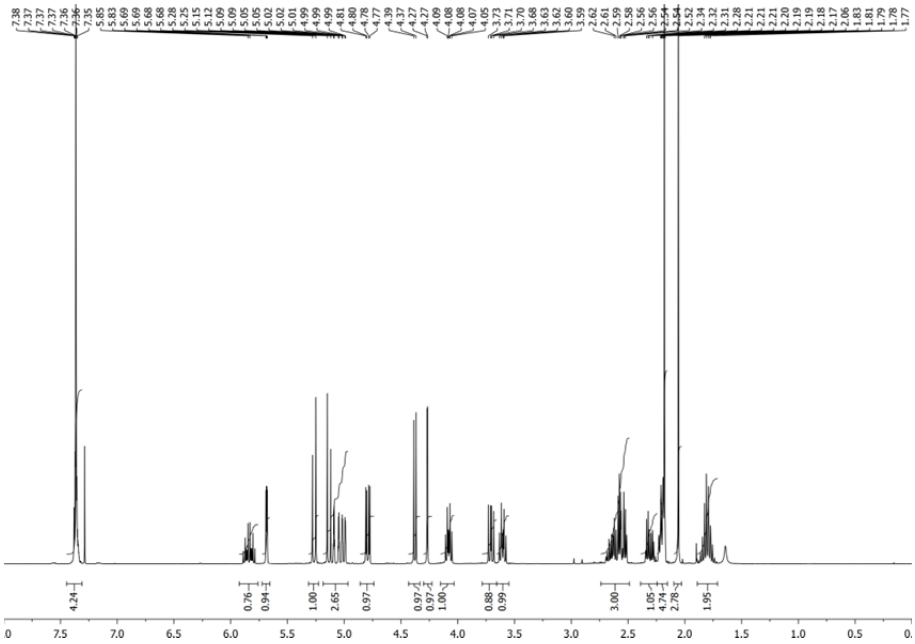
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **74β**



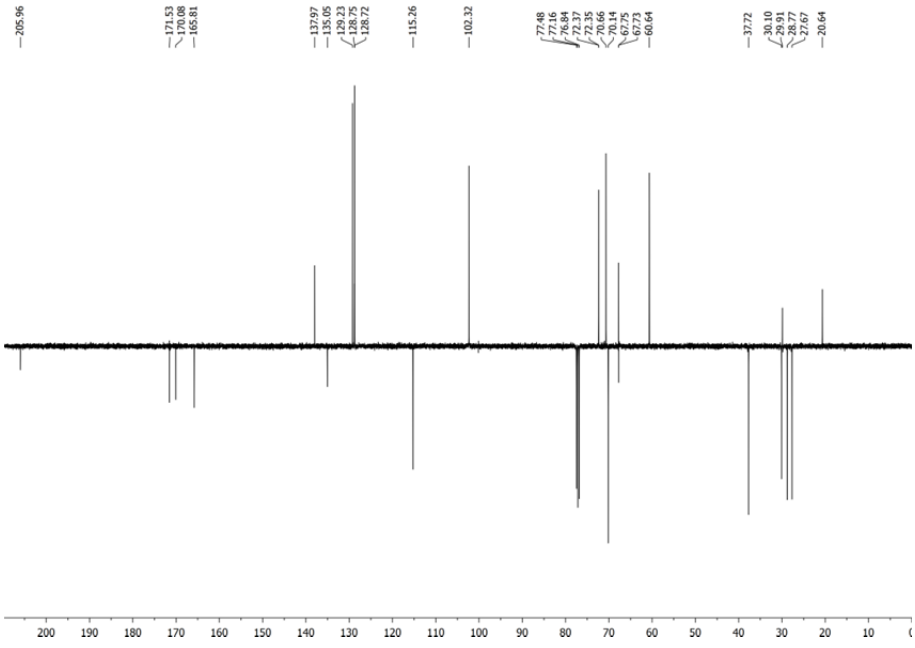
^{13}C NMR spectrum (100.6 Mhz, $CDCl_3$)



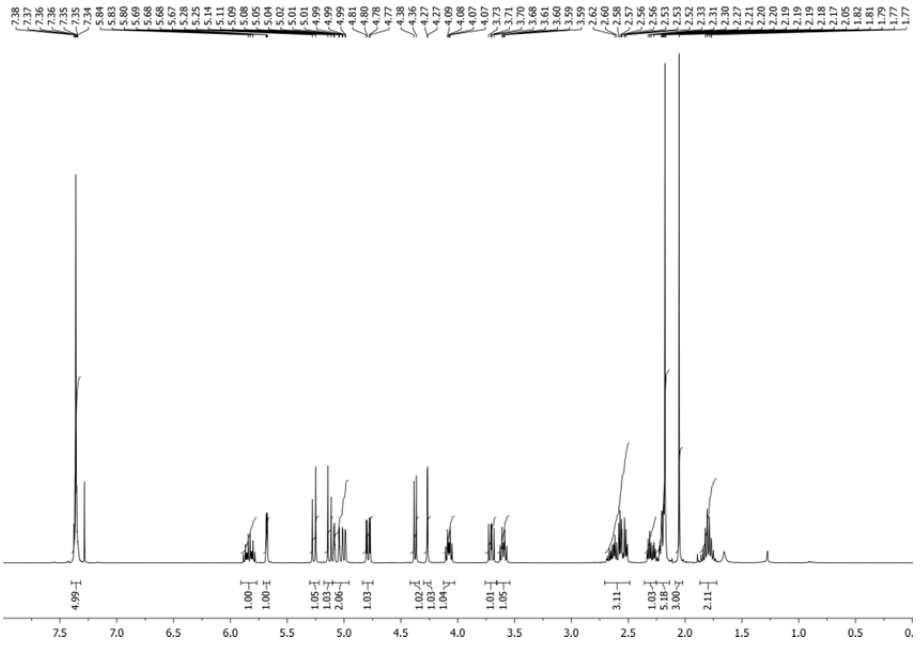
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **75β**



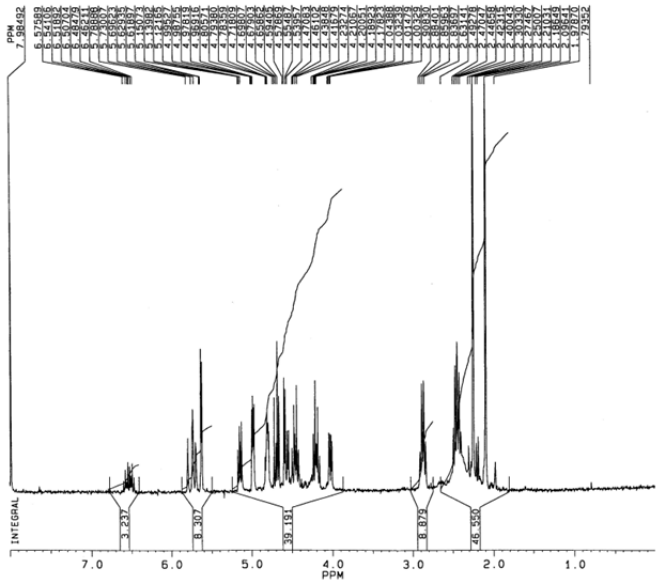
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



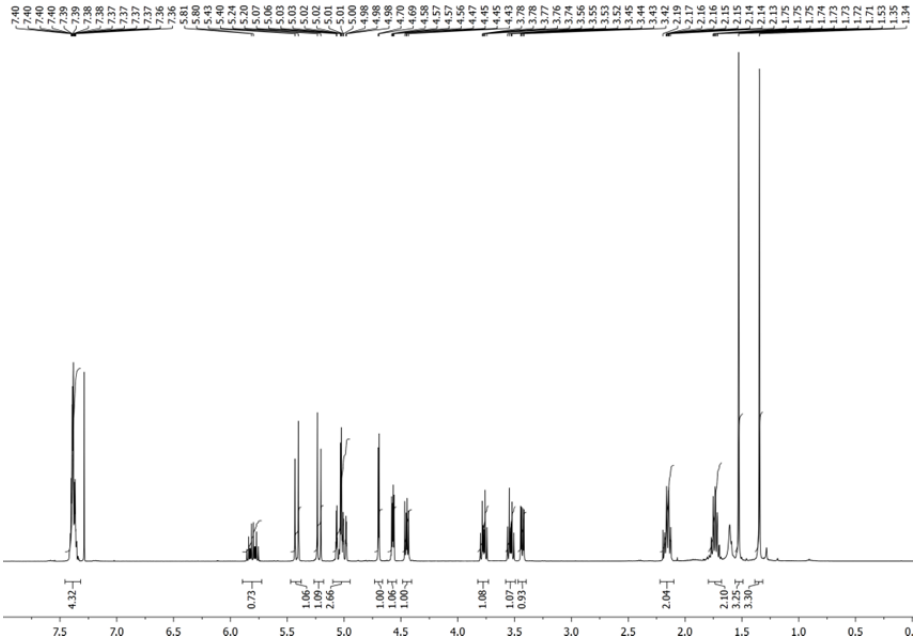
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **76β**



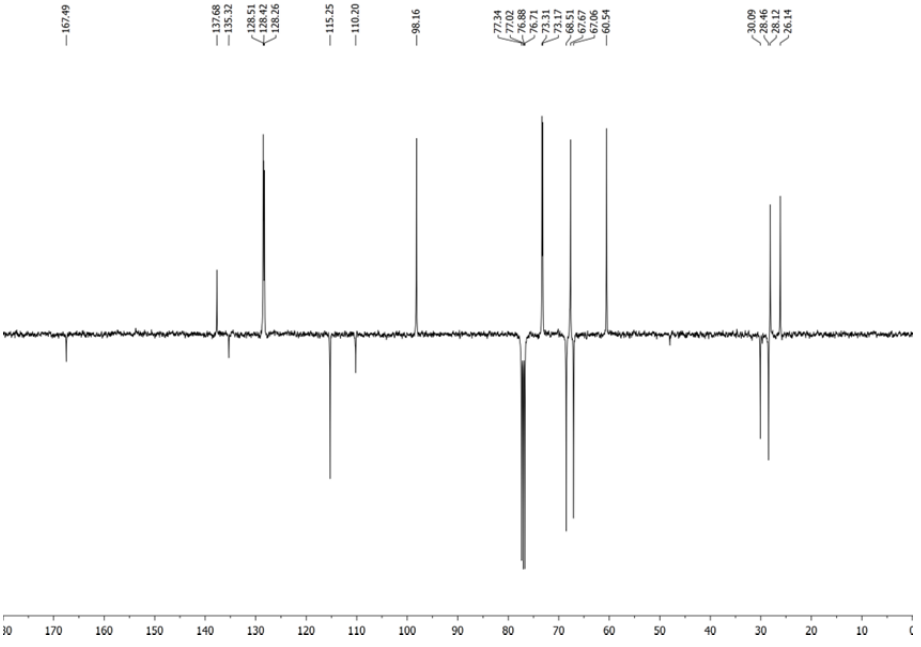
^{1}H NMR spectrum (300 Mhz, $CDCl_3$) of compound **80**



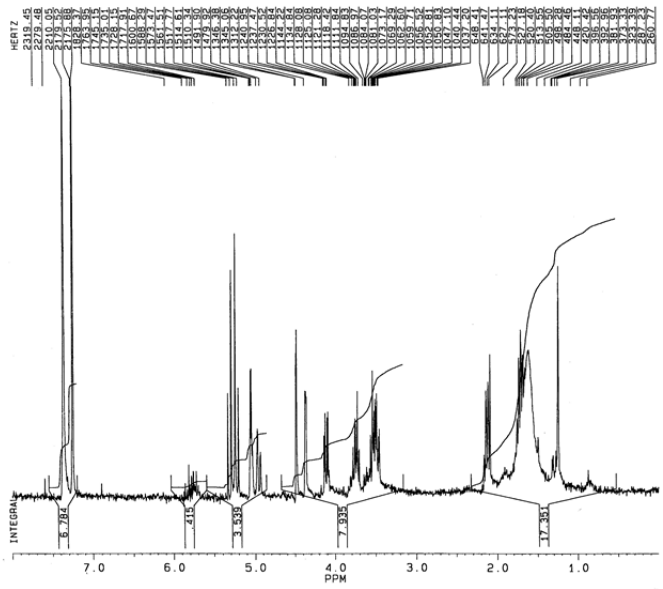
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **81**



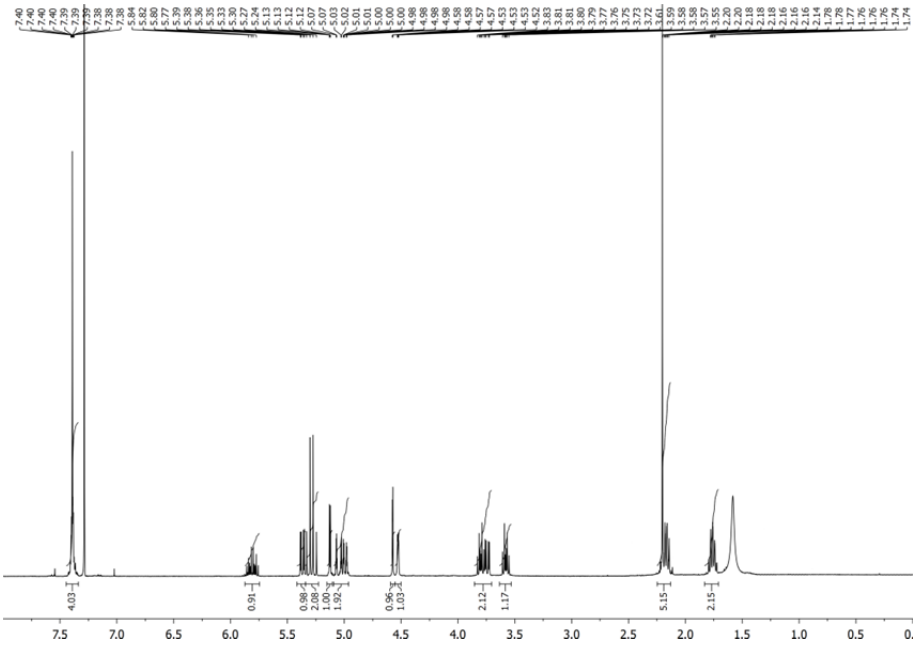
^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



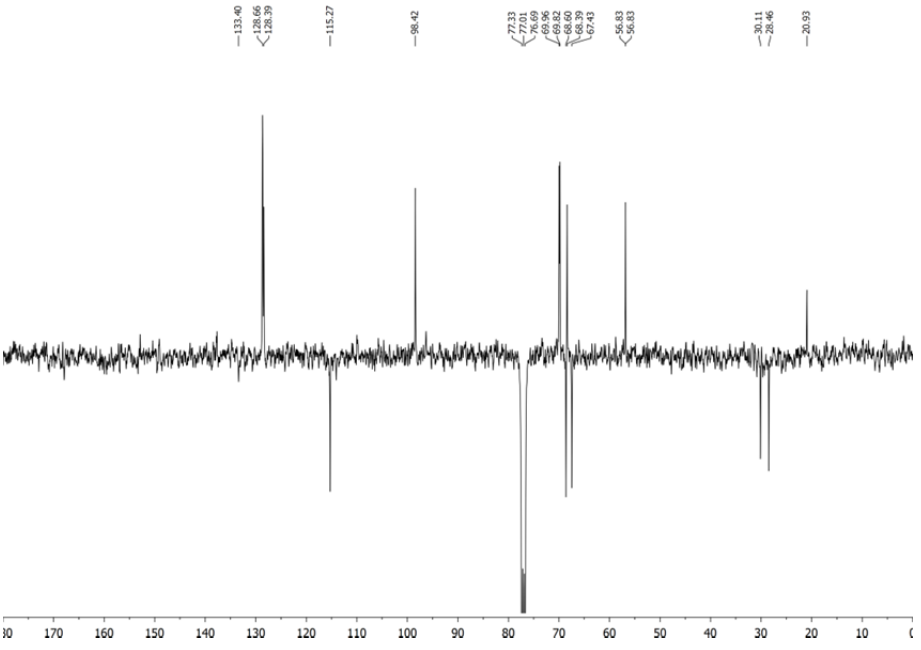
^{1}H NMR spectrum (300 Mhz, $CDCl_3$) of compound **82**



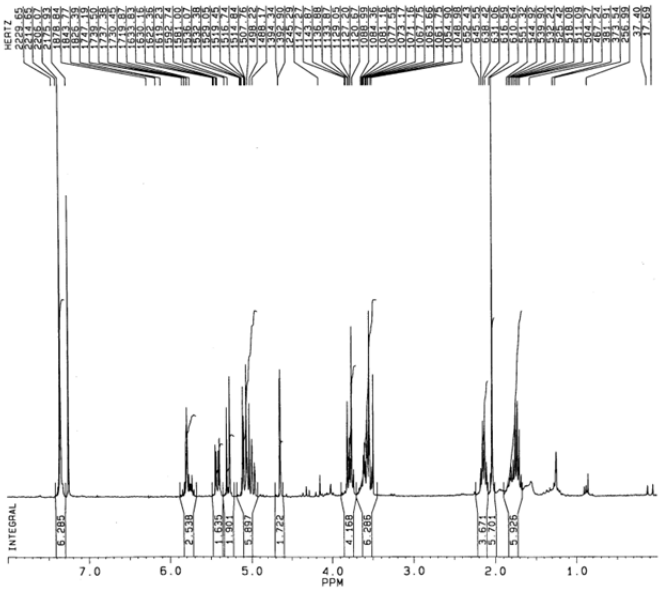
^{1}H NMR spectrum (400 Mhz, $CDCl_3$) of compound **70**



^{13}C NMR spectrum (100.6 MHz, $CDCl_3$)



^{1}H NMR spectrum (300 Mhz, $CDCl_3$) of compound **83**



References

1. Parkin, J.; Cohen, B. *Lancet* **2001**, *357*, 1777-1789.
2. Dochez, A.R.; Avery, O.T. *J. Exp. Med.* **1917**, *26*, 477-493.
3. Heidelberger, M.; Avery, O.T. *J. Exp. Med.* **1923**, *38*, 73-79.
4. Heidelberger, M.; Avery, O.T. *J. Exp. Med.* **1924**, *40*, 301-317.
5. Avery, O.T.; Goebel, W.F. *J. Exp. Med.* **1929**, *50*, 533-550.
6. Diethelm-Okita, B.M.; Raju, R.; Okita, D.K.; Conti-Fine, B.M. *J. Inf. Dis.* **1997**, *175*, 382-391.
7. Berti, F.; Adamo, R. *ACS Chem. Biol.* **2013**, *8*, 1653-1663.
8. Buskas, T.; Li, Y.; Boons, G.-J. *Chem. Eur. J.* **2004**, *10*, 3517-3524.
9. Costantino, P.; Rappuoli, R.; Berti, F. *Expert Opin. Drug Dis.* **2011**, *6*, 1045-1066.
10. Lundquist, J.J.; Toone, E.J. *Chem. Rev.* **2002**, *102*, 555-578.
11. Lee, R.T.; Lee, Y.C. In *Neoglycoconjugates. Preparation and Application*; Lee, Y.C., Lee, R.T., Eds.; Academic Press: London, 1994, p 23-50.
12. Krauss, I.J.; Joyce, J.G.; Finnefrock, A.C.; Song, H.C.; Dudkin, V.Y. *et al. J. Am. Chem. Soc.* **2007**, *129*, 11042-11044.
13. Ohta, T.; Miura, N.; Funitani, N.; Nakajima, F.; Niikura, K. *et al. Angew. Chem. Int. Ed.* **2003**, *42*, 5186-5189.
14. Pujol, A.M.; Cuillel, M.; Renaudet, O.; Lebrun, C.; Charbonnier, P. *et al. J. Am. Chem. Soc.* **2011**, *133*, 286-296.
15. Chabre, Y.M.; Roy, R. *Adv. Carbohydr. Chem. Biochem.* **2010**, *63*, 165-393.
16. Andre, S.; Pieters, R.J.; Vrasidas, I.; Kaltner, H.; Kuwabara, I. *et al. ChemBioChem* **2001**, *2*, 822-830.
17. Heidecke, C.D.; Lindhorst, T.K. *Chem. Eur. J.* **2007**, *13*, 9056-9067.
18. Mintzer, M.A.; Dane, E.L.; O'Toole, G.A.; Grinstaff, M.W. *Mol. Pharm.* **2012**, *9*, 342-354.
19. Touaibia, M.; Roy, R. *Mini-Rev. Med. Chem.* **2007**, *7*, 1270-1283.
20. Turnbull, W.B.; Stoddart, J.F. *J. Biotechnol.* **2002**, *90*, 231-255.

21. Kiessling, L.L.; Gestwicki, J.E.; Strong, L.E. *Angew. Chem. Int. Ed.* **2006**, *45*, 2348-2368.
22. Bes, L.; Angot, S.; Limer, A.; Haddleton, D.M. *Macromolecules* **2003**, *36*, 2493-2499.
23. Otsuka, I., Blanchard, B., Borsali, R., Imberty, A. and Kakuchi, T. *ChemBioChem* **2010**, *11*, 2399-2408.
24. Ponader, D., Wojcik, F., Beceren-Braun, F., Dernedde, J. and Hartmann, L. *Biomacromolecules* **2012**, *13*, 1845-1852.
25. Rieger, J.; Stoffelbach, F.; Cui, D.; Imberty, A.; Lameignere, E. *et al. Biomacromolecules* **2007**, *8*, 2717-2725.
26. Marradi, M.; Martin-Lomas, M.; Penades, S. *Adv. Carbohydr. Chem. Biochem.* **2010**, *64*, 211-290.
27. Wang, X.; Matei, E.; Deng, L.Q.; Ramstrom, O.; Gronenborn, A.M. *et al. Chem. Commun.* **2011**, *47*, 8620-8622.
28. Cecioni, S.; Oerthel, V.; Iehl, J.; Holler, M.; Goyard, D. *et al. Chem. Eur. J.* **2011**, *17*, 3252-3261.
29. Nierengarten, J.F.; Iehl, J.; Oerthel, V.; Holler, M.; Illescas, B.M. *et al. Chem. Commun.* **2010**, *46*, 3860-3862.
30. Compain, P.; Decroocq, C.; Iehl, J.; Holler, M.; Hazelard, D. *et al. Angew. Chem. Int. Ed.* **2010**, *49*, 5753-5756.
31. Dondoni, A.; Marra, A. *Chem. Rev.* **2010**, *110*, 4949-4977.
32. Sansone, F.; Rispoli, G.; Casnati, A.; Ungaro, R. In *Synthesis and Biological Applications of Glycoconjugates*; Renaudet, O., Spinelli, N., Eds.; Bentham Science Publishers Ltd.: 2011, p 36-63.
33. Baldini, L.; Casnati, A.; Sansone, F.; Ungaro, R. *Chem. Soc. Rev.* **2007**, *36*, 254-266.
34. Cecioni, S.; Lalor, R.; Blanchard, B.; Praly, J.P.; Imberty, A. *et al. Chem. Eur. J.* **2009**, *15*, 13232-13240.
35. Andre, S.; Grandjean, C.; Gautier, F.M.; Bernardi, S.; Sansone, F. *et al. Chem. Commun.* **2011**, *47*, 6126-6128.

36. Hong, S.Y.; Tobias, G.; Al-Jamal, K.T.; Ballesteros, B.; Ali-Boucetta, H. *et al. Nat. Mat.* **2010**, *9*, 485-490.
37. Kikkeri, R.; Laurino, P.; Odedra, A.; Seeberger, P.H. *Angew. Chem. Int. Ed.* **2010**, *49*, 2054-2057.
38. Robinson, A.; Fang, J.M.; Chou, P.T.; Liao, K.W.; Chu, R.M. *et al. ChemBioChem* **2005**, *6*, 1899-1905.
39. Yang, Y.; Yu, M.; Yan, T.T.; Zhao, Z.H.; Sha, Y.L. *et al. Biorg. Med. Chem.* **2010**, *18*, 5234-5240.
40. Cobb, B.A.; Kasper, D.L. *Glycobiology* **2008**, *18*, 707-718.
41. Surana, N.K.; Kasper, D.L. *Immunol. Rev.* **2012**, *245*, 13-26.
42. Avci, F.Y.; Li, X.; Tsuji, M.; Kasper, D.L. *Semin. Immunol.* **2013**, *25*, 146-151.
43. Bloem, K.; Garcia-Vallejo Juan, J.; Vuist Ilona, M.; Cobb Brian, A.; van Vliet Sandra, J. *et al. Front. Immunol.* **2013**, *4*, 103.
44. Ruiz-Perez, B.; Chung, D.R.; Sharpe, A.H.; Yagita, H.; Kalka-Moll, W.M. *et al. Proc. Natl. Acad. Sci. USA* **2005**, *102*, 16753-16758.
45. Gallorini, S.; Berti, F.; Parente, P.; Baronio, R.; Aprea, S. *et al. J. Immunol.* **2007**, *179*, 8208-8215.
46. Tzianabos, A.O., Onderdonk, A.B., Rosner, B., Cisneros, R.L. and Kasper, D.L. *Science* **1993**, *262*, 416-419.
47. Mazmanian, S.K.; Kasper, D.L. *Nat. Rev. Immunol.* **2006**, *6*, 849-858.
48. Tzianabos, A.; Wang, J.Y.; Kasper, D.L. *Carbohydr. Res.* **2003**, *338*, 2531-2538.
49. De Silva, R.A., Wang, Q.L., Chidley, T., Appulage, D.K. and Andreana, P.R. *J. Am. Chem. Soc.* **2009**, *131*, 9622-9623.
50. De Silva, R.A.; Appulage, D.K.; Pietraszkiewicz, H.; Bobbitt, K.R.; Media, J. *et al. Cancer Immunol. Immunother.* **2012**, *61*, 581-585.
51. Bourgault, J.P.; Trabbic, K.R.; Shi, M.; Andreana, P.R. *Org. Biomol. Chem.* **2014**, *12*, 1699-1702.
52. Russ. J. Bioorg. Chem.Tesheva, A.M.; Aparin, P.G.; L'Vov, V.L. *Russ. J. Bioorg. Chem.* **2002**, *28*, 58-61.

53. Felix, A.; Pitt, R.M. *Lancet* **1934**, *II*, 186-191.
54. Santhanam, S.K.; Dutta, D.; Parween, F.; Qadri, A. *J. Infect. Dis.* **2014**, *210*, 79-88.
55. Virlogeux-Payant, I.; Popoff, M.Y. *Bull. Inst. Pasteur* **1996**, *94*, 237-250.
56. Szu, S.C.; Li, X.R.; Stone, A.L.; Robbins, J.B. *Infect. Immun.* **1991**, *59*, 4555-4561.
57. Levine, M.M. In *Vaccines*; 3rd ed.; Plotkin, S.A., Orenstein, W.A., Eds.; WB Saunders Co.: Philadelphia, 1999, p 781-815.
58. Crump, J.A., Ram, P.K., Gupta, S.K., Miller, M.A. and Mintz, E.D. *Epidemiol. Infect.* **2008**, *136*, 436-448.
59. Crump, J.A.; Mintz, E.D. *Clin. Infect. Dis.* **2010**, *50*, 241-246.
60. Lozano, R.; Naghavi, M.; Foreman, K.; Lim, S.; Shibuya, K. *et al. Lancet* **2012**, *380*, 2095-2128.
61. Muyembe-Tamfum, J.J.; Veyi, J.; Kaswa, M.; Lunguya, O.; Verhaegen, J. *et al. Travel Med. Inf. Dis.*, *7*, 40-43.
62. Baddam, R.; Kumar, N.; Thong, K.L.; Ngoi, S.T.; Teh, C.S. *et al. J. Bacteriol.* **2012**, *194*, 3565-3566.
63. Levine, M.M.; Black, R.E.; Lanata, C. *J. Infect. Dis.* **1982**, *146*, 724-726.
64. Gonzalez-Escobedo, G.; Marshall, J.M.; Gunn, J.S. *Nat. Rev. Microbiol.* **2011**, *9*, 9-14.
65. Bhutta, Z.A. *Br. Med. J.* **2006**, *333*, 78-82.
66. Typhoid vaccines: WHO position paper. *Wkly. Epidemiol. Rec.* **2008**, *83*, 49-59.
67. Fraser, A., Goldberg, E., Acosta, C.J., Paul, M. and Leibovici, L. *Cochrane Database Syst. Rev.* **2007**, CD001261.
68. DeRoeck, D.; Ochiai, R.L.; Yang, J.; Anh, D.D.; Alag, V. *et al. Expert Rev. Vaccines* **2008**, *7*, 547-560.
69. Guzman, C.A.; Borsutzky, S.; Griot-Wenk, M.; Metcalfe, I.C.; Pearman, J. *et al. Vaccine* **2006**, *24*, 3804-3811.
70. Fraser, A., Paul, M., Goldberg, E., Acosta, C.J. and Leibovici, L. *Vaccine* **2007**, *25*, 7848-7857.

71. Acharya, I.L.; Lowe, C.U.; Thapa, R.; Gurubacharya, V.L.; Shrestha, M.B. *et al. New Engl. J. Med.* **1987**, *317*, 1101-1104.
72. Klugman, K.P.; Koornhof, H.J.; Robbins, J.B.; Le Cam, N.N. *Vaccine* **1996**, *14*, 435-438.
73. Szu, S.C.; Taylor, D.N.; Trofa, A.C.; Clements, J.D.; Shiloach, J. *et al. Infect. Immun.* **1994**, *62*, 4440-4444.
74. Szu, S.C.; Robbins, J.B.; Schneerson, R.; Lin, F.-Y. In *New Generation Vaccines*, p 489-496.
75. Shah Nitin, K. *Indian Pediatr.* **2009**, *46*, 181-182.
76. Lin, F.Y.; Vo, A.H.; Phan, V.B.; Nguyen, T.T.; Bryla, D. *et al. Am. J. Trop. Med. Hyg.* **2000**, *62*, 644-648.
77. Mai, N.L.; Phan, V.B.; Vo, A.H.; Tran, C.T.; Lin, F.Y. *et al. New Engl. J. Med.* **2003**, *349*, 1390-1391.
78. Thiem, V.D.; Lin, F.Y.; Canh do, G.; Son, N.H.; Anh, D.D. *et al. Clin. Vaccine Immunol.* **2011**, *18*, 730-735.
79. Canh, D.G.; Lin, F.Y.; Thiem, V.D.; Trach, D.D.; Trong, N.D. *et al. Infect. Immun.* **2004**, *72*, 6586-6588.
80. Lin, F.Y.; Ho, V.A.; Khiem, H.B.; Trach, D.D.; Bay, P.V. *et al. New Engl. J. Med.* **2001**, *344*, 1263-1269.
81. Micoli, F.; Rondini, S.; Pisoni, I.; Proietti, D.; Berti, F. *et al. Vaccine* **2011**, *29*, 712-720.
82. Rondini, S.; Micoli, F.; Lanzilao, L.; Pisoni, I.; Di Cioccio, V. *et al. J. Infect. Dev. Ctries.* **2012**, *6*, 763-773.
83. Bhutta, Z.A.; Capeding, M.R.; Bavdekar, A.; Marchetti, E.; Ariff, S. *et al. Lancet Inf. Dis.* **2014**, *14*, 119-129.
84. Kiow Shi-Shun, L.; Mallet, J.-M.; Moreau, M.; Sinaÿ, P. *Tetrahedron* **1999**, *55*, 14043-14068.
85. Yang, L., Zhu, J., Zheng, X.J., Tai, G. and Ye, X.S. *Chemistry* **2011**, *17*, 14518-14526.
86. Yu, B.; Sun, J. *Chem. Commun.* **2010**, *46*, 4668-4679.
87. Goddard-Borger, E.D.; Stick, R.V. *Org. Lett.* **2007**, *9*, 3797-3800.

88. Fischer, N.; Goddard-Borger, E.D.; Greiner, R.; Klapotke, T.M.; Skelton, B.W. *et al. J. Org. Chem.* **2012**, *77*, 1760-1764.
89. Crich, D.; Jayalath, P. *Org. Lett.* **2005**, *7*, 2277-2280.
90. Schmidt, R.R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353-3356.
91. Shie, C.-R.; Tzeng, Z.-H.; Wang, C.-C.; Hung, S.-C. *J. Chin. Chem. Soc.* **2009**, *56*, 510-523.
92. Tani, S., Sawadi, S., Kojima, M., Akai, S. and Sato, K.-i. *Tetrahedron Lett.* **2007**, *48*, 3103-3104.
93. Sakagami, M.; Hamana, H. *Tetrahedron Lett.* **2000**, *41*, 5547-5551.
94. Gavard, O.; Hersant, Y.; Alais, J.; Duverger, V.; Dilhas, A. *et al. Eur. J. Org. Chem.* **2003**, *2003*, 3603-3620.
95. Clausen, M.H.; Madsen, R. *Chemistry* **2003**, *9*, 3821-3832.
96. Zhao, M.; Li, J.; Mano, E.; Song, Z.; Tschaen, D.M. *et al. J. Org. Chem.* **1999**, *64*, 2564-2566.
97. Mootoo, D.R.; Date, V.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 2662-2663.
98. van den Bos, L.J.; Codée, J.D.C.; Litjens, R.E.J.N.; Dinkelaar, J.; Overkleeft, H.S. *et al. Eur. J. Org. Chem.* **2007**, *2007*, 3963-3976.
99. Codée, J.C., Christina, A., Walvoort, M.C., Overkleeft, H. and van der Marel, G. In *Reactivity Tuning in Oligosaccharide Assembly*; Fraser-Reid, B., Cristóbal López, J., Eds.; Springer Berlin Heidelberg: 2011; Vol. 301, p 253-289.
100. Pourcelot, M.; Barbier, M.; Landoni, M.; S. Couto, A.; Grand, E. *et al. Curr. Org. Chem.* **2011**, *15*, 3523-3534.
101. Wadouachi, A.; Kovensky, J. *Molecules* **2011**, *16*, 3933-3968.
102. Kalikanda, J.; Li, Z. *J. Org. Chem.* **2011**, *76*, 5207-5218.
103. Cipolla, L., La Ferla, B., Lay, L., Peri, F. and Nicotra, F. *Tetrahedron: Asymmetry* **2000**, *11*, 295-303.
104. Lee, D.; Taylor, M.S. *J. Am. Chem. Soc.* **2011**, *133*, 3724-3727.
105. Still, W.C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.
106. Gottlieb, H.E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512-7515.

107. Lemieux, R.U.; Ratcliffe, R.M. *Can. J. Chem.* **1979**, *57*, 1244-1251.
108. Guazzelli, L.; Catelani, G.; D'Andrea, F.; Giannarelli, A. *Carbohydr. Res.* **2009**, *344*, 298-303.
109. Parry, A.L.; Clemson, N.A.; Ellis, J.; Bernhard, S.S.R.; Davis, B.G. *et al. J. Am. Chem. Soc.* **2013**, *135*, 9362-9365.
110. Nagel, L.; Budke, C.; Erdmann, R.S.; Dreyer, A.; Wennemers, H. *et al. Chem. Eur. J.* **2012**, *18*, 12783-12793.
111. Rowan, A.S.; Nicely, N.I.; Cochrane, N.; Wlassoff, W.A.; Claiborne, A. *et al. Org. Biomol. Chem.* **2009**, *7*, 4029-4036.
112. Herzner, H., Eberling, J., Schultz, M., Zimmer, J. and Kunz, H. *J. Carbohydr. Chem.* **1998**, *17*, 759-776.
113. Myers, A.G.; Gin, D.Y.; Rogers, D.H. *J. Am. Chem. Soc.* **1994**, *116*, 4697-4718.
114. Allen, J.R.; Harris, C.R.; Danishefsky, S.J. *J. Am. Chem. Soc.* **2001**, *123*, 1890-1897.
115. Svarovsky, S.A.; Barchi Jr, J.J. *Carbohydr. Res.* **2003**, *338*, 1925-1935.