Synthesis of Oligosaccharide Antigens as Glycoconjugate Vaccine Candidates against Bacterial Pathogens

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by

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Date of defense: January 6, 2022

Declaration

This is to certify that the entire work in this thesis has been carried out by Shuo Zhang. The assistance and help received during the course of investigation have been fully acknowledged.

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(Date, Place) (Signature)

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List of publications

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List of abbreviations

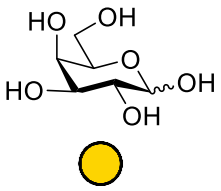
Å	Angstrom, 10^{-10} m
Ac	acetyl
AIBN	azobisisobutyronitrile
APC	antigen-presenting cell
BAIB	bis(acetoxy)iodobenzene
BCR	B cell receptor
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Bu	butyl
BSP	1-benzenesulfinyl piperidine
Bz	benzoyl
CAN	ceric (IV) ammonium nitrate
Cbz	benzyloxycarbonyl
CRM_{197}	non-toxic mutant of diphtheria toxin
CSA	camphorsulfonic acid
CPS	capsular polysaccharide
CWPS	cell wall polysaccharide
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DT	diphtheria toxin
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

EPS	exopolysaccharide
ESI	electrospray ionization
Et	ethyl
Fmoc	9-fluorenylmethyloxycarbonyl
FucNAc	*N*-acetyl-fucosamine
Gal	galactose
Glc	glucose
Glcol	glucitol
HDMS	1,1,1,3,3,3-hexamethyldisilazane
HMBC	heteronuclear multiple bond coherence
HRMS	high resolution mass spectroscopy
HPLC	high-performance liquid chromatography
HSQC	heteronuclear single quantum coherence spectroscopy
ICU	intensive care unit
Ig	immunoglobulin
IR	infrared spectroscopy
Lev	levulinoyl
LG	leaving group
LPS	lipopolysaccharide
MALDI	matrix assisted laser desorption/ionization
Man	mannose
Man*p*NAcA	*N*-acetyl-mannosaminouronate
MDR	multidrug-resistant
Me	methyl
MHC	major histocompatibility complex
MS	molecular sieves
Nap	2-naphthylmethyl
NBS	*N*-bromosuccinimide

NHS	*N*-hydroxysuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magentic resonance
Nu	nucleophile
OECD	Organisation for Economic Co-operation and Development
OMP	outer membrane protein
PD	protein D of non-typeable *Haemophilus influenzae*
PG	protecting group
Ph	phenyl
Piv	pivaloyl
Pyr	pyridine
Rha	rhamnose
TA	teichoic acids
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBS	*tert*-butyldimethylsilyl
TCA	trichloroacetimidate
TEA	triethylamine
TEAB	triethylammonium bicarbonate
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl
TfOH	trifluoromethanesulfonic acid
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Tol	toluene
Ts	tosyl
TT	tetanus toxoid

TTBP	2,4,6-tri-*tert*-butylpyrimidine
***p*-TsOH**	*para*-toluenesulfonic acid
PB	printing buffer
UV	ultraviolet
WHO	World Health Organization

Symbols



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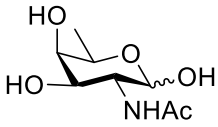
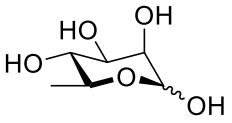


***N*-Acetyl-D-fucosamine (FucNAc)**



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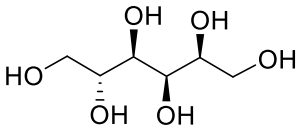

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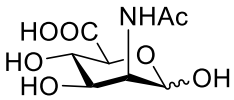
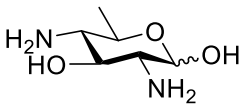


***N*-Acetyl-D-mannosaminouronate (ManNAcA)**



D-Bacillosamine (Bac)

Summary

Infections caused by bacteria are increasing in frequency with high morbidity and mortality in the past decades. Antibiotics are efficient to treat bacterial infections and have saved millions of lives worldwide. However, the resistance crisis has arisen and been attributed to abuse of these antibiotics as well as a lack of novel antibacterial means development. In the situation that development of new medications is declining while resistance is rising, vaccines become attractive alternatives against bacterial infections.

Capsular polysaccharides (CPSs) from the bacterial cell surface are important virulence factors and play an essential role for bacterial survival. CPSs are also important antigens capable of inducing a specific immune response rending these structurally unique glycans attractive targets for antibacterial vaccines development. Capsular polysaccharides are generally conjugated to carrier proteins to produce glycoconjugate vaccines as CPSs alone are poorly immunogenic in children under two years old, elderly and immunocompromised people. The conjugate is a T-cell-dependent antigen that can trigger the production of IgG antibodies and a long-lasting immunity.

Glycoconjugate vaccines met with great success to prevent infections caused by *Haemophilus influenzae*, *Neisseria meningitides* and *Streptococcus pneumoniae*. These marketed vaccines are produced from isolated and depolymerized natural capsular polysaccharides, other types of polysaccharides from bacterial cell wall may lead to decreased immunogenicity. Alternatively, glycoconjugate vaccines based on pure, defined and homogenous synthetic oligosaccharides have shown flexible design, indicating a new direction for vaccine development.

The first part of my research (Chapter 2) describes the construction of a collection of 15 novel synthetic oligosaccharides resembling the capsular polysaccharides of *Streptococcus suis* serotype 9. Differently protected monosaccharide building blocks were synthesized and employed into glycosylations to assemble the oligosaccharides. Neighboring and remote participations ensured the stereoselectivity of glycosidic bond formation. An optimized phosphorylation strategy gave access to the desired compounds with labile phosphodiesters. These oligosaccharides were printed onto glycan arrays and screened with sera from infected pigs as well as immunized rabbits. With the assistance of glycan microarrays, identification of the immunogenic epitope, the key step in

development of semisynthetic glycoconjugate vaccines, was performed. The phosphorylated trisaccharide **2-6** was established as the antigen lead.

The second part of my research focused on a multi-drug resistant bacterium *Acinetobacter baumannii*. *A. baumannii* is responsible for about 10% hospital and community acquired infections, some strains including AB5075 are resistant to most first-line antibiotics. In Chapter 3, the synthesis of repeating unit of CPS from *A. baumannii* AB5075 as well as two analogues is described. A number of synthetic challenges associated with the complicated trisaccharides were tackled, including *β*-mannoside synthesis, introduction of (*S*)-3-hydroxybutanoyl and the incorporation of labile glycosidic bonds. A double-serial inversion strategy was employed to build 1,2-*cis* linkages between rare sugars. The synthetic oligosaccharides bearing an amine linker are ready for glycan microarray study, identification of the minimal epitope and conjugation.

Zusammenfassung

Durch Bakterien verursachte Infektionen haben in den letzten Jahrzehnten mit hoher Morbidität und Mortalität zugenommen. Antibiotika sind wirksam bei der Behandlung von bakteriellen Infektionen und haben weltweit Millionen Menschenleben gerettet. Es ist jedoch eine Resistenzkrise entstanden, die dem Missbrauch dieser Antibiotika sowie dem Fehlen der Entwicklung neuer antibakterieller Mittel zugeschrieben wird. In der Situation, dass die Entwicklung neuer Medikamente zurückgeht und die Resistenzen steigen, werden Impfstoffe zu attraktiven Alternativen gegen bakterielle Infektionen.

Kapselpolysaccharide (engl. *capsular polysaccharides*, CPSs) von der Zelloberfläche von Bakterien sind wichtige Virulenzfaktoren und spielen eine wesentliche Rolle für das Überleben von Bakterien. CPSs sind auch wichtige Antigene, die in der Lage sind, eine spezifische Immunantwort zu induzieren, die diese strukturell einzigartigen Glykane zu attraktiven Zielstrukturen für die Entwicklung antibakterieller Impfstoffe macht. Kapselpolysaccharide werden im Allgemeinen mit Trägerproteinen konjugiert, um Glykokonjugatimpfstoffe herzustellen, da CPSs alleine für Kinder unter zwei Jahren, ältere Menschen und Menschen mit geschwächtem Immunsystem schlecht immunogen sind. Der Konjugat ist ein T-Zell-abhängiges Antigen, das eine Produktion von IgG-Antikörpern und eine langanhaltende Immunität auslösen kann.

Glykokonjugat-Impfstoffe waren bei der Vorbeugung von Infektionen durch *Haemophilus influenzae*, *Neisseria meningitides* und *Streptococcus pneumoniae* sehr erfolgreich. Diese auf dem Markt befindlichen Impfstoffe werden aus isolierten und depolymerisierten natürlichen Kapselpolysacchariden hergestellt. Andere Arten von Polysacchariden, die während der Verarbeitungsphase eingeführt werden, können zu einer verminderten Immunogenität führen. Eine Alternative sind Glykokonjugat-Impfstoffe, auf Basis reiner, definierter und homogener synthetischer Oligosaccharide, die eine flexible Gestaltungsfähigkeit gezeigt haben, was eine neue Richtung für die Impfstoffentwicklung anzeigt.

Der erste Teil meiner Arbeit (Kapitel 2) beschreibt die Synthese von 15 neuartigen synthetischen Oligosacchariden, die den Kapselpolysacchariden der *Streptococcus suis* Serotypen 9 ähneln. Es wurden unterschiedlich geschützte Monosaccharid-Bausteine hergestellt und in Glykosylierungen

verwendet, um Oligosaccharide aufzubauen, wobei Nachbar- und Fern-Schutzgruppen-Beteiligungen die Stereoselektivität der glykosidischen Bindungen sicherstellten. Eine optimierte Phosphorylierungsmethode ermöglichte den Zugang zu gewünschten Verbindungen mit labilen Phosphodiestern. Diese Oligosaccharide wurden auf Glycan-Arrays gedruckt und mit Seren von infizierten Schweinen und immunisierten Kaninchen gescreent. Mithilfe der Glykan-Mikroarray-Studien wurde die Identifizierung des immunogenen Epitops durchgeführt - der Schlüsselschritt in der Entwicklung halbsynthetischer Glykokonjugat-Impfstoffe - und die phosphorylierten Trisaccharide **2-6** als Leit-Antigen etabliert.

Der zweite Teil meiner Arbeit beschäftigte sich mit einem multiresistenten Bakterium *Acinetobacter baumannii*. *A. baumannii* ist für etwa 10% der im Krankenhaus und ambulant erworbenen Infektionen verantwortlich - einige Stämme, darunter AB5075, sind gegen die meisten Erstlinien-Antibiotika resistent. In Kapitel 3 wird die Synthese der Wiederholungs-Einheit des CPS von *A. baumannii* AB5075 sowie zweier Analoga beschrieben. Eine Reihe von synthetischen Herausforderungen im Zusammenhang mit komplizierten Trisacchariden wurden in Angriff genommen, darunter die β-Mannosid-Synthese, die Einführung der (*S*)-3-Hydroxybutanoyl-Gruppe und der Einbau labiler glykosidischer Bindungen. Eine doppelt-serielle Inversionsstrategie wurde verwendet, um 1,2-*cis*-Bindungen zwischen seltenen Zuckern aufzubauen. Die synthetischen Oligosaccharide, die einen Amin-Linker tragen, sind bereit für die Glykan-Mikroarray-Studien, die Identifizierung des minimalen Epitops und die Konjugation.

Chapter 1

Introduction

1.1 Threat of bacterial infections

1.1.1 Bacterial pathogenesis

Bacteria are ancient single-celled organisms that are widely distributed in the air, soil and water. They can also form symbiotic relationships with other organisms.[1] Since bacteria were first discovered by Leeuwenhoek in 1674,[2] and named by Ehrenberg in 1828,[3] bacteria have always played an important role in human life. Most bacteria are harmless or beneficial to human health, such as *Lactobacillus*, *Enterococcus* and *Bifidobacterium*. These microbes can synthesize essential vitamins and promote the health of digestive, urinary and genital systems.[4]

Bacteria that can cause human diseases are called pathogenic bacteria. These bacteria utilise a diverse range of mechanisms to infect humans and cause disease.[5] Bacterial pathogens express a series of molecules that can bind to host cells, inducing a variety of host responses. One such mechanism is toxigenesis, which is the ability of bacteria to produce toxins, generally classified into two categories: exotoxins and endotoxins.[6]

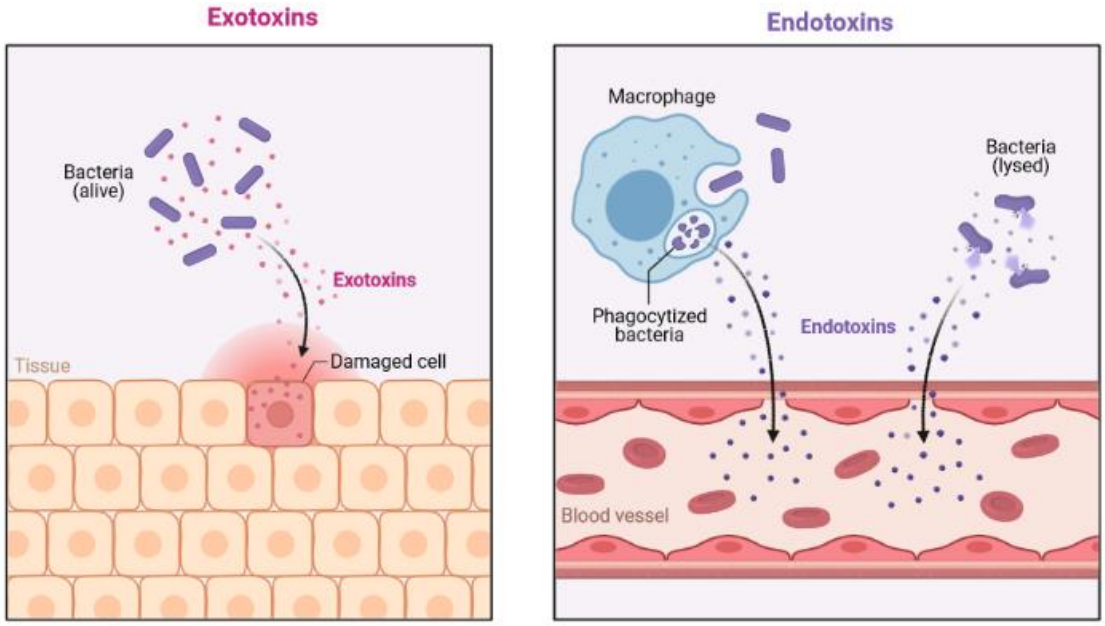


Figure 1.1 Exotoxins and endotoxins (Drawn on BioRender.com)

Bacterial exotoxins are often secreted polypeptides or proteins by Gram-positive or Gram-negative bacteria,[7] they are released into the surrounding medium and can efficiently poison host cells (Figure 1.1).[8] Endotoxins are lipopolysaccharides that constitute a major component of the outer membrane of Gram-negative bacteria.[9] Endotoxins can be released when the bacteria are lysed or phagocytized by host cells and are then transported by blood and lymph, causing meningococcemia or sepsis (Figure 1.1).[10,11]

1.1.2 Antibiotics and multi-antimicrobial resistance

Since the early days of human society, bacterial infectious diseases have had big effects on the progress of human civilization. In the agricultural revolution, humans became closer to animals and poultry, which contributed to the spread of microbes and new zoonotic diseases.

Around 1350, a plague caused by *Yersinia pestis* swept across Europe, claiming tens of millions of lives and reducing the population of Europe by a third, known popularly as the Black Death.[12] Cholera is an acute diarrheal infectious disease caused by the contamination of food or water with *Vibrio cholerae*.[13,14] Every year, there are an estimated 3-5 million cases of cholera and around ten thousand deaths.[15] For public health, cholera remains an emergency threat, especially in developing countries. In a sense, the history of human civilization is a history of constant struggle against the bacteria.

In the process of fighting against bacteria, humans had always been the weak side until 1928, when Alexander Fleming discovered penicillin.[16] After that, a variety of new antibiotics were discovered and synthesized (Figure 1.2), which enabled doctors to treat bacterial infections effectively. Antibiotics kill bacteria by a variety of mechanisms including: inhibition of bacterial cell wall synthesis, enhancing the permeability of bacterial cell membranes, selectively interfering with protein synthesis, and inhibiting nucleic acid replication and transcription.[17]

However, no matter how powerful the antibiotics humans can produce, bacteria can eventually develop resistance. In fact, the abuse of antibiotics is considered to be responsible for the evolution of bacteria. Between 2000 and 2015, expressed in defined daily doses, antibiotic consumption increased by 65% and antibiotic consumption rate increased by 39%.[18] Overuse of antimicrobial has contributed to the emergence of several multidrug-resistant (MDR) bacteria species.[19,20]

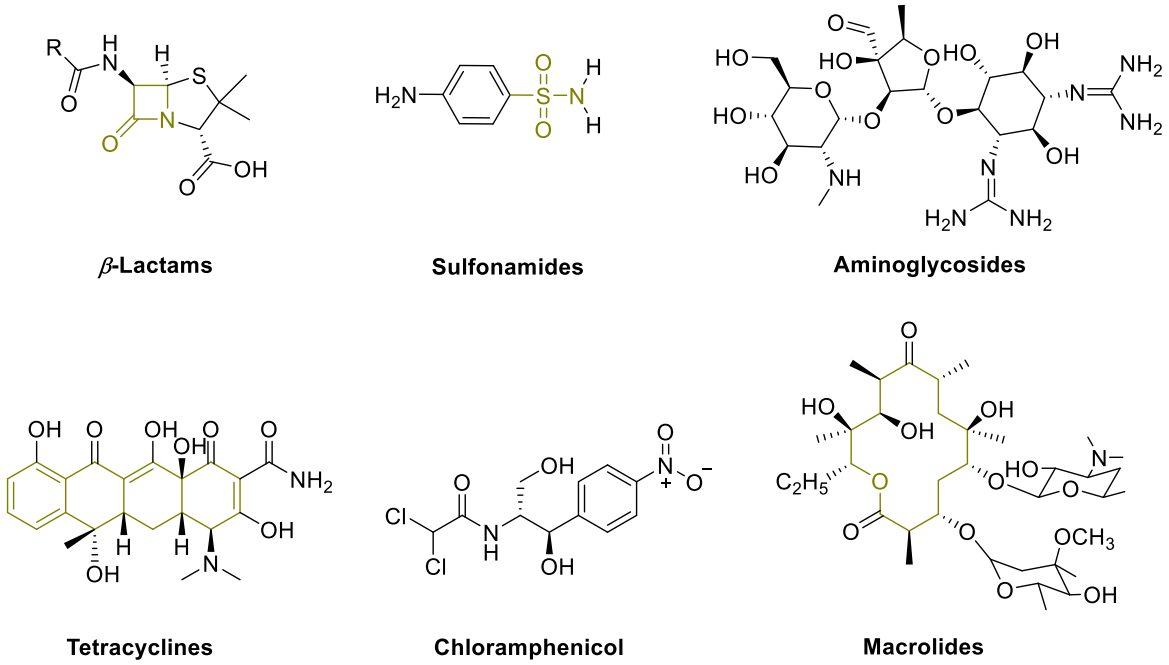


Figure 1.2 Different classes of commonly used antibiotics

Nowadays, multidrug-resistant bacteria are not only found in hospitals, but also often identified in community settings,[21,22] demonstrating the antimicrobial resistance has become a serious and wide spread public-health crisis. However, it is difficult to develop new drugs to keep up with the evolutionary speed of bacteria. There is an urgent need to develop new therapies against bacterial infections.

1.1.3 Vaccines against bacterial infections

Vaccination is an effective way to prevent bacterial infections. The principle is to introduce bacterial antigens into human body to induce the immune system to produce specific neutralizing antibodies and a long-lasting protective immune memory, so that upon exposure to the same antigens again, our body can better handle the infection, and reduce the chance of serious illness and death.

The discovery of vaccine was a landmark event in human history. In 1796, Edward Jenner observed that inoculation with vaccinia mucus was an effective preventive method against smallpox,[23] so that the first vaccine was born. In the following two centuries, dozens of vaccines have saved millions of lives.[24,25] Vaccines can significantly reduce bacterial infections and thus reduce the use of antibiotics, thereby slowing down the emergence of resistance.

1.2 Carbohydrate-based vaccines

1.2.1 Bacterial capsular polysaccharides

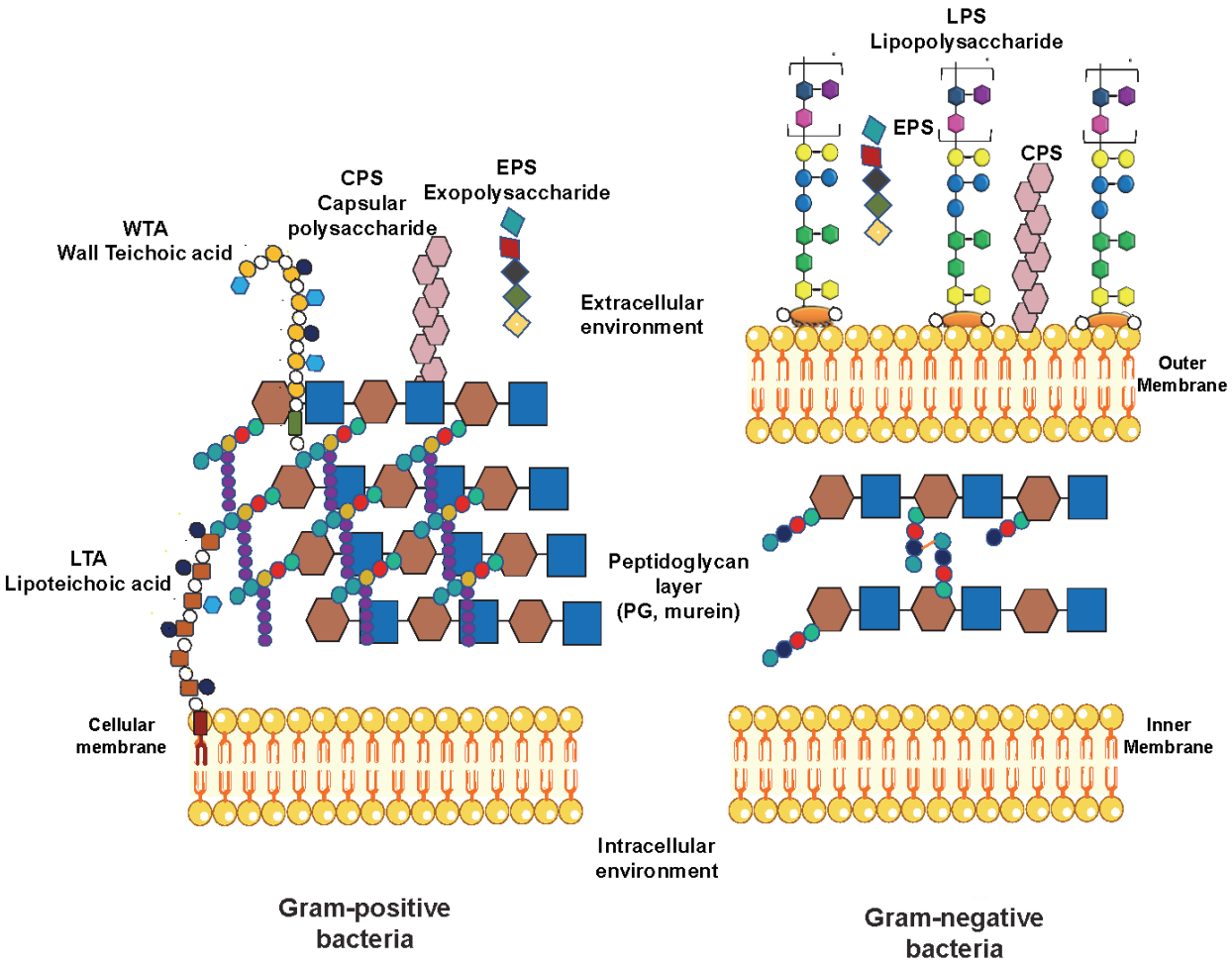


Figure 1.3 Schematic diagram of the cell surface of Gram-positive (left) and Gram-negative bacteria (right)*

The cell surface of a broad range of bacterial species is surrounded by a capsule made of polysaccharides. These surface polysaccharides are classified into different types: capsular polysaccharide (CPS), teichoic acids (TA), lipopolysaccharide (LPS) and exopolysaccharide (EPS), which constitute the interface and mediate interactions between the bacteria and enviroment.[26,27] In Gram-positive bacteria, CPSs are attached to peptidoglycan via glycosidic bonds.[28] For Gram-negative bacteria, CPSs locate on the outer membrane and linked to the cell

* https://www.glycopedia.eu/e-chapters/bacterial-polysaccharides-integrated-use-of-databases-and-computational-tools/article/microbial-glycoconjugate-structures

surface at their reducing ends via covalent bonds to phospholipid or lipid A molecules (Figure 1.3).[29]

As the outmost layer of bacterial cell, it has been demonstrated that capsular polysaccharides are important virulence factors for many bacterial pathogens.[30–32] The capsule prevents the cell from suffering desiccation and disinfection regimes to confer survival rate of bacteria,[33] and also promotes the occurrence and development of invasive diseases. CPSs increase adherence of bacteria to other cells, enabling the formation of biofilms and colonisation.[34] Besides, capsule may also promote the resistance to the non-specific and specific immunity of the host.[35,36]

Capsular polysaccharides are highly hydrated and consists of more than 95% water.[37] Structurally, CPSs are homopolysaccharides or heteropolysaccharides with a linear or branched structure, which contain a number of monosaccharides that repeat regularly to form a high molecular weight polymer (10^5-10^6 Da).[38]

1.2.2 Vaccines based on capsular polysaccharides

It is widely established that the capsular polysaccharides are not only important virulence factors for bacteria to infect the hosts, but also act as pathogenic antigens to induce specific immune responses.[39–41] Although the structures of CPS vary significantly between bacterial species, serotypes and even different strains of the same bacterium, the CPS repeating unit structure of a certain serotype or strain is unique. Therefore, due to the diversity and specificity, capsular polysaccharides have become attractive targets for the development of antibacterial vaccines.

Polysaccharide-based vaccines have a long history. The first vaccine dates back to 1930, when Thomas Francis injected pure capsular polysaccharide of *Streptococcus pneumoniae* to patients and observed CPS-specific antibodies.[42] After further studies and clinical tests, Heidelberger finally developed two variants of pneumococcal vaccines that contained six different serotypes.[43] The following 23-valent pneumococcal CPS vaccine licensed in 1983 also showed effective protection against pneumonia.[44] Inspired by the success of *Streptococcus pneumoniae* vaccines, anti-bacterial CPS vaccines developed rapidly and approved for marketing successively.

Due to the weak immune system in children under two years of age and the elderly, capsular polysaccharides alone are not very immunogenic in them as well as immunocompromised people.[45–47] To obtain longer immune response and higher immunity, bacterial polysaccharides are

generally linked to non-toxic carrier proteins to form conjugated vaccines, which can stimulate the human immune response more efficiently. Variety of glycoconjugate vaccines were developed and some licensed vaccines are listed in Table 1.1.[48]

Table 1.1 Licensed glycoconjugate vaccines by FDA (Adapted from: *Recent Trends Carbohydr. Chem.* 285–313 (2020))

Manufacturer	Vaccine name	Target infection	Carrier	Conjugation chemistry/carbohydrate source
Connaught Laboratories	ProHIBIT	*Haemophilus influenzae* type B	DT	Cyanylation/size-reduced PS
Wyeth Pharmaceuticals	HibTiter		CRM197	Reductive amination/oligosaccharide
Merck	PedvaxHIB		OMPs	Thioalkylation/size-reduced PS
Sanofi Pasteur	ActHib		TT	Carbodiimide chemistry/native PS
Chiron	VaxemHib		CRM197	Active ester/oligosaccharide
GSK	Hiberix		TT	Carbodiimide/native PS
Center for Genetic Engineering and Biotechnology (Cuba)	QuimiHib		TT	Thiol-maleimide/synthetic oligosaccharide
Wyeth	Meningitec	*Neisseria meningitidis* (group C)	CRM197	Reductive amination/oligosaccharide
Chiron	Menjugate		CRM197	Active ester/oligosaccharide
Baxter	NeisVac-C		TT	Reductive amination/de-*O*-acetylated polysaccharide
Serum Institute of India	MenAfrivac	*Neisseria meningitidis* (group A)	TT	Reductive amination, native PS
Sanofi Pasteur	Menactra	*Neisseria meningitidis* (group A, C, W135 and Y)	DT	Reductive amination/oligosaccharide
Novartis	Menveo		CRM197	Active ester/oligosaccharide
GSK	Nimenrix		TT	Cyanylation, carbodiimide chemistry /sized polysaccharide
GSK	MenHibrix	*Neisseria meningitidis* (group C,Y) + *Haemophilus influenzae* type B	TT	Cyanylation Carbodiimide chemistry, native Hib
Pfizer	Prevnar Prevnar13	*Streptococcus pneumoniae*	CRM197	Reductive amination/native or size-reduced polysaccharide
GSK	Synflorix		TT, DT, PD	Cyanylation (10 valent: 1, 4, 5, 6B, 7, 9V, 14, 18C, 19F and 23F, size-reduced PS except 6B and 23F (native PS)
Bharat Biotech Ltd.	Typbar-TVC	*Salmonella typhi*	TT	Carbodiimide chemistry/native polysaccharide
BioMed	PedaTyph		TT	Carbodiimide chemistry/native polysaccharide

1.2.3 Immunology of glycoconjugate vaccines

Non-protein conjugated capsular polysaccharides are poorly immunogenic in the high-risk groups (mentioned in 1.2.2), as CPSs are T-cell-independent antigens that can directly induce an immune response without help of T cells (Figure 1.4a). The polysaccharide is recognized by B cell receptor (BCR), the cross-linking with BCR drives the differentiation of plasma cells and production of antibodies, mostly of IgG2 and IgM. New memory B cells are not produced in this process, so that the responses are short-lived and no immunological memory is obtained.[49,50]

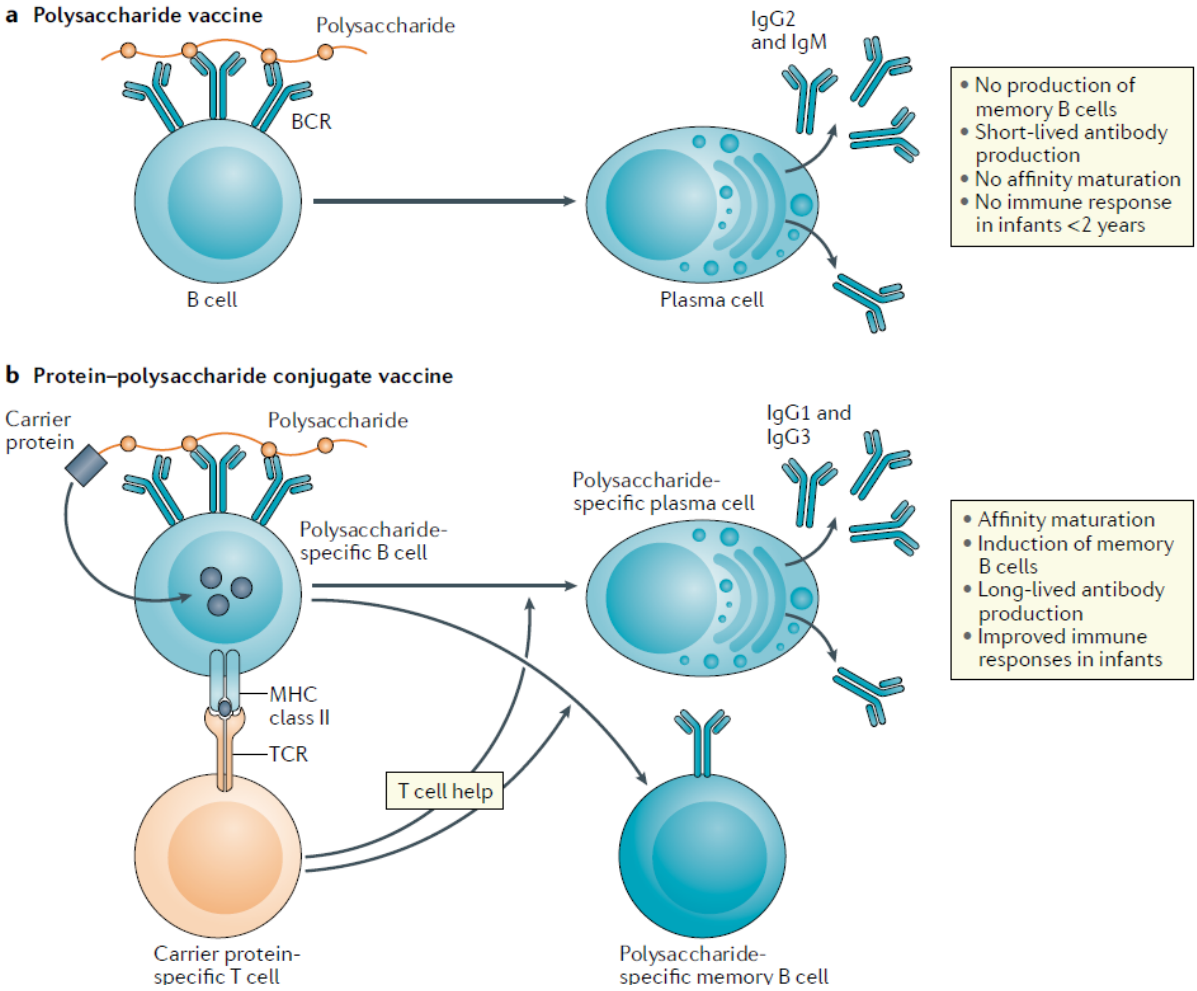


Figure 1.4 Simplified mechanism for immune responses to polysaccharide and polysaccharide-protein conjugate vaccine (Reprinted from: *Nat. Rev. Immunol.* 21, 83–100 (2021))

Beginning in early 1980s, bacterial polysaccharides were coupled to carrier proteins to create glycoconjugate vaccines. Due to the ability of carrier protein to activate T cells, a different immune response associated by T cells will be elicited in this case, B cells will play a role of antigen-presenting cells (APCs).[51,52] First, the glycoconjugate is recognized by receptor on polysaccharide specific B cells, the carrier protein is then processed into small peptides or glycopeptides by internalization (Figure 1.4b). These fragments are loaded onto major histocompatibility complex (MHC) class II and presented to carrier-peptide-specific T cells, leading to affinity maturation and class switching of immunoglobulin gene in B cells to IgG isotype.[24] Polysaccharide specific B cells differentiate into plasma cells that secret highly antigen-specific IgG1 and IgG3 antibodies. Some B cells, meanwhile, differentiate into memory B cells to promote a rapid and efficient immune response in case of re-infection.

1.2.4 Design of semi-synthetic glycoconjugate vaccines

Traditional glycoconjugate vaccines are manufactured by combining natural or depolymerized capsular polysaccharides with carrier proteins via reductive amination.[53] The polysaccharides are isolated from bacterial cultures followed by a number of purification, depolymerization and activation steps. During the isolation process, other polysaccharides on the cell surface will mix with the CPS, making the separation and purification difficult. Since the role of polysaccharide impurities in the immune response is still unclear, the vaccine produced with low-purity CPS leads to manufacturing issues and decreased immunogenicity.[54,55] Isolated polysaccharides have multiple conjugating sites that may lead to a variety of conjugates. Furthermore, the functionalization of unstable groups in some capsular polysaccharides during the isolation process will further exacerbate the decline in vaccine efficiency and reproducibility.

In order to overcome these shortcomings, the use of chemically synthesized oligosaccharides to produce vaccines has become an attractive alternative. With the development and progress of chemical synthesis technology, compared to the isolated capsular polysaccharides, synthetic oligosaccharides are well-defined and easier to be characterized by nuclear magnetic resonance (NMR), matrix-assisted laser desorption/ionization (MALDI), infrared spectroscopy (IR) as well as other tools of organic chemistry. Due to the controllability of chemical synthesis, a linker with an amino group can be introduced to synthetic oligosaccharides, they can be conjugated specifically to the carrier protein to obtain pure and defined glycoconjugates.[56]

The development of novel semisynthetic glycoconjugate vaccines commences with the determination of the structure of bacterial capsular polysaccharides (Figure 1.5). Unlike the isolated capsular polysaccharides, synthetic oligosaccharides are smaller, generally only one or two repeating units, so it is important to identify minimal immunogenic epitopes that induce the specific immune response and antibody production.[57]

Based on the structure of CPS repeating unit, a collection of oligosaccharides antigens can be constructed by chemical synthesis (Figure 1.5). Normally, this collection includes one or two complete repeating units of CPS and fragments containing different monosaccharide units. The reducing end of these oligosaccharides are bound to a linker that has an amino group on the other end. The linker makes it possible to conjugate the oligosaccharide to glycan array surface or carrier proteins for further immunization and biological evaluation.

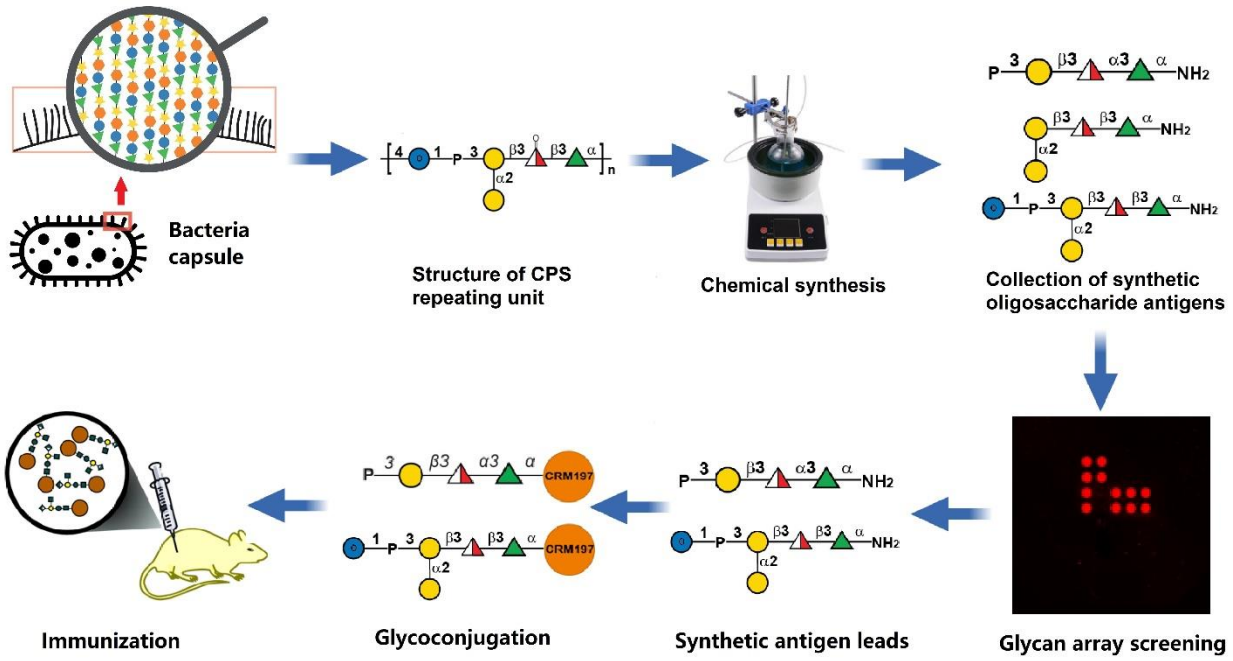


Figure 1.5 Development of synthetic glycoconjugate vaccines

Glycan microarrays have become a powerful technology for rapid, sensitive and high-throughput interactions studies of glycan antigens and antibodies during infections.[58,59] The amine binding microarray glass slides surface is modified with *N*-hydroxysuccinimide (NHS) esters (Figure 1.6), the synthetic oligosaccharides bearing amine terminated linker play a role as nucleophiles, that allows for the covalent attachment to microarray via amide bonds. The resulting glycan microarray is incubated with sera and fluorescence-labeled secondary antibody. Binding is detected by an image of fluorescent spots. The binding intensity of the carbohydrate antigens to the antibody can be quantified by the signal strength of fluorescence dots.[60]

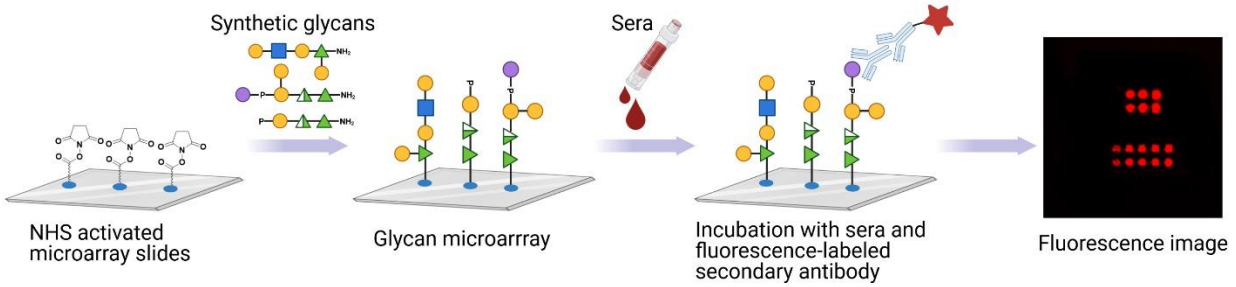
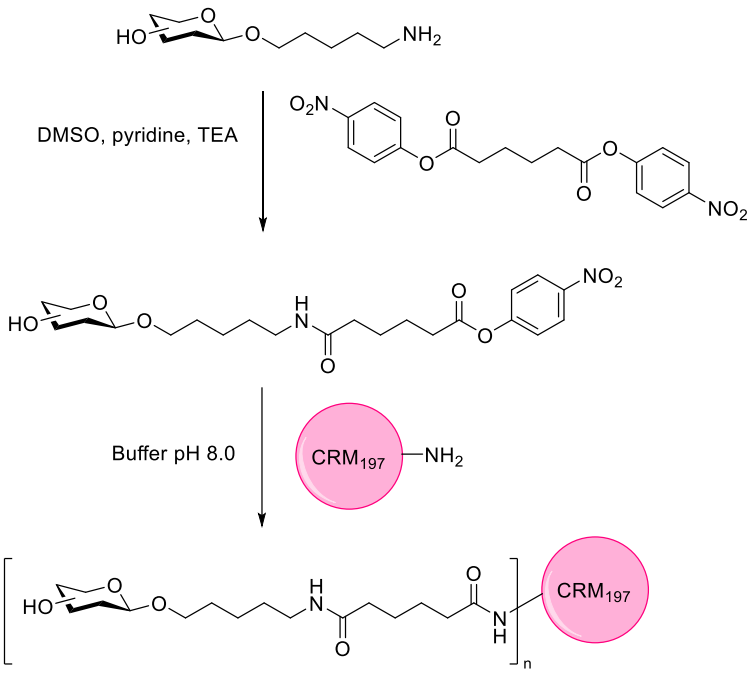


Figure 1.6 Workflow of a typical glycan microarray screening experiment

After the glycan microarray study, the oligosaccharides showed strong binding with antibodies will be covalently conjugated to non-toxic carrier proteins to form glycoconjugates. Currently, five

proteins are widely used in licensed glycoconjugate vaccines: cross reacting material (CRM_{197}), diphtheria toxin (DT), tetanus toxoid (TT), outer membrane protein (OMP) and protein D of nontypeable *Haemophilus influenzae* (PD).[61] Taking CRM_{197} as an example, reaction of oligosaccharide with homobifunctional adipic acid *p*-nitrophenyl diesters affords an amide half ester (Scheme 1.1), substituent conjugation with CRM_{197} under mild condition will produce the corresponding neoglycoproteins.



Scheme 1.1 Preparation of glycoconjugate using CRM_{197}

The last stage of vaccine development is the immunological evaluation. The glycoconjugates are formulated with adjuvants ($Al(OH)_3$ or $AlPO_4$) and injected into mice, rabbits or pigs (Figure 1.5).[62] A second injection will be performed after two weeks to boost the immunity. The titer of IgG antibodies against glycoconjugates is indicated by glycan microarray and enzyme-linked immunosorbent assays (ELISA).[63] Vaccine candidates that can effectively trigger an immune response will be tested on higher-order animal models and then enter into clinical trials. The data obtained will be used to evaluate the possibility of entering the market.

1.3 Synthesis of oligosaccharides

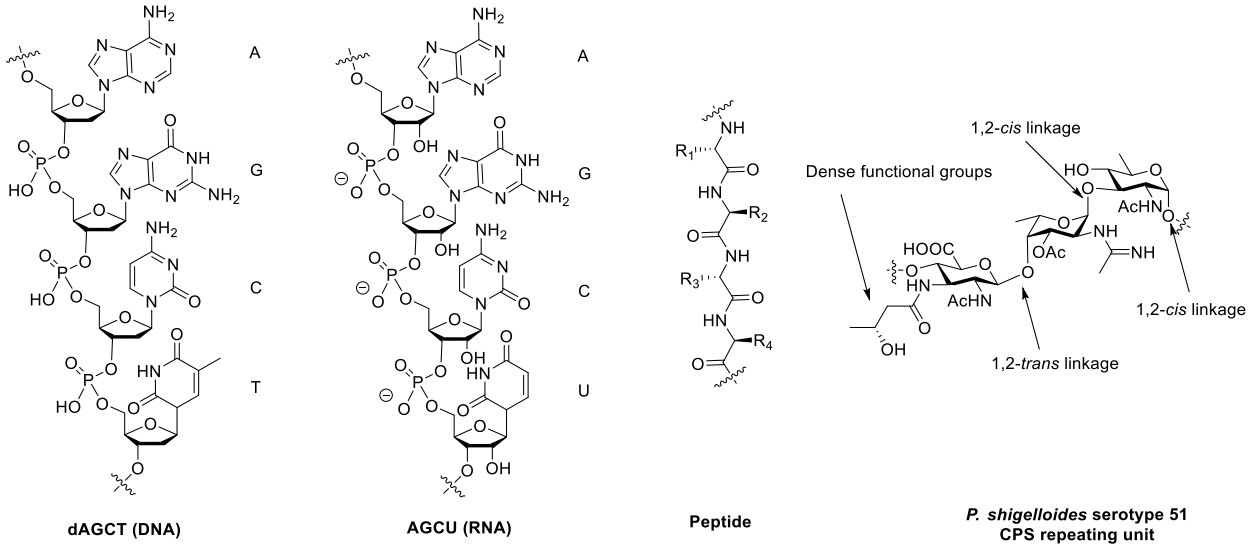
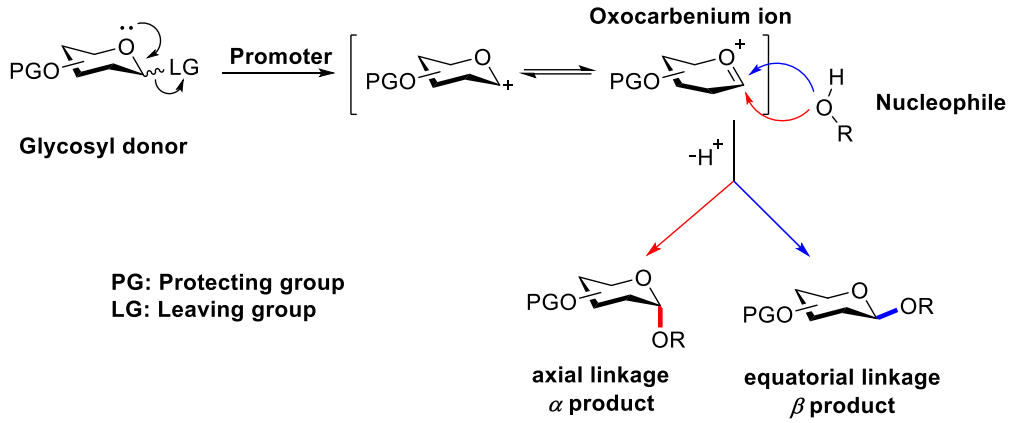


Figure 1.7 Chemical structures of DNA oligonucleotide dAGCT, RNA oligonucleotide AGCU, a peptide and *P. shigelloides* serotype 51 CPS repeating unit

Nucleic acids, proteins and carbohydrates are the three major biopolymers in nature. For polypeptides and oligonucleotides, amino acids and nucleotide units are connected through amide and phosphodiester bonds, respectively (Figure 1.7).[64,65] Thus, efficient and universal bond formation methods can be applied to synthesize them. However, oligosaccharides contain more functional groups, so that complex protection and deprotection operations are required. Furthermore, unlike the linear structure of oligonucleotide and peptide, oligosaccharides are usually branched as each monosaccharide has multiple hydroxyl groups that can become chain elongation sites. Besides, the monosaccharide can coupled with another monomer via 1,2-*cis* or 1,2-*tran* glycosidic bond, which greatly increases the diversity of oligosaccharides and difficulty of synthesis.[66] Due to the complexity of carbohydrates, synthesis of oligosaccharides has become the bottleneck of glycoconjugate vaccines development.

1.3.1 Glycosylation



Scheme 1.2 General mechanism of glycosylation

Formation of glycosidic bond by chemical glycosylation is the most important step in the synthesis of oligosaccharides. Since Arthur Michael reported the first chemical glycosylation in 1879,[67] many methods have been developed. In general, the glycosylation involves the coupling of a glycosyl donor with a glycosyl acceptor under the activation of a promoter (Scheme 1.2). With the catalysis of promoter, the leaving group on glycosyl donor is activated to give an oxocarbenium ion intermediate, the glycosyl acceptor can perform the nucleophilic attack form either top or bottom face to generate the glycosidic bond, leading to α and β products, respectively.

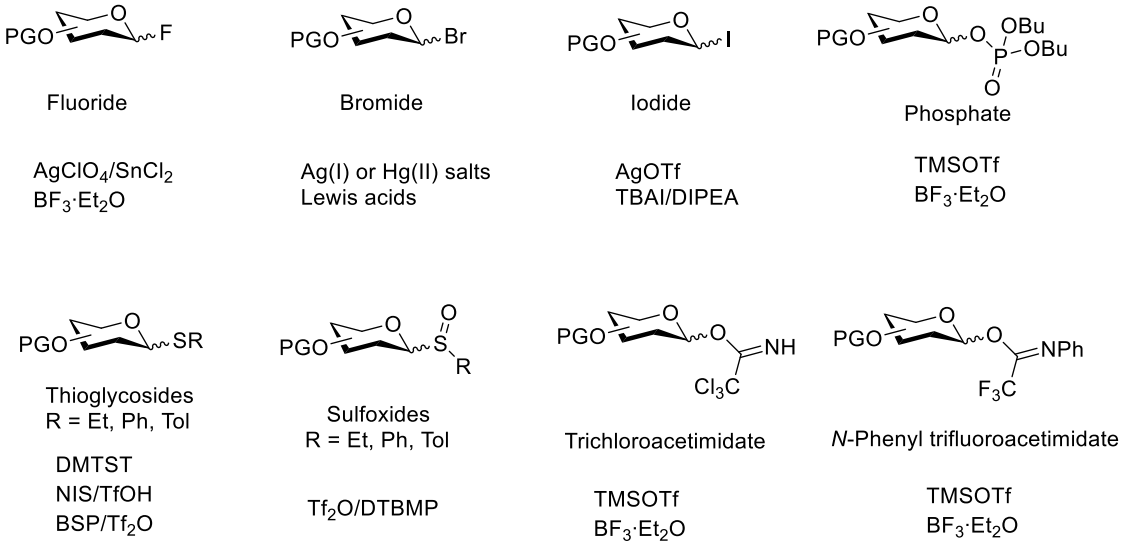


Figure 1.8 Common glycosylating agents and their promoters

As a necessary element for glycosylation, the characteristics of a good glycosylating agent should include: 1) easy to access; 2) selective activation by promoter; 3) high reactivity.[68,69] In the past decades, a variety of glycosyl donors have been developed as listed in Figure 1.8, including glycosyl halides, phosphate, thioglycosides, sulfoxides and acetimidates.[70] Thioglycosides stand out due to their high reactivity during glycosylation and stability during storage, which are mainly used in the work of this thesis.

Despite the research progress concerning chemical glycosylation, the mechanism is still not entirely understood. Currently, the glycosylation is regarded as a nucleophilic substitution with both S_N1 and S_N2 features.[71] The activation of glycosyl donor by promoter gives a series of intermediates, α- and β-covalent adducts can be formed, they are in equilibrium with more reactive and less stable oxocarbenium ion (like) species (Figure 1.9). Such an oxocarbenium ion may react with another counterion forming closed contact ion-pair or be separated from their counterion by solvent to give solvent-separated ion-pairs.[72–74] The nucleophilic attack by a glycosyl acceptor to these reactive intermediates follows a mechanism ranging from S_N1-like to S_N2-like. The mechanism is influenced by multiple factor that even a change of a protecting group may alter the reaction pathway.[75,76]

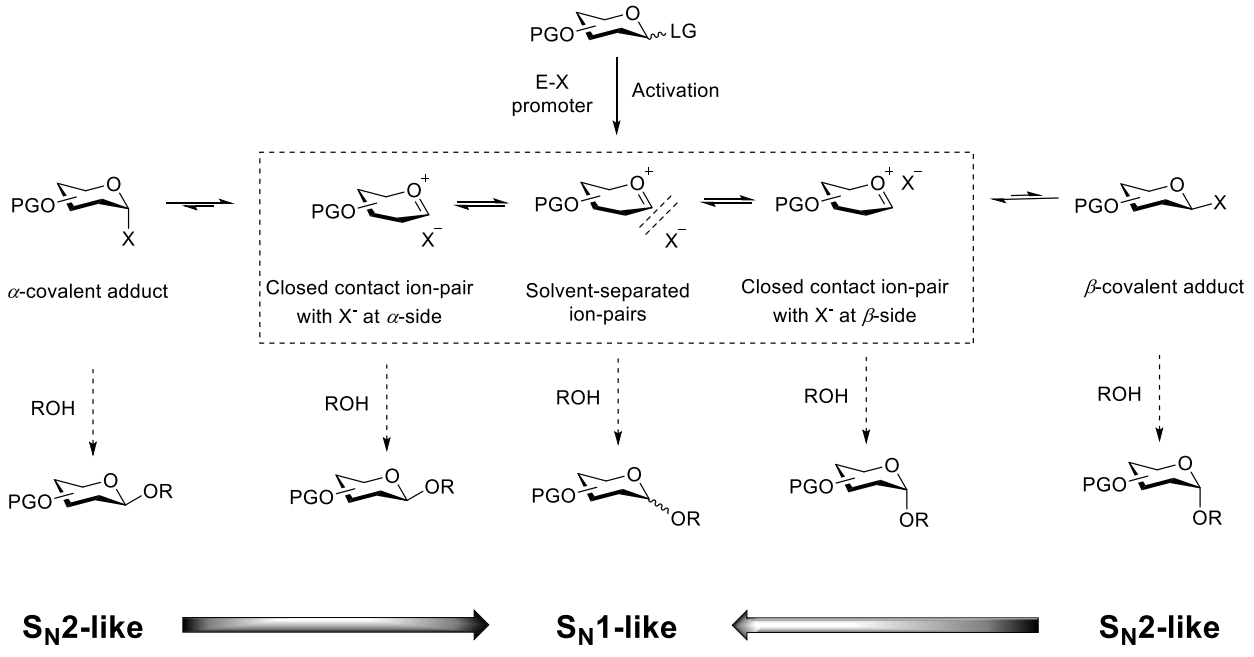
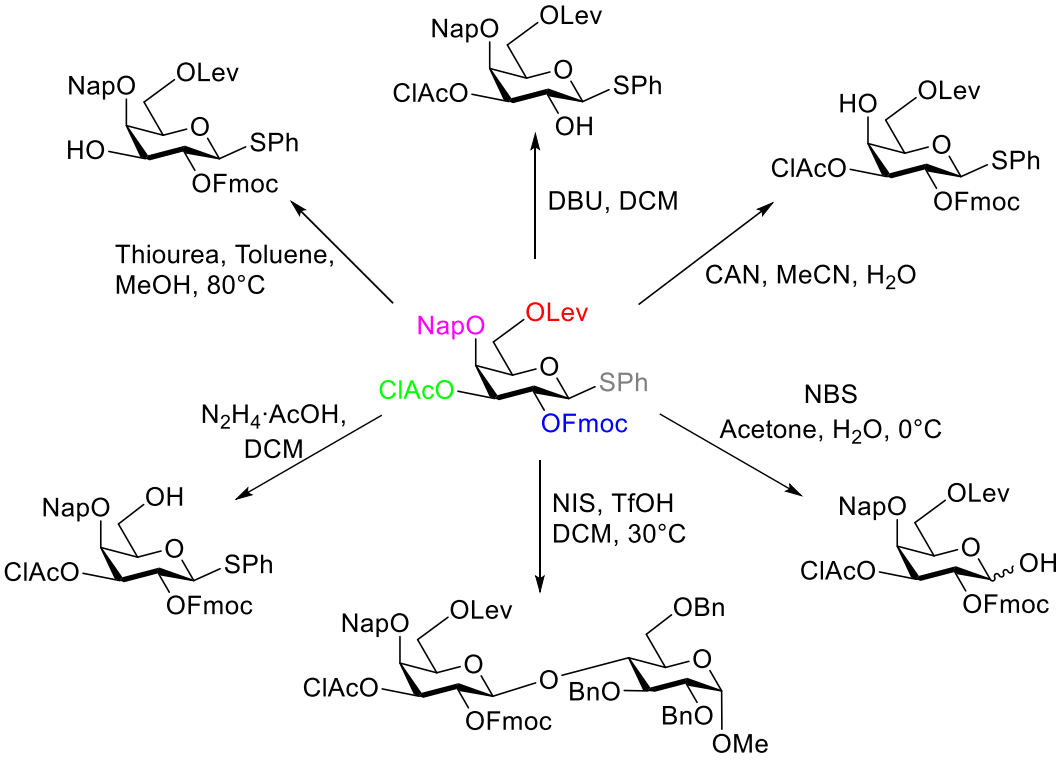


Figure 1.9 Possible glycosylation mechanisms

1.3.2 Regioselectivity of glycosylation

The most challenging part in the synthesis of oligosaccharides is selectivity, including regioselectivity and stereoselectivity. Regioselectivity challenges come from the structure of carbohydrate with multiple hydroxyl groups, the hydroxyl groups are unprotected on natural sugars, and glycosylation using these sugars will result in the coupling of acceptors with multiple donors. It is important to proceed glycosylation between the designated hydroxyl group with glycosyl donor, which is the so-called regioselectivity.



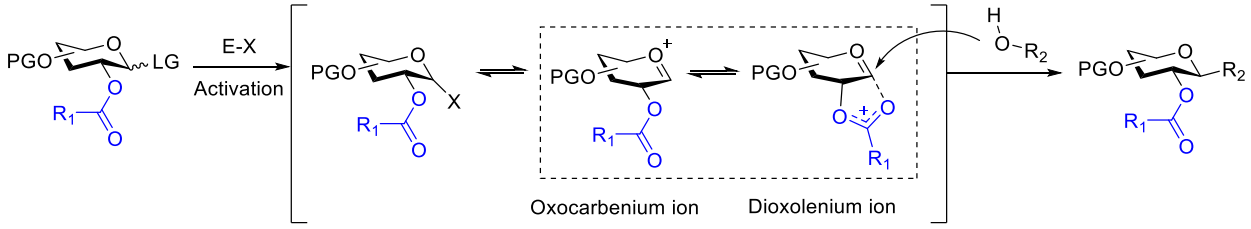
Scheme 1.3 Orthogonal protecting groups on a monosaccharide scaffold

In the synthesis of oligosaccharides, the hydroxyl groups and other nucleophilic groups on the building blocks are usually masked with temporary or permanent protecting groups. Based on the steric configuration of the sugar ring and different nucleophilicity between hydroxyl groups,[77] orthogonal protecting groups can be installed. Scheme 1.3 illustrates a set of four orthogonal groups (Fmoc, ClAc, Nap and Lev) on a monosaccharide.[78] Fmoc on C2 can be removed with the treatment of DBU while the other protecting groups could survive under this weak basic condition. ClAc, Nap and Lev follow the similar principle. Thus, we can obtain the envisioned glycosyl acceptor by removing the protecting group at the designated position and realize regioselective

glycosylation. Furthermore, these orthogonal groups can remain stable during glycosylation, and the selective cleavage of protecting groups on disaccharides provides a theoretical basis for the regioselective synthesis of oligosaccharides.

1.3.3 Stereoselectivity of glycosylation

Compared to the regioselectivity, control of the anomeric stereoselectivity for glycosylations is critical and more challenging. Glycosidic bonds exist either as 1,2-*trans* or 1,2-*cis* diastereomers.[79] 1,2-*trans* glycosidic linkages can be installed under assistance of neighboring participating groups (Scheme 1.4).[80,81] Activation of a glycosyl donor forms an oxocarbenium ion, the participation of an ester-type protecting group at C2 can generate a bicyclic C1,C2 dioxolenium ion intermediate that blocks the nucleophilic attack from the covered face, leading to the formation of 1,2-*trans* glycosidic bond.

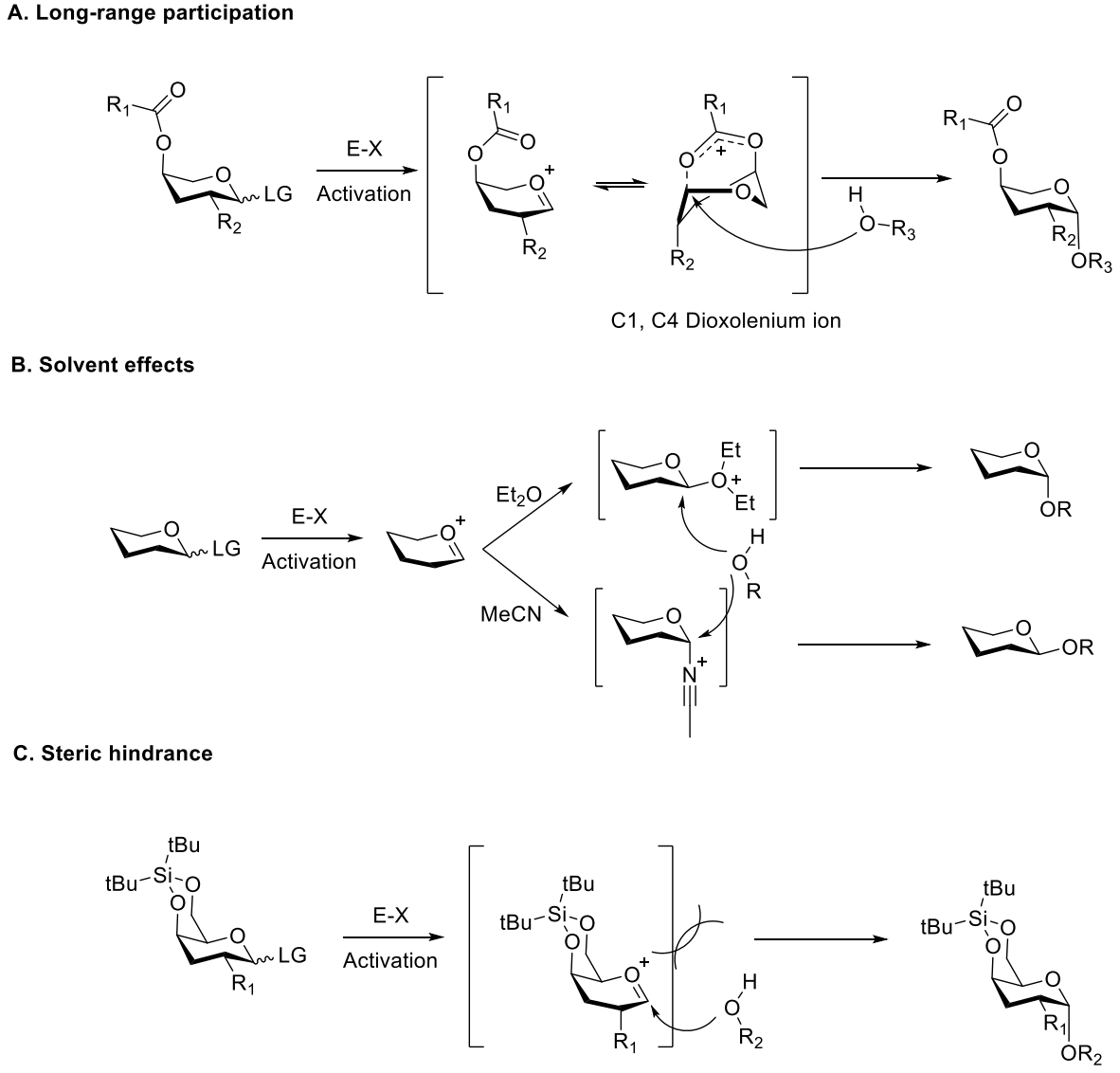


Scheme 1.4 Mechanism of 1,2-*trans* glycosidic bond construction via neighboring group participation

1,2-*cis* Linkages exist in many capsular polysaccharides,[82–85] however, unlike the efficient anomeric stereochemical glycosylation to construct 1,2-*trans* linkages, there is no generally accepted methods for the installation of 1,2-*cis* glycosidic bond. The basic principle to construct the 1,2-*cis* linkages is designing the protecting group and reaction conditions to turn 1,2-*cis* glycoside into the favored product of glycosylation. Various reaction conditions and methods have been investigated and the most commonly used strategies are shown in Scheme 1.5.[81,86–89]

It was considered that the effect of remote substituents is less important than C2 neighboring substituents. However, several groups have reported the assistance of ester groups on C6 and C4 in synthesis of α-galactosides,[81,90,91] as well as remote participation of groups on C6 and C3 to access *a*-glucosides.[81,92] Long-range participation by ester group on C4 may afford a C1,C4 dioxolenium ion and nucleophile can attack from α face to get the 1,2-*cis* glycoside (Scheme 1.5A). This also works when the participating groups are located on C3 and C6.[81]

Solvent effects are also important to the glycosylation. In general, the glycosylation in ether type solvent such as diethyl ether or dioxane can form an equatorial intermediate (Scheme 1.5B), while in polar solvent like acetonitrile tends to form an axial intermediate, leading to a stereoselective formation of α and β linkage, respectively. This strategy allows for the synthesis of 1,2-*cis* glucosides in high selectivity even without the participation of substituent.[93]



Scheme 1.5 Strategies to generate 1,2-*cis* glycosidic bonds

In addition to electronic effects, steric effects can also favor the formation of 1,2-*cis* glycosides. For example, introduction of a remote bulky group (4,6-*O*-di-*tert*-butylsilydene) blocks the

nucleophilic attack from top face, which makes α glycoside be the favored product (Scheme 1.5C). This strategy was used in the synthesis of building blocks in Chapter 3.

Considering the difficulty to synthesize an oligosaccharide containing multiple 1,2-*cis* linkages, it is crucial to design the monosaccharide building blocks with suitable protecting groups, that will facilitate the stereoselective synthesis. However, it is always difficult to predict if the protecting groups will work well for the selectivity or not, rendering a lot of iteration and protecting group optimization, that discloses the challenges in the synthesis of 1,2-*cis* linkages containing oligosaccharides.

1.4 Aim of this thesis

The goal of the thesis is to contribute to the development of novel synthetic glycoconjugate vaccines against pathogenic bacteria. Due to the emergence of bacterial antibiotic resistance, it is urgent to find alternative methods to fight bacterial infections. Capsular polysaccharides are important immunogens of bacteria, CPS-based glycoconjugate vaccines have become attractive options for acquiring long-term immunity and reducing the use of antibiotics. The focus of my research this thesis are *Streptococcus suis* (*S. suis*) serotype 9 and *Acinetobacter baumannii* (*A. baumannii*) AB5075.

Using synthetic organic chemistry, a number of oligosaccharides related to the repeating unit of *Streptococcus suis* serotype 9 were synthesized and described in Chapter 2. *S. suis* is a common pathogen of pigs and is causing severe financial losses to swine industry. Therefore, we sought to produce novel veterinary vaccine candidates using synthetic glycans mimicking the CPS repeating unit of *S. suis*. A collection of oligosaccharides were assembled and were screened using glycan microarrays to identify the minimal epitope of antibodies from infected pigs and immunized rabbit.

Chapter 3 describes a multi-drug resistant bacterium, *Acinetobacter baumannii*. *A. baumannii* AB5075 is capable of causing severe infections with high morbidity and mortality. As a result of its high adaptability, AB5075 can survive the treatment of almost all antibiotics. The objective of Chapter 3 is to synthesize the repeating units of the capsular polysaccharide on *A. baumannii*. Furthermore, two analogues were prepared as the conjugate-ready oligosaccharides. These vaccine candidates are ready for glycan microarray study, conjugation and further immunological evaluations.

Chapter 2

Discovery of oligosaccharide antigens for semi-synthetic glycoconjugate vaccine leads against *Streptococcus suis* serotype 9

This chapter is based on the following article:

Zhang, S.; Sella, M.; Sianturi, J.; Priegue, P.; Shen, D.; Seeberger, P. H. Discovery of Oligosaccharide Antigens for Semi-Synthetic Glycoconjugate Vaccine Leads against *Streptococcus suis* Serotypes 2, 3, 9 and14. *Angew. Chemie Int. Ed.* **2021**, *60* (26), 14679–14692.[94] https://doi.org/10.1002/anie.202103990.

2.1 Introduction

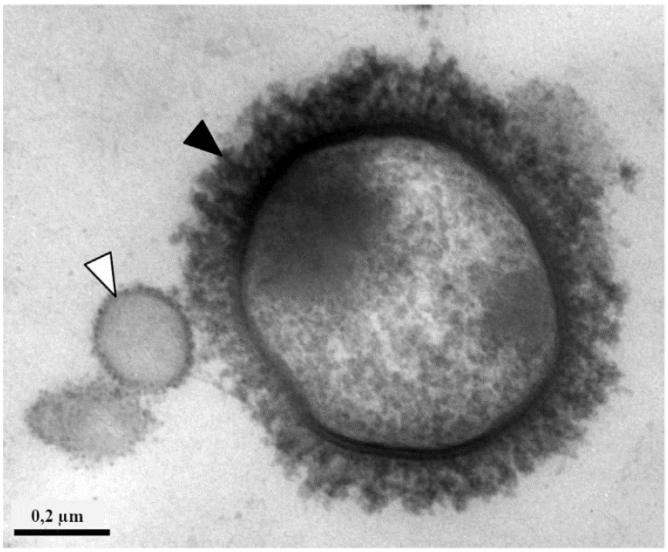


Figure 2.1 Transmission electron micrograph of *Streptococcus suis*[95]

Streptococcus suis is an encapsulated Gram-positive bacterium (Figure 2.1) and is not only an important pathogen of pigs. *S. suis* causes bacterial infections in farm pigs globally,[96–98] but also a commensal bacterium that commonly inhabits the upper respiratory, digestive and reproductive systems of pigs.[99] Virulent strains can infect the bloodstream and eventually result in septic shock and meningitis in pigs,[100–103] causing severe economic losses to swine industry. Moreover, as an emerging zoonotic pathogen, *S. suis* is also capable of transmission to humans through exposure to carrier pigs or contaminated meat products to cause septicemia and meningitis in humans.[104–106]

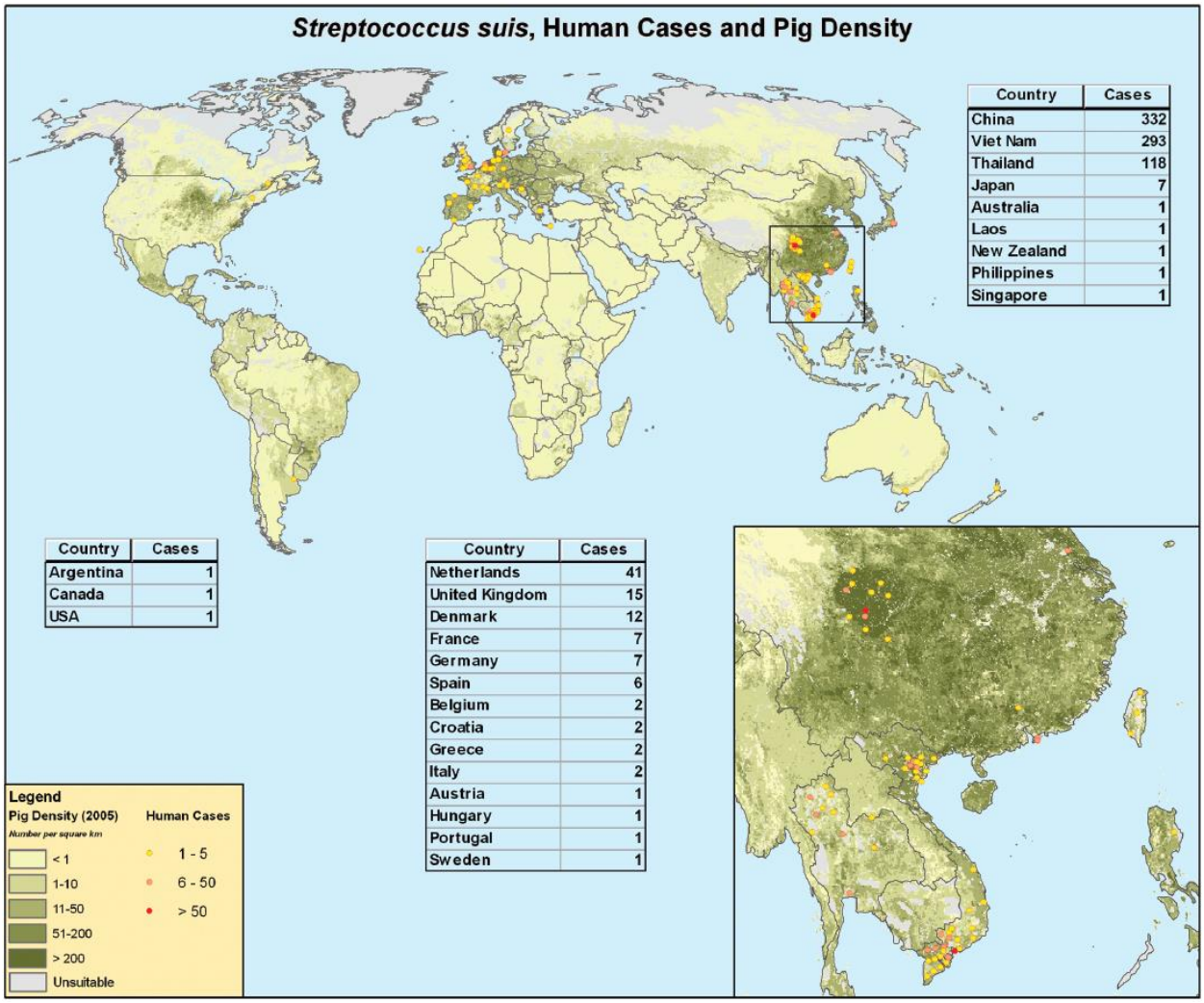


Figure 2.2 World map of human *Streptococcus suis* cases with background pig density data (Reprinted form: *Clin. Infect. Dis.* 48, 617–625 (2009))

In the past decades, *Streptococcus suis* infections have been considered a global problem worldwide, especially for the people working with pigs or pork-derived products.[107] After the first case of human infection was reported in Denmark, several outbreaks of human *S. suis* infections were reported in recent years. The largest one occurred in the summer of 2005 in Sichuan province, China. 215 people were infected and 38 cases died.[104,108] Currently, there are hundreds of people infected by *S. suis* worldwide and the number is still increasing (Figure 2.2).

S. suis is surrounded by a layer of polysaccharides forming the bacterial capsules that play a fundamental role for pathogen survival,[33] protect the bacterium and are important virulence factors. CPSs are able to trigger an adaptive immune response resulting in the production of specific antibodies rendering polysaccharides attractive targets for antibacterial vaccine development.[109] *S.*

suis are distinguished to 35 different serotypes based on the chemical composition of the capsules, they are named with serotypes 1 to 34 and serotype ½.[107,110]

The majority of *S. suis* infections are caused by the strains relatively a part of the serotypes. Globally, the *S. suis* serotypes isolated from clinical cases are serotype 2, 9, 3 ½ and 7, in decreasing order.[98] Most reported studies have focused on serotype 2, the most prevalent serotype worldwide. Serotype 9, the most invasive serotype in Europe,[111,112] however, has received only limited attention.

Immune prophylaxis against *S. suis* serotype 9 is challenging that antibiotics are so far the only treatment option in the case of infections caused by *S. suis* serotype 9.[113] However, the abuse of antibiotics will lead to bacterial resistance and other side effects.[114,115] To overcome these deficiencies, veterinary vaccines are an effective strategy to limit disease in farm animals and reduce the spread of pathogens from animals to humans. Vaccinations help to reduce antibiotic consumption and slow the development of antimicrobial resistance.[116] All currently used antibacterial veterinary vaccines are prepared from live attenuated or inactivated bacteria that suffer shortcomings in terms of safety, stability and in some cases limited immunogenicity.[116–118] While glycoconjugate vaccines in humans are very successful, veterinary glycoconjugate vaccines remain a largely unexplored opportunity.[119]

Identification of the immunogenic epitope is the key step in the development of semisynthetic glycoconjugate vaccines.[120] Synthetic antigen candidates based on CPS are important tools to investigate the structures of glycan epitope. Structural features including branching, terminal sugar moiety, overall sequence and length must be considered for they may affect the activity or stability of the antigen.[121] In this chapter, the first synthesis of a series of oligosaccharides related to the natural *S. suis* serotype 9 CPS repeating unit as the basis for further immunological studies is described.

2.2 Results and discussion

2.2.1 Synthetic strategy

The structure of *S. suis* serotype 9 capsular polysaccharide repeating unit was assigned in 2016[122] following the sequence [→4)-Glcol-1-P-3-[*α*-D-Gal(1→2)]-*β*-D-Gal(1→3)-*β*-D-Sug(1→3)-*α*-L-Rha(1→]. The repeating unit consists of a branched tetrasaccharide with a phosphorylated D-

glucitol residue (Figure 2.3). The tetrasaccharide contains a core D-galactose that is linked to another D-galactose at C2 and the D-glucitol residue is attached to C3 via a phosphodiester bond. At the anomeric position of the core D-galactose is a disaccharide consisting of a rare sugar, 2-acetamido-2,6-dideoxy-D-xylo-hexopyranos-4-ulose linked to the C3 of L-rhamnose. The presence of the labile C4-keto sugar, stereoselective glycosidic bonds and the phosphodiester group pose considerable synthetic challenges to access this molecule.

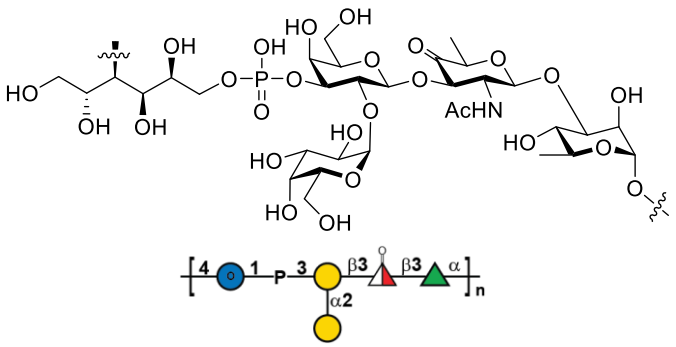


Figure 2.3 Structure of the repeating unit of *S. suis* serotype 9 capsular polysaccharide

Previous experience gained from similar structures synthesized by the Seeberger group[123] indicates instability of synthetic C4-keto sugars (Figure 2.4). Attempts to synthesize the natural ketone-containing repeating unit were not successful because the ketone was proved highly unstable and could not be isolated in pure form. However, the corresponding reduced forms exhibited improved stability, while maintaining the ability to induce an immune response against the CPS. This modification represents an isosteric chemical modification useful in view of a subsequent synthetic scale-up as well.

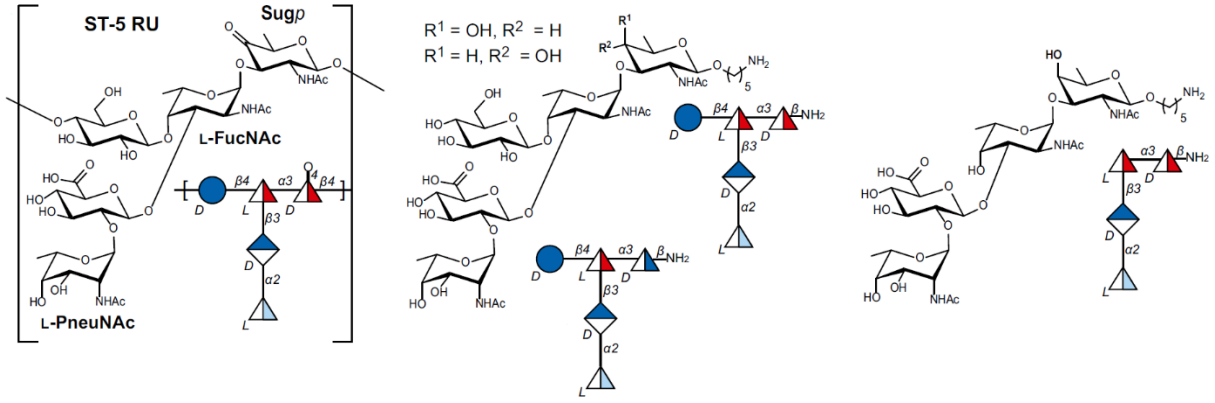


Figure 2.4 Structure of *Streptococcus pneumoniae* serotype 5 CPS repeating unit and related synthetic antigens

Looking back at the repeating unit of *S. suis* serotype 9, it has a same rare C4-keto sugar as *Streptococcus pneumoniae* serotype 5. To make sure the synthetic oligosaccharides are stable enough during purification, glycan array screening and conjugation, this research focused on the synthesis of the C4-axial reduced form of *S. suis* serotype 9 CPS repeating unit and its analogues (Figure 2.5).

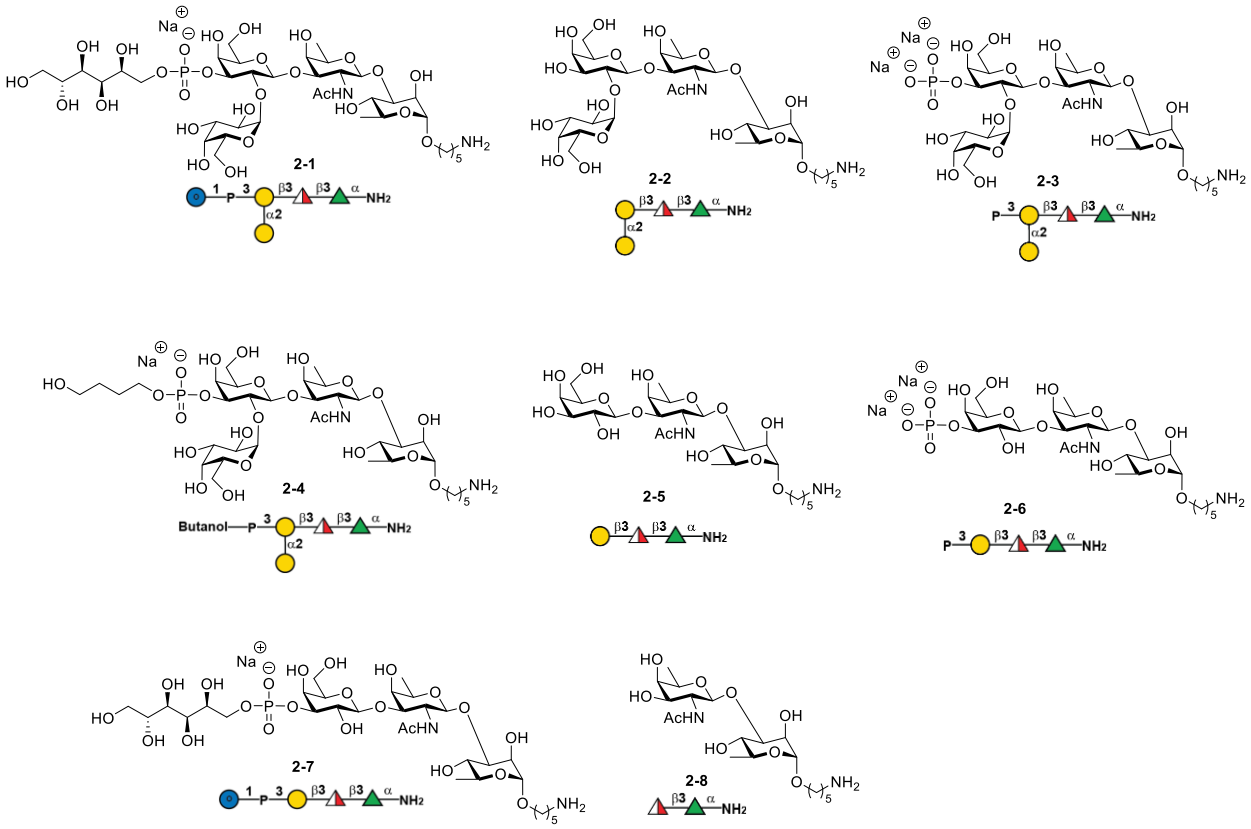
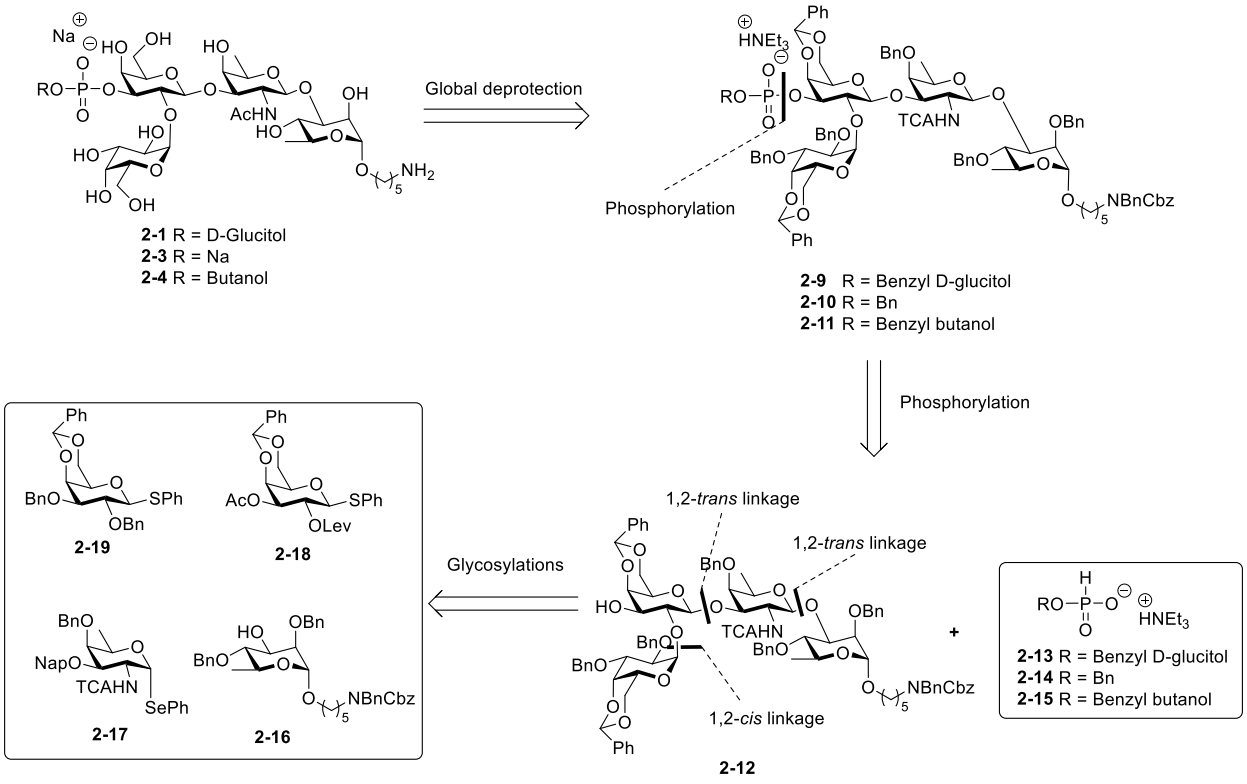


Figure 2.5 A collection of synthetic oligosaccharide antigens resembling the *S. suis* serotype 9 CPS

The library of synthetic oligosaccharide antigens includes four tetrasaccharides, three trisaccharides and one disaccharide, all the sugars carry an aminopentyl linker at the reducing ends. The variety of synthetic antigens allows for the identification of antibody epitopes and selection of vaccine candidate leads.

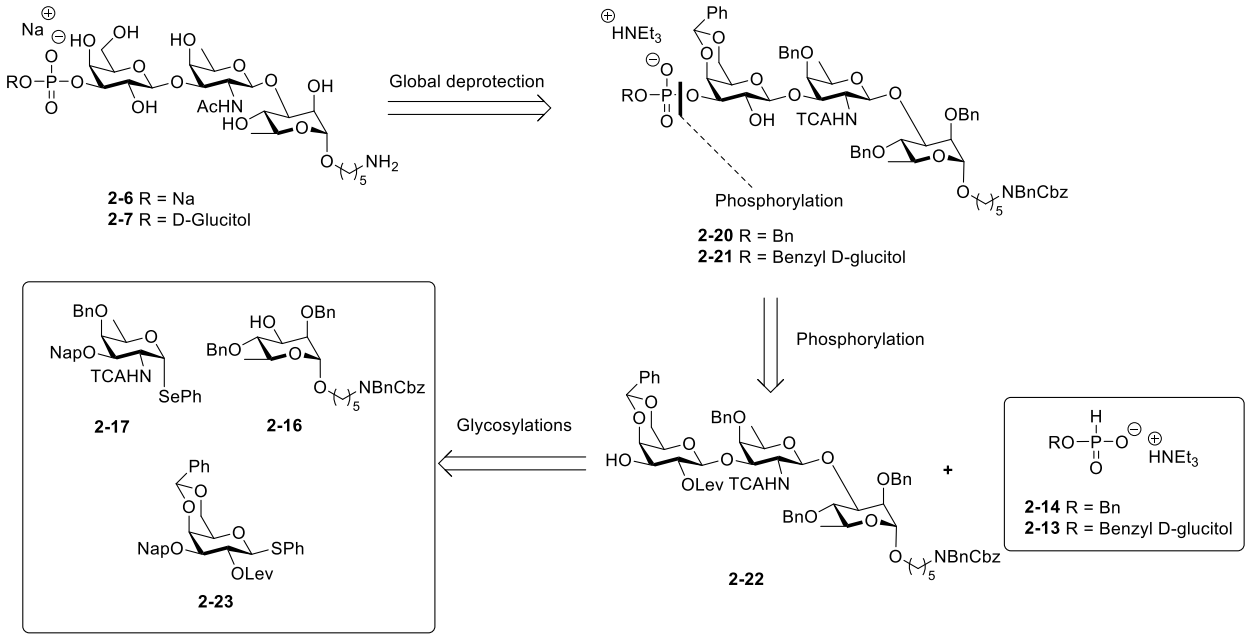
Retrosynthetic analysis of representative tetrasaccharides (Scheme 2.1) reveals that **2-1**, **2-3** and **2-4** can be obtained from global deprotection of the fully protected compounds **2-9**, **2-10** and **2-11**, respectively. Phosphorylation of tetrasaccharide backbone **2-12** with three *H*-phosphonates (**2-13**, **2-14**, **2-15**) would introduce corresponding phosphodiester bonds. Glycosylations of four differently protected monosaccharide building blocks provide the tetrasaccharide **2-12**. Benzyl

ethers serve as permanent protecting groups while trichloroacetyl group (TCA) and levulinoyl ester (Lev) ensure neighboring participation as C2 protective groups to construct 1,2-*trans* linkages. The 1,2-*cis* linkage between galactoses relies on solvent effect. Hydrogenolysis of the intermediates in the synthesis of **2-12** will give compounds **2-2**, **2-5** and **2-8**.



Scheme 2.1 Retrosynthetic analysis of tetrasaccharides 2-1, 2-3 and 2-4

As for the trisaccharides **2-6** and **2-7**, slightly different synthetic routes were designed and shown in Scheme 2.2. A 2-naphthylmethyl protecting group (Nap) at C3 of **2-13** ascertains survival of Lev in the preparation stage before phosphorylation.

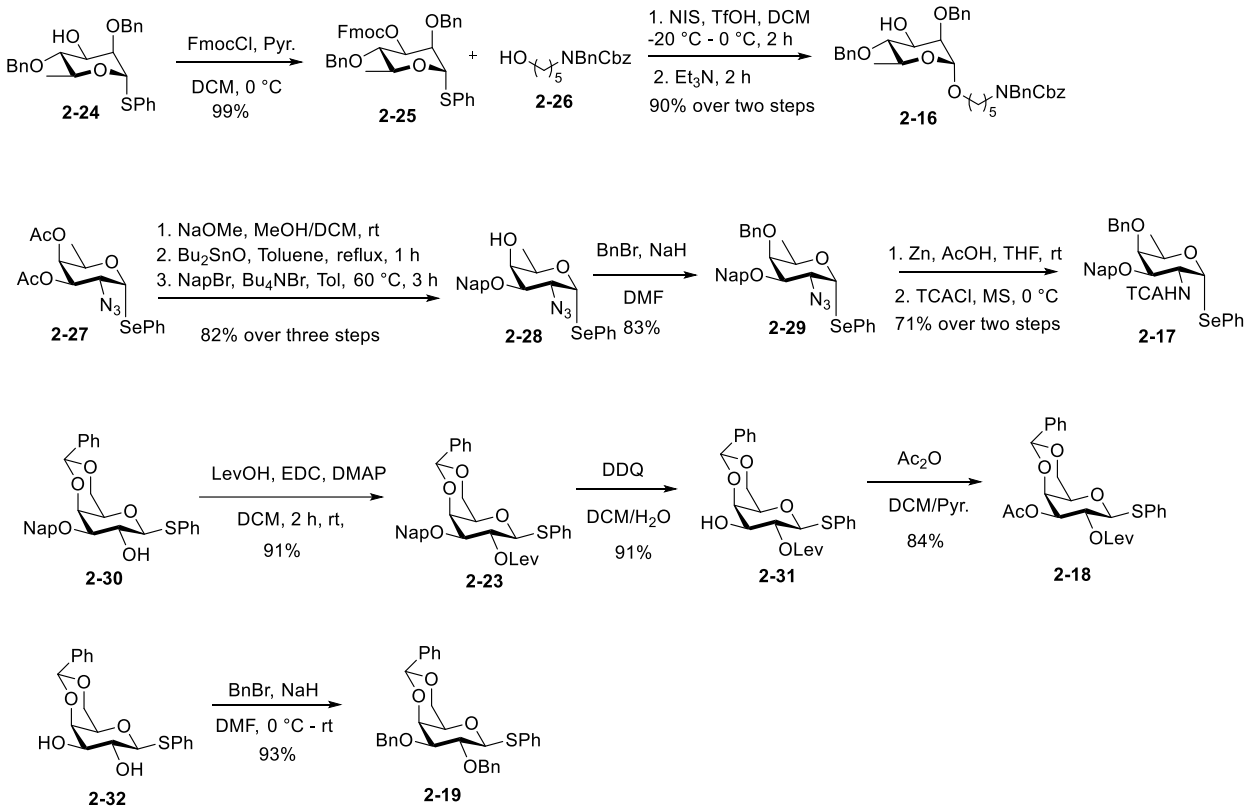


Scheme 2.2 Retrosynthetic analysis of trisaccharides 2-6 and 2-7

2.2.2 Synthesis of oligosaccharides 2-1 - 2-8

To synthesize the oligosaccharide targets, building blocks and *H*-phosphonates were prepared first. The synthesis of building block **2-16** commenced with known thioglycoside **2-24**[124,125] (Scheme 2.3), treatment of **2-24** with fluorenylmethyloxycarbonyl chloride (FmocCl) in the presence of pyridine produced **2-25** in high yield. Subsequently, the glycosylation of **2-25** with aminopropyl linker **2-26** using NIS/TfOH as a promoter gave the α-linked glycoside intermediate, triethylamine was added in the same reaction pot to cleave Fmoc protecting group, forming the desired building block **2-16** in 90% yield over two steps.

The acetyl groups on D-fucosamine derivative **2-27**[123,126,127] were removed with sodium methoxide, following regioselective protection of Nap on C3 afforded the selenoglycoside **2-28**. Then, **2-29** was obtained after the hydroxyl group at C4 was protected with benzyl ether. At last, azide group was reduced by excess zinc, and the resulted amine was masked with TCA group using trichloroacetyl chloride, forming building block **2-17** in 71% yield (Scheme 2.3).

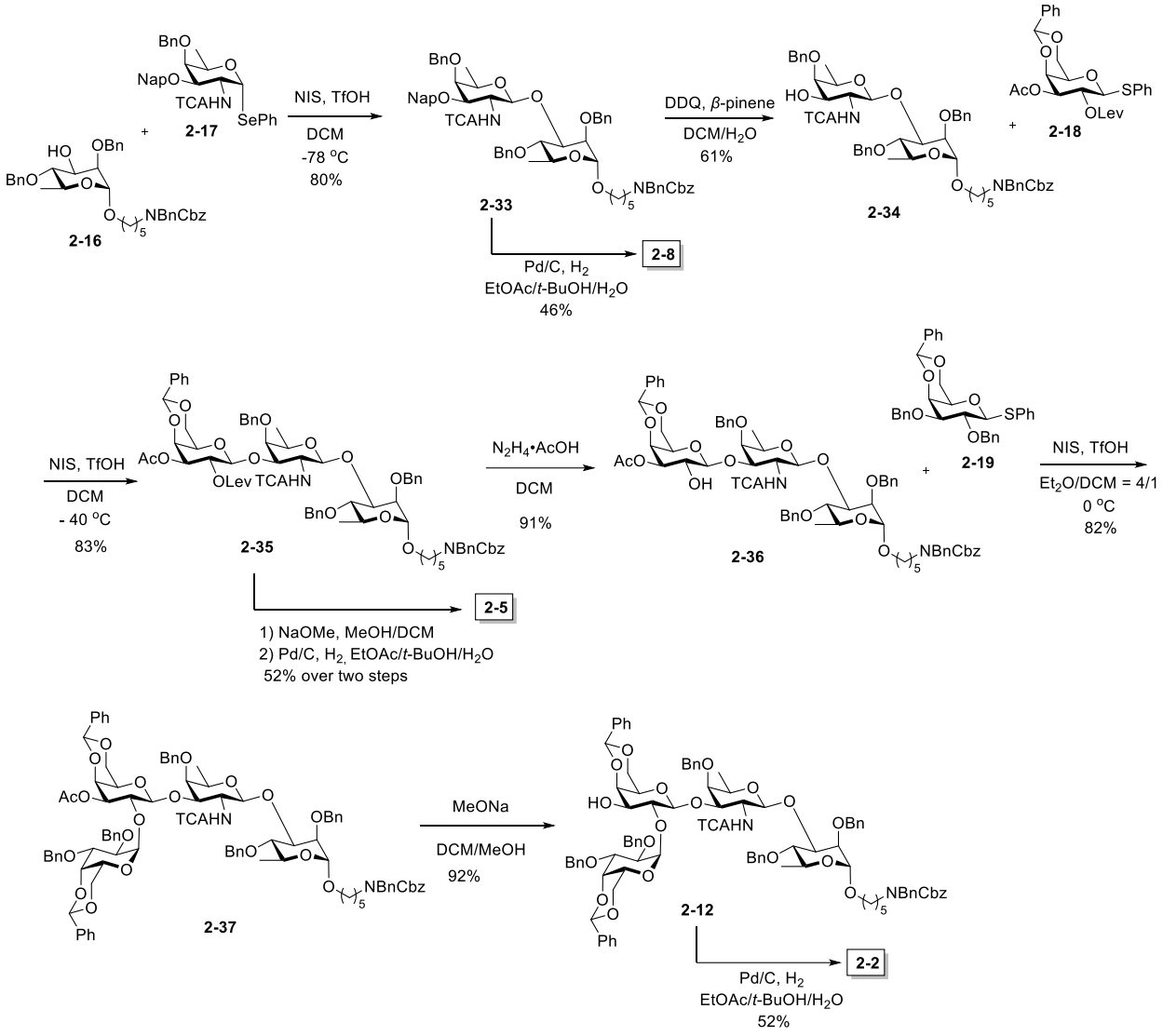


Scheme 2.3 Synthesis of building blocks 2-16, 2-17, 2-18, 2-19 and 2-23

Synthesis of building block **2-18** started from the Lev protection of C2-OH on 4,6-*O*-benzylidene-1-thio-*β*-D-galactopyranoside **2-30**.[128,129] Cleavage of Nap on **2-23** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in DCM/H_2O mixed solvent provided **2-31** in 91% yield. The free hydroxyl group of **2-31** was protected with acetyl group to give **2-18** in good yield. As for building block **2-19**, benzylation of **2-32** with benzyl bromide and sodium hydride in DMF afforded the product in 93% yield.[130]

With the building blocks in hand, I focused my attention on assembling the tetrasaccharide core **2-12**. Building block **2-16** was coupled with selenoglycoside **2-17** in the presence of NIS/TfOH as promoter to afford disaccharide **2-33** in good stereoselectivity (α/β = 1/6) (Scheme 2.4). Cleavage of the Nap ether on **2-33** with DDQ provided disaccharide **2-34** in very low yield, around 13%. Addition of *β*-pinene significantly increased the yield of this step (61%).[131] Disaccharide **2-34** reacted with thioglycoside **2-18** to form trisaccharide **2-35** in 83% yield with complete *β* selectivity. Removal of the temporary Lev group with hydrazine acetate yielded trisaccharide **2-36** as the acceptor for the subsequent glycosylation. Under the catalysis of NIS and TfOH, **2-19** was installed to **2-36** to produce tetrasaccharide **2-37** in 83% yield. Control over stereoselectivity via solvent

effect yielded consistent results, where the role of dichloromethane (DCM) in the mixed solvent was to help dissolve the reactants. Acetyl ester cleavage with sodium methoxide finished the synthesis of tetramer core **2-12**.

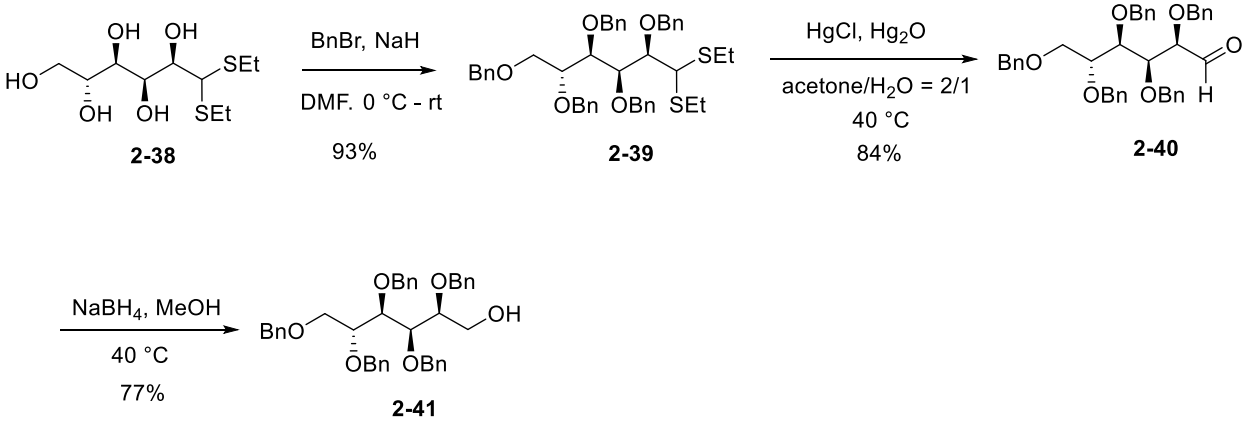


Scheme 2.4 Assembly of tetrasaccharide core 2-12 and target oligosaccharides 2-8, 2-5 and 2-2

After tetrasaccharide **2-12** was obtained, the stage was set to perform the challenging phosphorylation. Phosphorylation methods are adopted from the well-established protocols for the synthesis of oligonucleotides.[132–134] The two most commonly used strategies are both based on phosphitylation that is followed by oxidation.[135] One of them relies on phosphitylation of the hydroxyl group in oligosaccharides with phosphoramidites activated with 1*H*-tetrazole, followed

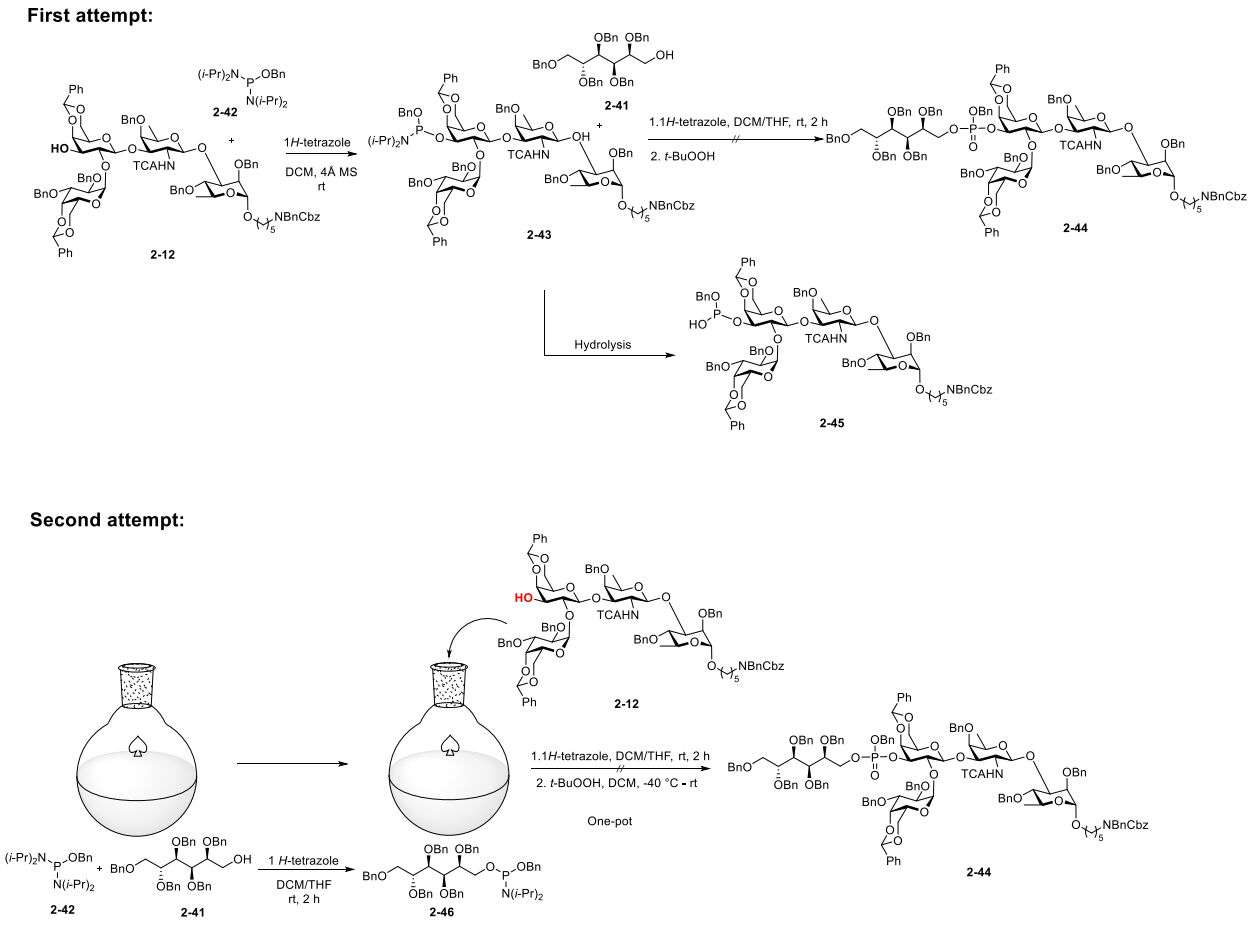
by oxidation with *tert*-butyl hydroperoxide or peroxides. This strategy was chosen initially to introduce the phosphodiester bonds.

Assembly of compound **2-1** with a D-glucitol was attempted. First, benzyl ether protected D-glucitol was synthesized from commercially available D-glycose diethyl mercaptal **2-38** (Scheme 2.5). Benzyl bromide and sodium hydride introduced benzyl groups to the hydroxyl groups and obtained **2-39** in 93% yield, followed by oxidation with mercury(II) chloride and mercury(I) oxide at 40 °C giving the aldehyde **2-40**.[136] **2-41** was furnished after the reduction of the aldehyde into alcohol with sodium borohydride in 77% yield.[137]



Scheme 2.5 Synthesis of D-glucitol 2-41

Next, conversion of **2-12** into phosphoramidite **2-43** using bis(diisopropylamino)-phosphite in the presence of 1*H*-tetrazole was performed in DCM, and 4Å molecular sieves were used to offer an anhydrous environment. However, the first phosphorylation was in very low efficiency and majority of starting material **2-12** did not react. The second phosphorylation using **2-41** and oxidation did not give the desired phosphate **2-44**. The phosphoramidite intermediate **2-43** was hydrolyzed due to its high reactivity. Then, the order of phosphorylations were changed and glucitol phosphoramidite **2-46** was prepared first, it was observed **2-46** was also super reactive that it would be hydrolyzed in the air. In order to reduce the hydrolysis ratio of **2-46** to promote the coupling with **2-12**, **2-12** was added immediately to the reaction mixture after **2-46** was formed. Unfortunately, the one-pot reaction failed to give desired **2-44**, tetrasaccharide **2-12** can be recovered and **2-46** was hydrolyzed (Scheme 2.6).

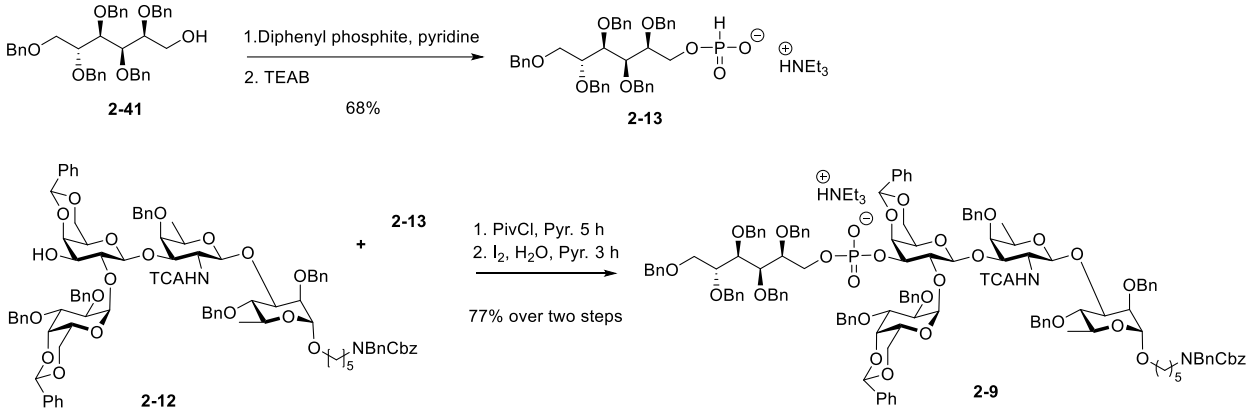


Scheme 2.6 Attempts to synthesize phosphate 2-44 employing phosphitylation/oxidation protocol using phosphoramidite

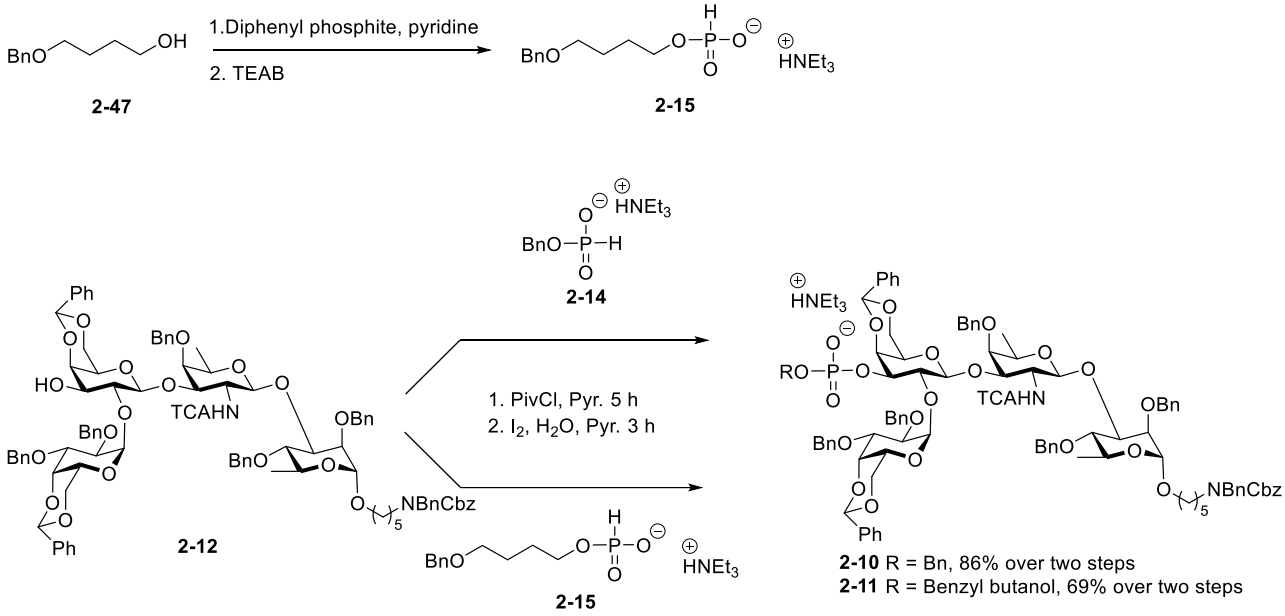
These results indicate that phosphorylation using phosphoramidite was hard to control because of the high reactivity and sensitivity of phosphoramidite intermediates, furthermore, the nucleophilicity of hydroxyl group on **2-12** was too weak to complete the second phosphorylation. Thus, another commonly used method is worthy trying. This method is using *H*-phosphonates to perform the phosphitylation of alcohol under the activation with pivaloyl chloride and pyridine followed by oxidation with iodine in pyridine and water.[138–140]

Treatment of **2-41** with diphenyl phosphite in pyridine and wash with triethylammonium bicarbonate (TEAB) buffer gave the D-glucitol *H*-phosphonate **2-13** in 68% yield. To my delight, the coupling of **2-13** with the hydroxyl group on **2-12** in the presence of pivaloyl chloride worked efficiently to give intermediate *H*-phosphonate diesters.[141] Subsequent oxidation with iodine and water in the same pot furnished the phosphate **2-9** as a triethylammonium salt in 77% yield (Scheme 2.7). Similarly, 4-benzyl-1-butanol *H*-phosphonate **2-14** was synthesized from **2-47** by

treatment of diphenyl phosphite in pyridine. **2-14**[138] and **2-15** were coupled with **2-12** to afford corresponding phosphorylated oligosaccharides **2-10** and **2-11** in good yields (Scheme 2.8).



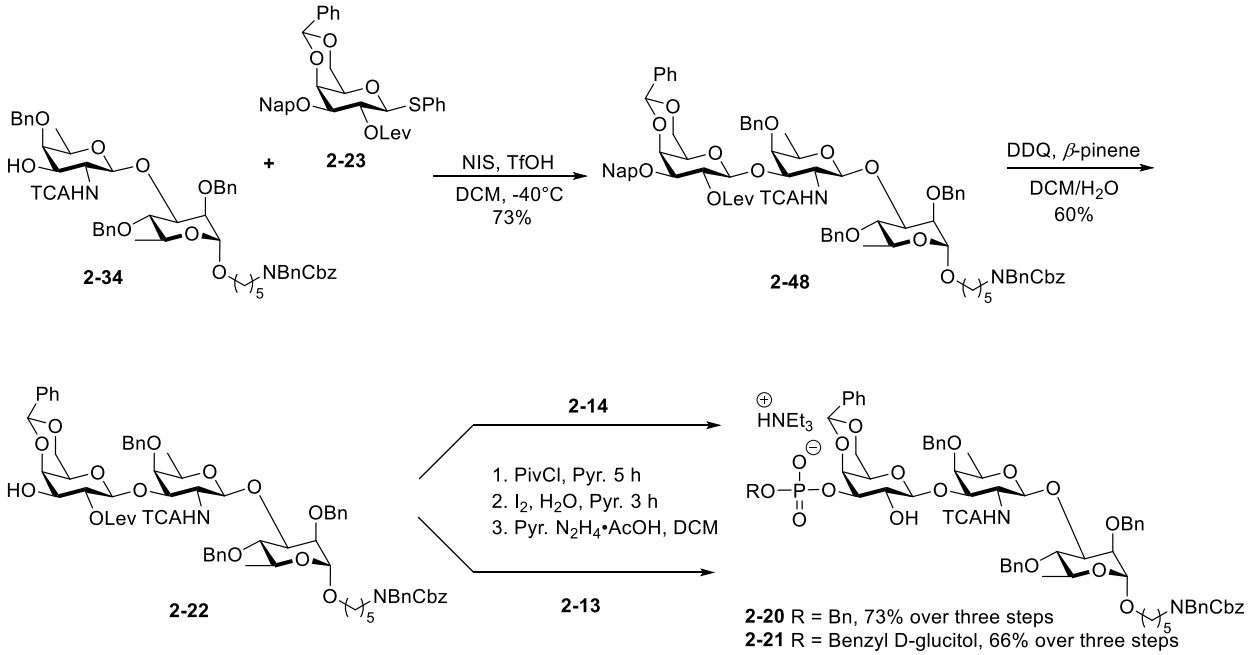
Scheme 2.7 Synthesis of tetrasaccharide phosphate 2-9 employing *H*-phosphonate strategy



Scheme 2.8 Synthesis of tetrasaccharide phosphates 2-10 and 2-11

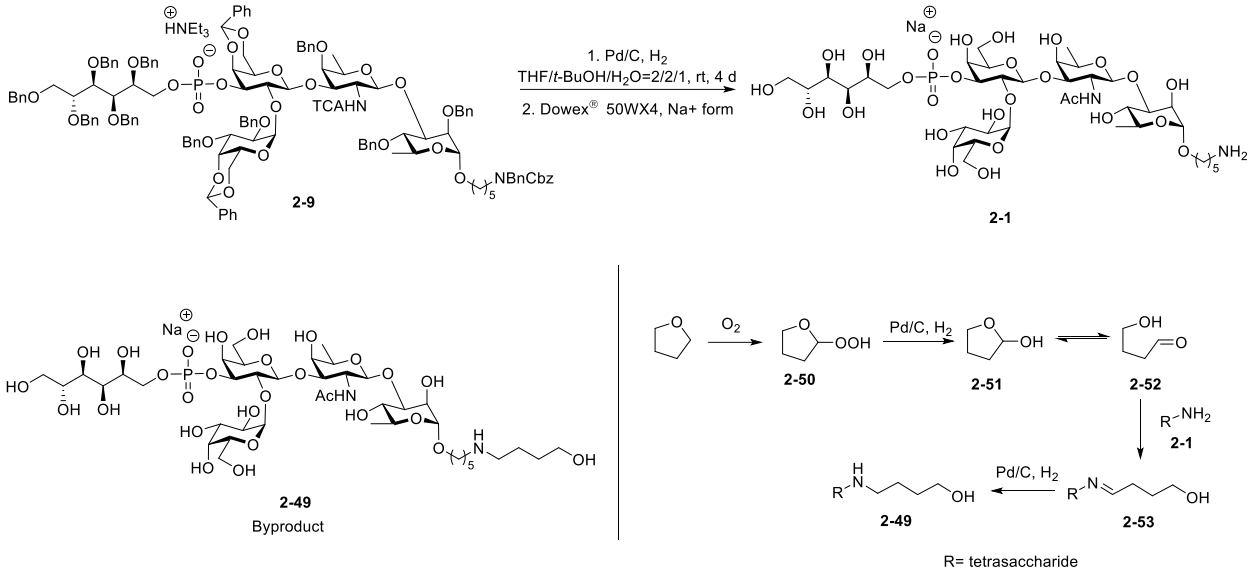
For the synthesis of trisaccharide phosphates (Scheme 2.9), with promotion of NIS and TfOH, glycosylation between **2-34** and **2-23** yielded trisaccharide **2-48** in 73% yield with complete *β*-selectivity, which was ascertained by the neighboring participation of 2-OLev group on **2-23**. Removal of Nap ether with DDQ and *β*-pinene provided **2-22** with a free C-3 hydroxyl group to couple with *H*-phosphonates **2-14** and **2-13**. After the coupling reaction was finished, levulinoyl

esters were cleaved to afford trisaccharide phosphate diesters **2-20** (73% yield) and **2-21** (66% yield).



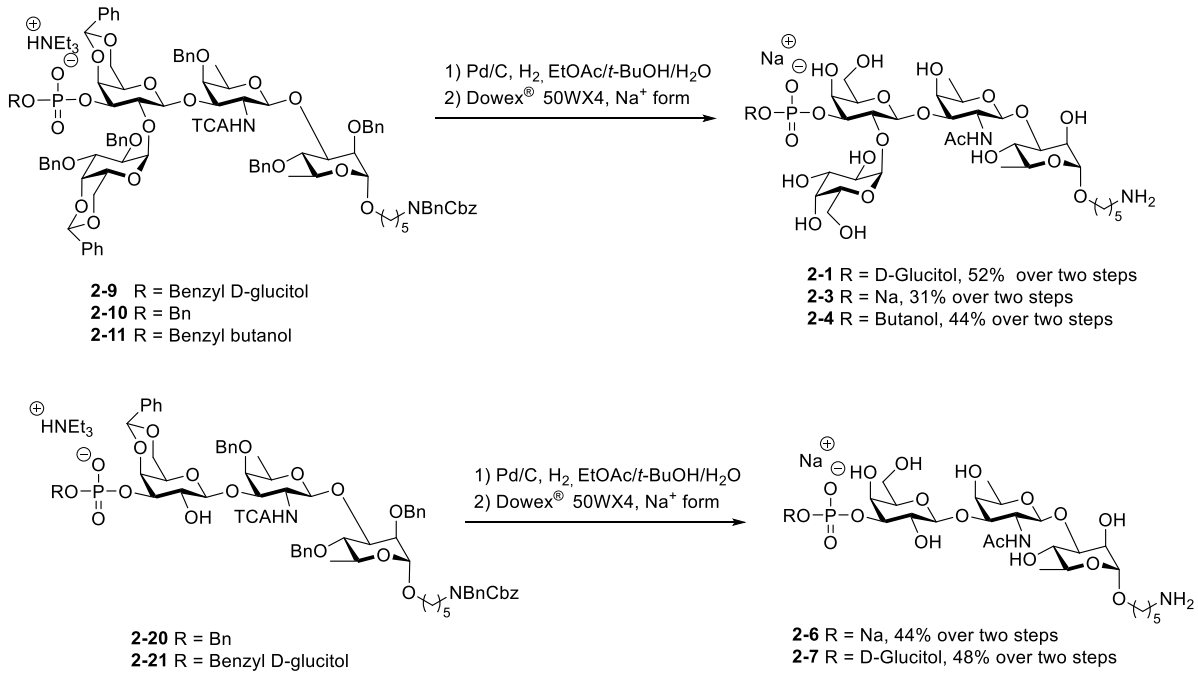
Scheme 2.9 Synthesis of trisaccharide phosphates 2-20 and 2-21

With all oligosaccharides in hand, global deprotection was carried out by Pd/C-catalyzed hydrogenolysis. Phosphate **2-9** was dissolved in THF/*t*-BuOH/H_2O=2/2/1 mixture and kept in hydrogen atmosphere for two days. Although desired product **2-1** was obtained, the yield was very low as another major product with a mass 72 bigger than **2-1** was detected by MALDI-TOF. It is possible that THF was covalently bound to **2-1** to give the byproduct. The possible structure of **2-49** and mechanism are shown in Scheme 2.10. 2-Tetrahydrofuryl hydroperoxide **2-50** was generated in presence of oxygen, followed by reduction into 2-hydroxytetrahydrofuran **2-51** with hydrogen. **2-51** was in an equilibration with 4-hydroxybutanal **2-52** that could couple with the amino group on **2-21** to give compound **2-53**. The reduction of C=N bond finally furnished byproduct **2-49**.



Scheme 2.10 Global deprotection in THF and possible mechanism for formation of byproduct

This problem was solved by changing solvent from THF to ethyl acetate (Scheme 2.11). Subsequently, the ion exchange column chromatography furnished target phosphates as sodium salts in moderate yields. As for the other target compounds (Figure 2.5), as designed, global deprotection of oligosaccharides **2-33**, **2-35** and **2-12** gave the corresponding non-phosphorylated compounds **2-8**, **2-5** and **2-2** (Scheme 2.4).



Scheme 2.11 Global deprotection of oligosaccharides

2.2.3 Synthesis of *α*-linked analogues 2-54 - 2-60

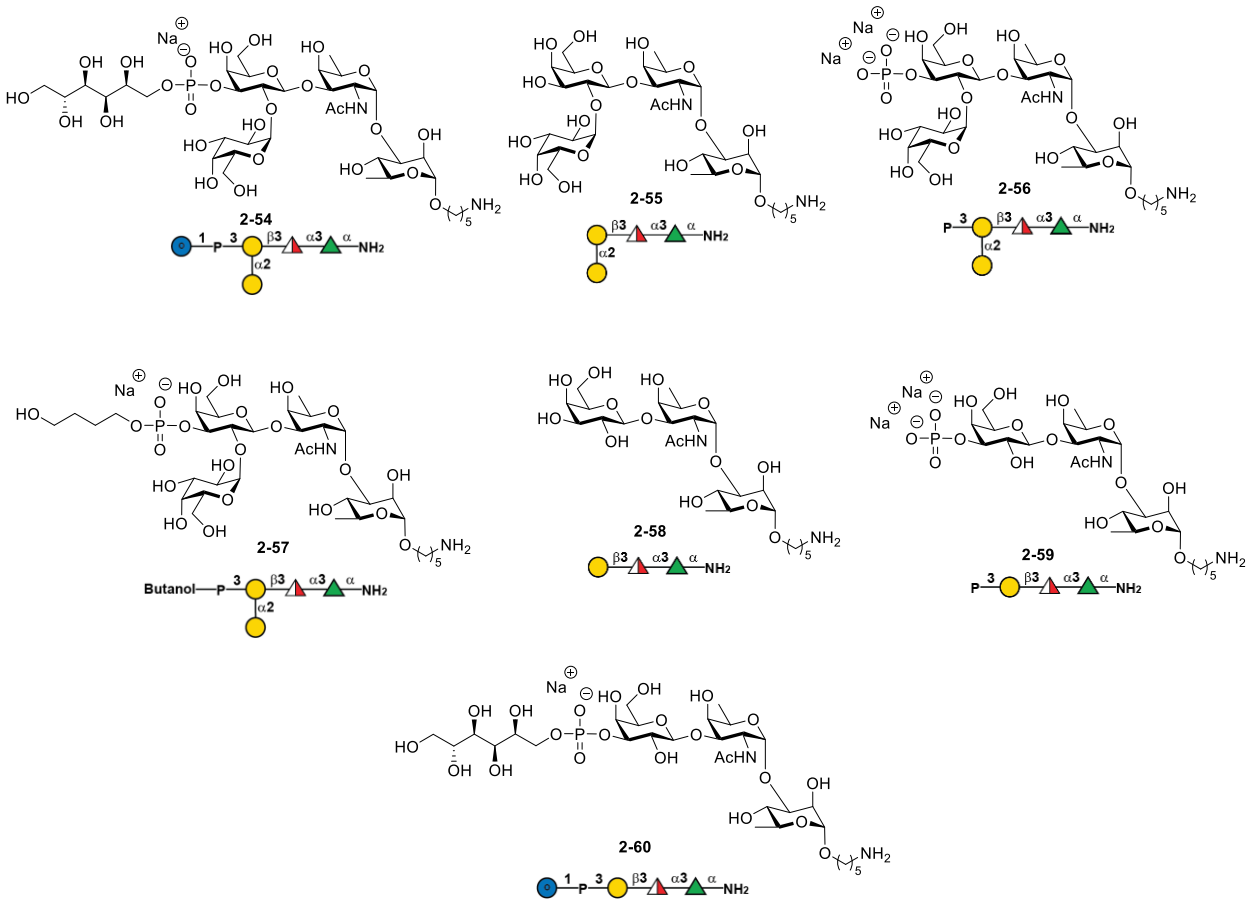
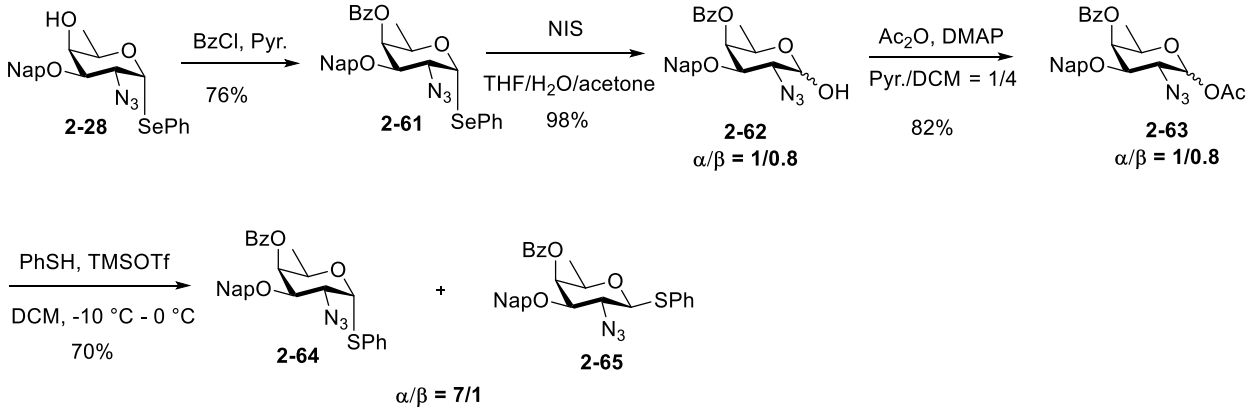


Figure 2.6 Oligosaccharide antigens resembling *S. suis* serotype 9 CPS

The antibody-binding activity of a synthetic oligosaccharide antigen depends on the structure and configuration of the antigen.[142] *α* and *β* configurations are intuitive embodiments in oligosaccharide synthesis. In order to investigate the importance of the *β* linkage in the *S. suis* serotype 9 CPS, oligosaccharides **2-54 – 2-60** bearing an *α* glycosidic linkage in place of the native *β* glycosidic bond between D-fucosamine and L-rhamnose based on the *S. suis* serotype 9 CPS were prepared (Figure 2.6).

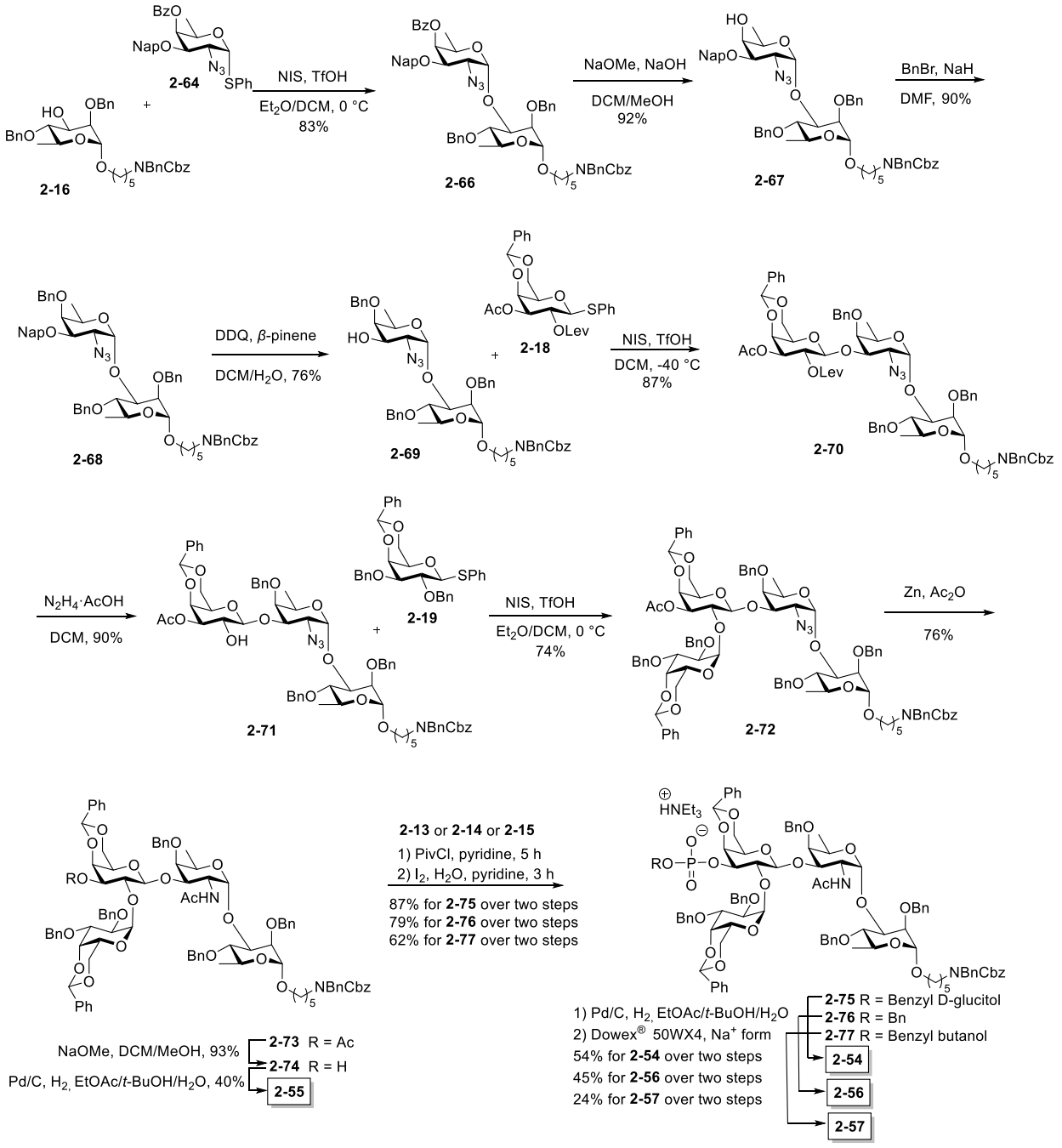
To install the *α* linkage between D-fucosamine and L-rhamnose, a new building block **2-64** was prepared (Scheme 2.12). Benzoyl ester was introduced to C4-OH on selenoglycoside **2-28** using benzoyl chloride in pyridine to provide **2-61** in 76 % yield. **2-61** was directly used as a donor for glycosylation with **2-16**, however, the reaction exhibited poor stereoselectivity (*α*/*β* = 3/2) due to the high reactivity of selenoglycoside. This also offers an explanation from the side why in the

synthesis of disaccharide **2-33**, 1,2-*cis* glycosidic bond was formed even with the neighboring participation of 2-NHTCA. In order to reduce the reactivity of selenoglycoside in the activation stage of glycosylation, **2-61** was hydrolyzed with NIS and water to offer hemiacetal **2-62**. The free hydroxyl moiety at anomeric position was protected with acetyl group and then converted to thiophenyl group to obtain α-linked **2-64** as the major product in 70% yield.



Scheme 2.12 Synthesis of building block 2-64

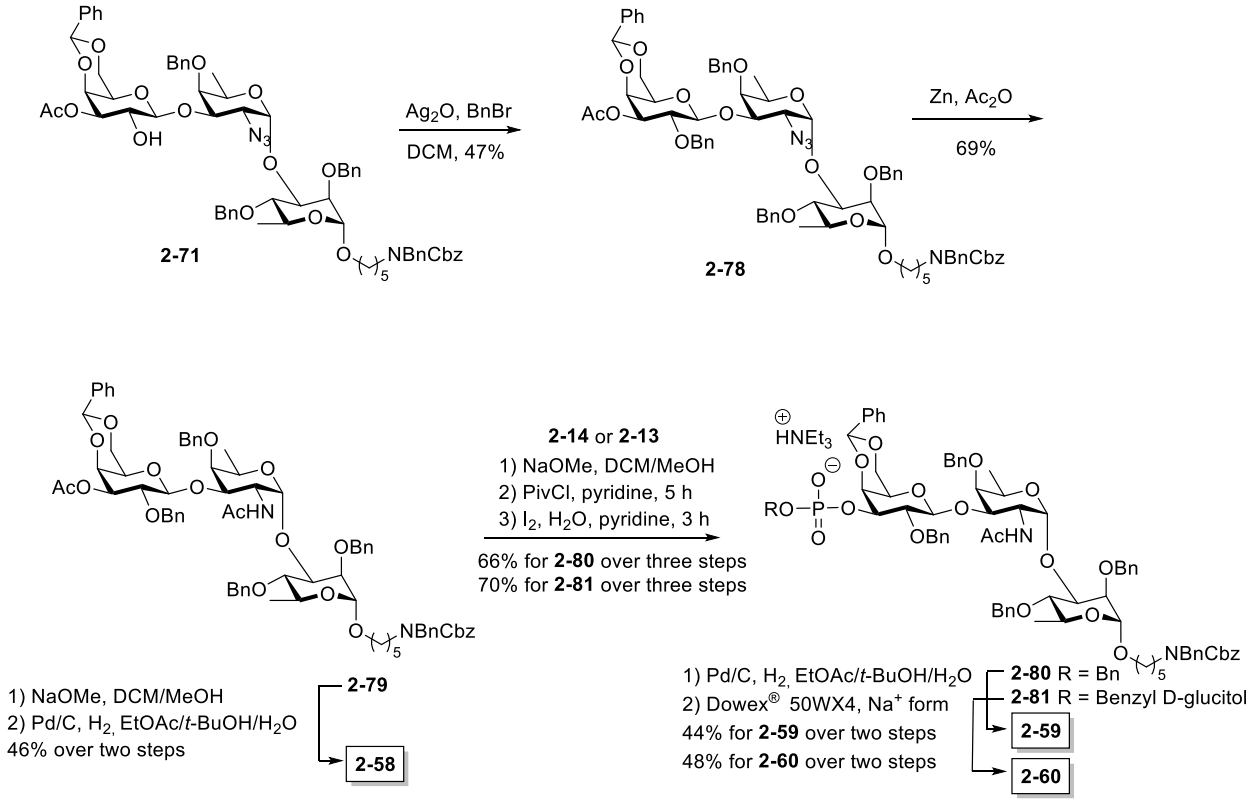
Following the assembly sequence used for the β-linked oligosaccharides, synthesis of α-tetrasaccharide analogues started with the union between of **2-16** and **2-64** at 0 °C produced disaccharide **2-66** with high α selectivity (Scheme 2.13), the remote participation of the benzoyl ester is considered to be the main dominant factor for stereoselectivity.[143–145] Conversion of the benzoyl ester to the benzyl ether over two steps followed by the cleavage of the Nap ether using DDQ and β-pinene afforded disaccharide acceptor **2-69**. Under promotion of NIS and TfOH, coupling of thioglycoside **2-18** with **2-69** produced exclusively β product **2-70** in 87% yield. After levulinoyl ester cleavage with hydrazine acetate furnished **2-71** in 90% yield, NIS/TfOH-mediated glycosylation of **2-71** with **2-19** produced tetrasaccharide **2-72** with high α selectivity (α/β > 20/1). The azide moiety of **2-72** was reduced by zinc and the resulting amine was acetylated to give **2-73** in 76% yield. Next, tetrasaccharide **2-74** with a free hydroxyl was obtained after saponification of the acetyl ester and following hydrogenolysis gave **2-55**. Furthermore, tetrasaccharide **2-74** was smoothly coupled with three different *H*-phosphonates **2-13**, **2-14** and **2-15** to produce corresponding phosphates **2-75**, **2-76** and **2-77** in good yields. Subsequent hydrogenolysis and sodium ion exchange chromatography afforded pure **2-54**, **2-56** and **2-57**, respectively.



Scheme 2.13 Synthesis of *α*-analogues (2-54, 2-55, 2-56, and 2-57) of *S. suis* serotype 9 CPS repeating unit

The presence of an acetyl ester of **2-71** rendered benzylation under basic conditions not feasible. Treatment of **2-71** with freshly prepared silver oxide and benzyl bromide[146] produced **2-78** in 47% yield (Scheme 2.14). The azide was converted to the corresponding acetamide using zinc and acetic anhydride to yield **2-79** (69%). The synthesis of **2-58** was achieved after removal of the acetyl group and hydrogenolysis of the benzyl ethers on **2-79** in 46% yield over two steps. To set the

stage for phosphorylation, deacetylation of **2-79** yielded the requisite free hydroxyl group that was coupled with **2-14** and **2-13** to furnish phosphates **2-80** and **2-81** as triethylammonium salts. Global deprotection of these phosphates afforded the corresponding final glycosides **2-59** and **2-60**.



Scheme 2.14 Synthesis of *α*-analogues 2-58, 2-59 and 2-60

2.2.4 Glycan microarray screening*

To date, no capsular polysaccharide based glycoconjugate vaccine against *S. suis* serotype 9 has been described. A reported *S. suis* serotype 9 bacterial vaccine candidate did not induce a high anti-CPS antibody response in piglets.[17] To develop novel glycoconjugate vaccines, a glycan microarray study was performed to identify the glycan epitope and attractive antigen leads.

Sera collected from pigs infected with *S. suis* serotype 9, immunized rabbit and humans vaccinated against *S. pneumoniae* (007sp WHO reference sera)[147] were screened for antibodies binding to synthetic oligosaccharides and isolated *S. suis* serotype 9 CPS using glycan microarrays.

* Glycan microarray evaluation and data analysis were performed by Patricia Priegue.

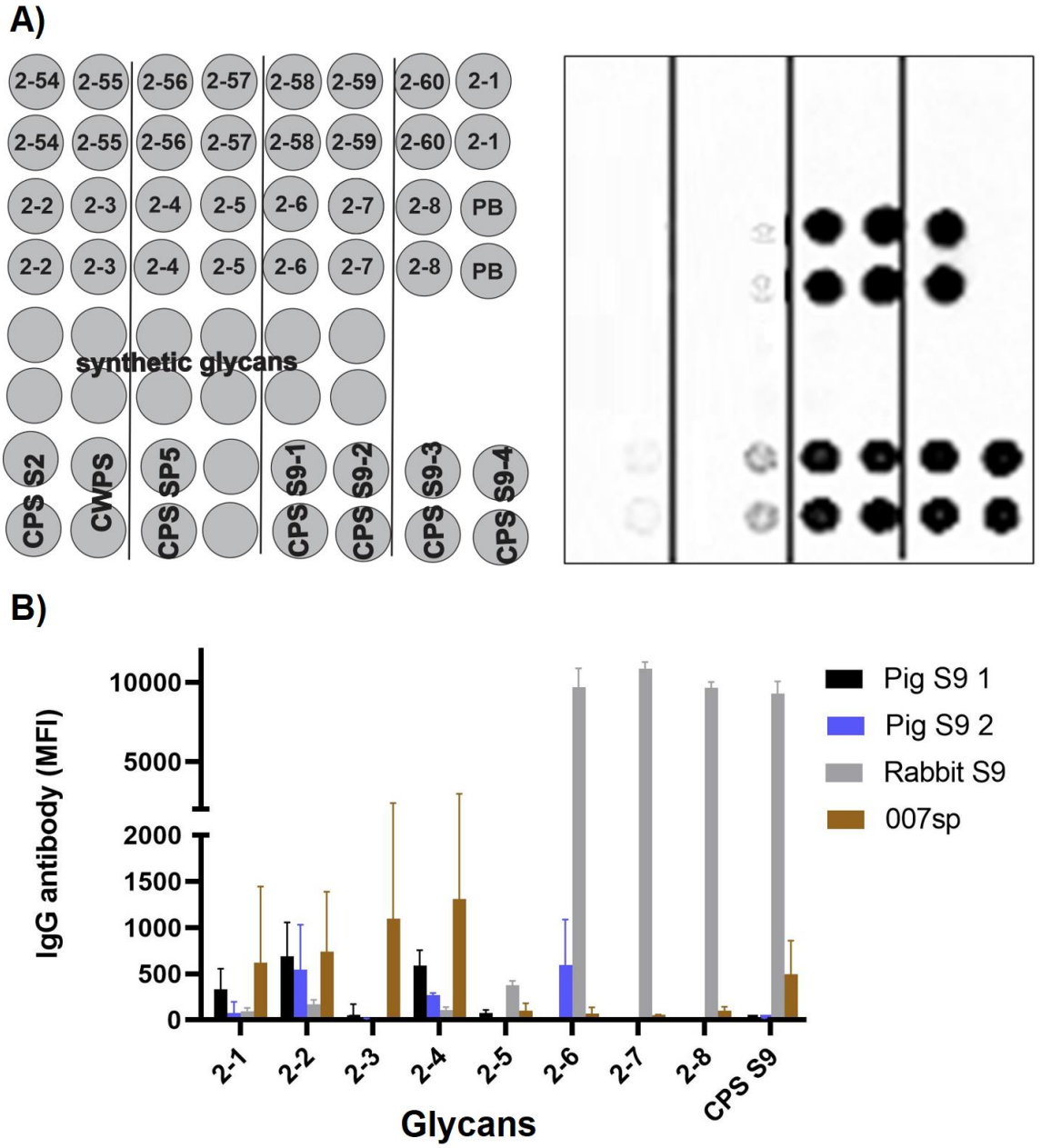


Figure 2.7 Glycan array analysis of *S. suis* serotype 9 oligosaccharides and native CPS A) Printing pattern of microarray and binding of rabbit serum to immobilized glycans; B) IgG antibody binding to glycans. A serum dilution of 1:100 was used. MFI, mean fluorescence intensity (mean ± standard deviation); PB, printing buffer; CWPS, cell wall polysaccharide.

IgG antibodies present in pig sera bound weakly to oligosaccharides revealing a complex binding pattern. Rabbit sera on the other hand showed a clearer picture (Figure 2.7). Rabbit IgGs recognized strongly phosphorylated trisaccharides **2-6** and **2-7**, disaccharide **2-8**, and similarly the

native CPS. Trisaccharide **2-6** includes both the sugar sequence of **2-8** and a terminal phosphate monoester. This functional group appears to be essential since the absence of phosphorylation on **2-5** strongly reduced binding. On the other hand, the glucitol chain on **2-7** did not show a significant effect. Branched oligosaccharides **2-1 - 2-4** were not bound specifically (Figure 2.7). These observations suggest that the minimal glycotope contains L-rhamnose, D-fucosamine and a phosphate moiety, indicating that trisaccharide **2-6** is the minimum glycotope useful to elicit an immune response.

Antibodies in sera from vaccinated humans (007sp serum) also bound to the longer oligosaccharides due to cross-reactivity with CPS *S. pneumoniae*, probably because of the branched α-galactose.[148,149] It had been shown previously, that other *S. suis* serotypes cross-react with some serotypes of *S. pneumoniae*.[82,148] The IgG response was higher than that of IgM, as IgM antibodies showed low or no binding for most cases (Figure 2.8) indicating that isotype switching after infection in pigs or immunization in rabbits and humans was induced.

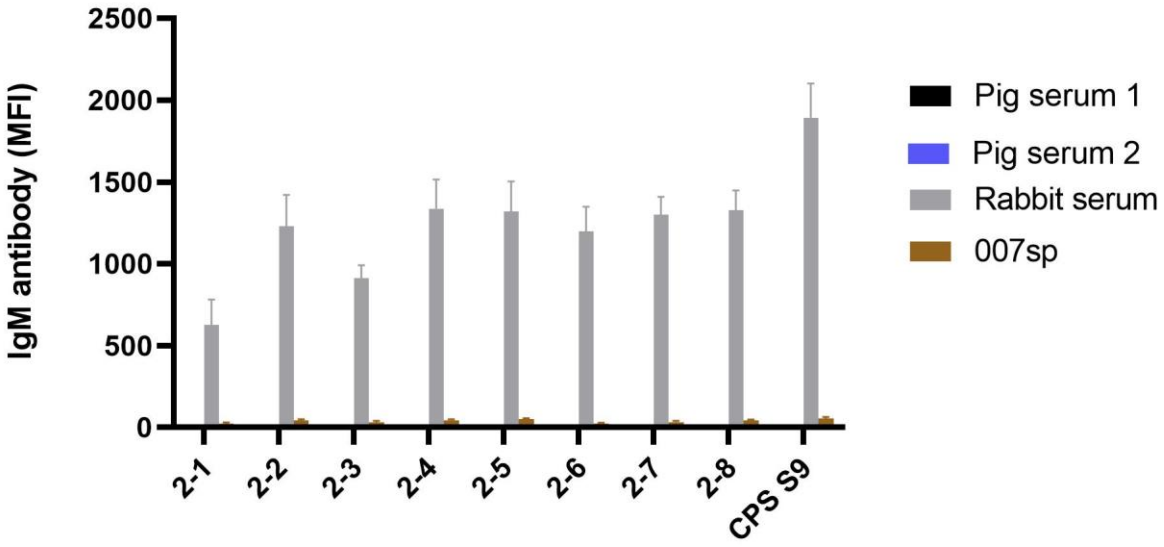


Figure 2.8 Glycan array analysis of *S. suis* serotype 9 oligosaccharides 2-1 – 2-8 and native CPS: IgM antibody binding to glycans

Oligosaccharides with an α- in place of a β-linkage between D-fucosamine and L-rhamnose (**2-54 – 2-60**) were found to be bound much weaker (Figure 2.9). This finding suggests that the β-linkage, present in the native CPS,[122] has an important role for the recognition by antibodies.[142]

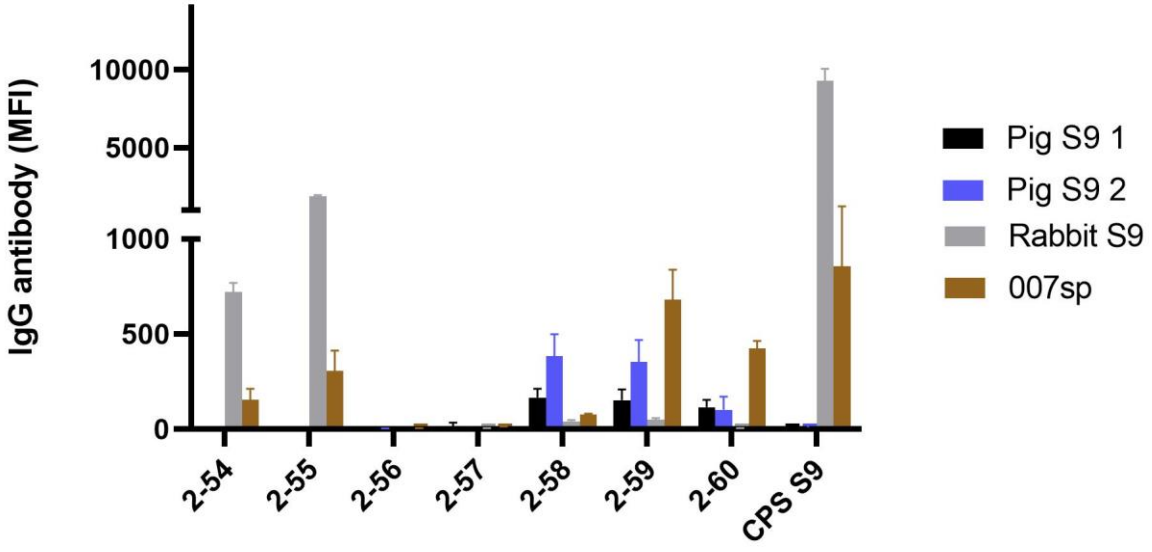


Figure 2.9 Glycan array analysis of *S. suis* serotype 9 oligosaccharides 2-54 - 2-60 and native CPS: IgG antibody binding to glycans

In conclusion, trisaccharide **2-6** is an attractive lead for the development of a glycoconjugate vaccine against *S. suis* serotype 9.

2.3 Conclusion and outlook

This chapter describes the synthesis of a collection of 15 novel oligosaccharides resembling the capsular polysaccharides related to *Streptococcus suis* serotype 9. The syntheses tackled challenges associated with complex glycan targets, including the introduction of 1,2-*cis* glycosidic bonds and labile phosphodiesters.

The synthetic, conjugation-ready glycans were printed onto an array surface to give rise to glycan microarrays. The glycan microarrays were used to screen the sera of pigs infected with *S. suis* as well as sera from immunized rabbit. Based on the glycan array studies, the glycan epitopes of lead antigens for the development of semi-synthetic glycoconjugate vaccines to protect from *S. suis* serotype 9 were identified.

Currently, vaccination of pigs for challenge studies is being prepared in an effort to develop efficacious vaccines to protect pigs as well as people working in the swine industry while reducing the use of antibiotics.

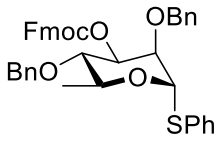
2.4 Experimental section

2.4.1 General information

Commercial grade solvents and reagents were used without further purification. Sugar building blocks indicated as commercially available were purchased from GlycoUniverse GmbH. Anhydrous solvents were obtained from a solvent drying system (JCMeyer) or dried according to reported procedures. Analytical TLC was performed on Kieselgel 60 F254 glass (Macherey-Nagel). Spots were visualized with UV light, sulphuric acid stain [1 mL of 3-methoxyphenol in 1 L of EtOH and 30 mL H_2SO_4] or ceric ammonium molybdate stain [0.5 g $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$, 12 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and 15 mL H_2SO_4 in 235 mL H_2O]. Flash chromatography was performed on Kieselgel 60 230-400 mesh (Sigma-Aldrich). Preparative HPLC purifications were performed with an Agilent 1200 Series or Agilent 1260 Infinity II. NMR spectra were recorded on a Varian 400 MHz spectrometer (Agilent), Ascend 400 MHz (cryoprobe, Bruker) or Varian 600 MHz (Agilent) at 25 °C unless indicated otherwise. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks ($CHCl_3$: δ 7.26 in ^1H and 77.16 in ^{13}C; HDO δ 4.79 in ^1H). Bidimensional and non-decoupled experiments were performed to assign identities of peaks showing relevant structural features. Configurations of sialic acid derivatives were determined by bidimensional HMBC and EXSIDE.[150] The following abbreviations are used to indicate peak multiplicities: *s* (singlet), *d* (doublet) *dd* (doublet of doublets), *t* (triplet), *dt* (doublet of triplets), *td* (triplet of doublets), *q* (quartet), *p* (pentet), *m* (multiplet). Additional descriptors *b* (broad signal) and *app* (apparent first-order multiplet) are also employed when required. Coupling constants (*J*) are reported in Hertz (Hz). NMR spectra were processed using MestreNova 14.1 (MestreLab Research). Specific rotations were measured with a UniPol L1000 polarimeter (Schmidt & Haensch) at λ = 589 nm. Concentration (*c*) is expressed in g/100 mL in the solvent noted in parentheses. IR spectra were measured with a Perkin Elmer 100 FTIR spectrometer. High-resolution mass spectra (ESI-HRMS) were recorded with a Xevo G2-XS Q-Tof (Waters).

2.4.2 Experimental procedures

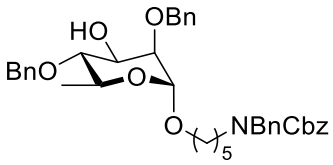
Phenyl 2,4-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-*α*-L-rhamnopyranoside (2-25)



To a solution of **2-24**[124,125] (115 mg, 0.26 mmol) and pyridine (85 µL, 1.06 mmol) in anhydrous DCM (2.5 mL), FmocCl (170 mg, 0.66 mmol) was added and the mixture was stirred overnight. After the starting material was consumed, the solution was diluted with DCM (10 mL) and the organic layer was washed with 0.01 M HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 10/1) to give **2-25** as a white solid (172 mg, 99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 7.6, 2.8 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.47 – 7.19 (m, 19H), 5.50 (d, *J* = 1.8 Hz, 1H), 5.04 (ddd, *J* = 9.5, 3.4, 1.2 Hz, 1H), 4.85 (d, *J* = 11.0 Hz, 1H), 4.72 – 4.63 (m, 2H), 4.52 (d, *J* = 12.1 Hz, 1H), 4.41 – 4.28 (m, 2H), 4.28 – 4.19 (m, 2H), 4.19 – 4.16 (m, 1H), 3.77 (t, *J* = 9.5 Hz, 1H), 1.36 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 154.5, 143.4, 143.3, 141.41, 141.39, 138.1, 137.5, 134.4, 131.5, 129.2, 128.5, 128.1, 128.03, 128.01, 127.0, 127.9, 127.5, 127.3, 127.28, 125.27, 125.26, 120.2, 85.3, 78.9, 78.1, 75.4, 72.6, 70.0, 69.2, 46.8, 18.0; HRMS (ESI) calculated for $C_{41}H_{38}O_6S$ $[M+Na]^+$: 681.2281, found: 681.2065.

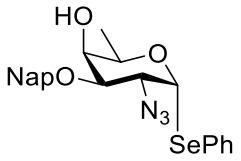
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-16)**



A mixture of **2-25** (100 mg, 0.15 mmol), aminopentyl linker **2-26** (99 mg, 0.30 mmol) and 4 Å molecular sieves in anhydrous DCM (3 mL) was stirred at room temperature for 30 min. The solution was cooled to −20 °C, NIS (38.3 mg, 0.17 mmol) and TfOH (1.51 μL, 0.02 mmol) were added. The reaction mixture was warmed to 0 °C and stirred for 2 h. After complete consumption of staring material, Et_3N (2 mL) was added and the mixture was stirred for another 2 h. Then the reaction was diluted with DCM (20 mL) and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. Crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 5/1) to give **2-16** as a colorless syrup (88 mg, 90% over 2 steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 6.97 (m, 20H), 5.07 (d, *J* = 11.7 Hz, 2H), 4.80 (d, *J* = 11.0 Hz, 1H), 4.69 – 4.60 (m, 2H), 4.55 (d, *J* = 11.0 Hz, 1H), 4.48 (d, *J* = 11.8 Hz, 1H), 4.39 (d, *J* = 8.3 Hz, 2H), 3.86 – 3.78 (m, 1H), 3.63 – 3.40 (m, 3H), 3.25 – 3.04 (m, 4H), 1.50 – 1.29 (m, 4H), 1.22 (d, *J* = 6.3 Hz, 3H), 1.19 – 1.10 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.8, 156.2, 138.5, 137.96, 137.8, 136.9, 136.8, 128.68, 128.65, 128.58, 128.53, 128.16, 128.13, 128.09, 128.02, 127.9, 127.8, 127.4, 127.3, 127.2, 96.8, 82.4, 78.8, 75.2, 73.1, 71.7, 67.3, 67.26, 67.24, 67.1, 60.5, 50.5, 50.2, 47.1, 46.1, 29.2, 28.0, 27.5, 23.5, 21.2, 18.1, 14.3; HRMS (ESI) calculated for $C_{40}H_{47}NO_7$ $[M+Na]^+$: 676.3245, found: 676.3242.

Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-seleno-*α*-D-fucopyranoside (2-28)

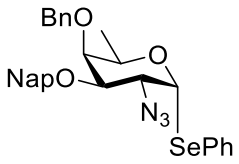


To a solution of **2-27**[123,126,127] (1.43 g, 3.47 mmol) in MeOH (50 mL), NaOMe was added slowly until the solution reached a pH of 11. The reaction mixture was stirred for 4 h, neutralized with Amberlite, filtered and concentrated under reduced pressure. The resulting white solid was co-evaporated with anhydrous toluene and dried under high vacuum for 30 min. Then anhydrous toluene (50 mL) was added under nitrogen, followed by Bu_2SnO (1.30 g, 5.21 mmol) and 4 Å molecular sieves. The reaction was stirred for 1 h under reflux. Then the reaction was cooled down

to room temperature, NapBr (1.53g, 6.94 mmol) and TBAB (1.68g 5.21 mmol) were added and left stir for 3 h at 60 °C. The reaction was filtered and the solvent was removed under reduced pressure, residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 5/1) to give product **2-28** as slightly yellow syrup (1.33 g, 82% over three steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.82 (m, 4H), 7.61 – 7.48 (m, 5H), 7.32 – 7.26 (m, 3H), 5.90 (d, *J* = 5.3 Hz, 1H), 4.98 – 4.83 (m, 2H), 4.30 (dd, *J* = 6.6, 1.3 Hz, 1H), 4.21 (dd, *J* = 10.2, 5.3 Hz, 1H), 3.95 – 3.87 (m, 1H), 3.76 (dd, *J* = 10.2, 3.1 Hz, 1H), 2.38 (s, 1H), 1.26 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.55, 134.50, 133.32, 129.25, 128.80, 128.60, 128.12, 127.95, 127.91, 127.18, 126.54, 126.46, 125.82, 85.29, 79.35, 72.41, 68.68, 60.37, 16.20; HRMS (ESI-TOF) m/z Calcd for $C_{23}H_{23}N_3O_3Se$ $[M+Na]^+$: 492.0797, found: 492.0801.

Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-benzyl-1-seleno-*α*-D-fucopyranoside (2-29)

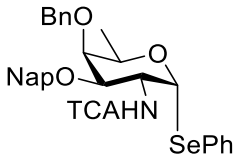


To a solution of **2-28** (70 mg, 0.15 mmol) in anhydrous DMF (2 mL) at 0 °C, NaH (as a 60% mineral oil dispersion, 12 mg, 0.30 mmol) was added and the resulting solution was stirred for 10 min, then BnBr (35.4 μL, 0.30 mmol) was added, the mixture was warmed to room temperature and stirred for 2 h. Excess NaH was quenched using methanol, and the bulk of the DMF removed under reduced pressure. The syrup was redissolved in DCM (50 mL) and washed twice with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash silica column chromatography (hexane/ethyl acetate = 10/1) to afford **2-29** as a white solid (70 mg, 83%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.91 – 7.82 (m, 4H), 7.60 – 7.53 (m, 3H), 7.52 – 7.47 (m, 2H), 7.35 – 7.20 (m, 8H), 5.95 (d, *J* = 5.3 Hz, 1H), 4.99 – 4.87 (m, 3H), 4.65 (d, *J* = 11.5 Hz, 1H), 4.40 (dd, *J* = 10.3, 5.3 Hz, 1H), 4.22 (q, *J* = 6.5 Hz, 1H), 3.79 (dd, *J* = 10.4, 2.7 Hz, 1H), 3.73 (d, *J* = 2.7 Hz, 1H), 1.13 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 138.2, 135.0, 134.48, 133.41, 133.2,

129.1, 128.8, 128.5, 128.4, 128.2, 128.1, 127.87, 127.86, 127.76, 126.72, 126.3, 126.2, 125.7, 85.6, 80.7, 76.0, 75.1, 72.7, 69.5, 61.1, 16.6.; HRMS (ESI) calculated for $C_{30}H_{29}N_3O_3Se$ $[M+Na]^+$: 582.1266, found: 582.1279.

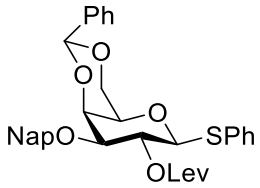
Phenyl 2-trichloroacetamido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-benzyl-1-seleno-*α*-D-fucopyranoside (2-17)



Zinc dust (1.36 g, 20.85 mmol) and AcOH (0.97 mL) were added to a stirred solution of **2-29** (1.06 g, 1.90 mmol) in 20 mL of THF, and the solution was stirred at room temperature for 4 h. After complete conversion of the starting material, the reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue obtained after solvent removal was dissolved in THF (20 mL) and 4Å MS (3.0 g) were added and trichloroacetyl chloride (423 µL, 3.80 mmol) was added slowly at 0 °C. After stirring the reaction mixture at 0 °C for 4 h, it was diluted with DCM (20 mL) and washed with brine. The separated organic layer was dried over Na_2SO_4, concentrated and purified by flash silica column chromatography (hexane/ethyl acetate = 8/1) to afford **2-17** as a white solid (1.02 g, 79 %).

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.90 – 7.79 (m, 4H), 7.55 – 7.46 (m, 5H), 7.39 (d, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.31 – 7.22 (m, 4H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.06 (d, *J* = 4.8 Hz, 1H), 5.02 (d, *J* = 11.5 Hz, 1H), 4.90 (d, *J* = 12.0 Hz, 1H), 4.83 – 4.77 (m, 1H), 4.72 (d, *J* = 11.8 Hz, 2H), 4.23 (q, *J* = 6.4 Hz, 1H), 3.88 – 3.85 (m, 1H), 3.66 (dd, *J* = 11.0, 2.5 Hz, 1H), 1.29 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 161.6, 138.1, 134.6, 134.2, 133.4, 133.2, 129.4, 128.9, 128.8, 128.4, 128.3, 128.09, 128.05, 127.95, 127.92, 126.7, 126.5, 126.4, 125.5, 92.6, 89.2, 78.8, 77.3, 74.9, 74.6, 71.7, 70.6, 52.1, 16.9; HRMS (ESI) calculated for $C_{32}H_{30}Cl_3NO_4Se$ $[M+Na]^+$: 700.0298, found: 700.0312.

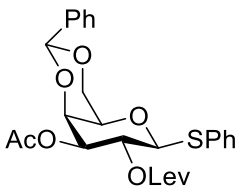
Phenyl 4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-(2-naphthylmethyl)-1-thio-*β*-D-galactopyranoside (2-23)



To a solution of **2-30**[128,129] (500.0 mg, 1 mmol) in DCM (30.0 mL), levulinic acid (232.2, 2 mmol), 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (383.4 mg, 2 mmol) and DMAP (12.2 mg, 0.1 mmol) were added at room temperature. The reaction mixture was stirred until TLC showed complete conversion of the starting material (2 h). The mixture was diluted with DCM (50 mL) and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 3/1) to give **2-23** as a white solid (544.2 mg, 91%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.64 (m, 4H), 7.53 – 7.46 (m, 2H), 7.42 – 7.35 (m, 3H), 7.34 – 7.28 (m, 2H), 7.28 – 7.22 (m, 3H), 7.20 – 7.10 (m, 3H), 5.25 (t, *J* = 9.7 Hz, 1H), 4.70 (d, *J* = 2.0 Hz, 2H), 4.51 (d, *J* = 9.8 Hz, 1H), 4.22 (dd, *J* = 12.3, 1.7 Hz, 1H), 4.07 (dd, *J* = 3.4, 1.0 Hz, 1H), 3.85 (dd, *J* = 12.3, 1.7 Hz, 1H), 3.55 (dd, *J* = 9.6, 3.4 Hz, 1H), 3.30 (q, *J* = 1.4 Hz, 1H), 2.67 – 2.60 (m, 2H), 2.55 – 2.48 (m, 2H), 2.05 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.5, 171.2, 137.6, 135.6, 133.5, 133.2, 133.1, 131.7, 129.1, 128.8, 128.33, 128.26, 128.23, 128.0, 127.9, 127.8, 126.7, 126.6, 126.3, 126.1, 125.8, 101.3, 85.4, 78.4, 73.4, 71.6, 70.0, 69.3, 68.7, 38.0, 30.0, 28.2; HRMS (ESI) calculated for $C_{35}H_{34}O_7S$ $[M+Na]^+$: 621.1917, found: 621.1910.

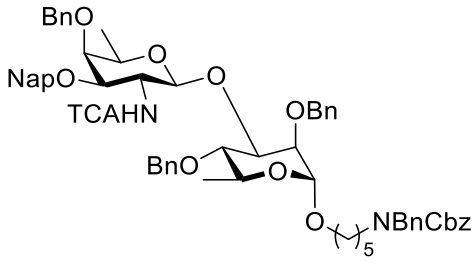
Phenyl 4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-acetyl-1-thio-*β*-D-galactopyranoside (2-18)



To a solution of **2-31**[151] (33 mg, 0.072 mmol) in 4:1 (v/v) anhydrous DCM-pyridine (1mL), Ac_2O (68 µL, 0.72 mmol) was added dropwise at 0 °C under a nitrogen atmosphere. After addition of DMAP (cat.), the solution was stirred at room temperature. After complete conversion of the starting material, the mixture was diluted with DCM (10 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL) respectively. The organic layer was dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by flash silica column chromatography (hexane/ethyl acetate = 4/1) to give product **2-18** as a slightly yellow syrup (30.5 mg, 84%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.49 (m, 2H), 7.34 – 7.14 (m, 8H), 5.40 (s, 1H), 5.28 (t, *J* = 9.9 Hz, 1H), 4.95 (dd, *J* = 9.9, 3.4 Hz, 1H), 4.64 (d, *J* = 9.8 Hz, 1H), 4.34 – 4.26 (m, 2H), 3.95 (dd, *J* = 12.4, 1.7 Hz, 1H), 3.53 (q, *J* = 1.5 Hz, 1H), 2.83 – 2.70 (m, 1H), 2.67 – 2.42 (m, 3H), 2.10 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2, 171.1, 171.0, 137.5, 133.8, 131.2, 129.2, 128.8, 128.2, 126.6, 101.1, 85.1, 73.6, 73.0, 69.8, 69.1, 66.8, 37.8, 29.9, 28.0, 21.0; HRMS (ESI) calculated for $C_{26}H_{28}O_8S$ $[M+Na]^+$: 523.1397, found: 523.1400.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-trichloroacetamido-2-deoxy-3-_O_-(2-naphthylmethyl)-4-_O_-benzyl-_β_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-33)

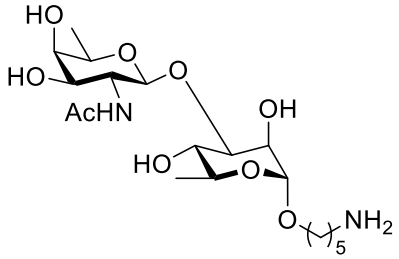


L-Rhamnose **2-16** (466 mg, 0.89 mmol) and D-fucose alcohol **2-17** (696 mg, 1.07 mmol) were coevaporated three times with toluene and the resulting mixture was dried under vacuum for 2 h. The mixture was dissolved in anhydrous DCM (10 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to −78 °C, NIS (240 mg, 1.07 mmol) and TfOH (9.42 µL, 0.11 mmol) were added. The reaction mixture was stirred at −78 °C

for 3 h. After complete consumption of staring material, Et_3N (0.1 mL) was added and the mixture was stirred for 10 min. Then the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 12/1) to give **2-33** as a colorless syrup (750 mg, 80%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.89 – 7.79 (m, 4H), 7.55 – 7.45 (m, 3H), 7.42 – 7.35 (m, 6H), 7.35 – 7.26 (m, 14H), 7.25 – 7.17 (m, 5H), 6.95 (s, 1H), 5.20 (d, *J* = 18.6 Hz, 2H), 5.11 (d, *J* = 8.2 Hz, 1H), 5.04 (d, *J* = 11.4 Hz, 1H), 4.88 – 4.81 (m, 3H), 4.75 (t, *J* = 10.9 Hz, 2H), 4.71 (d, *J* = 11.5 Hz, 1H), 4.58 (d, *J* = 11.5 Hz, 2H), 4.51 (d, *J* = 17.2 Hz, 2H), 4.30 (s, 1H), 4.17 (d, *J* = 8.7 Hz, 1H), 4.06 (dt, *J* = 10.9, 7.8 Hz, 1H), 3.86 (s, 1H), 3.73 (d, *J* = 2.7 Hz, 1H), 3.67 – 3.48 (m, 4H), 3.34 – 3.15 (m, 3H), 1.58 – 1.40 (m, 4H), 1.30 – 1.22 (m, 5H), 1.21 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 162.0, 156.8, 156.2, 138.8, 138.6, 138.05, 138.00, 137.0, 136.9, 135.2, 133.3, 133.1, 128.64, 128.57, 128.53, 128.4, 128.39, 128.30, 128.2, 128.1, 128.0, 127.9, 127.8, 127.76, 127.70, 127.5, 127.4, 127.35, 127.30, 126.8, 126.3, 126.1, 125.9, 99.5, 99.1, 92.7, 81.2, 77.8, 77.7, 75.4, 74.9, 74.5, 73.7, 72.3, 70.7, 67.8, 67.2, 56.0, 50.6, 50.3, 47.2, 46.2, 29.28, 29.23, 29.21, 27.9, 27.6, 23.49, 23.44, 18.3, 17.2; HRMS (ESI) calculated for $C_{66}H_{71}Cl_3N_2O_{11}$ $[M+Na]^+$: 1195.4015, found: 1195.4106.

5-Aminopentyl 2-acetamido-2-deoxy-*β*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside (2-8)

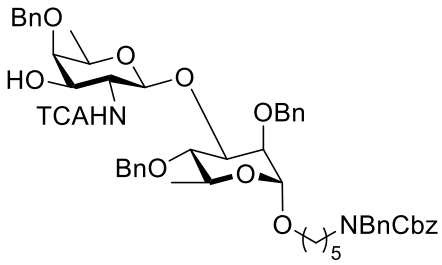


In a 20 mL vial, compound **2-33** (13 mg, 11.09 μmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 40 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of starting

material, the reaction mixture was filtered and the crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-8** (2.2 mg, 46%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 8.47 (s, 1H), 4.80 (d, *J* = 1.6 Hz, 1H), 4.61 (d, *J* = 8.4 Hz, 1H), 4.15 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.88 (dd, *J* = 10.4, 8.4 Hz, 1H), 3.80 – 3.65 (m, 6H), 3.56 (dt, *J* = 10.1, 6.2 Hz, 1H), 3.50 (t, *J* = 9.6 Hz, 1H), 3.05 – 3.00 (m, 2H), 2.05 (s, 3H), 1.75 – 1.61 (m, 4H), 1.52 – 1.40 (m, 2H), 1.32 – 1.25 (m, 6H); ^{13}C NMR (151 MHz, D_2O) δ 175.0, 170.9, 102.9, 99.1, 79.7, 70.98, 70.90, 70.5, 70.3, 69.9, 68.7, 67.4, 52.3, 39.2, 27.8, 26.4, 22.2, 22.1, 16.4, 15.5; HRMS (ESI) calculated for $C_{19}H_{36}N_2O_9$ $[M+H]^+$: 437.2494, found: 437.2492.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-trichloroacetamido-2-deoxy-4-_O_-benzyl-_β_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-34)

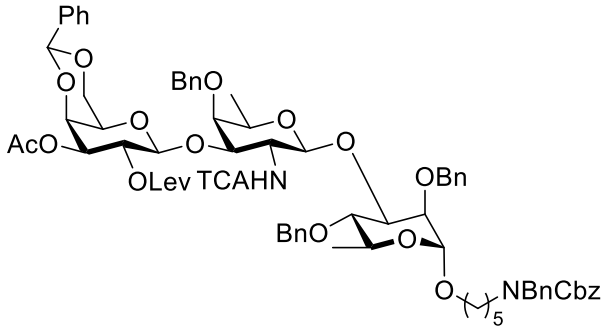


To a solution of **2-33** (335 mg, 0.29 mmol) in 20:1 (v/v) DCM-H_2O (10 mL), DDQ (97.3 mg, 0.43 mmol) and *β*-pinene (152 µL, 0.97 mmol) were added and the reaction mixture was stirred at room temperature for 5 h. The reaction was then diluted with DCM (10 mL) and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. Crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 4/1) to give **2-34** as a colorless syrup (180 mg, 61%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.45 – 7.14 (m, 25H), 6.80 (d, *J* = 19.2 Hz, 1H), 5.18 (d, *J* = 23.6 Hz, 2H), 4.88 – 4.77 (m, 5H), 4.73 (d, *J* = 12.1 Hz, 1H), 4.67 – 4.56 (m, 2H), 4.55 – 4.44 (m, 2H), 4.12 (dd, *J* = 8.6, 3.1 Hz, 1H), 3.99 – 3.91 (m, 1H), 3.88 – 3.75 (m, 2H), 3.68 – 3.48 (m, 5H), 3.32 – 3.13 (m, 3H), 2.61 (t, *J* = 9.6 Hz, 1H), 1.57 – 1.40 (m, 4H), 1.32 – 1.24 (m, 5H), 1.21 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 163.0, 156.8, 156.3, 138.9, 138.4, 138.1, 138.0, 137.0,

136.8, 128.7, 128.67, 128.60, 128.58, 128.51, 128.4, 128.3, 128.29, 128.25, 128.21, 128.14, 128.11, 128.0, 127.98, 127.93, 127.89, 127.85, 127.6, 127.5, 127.46, 127.40, 127.3, 100.6, 98.9, 92.5, 81.1, 79.2, 77.9, 76.2, 74.5, 74.4, 73.6, 72.4, 71.0, 67.96, 67.90, 67.28, 67.20, 57.3, 50.6, 50.3, 47.2, 46.1, 29.26, 29.20, 27.9, 27.6, 23.5, 23.4, 18.2, 17.0; HRMS (ESI) calculated for $C_{55}H_{63}Cl_3N_2O_{11}$ $[M+Na]^+$: 1055.3389, found: 1055.3453.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-_O_-benzylidene-2-_O_-levulinoyl-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-_O_-benzyl-_β_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-35)

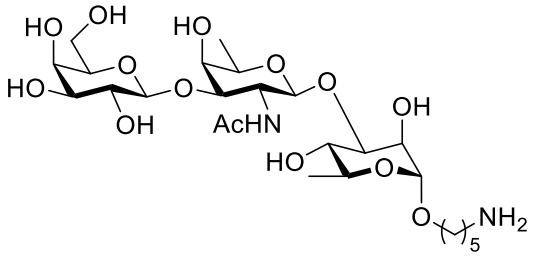


Disaccharide alcohol **2-34** (350 mg, 0.34 mmol) and thioglycoside **2-18** (203 mg, 0.41 mmol) were coevaporated three times with toluene and the resulting mixture was dried under vacuum for 2 h. The mixture was dissolved in anhydrous DCM (10 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to −40 °C, NIS (91.4 mg, 0.41 mmol) and TfOH (3.6 µL, 0.04 mmol) were added. The reaction mixture was stirred at −40 °C for 3 h. After complete consumption of the starting material, Et_3N (0.1 mL) was added and the mixture was stirred for 10 min. Then the reaction was then diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 12/1) to give **2-35** as a colorless syrup (399 mg, 83%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 8.74 – 8.62 (m, 1H), 7.49 – 7.27 (m, 20H), 7.26 – 7.14 (m, 10H), 5.56 – 5.49 (m, 2H), 5.43 (d, *J* = 8.2 Hz, 1H), 5.21 – 5.13 (m, 3H), 4.94 – 4.83 (m, 3H), 4.80 – 4.69 (m, 4H), 4.58 (d, *J* = 11.7 Hz, 1H), 4.48 (d, *J* = 13.8 Hz, 3H), 4.41 (d, *J* = 3.6 Hz, 1H), 4.32

(d, *J* = 12.3 Hz, 1H), 4.14 – 4.07 (m, 2H), 3.87 – 3.77 (m, 3H), 3.66 – 3.41 (m, 5H), 3.29 – 3.09 (m, 3H), 3.03 (dd, *J* = 18.6, 11.6 Hz, 1H), 2.84 – 2.75 (m, 1H), 2.50 (ddd, *J* = 18.5, 5.7, 2.9 Hz, 1H), 2.32 (ddd, *J* = 17.2, 5.7, 3.1 Hz, 1H), 2.04 (s, 3H), 2.04 (s, 3H), 1.55 – 1.36 (m, 4H), 1.26 – 1.11 (m, 5H), 1.09 (d, *J* = 4.7 Hz, 2H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 208.1, 171.2, 170.8, 162.3, 156.8, 156.2, 139.2, 139.1, 138.8, 138.0, 137.6, 137.0, 136.9, 129.2, 129.1, 128.9, 128.67, 128.65, 128.5, 128.4, 128.3, 128.2, 128.1, 128.08, 128.02, 127.9, 127.8, 127.5, 127.4, 127.38, 127.32, 126.4, 126.3, 101.5, 101.3, 98.9, 98.4, 92.8, 79.9, 79.2, 78.2, 77.7, 75.4, 75.0, 74.7, 73.4, 73.3, 71.5, 70.3, 69.05, 69.03, 67.8, 67.2, 67.1, 66.3, 57.2, 50.6, 50.3, 47.2, 46.2, 37.8, 30.0, 29.2, 28.0, 27.7, 27.6, 23.46, 23.42, 21.0, 18.1, 16.9; HRMS (ESI) calculated for $C_{75}H_{85}Cl_3N_2O_{19}$ $[M+Na]^+$: 1445.4704, found: 1445.4788.

5-Aminopentyl *β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*β*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside (2-5)

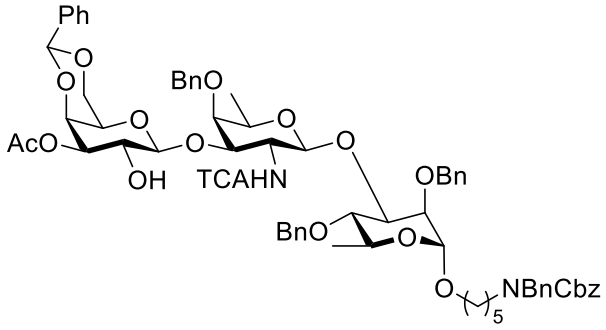


NaOMe was added slowly added to a solution of **2-35** (27 mg, 18.98 µmol) in MeOH/DCM = 1/1 (1 mL) until the solution reached pH 11. The reaction mixture was stirred for 1 h, neutralized with Amberlite, filtered and concentrated under reduced pressure. The residue was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and the crude product was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-5** (5.8 mg, 52%) as a white solid.

^{1}H NMR (700 MHz, D_2O) δ 8.46 (s, 1H), 4.67 (d, *J* = 8.5 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.16 – 4.12 (m, 1H), 4.02 – 3.97 (m, 2H), 3.93 – 3.87 (m, 2H), 3.81 – 3.60 (m, 8H), 3.58 – 3.47 (m,

3H), 3.02 (t, *J* = 7.6 Hz, 2H), 2.03 (s, 3H), 1.75 – 1.62 (m, 4H), 1.52 – 1.39 (m, 2H), 1.31 – 1.25 (m, 6H); ^{13}C NMR (176 MHz, D_2O) δ 175.0, 171.0, 104.8, 102.6, 99.2, 79.8, 79.7, 74.9, 72.4, 71.0, 70.6, 70.6, 70.3, 69.9, 68.7, 68.5, 67.4, 60.9, 51.3, 39.3, 27.9, 26.5, 22.3, 22.2, 16.5, 15.5; HRMS (ESI) calculated for $C_{25}H_{46}N_2O_{14}$ $[M+H]^+$: 599.3022, found: 599.3021.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-_O_-benzylidene-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-_O_-benzyl-_β_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-36)

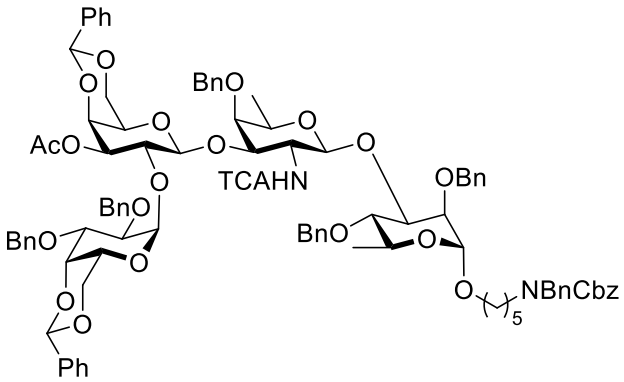


To a solution of **2-35** (20 mg, 0.014 mmol) in DCM (1 mL), N_2H_4·AcOH (12.9 mg, 0.14 mmol) was added and the mixture was stirred at room temperature for 12 h. The reaction was quenched with the addition of acetone (0.5 mL) and the solvent was removed under vacuum. The residue was purified by flash silica column chromatography (toluene/ethyl acetate = 4/1) to give **2-36** as a colorless syrup (17 mg, 91%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.49 – 7.14 (m, 30 H), 7.07 (s, 1H), 5.51 (s, 1H), 5.24 – 5.13 (m, 3H), 5.04 (d, *J* = 8.3 Hz, 1H), 4.86 – 4.68 (m, 5H), 4.66 – 4.56 (m, 2H), 4.54 – 4.38 (m, 5H), 4.32 (d, *J* = 12.4 Hz, 1H), 4.23 (s, 1H), 4.09 (d, *J* = 12.4 Hz, 1H), 4.02 (t, *J* = 9.0 Hz, 1H), 3.94 (q, *J* = 8.9 Hz, 1H), 3.89 (s, 1H), 3.81 (s, 1H), 3.68 – 3.48 (m, 5H), 3.32 – 3.13 (m, 3H), 2.13 (s, 3H), 1.57 – 1.39 (m, 4H), 1.32 – 1.24 (m, 2H), 1.23 – 1.15 (m, 6H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 171.1, 162.4, 156.8, 156.3, 138.9, 138.5, 138.0, 137.8, 137.0, 136.9, 129.2, 129.1, 128.67, 128.60, 128.56, 128.54, 128.4, 128.38, 128.35, 128.2, 128.0, 127.9, 127.8, 127.5, 127.4, 127.3, 126.4, 125.4, 104.3, 101.3, 99.2, 98.8, 92.5, 78.5, 78.2, 78.0, 75.1, 74.0, 73.6, 73.1, 70.7, 69.1, 68.9, 67.8, 67.4, 67.3,

67.2, 66.4, 50.6, 50.3, 47.2, 46.2, 29.8, 29.3, 28.0, 27.6, 23.5, 23.4, 21.5, 21.2, 18.5, 16.9, 14.2; HRMS (ESI) calculated for $C_{70}H_{79}Cl_3N_2O_{17}$ $[M+Na]^+$: 1347.4336, found: 1347.4397.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-_O_-benzyl-4,6-_O_-benzylidene-_α_-D-galactopyranosyl-(1→2)-4,6-_O_-benzylidene-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-_O_-benzyl-_β_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-37)

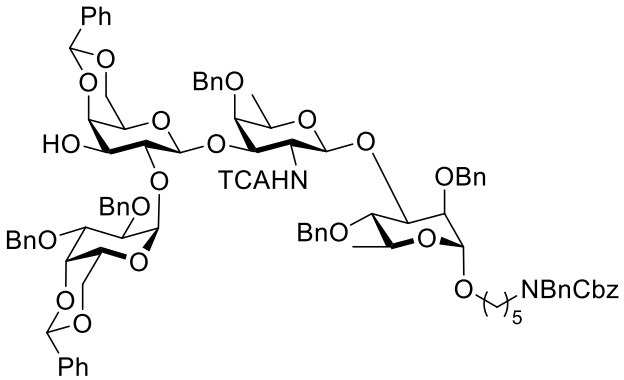


Trisaccharide alcohol **2-36** (200 mg, 0.15 mmol) and thioglycoside **2-19** (122 mg, 0.23 mmol) were coevaporated three times with toluene and the resulting mixture was dried under vacuum for 2 h. Then the mixture was dissolved in anhydrous Et_2O/DCM=4/1 (10 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to 0 °C, NIS (51 mg, 0.23 mmol) and TfOH (2.0 μL, 0.023 mmol) were added. The reaction mixture was stirred at 0 °C for 4 h. After complete consumption of the staring material, Et_3N (0.1 mL) was added and the mixture was stirred for 10 min. Then the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 7/1) to give **2-37** as a colorless syrup (218 mg, 82%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 8.99 (d, *J* = 6.0 Hz, 1H), 7.52 (d, *J* = 7.0 Hz, 2H), 7.50 – 7.46 (m, 2H), 7.43 (d, *J* = 7.3 Hz, 2H), 7.39 – 7.21 (m, 32H), 7.20 – 7.14 (m, 4H), 7.09 (dt, *J* = 26.6, 7.4 Hz, 3H), 5.71 (d, *J* = 8.1 Hz, 1H), 5.50 (s, 1H), 5.40 (s, 1H), 5.22 – 5.15 (m, 3H), 5.12 (d, *J* = 11.8 Hz, 1H), 5.00 (s, 2H), 4.92 (d, *J* = 11.8 Hz, 2H), 4.76 (d, *J* = 8.0 Hz, 1H), 4.73 – 4.67 (m, 2H),

4.64 (dd, *J* = 10.6, 3.5 Hz, 1H), 4.60 (d, *J* = 11.7 Hz, 1H), 4.56 – 4.43 (m, 5H), 4.40 – 4.33 (m, 2H), 4.31 (d, *J* = 12.4 Hz, 1H), 4.24 – 4.19 (m, 2H), 4.19 – 4.11 (m, 3H), 4.08 (dd, *J* = 9.1, 2.9 Hz, 1H), 4.05 (d, *J* = 12.3 Hz, 1H), 3.99 (dt, *J* = 10.2, 6.8 Hz, 1H), 3.92 (d, *J* = 12.2 Hz, 1H), 3.86 (s, 1H), 3.82 – 3.74 (m, 2H), 3.65 (dt, *J* = 13.3, 6.2 Hz, 1H), 3.55 (q, *J* = 7.1, 6.6 Hz, 1H), 3.51 – 3.39 (m, 3H), 3.28 – 3.05 (m, 3H), 1.99 (s, 3H), 1.54 – 1.33 (m, 4H), 1.24 – 1.10 (m, 5H), 1.01 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 162.3, 156.8, 156.2, 139.19, 139.16, 138.9, 138.7, 138.47, 138.45, 138.1, 138.0, 137.9, 137.0, 136.2, 129.4, 129.3, 129.2, 129.17, 129.10, 129.0, 128.9, 128.8, 128.66, 128.65, 128.56, 128.53, 128.46, 128.42, 128.38, 128.34, 128.32, 128.29, 128.22, 128.1, 128.0, 127.99, 127.96, 127.93, 127.91, 127.84, 127.81, 127.79, 127.76, 127.71, 127.6, 127.53, 127.50, 127.4, 127.36, 127.31, 127.29, 127.21, 126.54, 126.51, 126.46, 126.43, 126.40, 103.7, 103.5, 101.7, 101.2, 101.1, 100.8, 100.1, 99.0, 98.9, 93.1, 80.2, 79.58, 79.55, 78.0, 77.5, 76.3, 76.2, 75.4, 75.29, 75.26, 74.9, 74.8, 74.6, 73.8, 73.6, 73.5, 73.2, 73.1, 73.0, 72.1, 71.8, 71.0, 70.8, 70.1, 69.5, 69.4, 68.8, 67.7, 67.2, 67.0, 66.7, 66.2, 63.6, 62.9, 58.5, 50.6, 50.3, 47.2, 46.2, 29.8, 29.1, 27.9, 27.5, 23.4, 21.2, 18.1, 16.9; HRMS (ESI) calculated for $C_{97}H_{105}Cl_3N_2O_{22}$ $[M+Na]^+$: 1777.6117, found: 1777.6249.

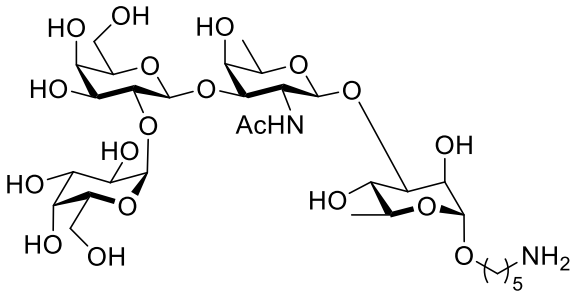
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-_O_-benzyl-4,6-_O_-benzylidene-_α_-D-galactopyranosyl-(1→2)-4,6-_O_-benzylidene-_β_-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-_O_-benzyl-_β_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-12)



Sodium methoxide was slowly added to a solution of **2-37** (20 mg, 0.011 mmol) in MeOH/DCM=1/1 (1 mL) until the solution reached pH 11. The reaction mixture was stirred for 1 h, neutralized with Amberlite, filtered and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (toluene/ethyl acetate = 4/1) to give **2-12** as a colorless syrup (18 mg, 92%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 9.03 (s, 1H), 7.57 (d, *J* = 7.3 Hz, 2H), 7.47 (d, *J* = 7.5 Hz, 2H), 7.43 (d, *J* = 7.4 Hz, 2H), 7.39 – 7.22 (m, 32H), 7.21 – 7.15 (m, 4H), 7.10 – 7.02 (m, 3H), 5.71 (d, *J* = 8.1 Hz, 1H), 5.57 (s, 1H), 5.39 (s, 1H), 5.24 – 5.14 (m, 3H), 5.08 (d, *J* = 3.1 Hz, 1H), 5.06 – 4.98 (m, 2H), 4.93 (dd, *J* = 22.3, 11.8 Hz, 2H), 4.72 (d, *J* = 11.6 Hz, 1H), 4.68 (d, *J* = 7.9 Hz, 1H), 4.62 (dd, *J* = 10.5, 3.6 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 4.54 – 4.44 (m, 4H), 4.36 (d, *J* = 7.1 Hz, 1H), 4.32 (dd, *J* = 17.3, 12.5 Hz, 2H), 4.27 (d, *J* = 2.9 Hz, 1H), 4.24 (s, 1H), 4.24 – 4.16 (m, 3H), 4.11 – 4.01 (m, 3H), 4.00 – 3.93 (m, 1H), 3.93 (d, *J* = 12.4 Hz, 1H), 3.85 (s, 1H), 3.79 (d, *J* = 3.6 Hz, 1H), 3.73 (t, *J* = 8.8 Hz, 1H), 3.66 (q, *J* = 6.5 Hz, 1H), 3.60 – 3.51 (m, 2H), 3.50 – 3.36 (m, 3H), 3.28 – 3.04 (m, 3H), 2.51 (d, *J* = 11.0 Hz, 1H), 1.55 – 1.32 (m, 4H), 1.24 – 1.09 (m, 5H), 0.99 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 162.3, 156.8, 156.2, 139.1, 138.7, 138.6, 138.5, 138.09, 138.05, 137.9, 137.6, 137.0, 136.5, 129.6, 129.2, 129.1, 128.9, 128.7, 128.64, 128.61, 128.58, 128.53, 128.50, 128.4, 128.37, 128.35, 128.27, 128.25, 128.14, 128.12, 128.0, 127.9, 127.8, 127.74, 127.70, 127.5, 127.2, 127.1, 126.5, 126.4, 125.4, 103.3, 102.0, 101.8, 100.8, 99.1, 98.9, 93.2, 80.4, 79.3, 76.7, 76.0, 75.1, 74.8, 73.3, 73.1, 72.9, 71.9, 71.5, 70.5, 70.1, 69.5, 69.0, 67.7, 67.27, 67.25, 67.0, 66.4, 63.0, 58.7, 50.6, 50.3, 47.2, 46.2, 29.1, 27.9, 27.5, 23.3, 21.5, 18.0, 16.9; HRMS (ESI) calculated for $C_{95}H_{103}Cl_3N_2O_{21}$ $[M+Na]^+$: 1735.6011, found: 1735.6144.

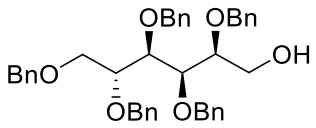
5-Aminopentyl *α*-D-galactopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*β*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside (2-2)



In a 20 mL vial, compound **2-12** (20 mg, 11.68 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 40 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and the crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-2** (5.1 mg, 58%) as a white solid.

^{1}H NMR (700 MHz, D_2O) δ 8.46 (s, 1H), 5.38 (d, *J* = 3.9 Hz, 1H), 4.68 – 4.64 (m, 2H), 4.24 (t, *J* = 6.3 Hz, 1H), 4.16 – 4.13 (m, 1H), 4.04 (s, 1H), 4.02 – 3.98 (m, 3H), 3.95 – 3.90 (m, 2H), 3.82 – 3.65 (m, 12H), 3.55 (dt, *J* = 10.0, 6.2 Hz, 1H), 3.50 (s, 1H), 3.01 (t, *J* = 7.7 Hz, 2H), 2.06 (s, 3H), 1.74 – 1.62 (m, 4H), 1.52 – 1.39 (m, 2H), 1.31 – 1.25 (m, 6H); ^{13}C NMR (176 MHz, D_2O) δ 175.0, 171.0, 103.6, 102.9, 99.1, 96.6, 79.8, 78.8, 74.9, 73.6, 71.5, 71.0, 70.7, 70.3, 70.0, 69.9, 69.4, 69.1, 68.79, 68.78, 68.4, 67.4, 60.9, 60.8, 51.2, 39.3, 27.9, 26.5, 22.5, 22.3, 16.5, 15.6; HRMS (ESI) calculated for $C_{31}H_{56}N_2O_{19}$ $[M+H]^+$: 761.3550, found: 761.3561.

2,3,4,5,6-Penta-*O*-benzyl-D-glucitol (2-41)

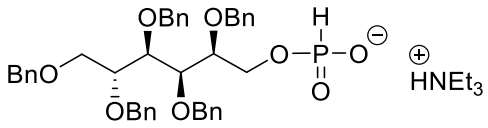


To a solution of **2-40**[137,152,153] (200 mg, 0.317 mmol) in MeOH (6 mL), $NaBH_4$ (24 mg, 0.635 mmol) was added and the mixture was stirred at 40 °C overnight. After the starting material was

consumed, it was quenched with aq. 1N HCl (10 mL) and stirred for 10 min. Then diluted with DCM (20 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. Crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 4/1) to give **2-41** as a colorless syrup (194 mg, 97%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.26 (m, 25H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.72 – 4.62 (m, 4H), 4.61 (s, 2H), 4.57 – 4.43 (m, 3H), 3.99 (t, *J* = 4.7 Hz, 1H), 3.94 – 3.83 (m, 3H), 3.78 – 3.65 (m, 3H), 3.57 – 3.47 (m, 1H), 1.90 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.4, 138.37, 138.34, 128.55, 128.53, 128.49, 128.43, 128.42, 128.40, 128.3, 128.2, 128.0, 127.8, 127.8, 127.77, 127.73, 127.6, 127.5, 79.4, 79.3, 79.0, 78.6, 74.8, 73.9, 73.48, 73.44, 72.8, 72.0, 69.6, 61.8; HRMS (ESI) calculated for $C_{41}H_{44}O_6$ $[M+Na]^+$: 655.3030, found: 655.3029.

Triethylammonium 2,3,4,5,6-penta-*O*-benzyl-D-glucityl *H*-phosphonate (2-13)

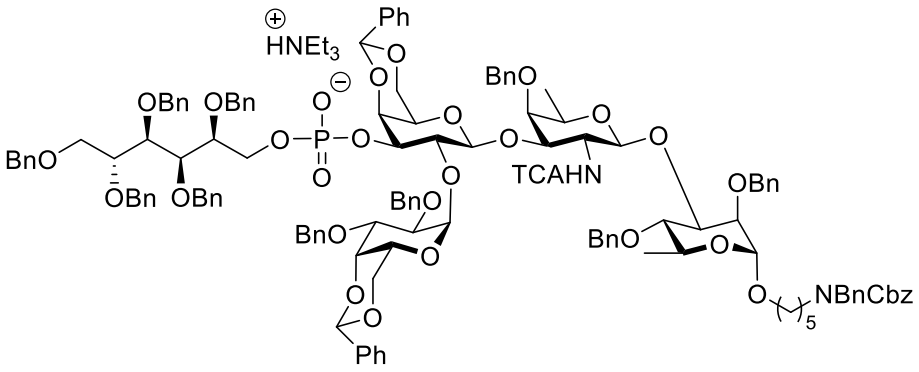


To a solution of **2-41** (70 mg, 0.110 mmol) in anhydrous pyridine (1.5mL), diphenyl phosphite (105 μL) was added. The mixture was stirred at room temperature for 2 h. TEAB buffer (2 mL) was added and reaction mixture was stirred for additional 2 h. The reaction was diluted with DCM (20 mL) and washed with TEAB buffer. The organic layer was dried over Na_2SO_4, concentrated and purified by flash column chromatography on silica gel which was neutralized with triethylamine (ethyl acetate/MeOH/H_2O=20/2/1) to afford the *H*-phosphonate **2-13** (60 mg, 68%) as a light yellow syrup[140].

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.64 (s, 1H), 7.41 – 7.21 (m, 25H), 4.87 – 4.43 (m, 10H), 4.23 (ddd, *J* = 11.4, 7.5, 4.1 Hz, 1H), 4.16 – 4.05 (m, 2H), 3.97 (q, *J* = 4.9 Hz, 1H), 3.94 – 3.85 (m, 3H), 3.77 (dd, *J* = 11.1, 6.4 Hz, 1H), 2.93 (q, *J* = 7.3, 2.9 Hz, 6H), 1.23 (t, *J* = 7.3 Hz, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.2, 138.8, 138.76, 138.74, 138.6, 138.4, 128.3, 128.23, 128.22, 128.20, 128.0, 127.9, 127.8, 127.6, 127.5, 127.49, 127.40, 127.38, 127.32, 118.6, 115.7, 79.3, 79.2, 79.1,

77.4, 77.3, 77.1, 76.8, 74.8, 74.0, 73.2, 72.7, 71.8, 70.0, 63.3, 63.2, 45.2, 8.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 4.76; HRMS (ESI) calculated for $C_{47}H_{60}NO_8P$ [M-Et_3N-H]$^-$: 695.2779, found: 695.2764.

Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*-D-galactopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-(2,3,4,5,6-penta-*O*-benzyl-D-glucityl phospho)-*β*-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-*O*-benzyl-*β*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-9)

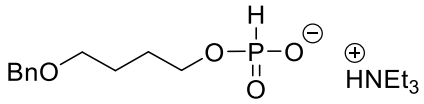


Tetrasaccharide alcohol **2-12** (30 mg, 17.5 μmol) and *H*-phosphonate **2-13** (27.9 mg, 35.0 μmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (10.7 μL, 87.6 μmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine (22.2 mg, 87.6 μmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with DCM, washed with saturated $Na_2S_2O_3$ solution and TEAB buffer, and then dried over Na_2SO_4. The crude product obtained after solvent removal under reduced pressure was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 80/2/1) to give **2-9** as a triethylammonium salt[141] (34 mg, 77%).

^{1}H NMR (700 MHz, Acetone-d_6) δ 12.40 (s, 1H), 9.17 (d, *J* = 6.2 Hz, 1H), 7.69 – 7.02 (m, 70H), 5.70 (d, *J* = 8.1 Hz, 1H), 5.57 (s, 1H), 5.36 (s, 1H), 5.23 (d, *J* = 11.6 Hz, 1H), 5.16 (q, *J* = 8.6, 7.9 Hz, 3H), 5.10 – 5.03 (m, 2H), 4.95 (dd, *J* = 24.1, 11.9 Hz, 2H), 4.88 (d, *J* = 11.0 Hz, 1H), 4.80 – 4.74 (m, 3H), 4.72 (dd, *J* = 11.6, 3.7 Hz, 2H), 4.68 (d, *J* = 6.3 Hz, 3H), 4.65 (d, *J* = 11.0 Hz, 1H), 4.57 (td, *J* = 12.8, 12.2, 4.6 Hz, 5H), 4.54 – 4.47 (m, 6H), 4.41 (dt, *J* = 9.7, 4.4 Hz, 3H), 4.28 (d, *J*

= 11.7 Hz, 2H), 4.15 – 3.96 (m, 14H), 3.89 – 3.77 (m, 4 H), 3.70 (s, 1H), 3.54 – 3.47 (m, 1H), 3.43 (t, *J* = 9.1 Hz, 1H), 3.22 (d, *J* = 20.5 Hz, 4H), 2.97 (q, *J* = 7.3 Hz, 8 H), 1.56 – 1.35 (m, 4H), 1.32 – 1.24 (m, 2H), 1.19 (t, *J* = 7.3 Hz, 12H), 1.17 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (176 MHz, Acetone-d_6) δ 172.5, 162.77, 162.70, 157.2, 156.6, 140.36, 140.33, 140.28, 140.26, 140.1, 140.09, 140.03, 139.8, 139.6, 138.4, 138.2, 130.0, 129.78, 129.70, 129.6, 129.59, 129.56, 129.4, 129.3, 129.29, 129.27, 129.23, 129.18, 129.12, 129.0, 128.9, 128.8, 128.77, 128.75, 128.72, 128.69, 128.62, 128.58, 128.54, 128.44, 128.40, 128.3, 128.29, 128.27, 128.20, 128.17, 128.16, 128.12, 128.0, 127.9, 127.7, 127.29, 127.26, 104.9, 102.2, 101.8, 100.9, 100.1, 99.7, 94.3, 81.05, 81.01, 80.5, 80.39, 80.37, 80.0, 78.9, 78.7, 78.3, 78.2, 77.4, 76.1, 76.0, 75.55, 75.50, 75.4, 75.3, 74.7, 74.1, 73.9, 73.5, 73.4, 72.7, 71.0, 70.9, 70.8, 70.5, 69.5, 68.6, 67.6, 67.5, 65.5, 63.7, 59.4, 59.3, 55.6, 51.0, 50.8, 47.8, 47.0, 46.3, 32.1, 28.7, 28.2, 27.8, 24.1, 21.2, 18.6, 18.4, 17.3, 9.1; ^{31}P NMR (162 MHz, Acetone-d_6) δ -1.13; HRMS (ESI) calculated for $C_{142}H_{161}Cl_3N_3O_{29}P$ [M-Et_3N-H]$^-$: 2405.8742, found: 2405.8267.

Triethylammonium 4-*O*-benzyl-butanyl *H*-phosphonate (2-15)

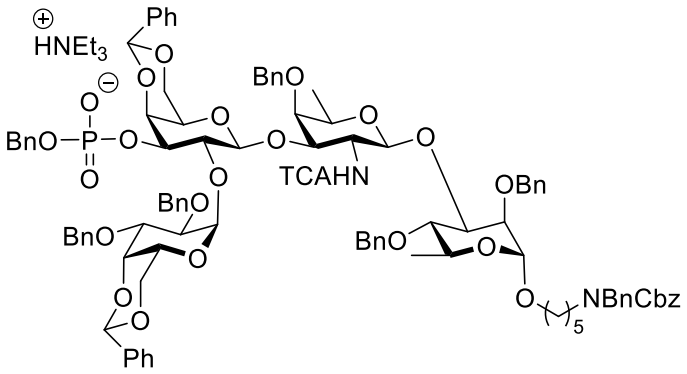


To a solution of 4-benzyloxy-1-butanol (1.0 g, 5.55 mmol) in anhydrous pyridine (30mL), diphenyl phosphite (5.3 mL) was added. The mixture was stirred at room temperature for 2 h. TEAB buffer (20 mL) was added and reaction mixture was stirred for additional 2 h. The reaction was diluted with DCM (20 mL) and washed with TEAB buffer. The organic layer was dried over Na_2SO_4, concentrated and purified by flash column chromatography on silica gel which was neutralized with triethylamine (ethyl acetate/MeOH/H_2O=10/2/1) to afford the *H*-phosphonate **2-15** (1.36 g, 71%) as a light yellow syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.34 (s, 1H), 7.33 – 7.09 (m, 5H), 4.42 (s, 2H), 3.90 – 3.79 (m, 2H), 3.45 – 3.36 (m, 2H), 2.99 (qd, *J* = 7.3, 4.0 Hz, 6H), 1.69 – 1.58 (m, 4H), 1.25 (t, *J* = 7.3 Hz, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 129.3, 128.3, 127.6, 127.5, 123.3, 120.67, 120.63, 72.8,

69.9, 63.9, 63.8, 45.4, 27.5, 27.4, 26.1, 8.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 4.68; HRMS (ESI) calculated for $C_{17}H_{32}NO_4P$ [M-Et_3N-H]$^-$: 243.0792, found: 243.0788.

Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*-D-galactopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-(benzyl phospho)-*β*-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-*O*-benzyl-*β*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-10)

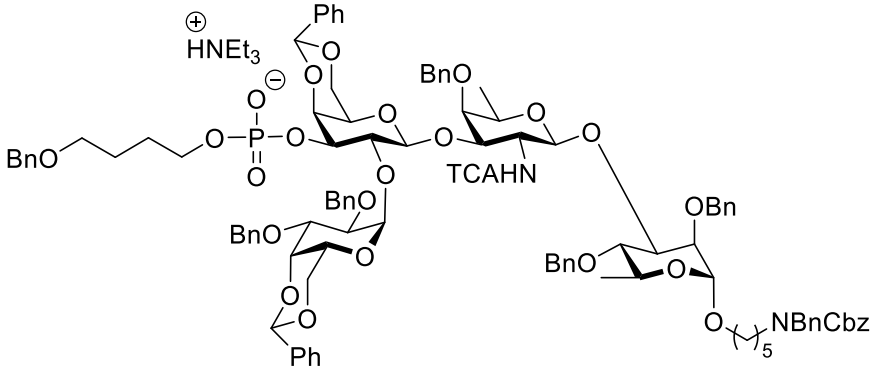


Tetrasaccharide alcohol **2-12** (30 mg, 17.5 μmol) and *H*-phosphonate **2-14** (9.6 mg, 35.0 μmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydous pyridine (1 mL). Pivaloyl chloride (10.7 μL, 87.6 μmol) was then added. After 5 h stirring at room temperature, a freshly prepared solution of iodine (22.2 mg, 87.6 μmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with DCM, washed with saturated $Na_2S_2O_3$ solution and TEAB buffer, and then dried over Na_2SO_4. The crude product obtained after solvent removal under reduced pressure was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 30/2/1) to give **2-10** as a triethylammonium salt (30 mg, 86%).

^{1}H NMR (700 MHz, Acetone-d_6) δ 12.27 (s, 1H), 9.19 (d, *J* = 6.2 Hz, 1H), 7.64 – 7.00 (m, 50H), 5.71 (d, *J* = 8.1 Hz, 1H), 5.54 (s, 1H), 5.48 (s, 1H), 5.26 (d, *J* = 11.6 Hz, 1H), 5.21 – 5.13 (m, 3H), 5.10 – 5.03 (m, 2H), 5.00 (dd, *J* = 12.2, 5.8 Hz, 2H), 4.96 – 4.90 (m, 2H), 4.86 (d, *J* = 7.9 Hz, 1H), 4.76 (d, *J* = 3.4 Hz, 1H), 4.67 (d, *J* = 11.6 Hz, 1H), 4.63 – 4.55 (m, 3H), 4.50 (d, *J* = 27.7 Hz, 3H), 4.41 (t, *J* = 7.4 Hz, 2H), 4.38 – 4.26 (m, 4H), 4.22 (d, *J* = 12.1 Hz, 1H), 4.13 – 3.96 (m, 6H), 3.88

(d, *J* = 3.9 Hz, 2H), 3.72 (s, 1H), 3.62 (s, 1H), 3.55 – 3.39 (m, 3H), 3.29 – 3.10 (m, 4H), 3.03 (q, *J* = 7.3 Hz, 8H), 1.56 – 1.37 (m, 4H), 1.35 – 1.27 (m, 2H), 1.24 (t, *J* = 7.3 Hz, 12H), 1.20 – 1.16 (m, 3H), 0.90 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (176 MHz, Acetone-d_6) δ 180.1, 172.5, 162.78, 162.71, 157.2, 156.6, 140.45, 140.40, 140.33, 140.30, 140.09, 140.05, 140.00, 139.6, 138.4, 138.2, 130.0, 129.8, 129.7, 129.57, 129.53, 129.4, 129.36, 129.30, 129.25, 129.21, 129.1, 129.03, 129.00, 128.88, 128.83, 128.78, 128.74, 128.72, 128.5, 128.4, 128.3, 128.28, 128.23, 128.13, 128.10, 128.0, 127.9, 127.8, 127.75, 127.70, 127.2, 104.8, 102.19, 102.11, 100.9, 100.2, 99.6, 94.18, 94.13, 81.5, 79.9, 78.9, 78.7, 78.2, 78.17, 78.12, 77.36, 76.35, 76.0, 75.58, 75.55, 75.3, 74.0, 73.5, 73.4, 73.3, 71.0, 70.7, 70.5, 69.78, 69.71, 68.60, 67.65, 67.63, 67.60, 67.5, 63.6, 59.4, 59.3, 55.6, 53.2, 51.0, 50.8, 47.8, 47.0, 46.4, 46.3, 46.2, 38.9, 32.1, 27.8, 24.1, 18.6, 17.3, 9.0; ^{31}P NMR (162 MHz, Acetone-d_6) δ -1.91; HRMS (ESI) calculated for $C_{108}H_{125}Cl_3N_3O_{24}P$ $[M\text{-}Et_3N\text{-}H]^-$: 1881.6179, found: 1881.6061.

Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*-D-galactopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-(4-*O*-benzyl-butanyl phospho)-*β*-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-*O*-benzyl-*β*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-11)

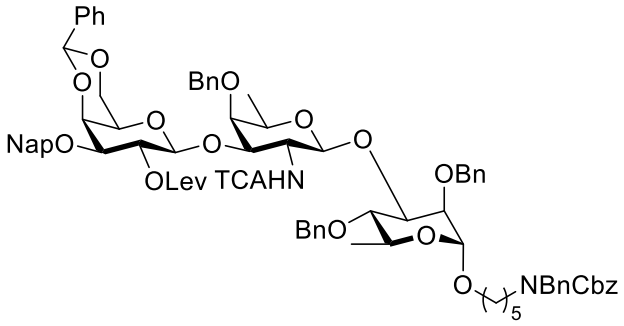


Tetrasaccharide alcohol **2-12** (30 mg, 17.5 µmol) and *H*-phosphonate **2-15** (12.1 mg, 35.0 µmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (10.7 µL, 87.6 µmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine

(22.2 mg, 87.6 μmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with DCM, washed with saturated $Na_2S_2O_3$ solution and TEAB buffer, and then dried over Na_2SO_4. The crude product obtained after solvent removal under reduced pressure was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 30/2/1) to give **2-11** as a triethylammonium salt (25 mg, 69%).

^{1}H NMR (700 MHz, Acetone-d_6) δ 12.09 (s, 1H), 9.19 (d, *J* = 6.2 Hz, 1H), 7.66 – 7.06 (m, 50H), 5.71 (d, *J* = 8.2 Hz, 1H), 5.62 (s, 1H), 5.52 (s, 1H), 5.25 (d, *J* = 11.6 Hz, 1H), 5.20 – 5.14 (m, 3H), 5.12 – 5.05 (m, 2H), 5.00 (d, *J* = 11.6 Hz, 1H), 4.94 (d, *J* = 12.2 Hz, 1H), 4.88 – 4.81 (m, 2H), 4.75 (d, *J* = 3.5 Hz, 1H), 4.65 – 4.35 (m, 11H), 4.32 – 4.18 (m, 3H), 4.16 – 3.98 (m, 7H), 3.94 – 3.83 (m, 4H), 3.71 (d, *J* = 7.0 Hz, 1H), 3.62 (s, 1H), 3.54 – 3.40 (m, 5H), 3.29 – 3.11 (m, 4H), 3.06 (q, *J* = 7.3 Hz, 8H), 1.73 – 1.58 (m, 4H), 1.54 – 1.37 (m, 4H), 1.35 – 1.28 (m, 2H), 1.25 (t, *J* = 7.3 Hz, 12H), 1.18 (d, *J* = 3.7 Hz, 3H), 0.91 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (176 MHz, Acetone-d_6) δ 180.0, 172.4, 162.78, 162.71, 157.2, 156.6, 140.35, 140.33, 140.2, 140.18, 140.17, 140.07, 140.04, 140.03, 139.6, 138.4, 138.2, 130.3, 130.0, 129.8, 129.7, 129.6, 129.59, 129.53, 129.4, 129.37, 129.32, 129.2, 129.19, 129.11, 129.0, 128.9, 128.84, 128.80, 128.77, 128.74, 128.47, 128.43, 128.39, 128.32, 128.29, 128.25, 128.21, 128.1, 128.0, 127.9, 127.76, 127.71, 127.4, 127.2, 104.8, 102.19, 102.10, 101.0, 100.2, 99.7, 94.2, 81.5, 80.0, 78.9, 78.7, 78.3, 78.1, 77.4, 76.3, 76.0, 75.4, 75.38, 75.32, 74.1, 73.5, 73.4, 73.3, 73.2, 71.0, 70.8, 70.7, 70.6, 69.78, 69.74, 68.61, 67.67, 67.60, 66.03, 66.00, 63.7, 59.4, 59.3, 55.6, 51.0, 50.8, 47.9, 47.8, 47.0, 46.4, 46.3, 38.9, 32.1, 28.8, 28.7, 27.8, 27.7, 27.2, 24.1, 18.6, 17.3, 9.0; ^{31}P NMR (162 MHz, Acetone-d_6) δ -2.21; HRMS (ESI) calculated for $C_{112}H_{133}Cl_3N_3O_{25}P$ $[M\text{-}Et_3N\text{-}H]^-$: 1953.6754, found: 1953.6492.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-_O_-benzylidene-2-_O_-levulinoyl-3-_O_-(2-naphthylmethyl)-_β_-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-_O_-benzyl-_β_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-48)

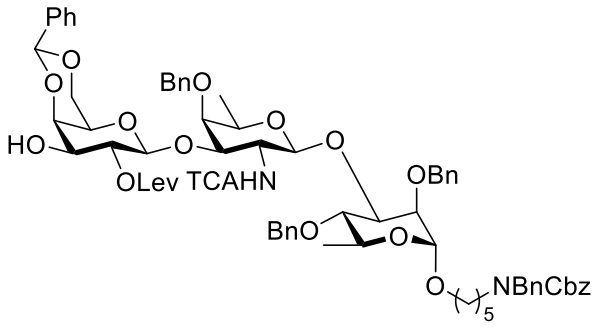


Disaccharide alcohol **2-34** (92 mg, 0.09 mmol) and thioglycoside **2-23** (80 mg, 0.13 mmol) were coevaporated three times with toluene and the resulting mixture was dried under vacuum for 2 h. The mixture was dissolved in anhydrous DCM (5 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to −40 °C, NIS (30.2 mg, 0.13 mmol) and TfOH (1.2 µL, 0.013 mmol) were added. The reaction mixture was stirred at −40 °C for 3 h. After complete consumption of staring material, Et_3N (0.1 mL) was added and the mixture was stirred for 10 min. Then the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 5/1) to give **2-48** as a colorless syrup (99 mg, 73%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 8.88 – 8.78 (m, 1H), 7.88 – 7.72 (m, 4H), 7.54 – 7.42 (m, 7H), 7.40 – 7.14 (m, 26H), 5.56 (dd, *J* = 10.3, 7.8 Hz, 1H), 5.50 (s, 1H), 5.46 (d, *J* = 8.2 Hz, 1H), 5.19 (d, *J* = 11.6 Hz, 3H), 4.93 (d, *J* = 12.1 Hz, 2H), 4.87 – 4.73 (m, 5H), 4.62 – 4.58 (m, 2H), 4.50 (d, *J* = 16.1 Hz, 3H), 4.29 (d, *J* = 12.2 Hz, 1H), 4.19 (d, *J* = 3.4 Hz, 1H), 4.17 – 4.12 (m, 1H), 4.04 (d, *J* = 12.2 Hz, 1H), 3.88 (s, 1H), 3.85 – 3.77 (m, 2H), 3.65 – 3.55 (m, 4H), 3.54 – 3.44 (m, 1H), 3.38 (s, 1H), 3.30 – 3.14 (m, 3H), 3.14 – 3.06 (m, 1H), 2.89 – 2.82 (m, 1H), 2.45 (dt, *J* = 18.8, 3.9 Hz, 1H), 2.32 (dt, *J* = 17.1, 4.0 Hz, 1H), 2.03 (s, 3H), 1.55 – 1.37 (m, 4H), 1.28 – 1.16 (m, 2H), 1.16 – 1.09 (m, 6H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 208.4, 171.2, 162.2, 156.7, 156.2, 139.2, 139.1, 138.8, 138.0, 137.7, 136.9, 136.9, 135.4, 133.2, 133.1, 129.3, 129.1, 128.68, 128.60, 128.55, 128.50, 128.4, 128.39, 128.30, 128.28, 128.24, 128.20, 128.1, 128.0, 127.97, 127.91, 127.90, 127.7,

127.5, 127.38, 127.31, 127.2, 126.5, 126.4, 126.3, 126.1, 125.6, 101.8, 101.5, 98.9, 98.3, 92.8, 79.8, 79.1, 77.9, 77.6, 76.5, 75.3, 74.8, 74.7, 73.5, 73.4, 71.5, 70.7, 70.2, 69.1, 67.8, 67.2, 67.08, 67.04, 66.5, 57.3, 50.6, 50.3, 47.2, 46.2, 37.8, 30.0, 29.1, 27.9, 27.8, 27.5, 23.4, 23.3, 18.0, 16.8; HRMS (ESI) calculated for $C_{84}H_{91}Cl_3N_2O_{18}$ $[M+Na]^+$: 1543.5224, found: 1543.5361.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-_O_-benzylidene-2-_O_-levulinoyl-_β_-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-_O_-benzyl-_β_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-22)

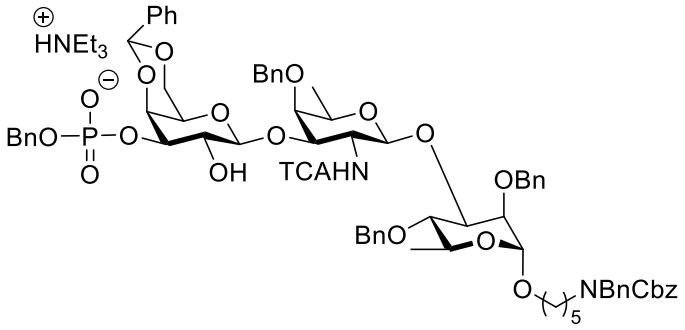


To a solution of **2-48** (20 mg, 0.013 mmol) in 20:1 (v/v) DCM-H_2O (1 mL), DDQ (4.5 mg, 0.020 mmol) and *β*-pinene (6.2 µL, 0.04 mmol) were added and the reaction mixture was stirred for 5 h. The reaction was then diluted with DCM (10 mL) and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 3/1) to give **2-22** as a colorless syrup (11 mg, 60%).-

^{1}H NMR (700 MHz, $CDCl_3$) δ 8.74 (dd, *J* = 16.2, 7.0 Hz, 1H), 7.53 – 7.12 (m, 30H), 5.56 (s, 1H), 5.45 (d, *J* = 8.2 Hz, 1H), 5.23 (t, *J* = 9.8, 7.7, 1.5 Hz, 1H), 5.16 (t, *J* = 13.3 Hz, 3H), 4.89 (dd, *J* = 12.0, 7.4 Hz, 2H), 4.75 (dd, *J* = 23.2, 11.7 Hz, 3H), 4.65 – 4.61 (m, 1H), 4.57 (d, *J* = 11.7 Hz, 1H), 4.51 – 4.46 (m, 3H), 4.34 (d, *J* = 12.4 Hz, 1H), 4.23 (d, *J* = 3.8 Hz, 1H), 4.15 – 4.09 (m, 2H), 3.85 (s, 1H), 3.83 – 3.78 (m, 2H), 3.71 (d, *J* = 10.2, 3.8 Hz, 1H), 3.63 (t, *J* = 7.3 Hz, 1H), 3.59 – 3.53 (m, 3H), 3.51 – 3.41 (m, 1H), 3.29 – 3.11 (m, 3H), 3.11 – 3.04 (m, 1H), 2.89 – 2.82 (m, 1H), 2.49 (dt, *J* = 16.7, 3.7 Hz, 1H), 2.41 (dt, *J* = 17.1, 4.1 Hz, 1H), 2.03 (s, 3H), 1.63 (d, *J* = 39.7 Hz, 1H), 1.54 – 1.35 (m, 4H), 1.31 – 1.17 (m, 2H), 1.16 – 1.02 (m, 6H); ^{13}C NMR (176 MHz, $CDCl_3$) δ

208.4, 172.1, 162.3, 156.8, 156.2, 139.2, 139.1, 138.8, 138.0, 137.4, 137.0, 129.5, 129.3, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.17, 128.13, 128.0, 127.9, 127.5, 127.48, 127.42, 127.3, 126.5, 101.8, 101.5, 98.9, 98.4, 92.8, 79.9, 79.1, 78.1, 77.7, 75.6, 75.5, 74.9, 74.8, 73.5, 72.6, 70.8, 70.3, 69.1, 67.8, 67.2, 67.1, 66.5, 57.4, 50.6, 50.3, 47.2, 46.3, 38.0, 30.0, 29.2, 28.0, 27.9, 27.6, 23.46, 23.43, 18.1, 16.9; HRMS (ESI) calculated for $C_{73}H_{83}Cl_3N_2O_{18}$ $[M+Na]^+$: 1403.4598, found: 1403.4697.

Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-*O*-benzylidene-3-*O*-(benzyl phospho)-*β*-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-*O*-benzyl-*β*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-20)

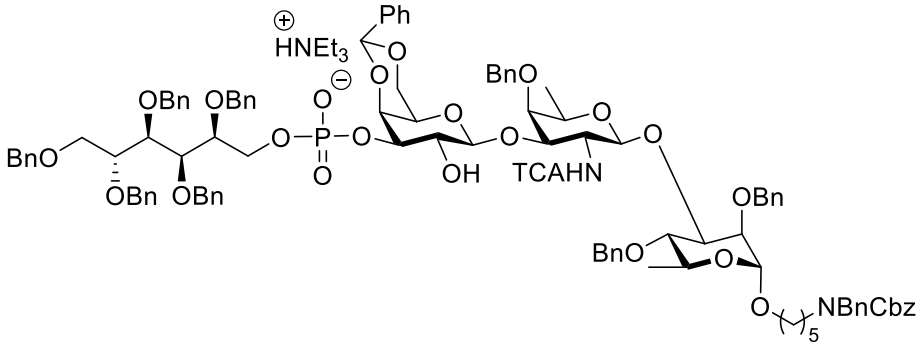


Trisaccharide alcohol **2-22** (14.5 mg, 10.5 µmol) and *H*-phosphonate **2-14** (5.7 mg, 21.0 µmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (6.4 µL, 52.5 µmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine (13.3 mg, 52.5 µmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with DCM, washed with saturated $Na_2S_2O_3$ solution and then dried over Na_2SO_4, the residue obtained after the removal of the solvent under reduced pressure was then dissolved in DCM (1 mL), pyridine (40 µL) and N_2H_4·AcOH (9.7 mg, 105.0 µmol) were added and the mixture was stirred at room temperature for 12 h. The reaction was quenched with the addition of acetone (0.5 mL) and diluted with DCM (20 mL), washed with TEAB buffer, the organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column

chromatography (ethyl acetate/MeOH/H_2O = 30/2/1) to give **2-20** as a triethylammonium salt (12 mg, 73%).

^{1}H NMR (700 MHz, Acetone-d_6) δ 11.99 (s, 1H), 8.49 (s, 1H), 7.55 – 7.18 (m, 35H), 5.52 (s, 1H), 5.25 (d, *J* = 11.7 Hz, 1H), 5.15 (d, *J* = 17.2 Hz, 2H), 5.06 – 4.87 (m, 5H), 4.76 (dd, *J* = 21.0, 11.8 Hz, 2H), 4.66 (d, *J* = 7.4 Hz, 1H), 4.62 – 4.47 (m, 4H), 4.37 (q, *J* = 9.8, 8.5 Hz, 2H), 4.20 (t, *J* = 11.7 Hz, 2H), 4.12 – 4.02 (m, 3H), 3.98 (s, 1H), 3.94 – 3.86 (m, 2H), 3.70 – 3.46 (m, 6H), 3.34 – 3.17 (m, 3H), 3.05 (q, *J* = 7.3 Hz, 13H), 1.60 – 1.43 (m, 4H), 1.36 – 1.30 (m, 2H), 1.24 (t, *J* = 7.3 Hz, 19H), 1.19 – 1.14 (m, 3H), 1.06 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (176 MHz, Acetone-d_6) δ 174.84, 163.19, 163.12, 157.2, 140.69, 140.66, 140.1, 140.0, 139.5, 138.4, 129.7, 129.4, 129.36, 129.33, 129.27, 129.21, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 128.3, 128.24, 128.20, 128.09, 128.04, 128.02, 127.6, 105.5, 102.7, 102.0, 99.8, 94.2, 81.2, 80.1, 79.3, 78.6, 76.5, 76.4, 76.17, 76.14, 75.8, 75.6, 74.1, 72.0, 71.2, 69.8, 69.7, 68.7, 68.1, 68.0, 67.6, 67.5, 58.5, 55.7, 55.6, 51.0, 50.8, 47.8, 46.9, 46.4, 46.3, 46.2, 34.4, 32.7, 32.0, 30.6, 30.4, 28.7, 28.2, 27.6, 25.8, 24.1, 23.4, 18.5, 17.3, 14.4, 8.9, 8.2; ^{31}P NMR (243 MHz, Acetone-d_6) δ 0.69; HRMS (ESI) calculated for $C_{75}H_{83}Cl_3N_2O_{19}P$ [M-Et_3N-H]$^-$: 1451.4399, found: 1451.4261.

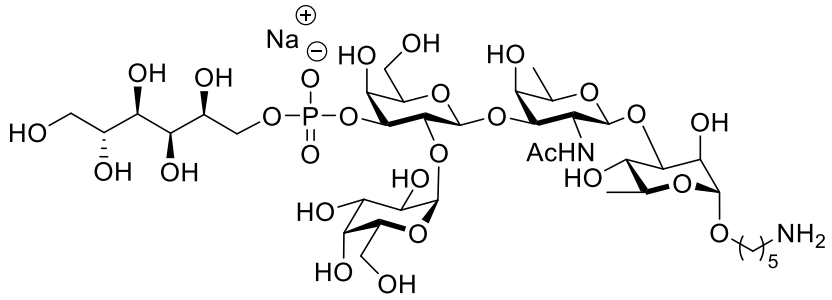
Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-*O*-benzylidene-3-*O*-(2,3,4,5,6-penta-*O*-benzyl-D-glucityl phospho)-*β*-D-galactopyranosyl-(1→3)-2-trichloroacetamido-2-deoxy-4-*O*-benzyl-*β*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-21)



Trisaccharide alcohol **2-22** (10 mg, 7.2 µmol) and *H*-phosphonate **2-13** (11.5 mg, 14.5 µmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (4.4 µL, 36.2 µmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine (9.2 mg, 36.2 µmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with DCM, washed with saturated $Na_2S_2O_3$ solution and then dried over Na_2SO_4, the residue obtained after the removal of the solvent under reduced pressure was then dissolved in DCM (1 mL), pyridine (40 µL) and N_2H_4·AcOH (6.7 mg, 72.4 µmol) were added and the mixture was stirred at room temperature for 12 h. The reaction was quenched with the addition of acetone (0.5 mL) and diluted with DCM (20 mL), washed with TEAB buffer, the organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 80/2/1) to give **2-21** as a triethylammonium salt (10 mg, 66%).

^{1}H NMR (600 MHz, Acetone-d_6) δ 12.02 (s, 1H), 8.36 (d, *J* = 6.8 Hz, 1H), 7.53 – 7.16 (m, 55H), 5.39 (s, 1H), 5.22 – 5.12 (m, 2H), 5.04 – 4.94 (m, 3H), 4.86 – 4.72 (m, 8H), 4.68 (dd, *J* = 11.5, 8.4 Hz, 2H), 4.63 – 4.50 (m, 8H), 4.42 (d, *J* = 7.5 Hz, 1H), 4.36 – 4.29 (m, 2H), 4.23 – 4.18 (m, 2H), 4.16 (t, *J* = 5.0 Hz, 1H), 4.14 – 4.07 (m, 3H), 4.07 – 3.98 (m, 4H), 3.91 – 3.85 (m, 3H), 3.85 – 3.78 (m, 2H), 3.66 – 3.59 (m, 1H), 3.59 – 3.47 (m, 3H), 3.31 – 3.18 (m, 3H), 3.12 (s, 1H), 3.00 – 2.93 (m, 12H), 1.50 (d, *J* = 38.5 Hz, 4H), 1.18 (t, *J* = 7.3 Hz, 17H), 1.15 (d, *J* = 6.0 Hz, 2H), 1.07 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (151 MHz, Acetone-d_6) δ 163.17, 163.11, 140.6, 140.5, 140.27, 140.23, 140.21, 140.1, 139.89, 139.86, 139.5, 129.7, 129.4, 129.37, 129.34, 129.31, 129.28, 129.22, 129.20, 129.16, 129.13, 129.0, 128.97, 128.95, 128.87, 128.81, 128.77, 128.73, 128.70, 128.66, 128.64, 128.56, 128.53, 128.51, 128.4, 128.37, 128.35, 128.26, 128.25, 128.23, 128.20, 128.17, 128.15, 128.12, 128.10, 128.0, 127.6, 105.2, 102.9, 101.9, 99.8, 94.2, 81.3, 80.57, 80.53, 80.51, 80.46, 80.42, 80.2, 80.1, 80.0, 79.9, 79.8, 79.3, 78.2, 76.4, 76.28, 76.24, 75.7, 75.5, 75.3, 75.1, 74.8, 74.7, 74.1, 73.9, 73.8, 73.7, 73.3, 72.7, 72.6, 72.2, 71.2, 71.1, 70.8, 69.7, 68.8, 67.6, 67.5, 67.3, 66.64, 66.60, 58.5, 55.6, 55.5, 51.0, 50.8, 46.9, 46.3, 34.4, 32.7, 28.7, 27.6, 25.8, 24.2, 23.4, 18.5, 17.3, 14.4, 8.9; ^{31}P NMR (162 MHz, Acetone-d_6) δ -1.26; HRMS (ESI) calculated for $C_{109}H_{119}Cl_3N_2O_{24}P$ $[M\text{-}Et_3N\text{-}H]^-$: 1975.6961, found: 1975.6693.

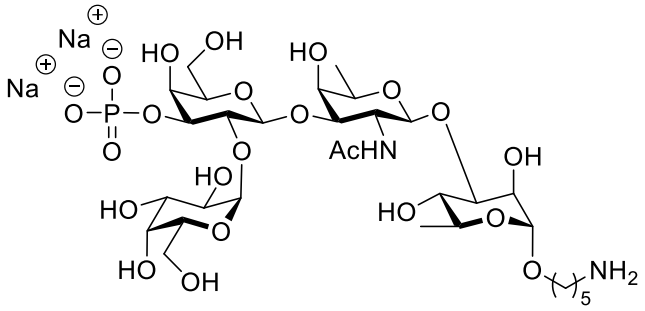
5-Aminopentyl *α*-D-galactopyranosyl-(1→2)-3-*O*-(D-glucityl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*β*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside sodium salt (2-1)



In a 20 mL vial, phosphate **2-9** (10 mg, 3.99 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-1** (2.2 mg, 52%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 5.37 (d, *J* = 3.9 Hz, 1H), 4.74 (d, *J* = 7.7 Hz, 1H), 4.66 (d, *J* = 7.3 Hz, 1H), 4.30 – 4.26 (m, 1H), 4.26 – 4.21 (m, 1H), 4.19 (d, *J* = 3.3 Hz, 1H), 4.15 (dd, *J* = 3.3, 1.8 Hz, 1H), 4.09 – 3.92 (m, 8H), 3.92 – 3.82 (m, 3H), 3.82 – 3.64 (m, 13H), 3.56 (dt, *J* = 10.0, 6.2 Hz, 1H), 3.51 (t, *J* = 9.6 Hz, 1H), 3.04 – 2.99 (m, 2H), 2.07 (s, 3H), 1.75 – 1.63 (m, 4H), 1.54 – 1.40 (m, 2H), 1.31 – 1.27 (m, 6H); ^{13}C NMR (151 MHz, D_2O) δ 175.0, 103.6, 102.9, 99.1, 96.4, 79.8, 79.2, 76.0, 75.9, 74.4, 71.78, 71.72, 71.18, 71.13, 71.11, 70.9, 70.8, 70.1, 69.9, 69.8, 69.3, 69.2, 68.7, 68.5, 67.5, 67.3, 66.47, 66.44, 62.7, 61.2, 60.6, 50.9, 39.2, 27.8, 26.4, 22.4, 22.3, 16.4, 15.6; ^{31}P NMR (243 MHz, D_2O) δ -0.47; HRMS (ESI) calculated for $C_{37}H_{68}N_2NaO_{27}P$ $[M\text{-}Na]^-$: 1003.3753, found: 1003.3690.

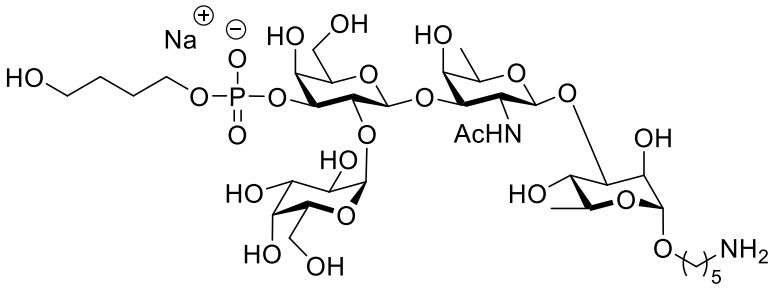
5-Aminopentyl *α*-D-galactopyranosyl-(1→2)-3-*O*-phospho-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*β*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside disodium salt (2-3)



In a 20 mL vial, phosphate **2-10** (10 mg, 5.04 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-3** (1.4 mg, 31%) as a white solid.

^{1}H NMR (700 MHz, D_2O) δ 5.24 (d, *J* = 4.0 Hz, 1H), 4.72 (d, *J* = 7.9 Hz, 1H), 4.65 (d, *J* = 8.3 Hz, 1H), 4.55 (dd, *J* = 8.1, 4.5 Hz, 1H), 4.26 (d, *J* = 3.3 Hz, 1H), 4.16 – 4.13 (m, 1H), 4.09 – 4.00 (m, 5H), 3.97 (dd, *J* = 10.9, 3.0 Hz, 1H), 3.82 – 3.62 (m, 11H), 3.58 – 3.52 (m, 1H), 3.50 (t, *J* = 9.6 Hz, 1H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.07 (s, 3H), 1.74 – 1.61 (m, 4H), 1.52 – 1.41 (m, 2H), 1.31 – 1.27 (m, 7H); ^{13}C NMR (176 MHz, D_2O) δ 174.9, 103.4, 103.0, 99.1, 97.5, 79.9, 79.4, 74.6, 74.1, 73.6, 71.0, 70.8, 70.1, 69.9, 69.7, 69.38, 69.36, 68.87, 68.81, 67.7, 67.4, 61.3, 61.0, 50.9, 39.3, 27.9, 26.6, 22.5, 22.4, 16.5, 15.7; ^{31}P NMR (162 MHz, D_2O) δ 2.56; HRMS (ESI) calculated for $C_{31}H_{55}N_2Na_2O_{22}P$ $[M-2Na+H]^-$: 839.3068, found: 839.3023.

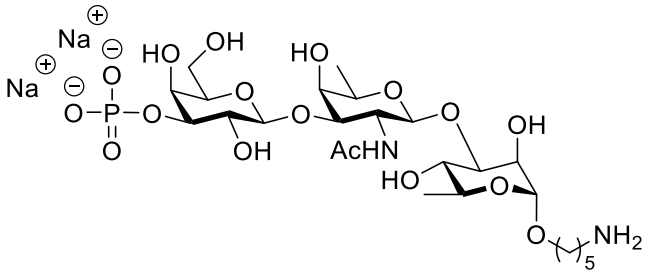
5-Aminopentyl *α*-D-galactopyranosyl-(1→2)-3-*O*-(butanyl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*β*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside sodium salt (2-4)



In a 20 mL vial, phosphate **2-11** (10 mg, 4.86 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-4** (2.1 mg, 44%) as a white solid.

^{1}H NMR (700 MHz, D_2O) δ 5.36 (d, *J* = 3.9 Hz, 1H), 4.73 (d, *J* = 7.7 Hz, 1H), 4.65 (d, *J* = 7.8 Hz, 1H), 4.31 – 4.27 (m, 1H), 4.20 (td, *J* = 9.4, 3.2 Hz, 1H), 4.17 – 4.12 (m, 2H), 4.06 (d, *J* = 2.2 Hz, 1H), 4.04 – 3.96 (m, 4H), 3.97 – 3.90 (m, 2H), 3.87 (dd, *J* = 9.8, 7.8 Hz, 1H), 3.81 – 3.65 (m, 10H), 3.67 – 3.62 (m, 2H), 3.55 (dt, *J* = 9.6, 6.2 Hz, 1H), 3.50 (t, *J* = 9.6 Hz, 1H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.07 (s, 3H), 1.75 – 1.60 (m, 8H), 1.52 – 1.39 (m, 2H), 1.28 (d, *J* = 6.2 Hz, 6H); ^{13}C NMR (176 MHz, D_2O) δ 175.0, 103.7, 102.9, 99.1, 96.5, 79.8, 79.3, 75.9, 75.9, 74.4, 71.2, 71.2, 71.0, 70.8, 70.2, 70.0, 69.9, 69.3, 69.2, 68.8, 68.6, 67.7, 67.4, 65.9, 65.8, 61.3, 61.2, 60.7, 51.0, 39.3, 27.9, 27.7, 26.56, 26.54, 26.52, 22.5, 22.4, 16.5, 15.7; ^{31}P NMR (243 MHz, D_2O) δ -0.40; HRMS (ESI) calculated for $C_{35}H_{64}N_2NaO_{23}P$ $[M\text{-}Na]^-$: 911.3643, found: 911.3583.

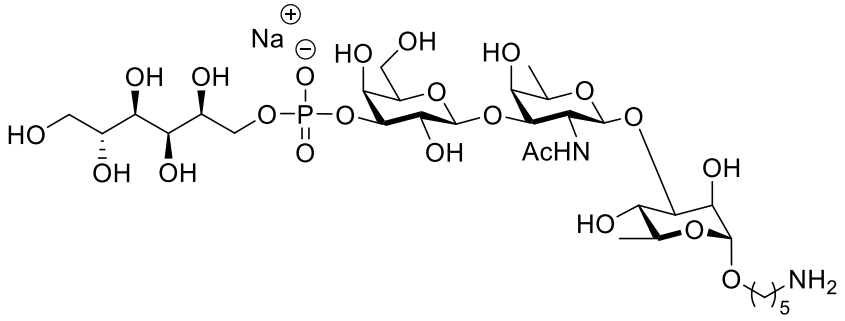
5-Aminopentyl 3-*O*-phospho-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*β*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside disodium salt (2-6)



In a 20 mL vial, phosphate **2-20** (10 mg, 6.26 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-6** (2.0 mg, 44%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 4.68 (d, *J* = 8.4 Hz, 1H), 4.54 (d, *J* = 7.9 Hz, 1H), 4.14 (dd, *J* = 3.3, 1.8 Hz, 1H), 4.13 – 4.11 (m, 1H), 4.06 – 3.97 (m, 3H), 3.93 (dd, *J* = 10.9, 3.2 Hz, 1H), 3.82 – 3.63 (m, 8H), 3.59 – 3.49 (m, 2H), 3.09 – 2.98 (m, 2H), 2.03 (s, 3H), 1.77 – 1.61 (m, 4H), 1.55 – 1.40 (m, 2H), 1.31 – 1.22 (m, 6H); ^{13}C NMR (151 MHz, D_2O) δ 174.9, 104.5, 102.5, 99.1, 79.8, 79.5, 75.97, 75.93, 74.7, 70.9, 70.3, 70.29, 70.25, 69.8, 68.7, 68.0, 67.4, 60.9, 51.0, 39.3, 27.8, 26.4, 22.3, 22.2, 16.4, 15.5; ^{31}P NMR (243 MHz, D_2O) δ 2.84; HRMS (ESI) calculated for $C_{25}H_{45}N_2Na_2O_{17}P$ $[M-2Na+H]^-$: 677.2540, found: 677.2524.

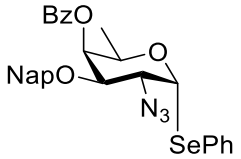
5-Amin-pentyl 3-*O*-(D-glucityl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*β*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside sodium salt (2-7)



In a 20 mL vial, phosphate **2-21** (12 mg, 5.78 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and then passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-7** (2.4 mg, 48%) as a white solid.

^{1}H NMR (700 MHz, D_2O) δ 4.81 (s, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.55 (d, *J* = 7.9 Hz, 1H), 4.20 – 4.14 (m, 2H), 4.14 – 4.07 (m, 2H), 4.06 – 3.97 (m, 4H), 3.95 – 3.90 (m, 2H), 3.86 (dd, *J* = 12.0, 2.9 Hz, 1H), 3.83 – 3.77 (m, 5H), 3.77 – 3.65 (m, 6H), 3.61 – 3.55 (m, 1H), 3.53 (t, *J* = 9.6 Hz, 1H), 3.04 (t, *J* = 7.7 Hz, 2H), 2.05 (s, 3H), 1.77 – 1.64 (m, 4H), 1.55 – 1.43 (m, 2H), 1.34 – 1.26 (m, 6H); ^{13}C NMR (176 MHz, D_2O) δ 175.0, 104.4, 102.6, 99.2, 80.2, 79.6, 77.55, 77.51, 74.5, 71.7, 71.6, 71.06, 71.03, 70.4, 70.3, 69.9, 69.67, 69.64, 69.5, 68.7, 67.7, 67.4, 66.6, 66.5, 62.8, 60.9, 51.1, 39.3, 27.9, 26.5, 22.4, 22.3, 16.5, 15.6; ^{31}P NMR (162 MHz, D_2O) δ -0.29; HRMS (ESI) calculated for $C_{31}H_{58}N_2NaO_{22}P$ $[M\text{-}Na]^-$: 841.3224, found: 841.3169.

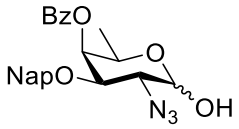
Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-benzoyl-1-seleno-*α*-D-fucopyranoside (2-61)



To a solution of compound **2-28** (804 mg, 1.71 mmol) in pyridine (17 mL), BzCl (1.6 mL, 13.71 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature until TLC showed complete conversion of the starting material. The reaction mixture was evaporated under reduced pressure to remove most pyridine. The resulting residue was diluted with DCM (50 mL) and washed with 1 N HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated to give crude product. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 11/1) to give **2-61** as a slightly yellow syrup (745 mg, 76%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 8.04 (m, 2H), 7.82 – 7.71 (m, 4H), 7.64 – 7.51 (m, 3H), 7.47 – 7.38 (m, 5H), 7.32 – 7.23 (m, 3H), 6.00 (d, *J* = 5.4 Hz, 1H), 5.73 (dd, *J* = 3.3, 1.2 Hz, 1H), 5.00 (d, *J* = 11.1 Hz, 1H), 4.73 (d, *J* = 11.1 Hz, 1H), 4.53 (dd, *J* = 6.4, 1.2 Hz, 1H), 4.27 (dd, *J* = 10.3, 5.4 Hz, 1H), 3.93 (dd, *J* = 10.3, 3.2 Hz, 1H), 1.16 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 134.7, 134.4, 133.5, 133.2, 133.1, 129.9, 129.5, 129.2, 128.6, 128.32, 128.30, 128.09, 128.08, 127.7, 127.2, 126.13, 126.11, 126.0, 85.0, 71.7, 69.4, 68.0, 60.6, 16.3; HRMS (ESI) calculated for $C_{30}H_{27}N_3O_4Se$ $[M+Na]^+$: 596.1059, found: 596.1066.

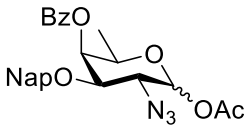
2-Azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-benzoyl-D-fucopyranose (2-62)



A solution of compound **2-61** (684 mg, 1.19 mmol) in a mixture of THF, water and acetone (2:2:1; 5 mL: 5 mL: 2.5 mL) was cooled to 0 °C under a nitrogen atmosphere. *N*-Iodosuccinimide (537 mg, 2.38 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction was then diluted with DCM (20 mL) and the organic layer was washed with saturated aqueous $Na_2S_2O_3$ (30 mL) and brine (30 mL) respectively. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 3/1) to give **2-62** (α/β=1/0.8) as a slightly yellow syrup (515 mg, 98%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 7.31 (m, 21H), 5.64 (dd, *J* = 3.2, 1.3 Hz, 1H), 5.51 (dd, *J* = 3.4, 1.0 Hz, 0.8H), 5.31 (d, *J* = 3.5 Hz, 1H), 4.90 (dd, *J* = 13.5, 11.5 Hz, 1.8H), 4.68 – 4.61 (m, 1.8H), 4.49 (d, *J* = 8.0 Hz, 0.8H), 4.36 – 4.26 (m, 1H), 4.10 – 3.95 (m, 1.8H), 3.79 (dd, *J* = 10.5, 3.5 Hz, 1H), 3.65 (tt, *J* = 10.3, 8.1 Hz, 2H), 3.47 (dd, *J* = 10.2, 3.4 Hz, 10.8H), 3.05 (s, 0.8H), 1.97 (s, 3H), 1.20 – 1.17 (m, 4H), 1.14 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.28, 166.26, 134.5, 134.4, 133.5, 133.4, 133.2, 133.17, 133.12, 133.10, 130.0, 129.9, 129.6, 129.4, 128.57, 128.55, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.24, 127.22, 126.15, 126.11, 126.08, 126.05, 126.02, 125.9, 96.1, 92.4, 77.5, 77.4, 74.2, 71.6, 71.4, 69.9, 69.8, 68.7, 65.3, 64.5, 64.0, 60.5, 60.0, 30.6, 29.7, 22.7, 21.14, 21.10, 19.1, 16.5, 16.4, 14.2, 13.7; HRMS (ESI) calculated for $C_{24}H_{23}N_3O_5$ $[M+Na]^+$: 456.1530, found: 456,1529.

Acetyl 2-Azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-benzoyl-D-fucopyranoside (2-63)

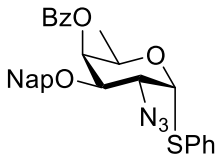


To a solution of hemiacetal **2-62** (568 mg, 1.31mmol) in 4:1 (v/v) anhydrous DCM-pyridine (20 mL), Ac_2O (1.24 mL, 13.1 mmol) was added dropwise at 0 °C under nitrogen. After addition of DMAP (cat.), the solution was stirred at room temperature. After complete conversion of the starting material, the mixture was was diluted with DCM (20 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL) respectively. The organic layer was dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by flash silica column chromatography (hexane/ethyl acetate = 4/1) to give **2-63** (α/β=1/0.8) as a slightly yellow syrup (510 mg, 82%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.06 (m, 4H), 7.83 – 7.71 (m, 8H), 7.64 – 7.54 (m, 2H), 7.52 – 7.40 (m, 10H), 6.31 (d, *J* = 3.6 Hz, 1H), 5.75 (dd, *J* = 3.2, 1.3 Hz, 1H), 5.62 (dd, *J* = 3.4, 1.1 Hz, 0.9H), 5.47 (d, *J* = 8.6 Hz, 0.9H), 4.98 (dd, *J* = 16.9, 11.4 Hz, 1.9H), 4.73 (dd, *J* = 11.4, 4.7 Hz, 1.9H), 4.20 (q, *J* = 6.4, 1.3 Hz, 1H), 4.08 (dd, *J* = 10.5, 3.2 Hz, 1H), 3.98 (dd, *J* = 10.5, 3.7 Hz, 1H), 3.91 – 3.80 (m, 1.8H), 3.63 (dd, *J* = 10.2, 3.3 Hz, 0.9H), 2.20 (s, 2.7H), 2.13 (s, 3H), 1.27 (d,

J = 6.4 Hz, 2.7H), 1.22 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.3, 169.2, 166.2, 134.5, 134.3, 133.6, 133.3, 133.26, 133.23, 133.20, 130.1, 130.0, 129.5, 129.4, 128.7, 128.6, 128.45, 128.40, 128.0, 127.81, 127.80, 127.3, 127.2, 126.26, 126.20, 126.14, 126.10, 92.9, 91.0, 77.8, 74.8, 71.9, 71.5, 70.7, 69.3, 68.7, 67.8, 61.6, 58.6, 21.22, 21.20, 16.5, 16.4; HRMS (ESI) calculated for $C_{26}H_{25}N_3O_6$ $[M+Na]^+$: 498.1635, found: 498,1636.

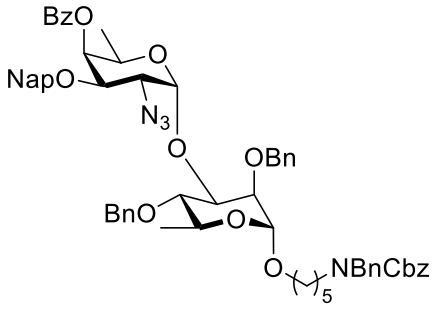
Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-benzoyl-1-thio-*α*-D-fucopyranoside (2-64)



The compound **2-63** (510 mg, 1.07 mmol) and thiophenol (165 µL, 1.61 mmol) were dissolved in anhydrous DCM (10 mL). The mixture was cooled to −10 °C and then TMSOTf (235 µL, 1.28 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h. After starting material was completely consumed, the reaction was quenched with Et_3N (0.5 mL). The solvent was evaporated, and the residue was purified by flash silica column chromatography (hexane/ethyl acetate = 4/1) to give **2-64** as a white solid (393 mg, 70%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.09 (m, 2H), 7.85 – 7.74 (m, 4H), 7.64 – 7.56 (m, 1H), 7.54 – 7.42 (m, 7H), 7.37 – 7.28 (m, 3H), 5.76 (dd, *J* = 3.3, 1.2 Hz, 1H), 5.70 (d, *J* = 5.5 Hz, 1H), 5.02 (d, *J* = 11.1 Hz, 1H), 4.76 (d, *J* = 11.1 Hz, 1H), 4.67 (qd, *J* = 6.4, 1.2 Hz, 1H), 4.35 (dd, *J* = 10.6, 5.5 Hz, 1H), 3.99 (dd, *J* = 10.6, 3.2 Hz, 1H), 1.23 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 134.5, 133.5, 133.3, 133.19, 132.15, 130.0, 129.6, 129.2, 128.6, 128.3, 128.1, 127.8, 127.7, 127.3, 126.1, 126.0, 87.5, 76.4, 71.7, 69.8, 66.5, 60.0, 16.4; HRMS (ESI) calculated for $C_{30}H_{27}N_3O_4S$ $[M+Na]^+$: 548.1614, found: 548.1617.

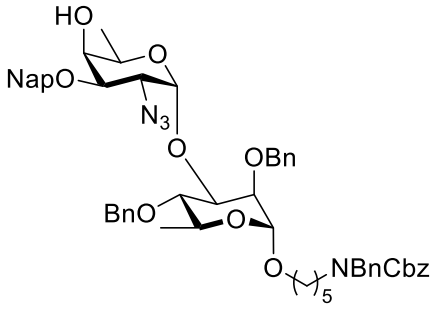
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-azido-2-deoxy-3-_O_-(2-naphthylmethyl)-4-_O_-benzoyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-66)



L-Rhamnose **2-16** (50.4 mg, 0.096 mmol) and D-fucose **2-64** (50 mg, 0.08 mmol) were coevaporated three times with toluene and the resulting mixture was dried under vacuum for 2 h. The mixture was dissolved in anhydrous Et_2O/DCM=4/1 (1 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to 0 °C, NIS (21.6 mg, 0.096 mmol) and TfOH (2.83 μL, 0.032 mmol) were added. The reaction mixture was stirred at 0 °C for 3 h. After complete consumption of the starting material, Et_3N (0.1 mL) was added and the mixture was stirred for 10 min. The reaction mixture was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 20/1) to give **2-66** as a colorless syrup (71 mg, 83%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 7.98 (m, 2H), 7.76 – 7.59 (m, 4H), 7.52 (t, *J* = 7.4 Hz, 1H), 7.44 – 7.04 (m, 25H), 5.30 (s, 1H), 5.11 (d, *J* = 12.4 Hz, 2H), 5.01 (d, *J* = 3.6 Hz, 1H), 4.83 (d, *J* = 11.2 Hz, 1H), 4.74 (d, *J* = 12.1 Hz, 1H), 4.70 – 4.50 (m, 5H), 4.43 (d, *J* = 7.8 Hz, 2H), 4.16 (q, *J* = 6.4 Hz, 1H), 3.98 (td, *J* = 9.5, 8.6, 3.1 Hz, 2H), 3.78 – 3.68 (m, 2H), 3.66 – 3.42 (m, 3H), 3.17 (ddt, *J* = 28.4, 15.0, 8.5 Hz, 3H), 1.54 – 1.33 (m, 4H), 1.25 (d, *J* = 6.0 Hz, 3H), 1.22 – 1.01 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 156.8, 156.2, 138.3, 138.1, 137.9, 136.9, 136.8, 134.6, 133.4, 133.2, 133.1, 129.9, 129.7, 128.6, 128.59, 128.54, 128.51, 128.3, 128.2, 128.06, 128.01, 127.9, 127.8, 127.7, 127.4, 127.3, 127.2, 126.15, 126.10, 126.0, 97.5, 93.8, 79.8, 75.3, 74.5, 74.2, 73.3, 72.8, 71.4, 69.9, 68.1, 67.4, 67.2, 65.1, 59.3, 50.5, 50.2, 47.1, 46.1, 32.0, 29.8, 29.5, 29.1, 27.9, 27.5, 23.4, 22.8, 18.1, 18.0, 16.2, 14.2; HRMS (ESI) calculated for $C_{64}H_{68}N_4O_{11}$ $[M+Na]^+$: 1091.4777, found: 1091.4767.

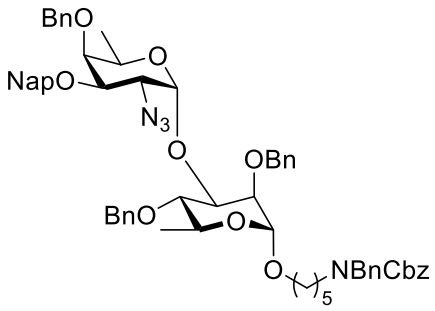
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-azido-2-deoxy-3-_O_-(2-naphthylmethyl)-α-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-α-L-rhamnopyranoside (2-67)



NaOMe was added slowly added to a solution of **2-66** (240 mg, 0.225 mmol) in 1:1 (v/v) DCM-MeOH (12 mL) until the solution reached pH 11. Then 1 M NaOH (0.1 mL) was added and the mixture was stirred at 40 °C overnight. After complete consumption of the starting material, the reaction was diluted with DCM (15 mL) and washed with H_2O and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 8/1) to give **2-67** as a colorless syrup (199 mg, 92%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 – 7.73 (m, 4H), 7.53 – 7.44 (m, 3H), 7.42 (d, *J* = 7.6 Hz, 2H), 7.39 – 7.12 (m, 18H), 5.17 (d, *J* = 16.2 Hz, 2H), 4.94 (d, *J* = 3.6 Hz, 1H), 4.83 – 4.67 (m, 5H), 4.67 – 4.58 (m, 2H), 4.49 (d, *J* = 9.8 Hz, 2H), 4.04 – 3.95 (m, 2H), 3.87 (dd, *J* = 10.4, 3.1 Hz, 1H), 3.77 (s, 1H), 3.72 – 3.63 (m, 2H), 3.62 – 3.46 (m, 3H), 3.35 – 3.11 (m, 3H), 1.56 – 1.41 (m, 4H), 1.31 – 1.23 (m, 5H), 1.06 (d, *J* = 6.6 Hz, 3H); 13C NMR (151 MHz, $CDCl_3$) δ 138.5, 138.1, 138.0, 134.6, 133.34, 133.32, 129.1, 128.7, 128.6, 128.59, 128.50, 128.4, 128.3, 128.2, 128.08, 128.05, 127.98, 127.95, 127.93, 127.8, 127.6, 127.4, 127.3, 127.2, 127.0, 126.4, 126.3, 125.8, 97.6, 93.8, 79.9, 76.3, 75.2, 74.4, 73.5, 72.8, 71.9, 68.8, 68.1, 67.5, 67.3, 65.5, 59.0, 50.6, 50.3, 47.2, 46.2, 29.8, 29.2, 28.0, 27.6, 23.4, 18.1, 16.0; HRMS (ESI) calculated for $C_{57}H_{64}N_4O_{10}$ $[M+Na]^+$: 987.4514, found: 987.4521.

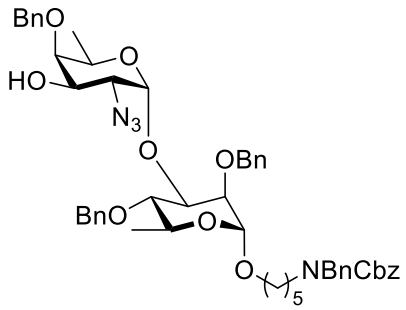
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-azido-2-deoxy-3-_O_-(2-naphthylmethyl)-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-68)



To a solution of **2-67** (33 mg, 0.0342 mmol) in anhydrous DMF (1 mL) at 0 °C, NaH (as a 60% mineral oil dispersion, 2.74 mg, 0.0684 mmol) was added and the resulting solution was stirred for 10 min, then benzyl bromide (8.2 µL, 0.0684 mmol) was added, the mixture was warmed to room temperature and stirred overnight. Excess NaH was quenched using MeOH, and the bulk of the DMF removed under reduced pressure. The syrup was redissolved in DCM (10 mL) and washed twice with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash silica column chromatography (toluene/ethyl acetate = 20/1) to afford **2-68** as a colorless syrup (30 mg, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.74 (m, 4H), 7.54 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.51 – 7.42 (m, 4H), 7.41 – 7.26 (m, 20H), 7.21 (dt, *J* = 10.7, 4.8 Hz, 3H), 5.20 (d, *J* = 11.1 Hz, 2H), 5.00 (d, *J* = 3.3 Hz, 1H), 4.93 (d, *J* = 11.5 Hz, 1H), 4.84 – 4.56 (m, 8H), 4.52 (d, *J* = 7.8 Hz, 2H), 4.08 – 3.87 (m, 4H), 3.80 (s, 1H), 3.74 – 3.64 (m, 1H), 3.65 – 3.50 (m, 2H), 3.45 (s, 1H), 3.25 (m, 3H), 1.63 – 1.40 (m, 4H), 1.31 (d, *J* = 6.1 Hz, 3H), 1.28 – 1.16 (m, 2H), 1.00 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.7, 156.2, 138.5, 138.3, 138.1, 137.9, 136.9, 136.8, 135.1, 133.3, 133.16, 133.12, 128.6, 128.56, 128.52, 128.48, 128.46, 128.39, 128.35, 128.28, 128.24, 128.20, 128.16, 128.10, 128.0, 127.98, 127.92, 127.8, 127.79, 127.77, 127.6, 127.4, 127.3, 127.2, 126.6, 126.3, 126.1, 125.7, 97.5, 93.9, 79.8, 77.5, 77.3, 76.2, 75.1, 75.0, 74.3, 73.3, 72.7, 72.1, 68.0, 67.46, 67.42, 67.2, 66.6, 59.6, 50.5, 50.2, 47.1, 46.1, 29.1, 27.9, 27.5, 23.4, 18.1, 16.5; HRMS (ESI) calculated for $C_{64}H_{70}N_4O_{10}$ $[M+Na]^+$: 1077.4984, found: 1077.4988.

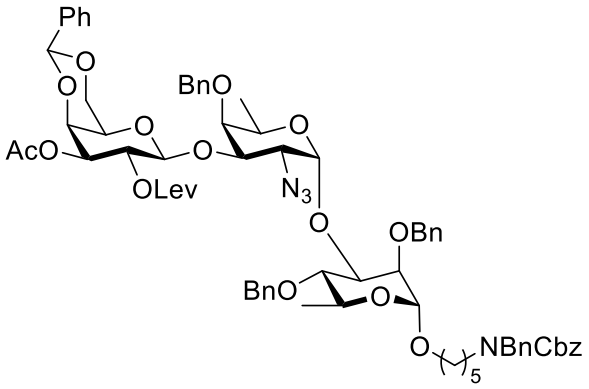
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-azido-2-deoxy-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-69)



To a solution of **2-68** (300 mg, 0.28 mmol) and *β*-pinene (152 µL, 0.97 mmol) in 20:1 (v/v) DCM-H_2O (10 mL), DDQ (120 mg, 0.53 mmol) was added and the reaction mixture was stirred for 5 h. The reaction was then diluted with DCM (20 mL) and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 15/1) to give **2-69** as a colorless syrup (200 mg, 76%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.16 (m, 25H), 5.20 (d, *J* = 12.5 Hz, 2H), 5.00 (d, *J* = 3.5 Hz, 1H), 4.86 – 4.67 (m, 6H), 4.60 (d, *J* = 11.6 Hz, 1H), 4.52 (d, *J* = 7.8 Hz, 2H), 4.09 – 3.93 (m, 3H), 3.80 (s, 1H), 3.75 – 3.67 (m, 1H), 3.59 (dt, *J* = 27.3, 9.3 Hz, 2H), 3.44 (d, *J* = 10.7 Hz, 1H), 3.37 – 3.14 (m, 4H), 2.22 (d, *J* = 9.6 Hz, 1H), 1.64 – 1.40 (m, 4H), 1.34 (d, *J* = 6.0 Hz, 3H), 1.31 – 1.17 (m, 2H), 1.07 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.7, 156.2, 138.5, 138.49, 138.46, 138.0, 137.95, 137.91, 137.8, 136.9, 136.7, 128.7, 128.6, 128.5, 128.49, 128.45, 128.42, 128.3, 128.26, 128.20, 128.09, 128.02, 127.9, 127.89, 127.83, 127.78, 127.70, 127.49, 127.40, 127.3, 127.2, 97.5, 93.89, 93.86, 80.1, 79.9, 76.1, 75.3, 74.3, 73.4, 72.7, 68.7, 68.0, 67.46, 67.41, 67.24, 67.22, 67.20, 66.4, 60.9, 50.5, 50.2, 47.1, 46.1, 29.1, 29.09, 29.08, 27.9, 27.5, 23.4, 18.0, 16.5; HRMS (ESI) calculated for $C_{53}H_{62}N_4O_{10}$ $[M+Na]^+$: 937.4358, found: 937.4378.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-_O_-benzylidene-2-_O_-levulinoyl-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-70)

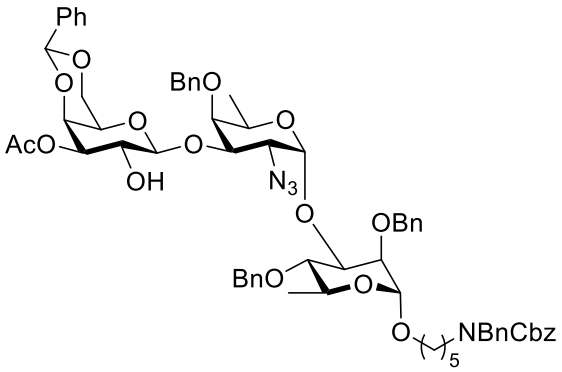


Disaccharide alcohol **2-69** (125 mg, 0.14 mmol) and thioglycoside **2-18** (82 mg, 0.16 mmol) were coevaporated three times with toluene and the resulting mixture was dried under vacuum for 2 h. Then the mixture was dissolved in anhydrous DCM (10 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to −40 °C, NIS (36.9 mg, 0.41 mmol) and TfOH (1.5 µL, 0.016 mmol) were added. The reaction mixture was stirred at −40 °C for 3 h. After complete consumption of the staring material, Et_3N (0.1 mL) was added and the mixture was stirred for 10 min. Then the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 5/1) to give **2-70** as a colorless syrup (155 mg, 87%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.10 (m, 30H), 5.48 (dd, *J* = 10.4, 7.8 Hz, 1H), 5.46 (s, 1H), 5.14 (d, *J* = 17.0 Hz, 2H), 5.05 (d, *J* = 11.5 Hz, 1H), 4.98 – 4.91 (m, 2H), 4.79 (d, *J* = 11.4 Hz, 1H), 4.74 – 4.53 (m, 6H), 4.45 (d, *J* = 11.0 Hz, 2H)zz, 4.32 (d, *J* = 3.5 Hz, 1H), 4.16 – 4.02 (m, 2H), 4.02 – 3.94 (m, 3H), 3.77 – 3.70 (m, 2H), 3.69 – 3.60 (m, 2H), 3.57 (t, *J* = 9.4 Hz, 1H), 3.54 – 3.46 (m, 1H), 3.37 (s, 1H), 3.29 – 3.07 (m, 3H), 2.81 (m, 1H), 2.73 – 2.47 (m, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 1.55 – 1.37 (m, 4H), 1.28 (d, *J* = 6.1 Hz, 3H), 1.26 – 1.12 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.4, 171., 171.22, 156.8, 156.2, 138.76, 138.71, 138.6, 138.09, 138.01, 137.4, 137.0, 136.8, 129.2, 128.7, 128.6, 128.5, 128.4, 128.34, 128.30, 128.19, 128.13, 128.0, 127.9, 127.6, 127.4, 127.38, 127.31, 127.1, 126.4, 102.4, 101.3, 97.4, 94.1, 79.9,

79.0, 78.4, 75.2, 75.1, 74.3, 73.4, 73.3, 72.6, 71.6, 68.9, 67.9, 67.5, 67.2, 66.5, 66.3, 59.7, 50.6, 50.3, 47.1, 46.2, 37.8, 29.89, 29.81, 29.7, 29.1, 28.0, 27.9, 27.5, 23.4, 21.0, 18.1, 16.3; HRMS (ESI) calculated for $C_{73}H_{84}N_4O_{18}$ $[M+Na]^+$: 1327.5673, found: 1327.5669.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-_O_-benzylidene-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-71)

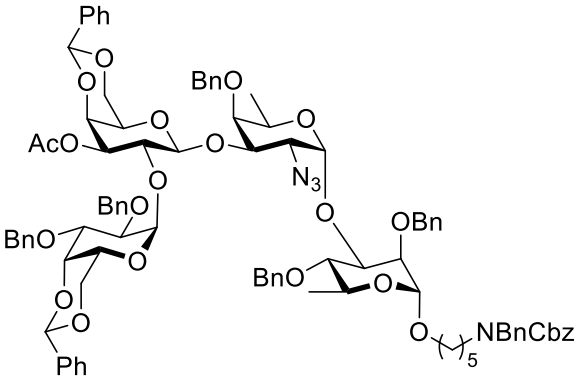


To a solution of **2-70** (150 mg, 0.12 mmol) in DCM (10 mL), N_2H_4·AcOH (21 mg, 0.23 mmol) was added and the mixture was stirred at room temperature for 6 h. The reaction was quenched by the addition of acetone (1 mL) and the solvent was removed under vacuum. The residue was purified by flash silica column chromatography (toluene/ethyl acetate = 5/1) to give **2-71** as a colorless syrup (123 mg, 90%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35 – 7.07 (m, 30H), 5.41 (s, 1H), 5.09 (d, *J* = 16.6 Hz, 2H), 4.98 (d, *J* = 11.6 Hz, 1H), 4.92 (d, *J* = 3.5 Hz, 1H), 4.80 (dd, *J* = 10.3, 3.5 Hz, 1H), 4.75 (d, *J* = 11.4 Hz, 1H), 4.71 – 4.54 (m, 5H), 4.47 (d, *J* = 7.5 Hz, 1H), 4.41 (d, *J* = 10.8 Hz, 2H), 4.29 (d, *J* = 3.5 Hz, 1H), 4.08 (d, *J* = 12.4 Hz, 1H), 4.05 – 3.88 (m, 5H), 3.79 (dd, *J* = 10.7, 3.5 Hz, 1H), 3.71 (s, 1H), 3.65 (s, 1H), 3.63 – 3.56 (m, 1H), 3.53 (t, *J* = 9.3 Hz, 1H), 3.50 – 3.40 (m, 1H), 3.32 (s, 1H), 3.26 – 3.04 (m, 3H), 2.58 (s, 1H), 2.05 (s, 3H), 1.50 – 1.31 (m, 4H), 1.24 (d, *J* = 6.1 Hz, 3H), 1.16 – 1.07 (m, 2H), 0.87 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.0, 156.8, 156.2, 138.76, 138.70, 138.08, 138.00, 137.7, 136.9, 136.8, 129.2, 128.6, 128.56, 128.51, 128.48, 128.43, 128.3, 128.2, 128.09, 128.02, 127.93, 127.92, 127.6, 127.5, 127.3, 127.2, 127.0, 126.3, 105.2,

101.3, 97.4, 94.0, 79.9, 79.8, 79.1, 75.1, 75.0, 74.5, 73.6, 73.3, 73.2, 72.6, 69.0, 68.8, 68.0, 67.5, 67.2, 66.49, 66.41, 59.7, 50.6, 50.3, 47.1, 46.2, 32.0, 29.8, 29.7, 29.6, 29.46, 29.42, 29.3, 29.2, 29.1, 28.0, 27.5, 23.4, 22.7, 21.1, 18.1, 16.3, 14.2; HRMS (ESI) calculated for $C_{68}H_{78}N_4O_{16}$ $[M+Na]^+$: 1229.5305, found: 1229.5300.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-_O_-benzyl-4,6-_O_-benzylidene-_α_-D-galactopyranosyl-(1→2)-4,6-_O_-benzylidene-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-72)

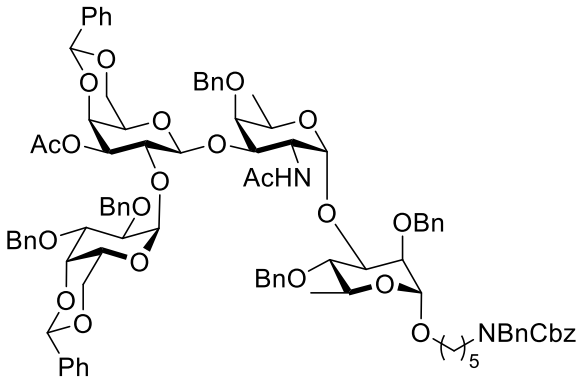


Trisaccharide alcohol **2-71** (140 mg, 0.12 mmol) and thioglycoside **2-19** (94 mg, 0.17 mmol) were coevaporated three times with toluene and the resulting mixture was dried under vacuum for 2 h. The mixture was dissolved in anhydrous Et_2O/DCM=4/1 (5 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to 0 °C, NIS (39 mg, 0.17 mmol) and TfOH (1.5 μL, 0.017 mmol) were added. The reaction mixture was stirred at 0 °C for 3 h. After complete consumption of the starting material, Et_3N (0.1 mL) was added and the mixture was stirred for 10 min. Then, the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 7/1) to give **2-72** as a colorless syrup (139 mg, 74%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 – 7.44 (m, 2H), 7.38 – 7.05 (m, 43H), 5.74 (d, *J* = 3.3 Hz, 1H), 5.40 (s, 1H), 5.37 (s, 1H), 5.09 (d, *J* = 16.6 Hz, 2H), 4.99 (d, *J* = 11.0 Hz, 1H), 4.92 – 4.83 (m,

3H), 4.77 – 4.50 (m, 10H), 4.40 (d, *J* = 10.9 Hz, 2H), 4.30 (d, *J* = 3.6 Hz, 1H), 4.27 – 4.19 (m, 2H), 4.15 (d, *J* = 12.3 Hz, 1H), 4.03 – 3.93 (m, 5H), 3.91 – 3.83 (m, 3H), 3.74 (s, 1H), 3.69 (d, *J* = 8.4 Hz, 2H), 3.63 – 3.56 (m, H), 3.53 (t, *J* = 9.3 Hz, 1H), 3.49 – 3.41 (m, 2H), 3.33 (s, 1H), 3.24 – 3.06 (m, 3H), 1.75 (s, 3H), 1.48 – 1.32 (m, 4H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.16 – 1.05 (m, 2H), 0.95 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.1, 170.0, 156.8, 156.3, 139.01, 139.00, 138.75, 138.74, 138.67, 138.63, 138.1, 138.0, 137.9, 137.82, 137.80, 137.0, 129.2, 129.1, 129.0, 128.66, 128.64, 128.58, 128.53, 128.52, 128.42, 128.41, 128.36, 128.34, 128.32, 128.29, 128.26, 128.24, 128.22, 128.06, 128.04, 128.00, 127.99, 127.95, 127.92, 127.91, 127.7, 127.67, 127.63, 127.4, 127.36, 127.34, 127.31, 127.2, 126.59, 126.57, 126.45, 126.44, 103.4, 101.2, 101.1, 97.4, 96.6, 94.2, 79.8, 79.7, 77.4, 77.3, 77.1, 77.0, 76.9, 75.8, 75.6, 75.4, 75.3, 75.2, 75.2, 74.8, 73.9, 73.3, 73.2, 73.19, 73.16, 72.59, 72.58, 72.45, 72.43, 70.1, 69.5, 68.9, 67.9, 67.5, 67.2, 66.4, 66.0, 62.9, 58.8, 50.6, 50.3, 47.2, 46.2, 32.0, 29.8, 29.7, 29.6, 29.49, 29.44, 29.3, 29.1, 28.0, 27.5, 27.3, 23.4, 22.8, 20.99, 20.97, 18.0, 16.36, 16.34, 14.2; HRMS (ESI) calculated for $C_{95}H_{104}N_4O_{21}$ $[M+Na]^+$: 1659.7085, found: 1659.7089.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-_O_-benzyl-4,6-_O_-benzylidene-_α_-D-galactopyranosyl-(1→2)-4,6-_O_-benzylidene-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-73)

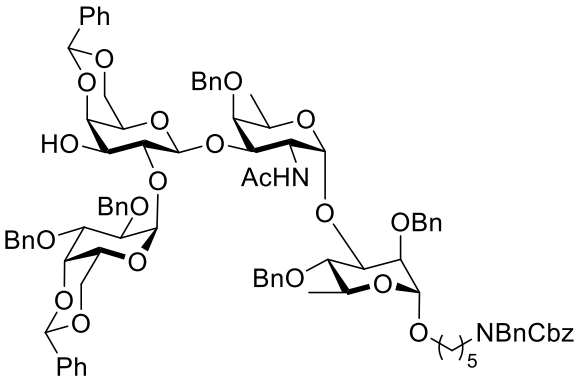


Zinc (100 mg) was added to a stirred solution of **2-72** (35 mg, 0.021 mmol) in acetic anhydride (1 mL) under nitrogen. The mixture was stirred for 12 h at room temperature. The solution was then

filtered and washed with sat. $NaHCO_3$ (20mL×2) and brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 1/1) to give **2-73** as colorless syrup (26 mg, 76%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 – 7.10 (m, 45H), 5.99 (d, *J* = 7.9 Hz, 1H), 5.53 (d, *J* = 3.0 Hz, 1H), 5.49 (s, 1H), 5.42 (s, 1H), 5.22 – 5.13 (m, 3H), 5.08 (d, *J* = 11.7 Hz, 1H), 4.93 – 4.86 (m, 1H), 4.75 – 4.46 (m, 12H), 4.38 – 4.29 (m, 3H), 4.21 – 4.11 (m, 4H), 4.09 – 3.96 (m, 5H), 3.91 (d, *J* = 12.3 Hz, 1H), 3.72 (t, *J* = 10.6 Hz, 3H), 3.68 – 3.63 (m, 1H), 3.61 – 3.47 (m, 1H), 3.45 – 3.36 (m, 2H), 3.31 – 3.11 (m, 3H), 1.96 (s, 3H), 1.66 (s, 3H), 1.55 – 1.41 (m, 4H), 1.28 (d, *J* = 8.4 Hz, 3H), 1.23 – 1.16 (m, 2H), 0.90 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.3, 156.8, 156.3, 139.19, 139.12, 138.8, 138.7, 138.3, 138.1, 138.0, 137.7, 136.9, 130.1, 129.8, 129.2, 128.9, 128.67, 128.61, 128.57, 128.54, 128.46, 128.41, 128.36, 128.33, 128.31, 128.2, 128.1, 128.0, 127.97, 127.96, 127.8, 127.7, 127.6, 127.58, 127.55, 127.48, 127.40, 127.2, 127.0, 126.59, 126.51, 126.4, 103.2, 101.2, 101.0, 97.5, 97.2, 80.0, 79.3, 76.6, 75.7, 75.6, 75.0, 74.6, 74.3, 74.0, 73.3, 73.0, 72.6, 71.6, 71.2, 69.3, 68.9, 67.9, 67.6, 67.2, 66.6, 66.2, 63.2, 50.6, 50.3, 49.3, 47.2, 46.3, 39.6, 37.1, 36.0, 32.07, 32.04, 30.1, 29.9, 29.88, 29.84, 29.79, 29.76, 29.66, 29.64, 29.5, 29.46, 29.42, 29.38, 29.35, 29.26, 29.22, 28.0, 27.6, 27.36, 27.33, 27.31, 27.0, 25.9, 25.6, 23.5, 22.8, 21.6, 21.2, 18.2, 16.5, 14.2; HRMS (ESI) calculated for $C_{97}H_{108}N_2O_{22}$ $[M+Na]^+$: 1675.7286, found: 1675.7280.

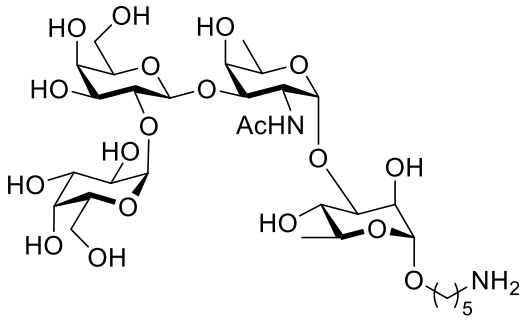
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-_O_-benzyl-4,6-_O_-benzylidene-_α_-D-galactopyranosyl-(1→2)-4,6-_O_-benzylidene-_β_-D-galactopyranosyl-(1→3)-2- acetamido-2-deoxy-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-74)



NaOMe was added slowly to a solution of **2-73** (130 mg, 0.079 mmol) in MeOH/DCM=1/1 (5 mL) until the solution reached pH 11. The reaction mixture was stirred for 1 h, neutralized with Amberlite, filtered and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (toluene/ethyl acetate = 2/3) to give **2-74** as a colorless syrup (118 mg, 93%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 – 6.98 (m, 45H), 5.94 (d, *J* = 6.2 Hz, 1H), 5.45 (s, 1H), 5.33 (s, 1H), 5.30 (d, *J* = 3.4 Hz, 1H), 5.24 – 5.19 (m, 1H), 5.09 (d, *J* = 16.7 Hz, 2H), 5.00 (d, *J* = 11.7 Hz, 1H), 4.65 (q, *J* = 12.3 Hz, 4H), 4.60 – 4.51 (m, 5H), 4.48 – 4.33 (m, 5H), 4.29 (d, *J* = 11.5 Hz, 1H), 4.19 – 4.10 (m, 2H), 4.10 – 4.01 (m, 4H), 3.99 – 3.88 (m, 5H), 3.85 (d, *J* = 12.5 Hz, 1H), 3.75 (t, *J* = 8.5 Hz, 1H), 3.69 – 3.63 (m, 2H), 3.59 – 3.53 (m, 1H), 3.50 – 3.39 (m, 1H), 3.35 (t, *J* = 9.8 Hz, 1H), 3.23 (s, 1H), 3.20 – 3.06 (m, 3H), 1.66 (s, 3H), 1.48 – 1.32 (m, 4H), 1.18 (d, *J* = 5.1 Hz, 3H), 1.14 – 1.06 (m, 2H), 0.86 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.8, 156.8, 156.2, 139.2, 138.9, 138.8, 138.59, 138.55, 138.0, 137.5, 137.0, 134.6, 130.0, 129.9, 129.4, 129.3, 129.17, 129.12, 129.0, 128.6, 128.5, 128.49, 128.46, 128.42, 128.3, 128.2, 128.0, 127.9, 127.8, 127.76, 127.73, 127.69, 127.63, 127.60, 127.5, 127.3, 127.1, 126.9, 126.5, 126.49, 126.45, 125.42, 102.6, 101.7, 101.1, 98.8, 97.7, 97.3, 79.9, 79.6, 79.2, 78.3, 76.7, 75.9, 75.6, 75.0, 74.9, 74.6, 74.0, 73.8, 72.9, 72.6, 71.9, 71.1, 71.0, 69.4, 69.0, 68.0, 67.6, 67.2, 66.6, 66.4, 63.1, 50.6, 50.3, 50.1, 47.2, 46.3, 33.7, 32.0, 29.8, 29.6, 29.4, 29.2, 28.0, 27.6, 23.7, 23.5, 22.8, 18.2, 16.6, 16.5, 14.2; HRMS (ESI) calculated for $C_{95}H_{106}N_2O_{21}$ $[M+Na]^+$: 1633.7180, found: 1633.7174.

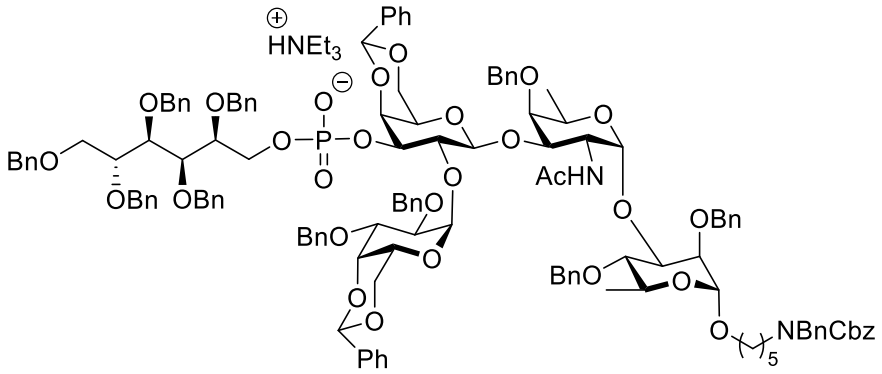
5-Amino-pentyl *α*-D-galactopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*α*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside (2-55)



In a 20 mL vial, compound **2-74** (10 mg, 6.2 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and the crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-55** (1.9 mg, 40%) as a white solid.

^{1}H NMR (700 MHz, D_2O) δ 8.46 (s, 1H), 5.35 (d, *J* = 4.0 Hz, 1H), 4.98 (d, *J* = 3.8 Hz, 1H), 4.80 (s, 1H), 4.69 (d, *J* = 7.4 Hz, 1H), 4.37 (q, *J* = 6.6 Hz, 1H), 4.32 (dd, *J* = 11.2, 3.8 Hz, 1H), 4.25 (t, *J* = 6.3 Hz, 1H), 4.22 (dd, *J* = 11.2, 3.0 Hz, 1H), 4.14 – 4.11 (m, 1H), 4.03 – 3.99 (m, 2H), 3.95 – 3.90 (m, 2H), 3.83 – 3.66 (m, 11H), 3.56 – 3.50 (m, 2H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.08 (s, 3H), 1.72 – 1.61 (m, 4H), 1.52 – 1.40 (m, 2H), 1.32 (d, *J* = 6.2 Hz, 3H), 1.23 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (176 MHz, D_2O) δ 174.3, 171.0, 103.5, 99.5, 97.0, 94.4, 76.4, 75.6, 74.9, 74.3, 71.5, 70.7, 70.3, 69.4, 69.1, 68.8, 68.59, 68.53, 67.5, 66.9, 66.3, 60.9, 60.8, 48.3, 39.4, 28.0, 26.7, 22.4, 22.1, 16.6, 15.4; HRMS (ESI) calculated for $C_{31}H_{56}N_2O_{19}$ $[M+H]^+$: 761.3550, found: 761.3560.

Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*-D-galactopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-(2,3,4,5,6-penta-*O*-benzyl-D-glucityl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4-*O*-benzyl-*α*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-75)

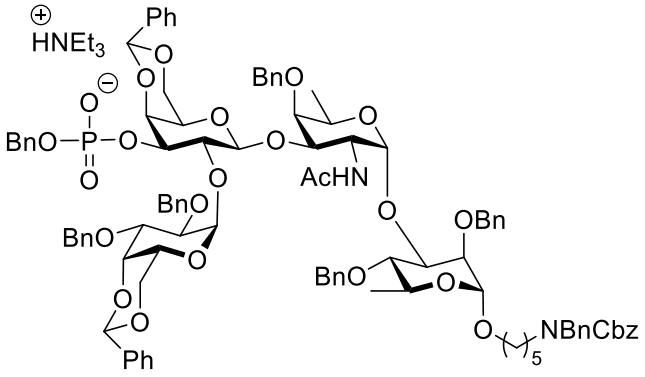


Tetrasaccharide alcohol **2-74** (30 mg, 18.6 μmol) and *H*-phosphonate **2-13** (29.7 mg, 37.3 μmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (11.4 μL, 93.1 μmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine (23.6 mg, 93.1 μmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with CH_2Cl_2, washed with saturated $Na_2S_2O_3$ solution and TEAB buffer, and then dried over Na_2SO_4. The crude product obtained after solvent removal under reduced pressure was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 60/2/1) to give **2-75** as a triethylammonium salt (39 mg, 87%).

^{1}H NMR (700 MHz, Acetone-d_6) δ 12.38 (s, 1H), 7.54 – 7.06 (m, 70H), 6.76 (d, J = 8.6 Hz, 1H), 5.79 (d, J = 3.3 Hz, 1H), 5.53 (s, 1H), 5.38 (s, 1H), 5.21 (d, J = 11.4 Hz, 1H), 5.19 – 5.08 (m, 3H), 4.88 (dd, J = 16.9, 10.0 Hz, 2H), 4.85 – 4.63 (m, 12H), 4.64 – 4.39 (m, 16H), 4.30 – 4.16 (m, 3H), 4.13 – 4.00 (m, 6H), 4.01 – 3.95 (m, 3H), 3.95 – 3.90 (m, 1H), 3.89 – 3.81 (m, 2H), 3.81 – 3.72 (m, 3H), 3.68 – 3.61 (m, 1H), 3.62 – 3.50 (m, 1H), 3.47 (t, J = 9.4 Hz, 1H), 3.41 – 3.18 (m, 5H), 3.02 (q, J = 7.3 Hz, 12H), 1.72 (s, 3H), 1.57 – 1.43 (m, 4H), 1.30 – 1.28 (m, 2H), 1.27 (d, J = 6.2 Hz, 3H), 1.24 (t, J = 7.3 Hz, 18H), 0.91 (d, J = 6.4 Hz, 3H); ^{13}C NMR (176 MHz, Acetone-d_6) δ 172.2, 170.4, 141.1, 140.7, 140.6, 140.36, 140.33, 140.31, 140.2, 140.1, 140.0, 139.99, 139.91, 139.6, 138.4, 129.5, 129.4, 129.3, 129.29, 129.25, 129.19, 129.16, 129.12, 129.10, 129.0, 128.9,

128.8, 128.79, 128.73, 128.69, 128.66, 128.63, 128.61, 128.5, 128.49, 128.47, 128.44, 128.41, 128.37, 128.32, 128.27, 128.23, 128.18, 128.13, 128.0, 127.9, 127.87, 127.85, 127.80, 127.6, 127.4, 127.3, 105.2, 101.6, 101.2, 98.3, 97.5, 94.2, 81.0, 80.9, 80.7, 80.6, 80.4, 80.2, 77.4, 76.4, 76.3, 76.0, 75.7, 75.7, 75.5, 75.3, 75.0, 74.9, 74.8, 74.7, 74.5, 73.9, 73.5, 73.2, 72.7, 72.6, 71.9, 70.9, 70.1, 69.6, 68.8, 68.0, 67.5, 67.2, 67.0, 65.8, 63.7, 51.0, 50.8, 50.1, 47.8, 47.0, 46.3, 34.6, 32.7, 30.7, 30.7, 30.5, 30.52, 30.50, 30.48, 30.46, 30.43, 30.39, 30.34, 30.32, 29.5, 28.8, 28.2, 25.9, 24.2, 23.8, 23.4, 20.9, 18.7, 17.1, 14.4, 9.0, 8.9, 8.8, 8.3; ^{31}P NMR (162 MHz, Acetone-d_6) δ -1.28; HRMS (ESI) calculated for $C_{142}H_{164}N_3O_{29}P$ [M-Et_3N-H]$^-$: 2303.9911, found: 2303.9722.

Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*-D-galactopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-(benzyl phospho)-*β*-D-galactopyranosyl-(1→3)-2- acetamido-2-deoxy-4-*O*-benzyl-*α*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-76)

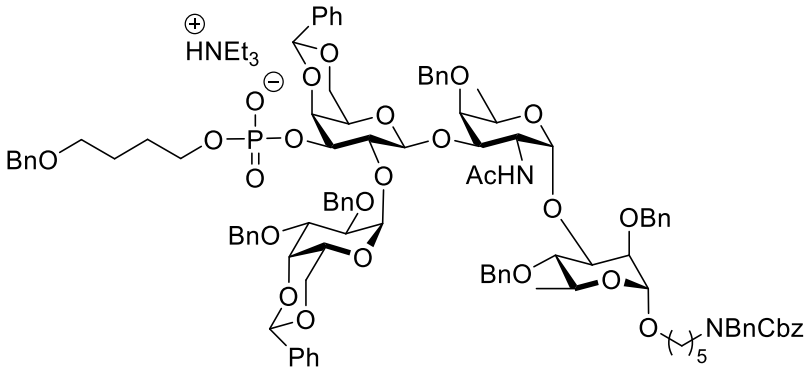


Tetrasaccharide alcohol **2-74** (30 mg, 18.6 µmol) and *H*-phosphonate **2-14** (10.2 mg, 37.3 µmol) were coevaporated three time with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (11.4 µL, 93.1 µmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine (23.6 mg, 93.1 µmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with CH_2Cl_2, washed with saturated $Na_2S_2O_3$ solution and TEAB buffer, and then dried over Na_2SO_4. The crude product obtained after solvent removal under reduced pressure was purified by

flash silica column chromatography (ethyl acetate/MeOH/H_2O = 30/2/1) to give **2-76** as a triethylammonium salt (28 mg, 79%).

^{1}H NMR (700 MHz, Acetone-d_6) δ 12.11 (s, 1H), δ 7.49 – 7.14 (m, 50H), 6.86 (d, *J* = 8.7 Hz, 1H), 5.81 (d, *J* = 3.3 Hz, 1H), 5.52 (d, *J* = 21.8 Hz, 2H), 5.25 (d, *J* = 11.3 Hz, 1H), 5.21 – 5.03 (m, 4H), 5.03 – 4.92 (m, 2H), 4.87 (d, *J* = 12.4 Hz, 1H), 4.79 – 4.74 (m, 2H), 4.74 – 4.66 (m, 3H), 4.66 – 4.57 (m, 3H), 4.56 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.54 – 4.43 (m, 5H), 4.43 – 4.35 (m, 2H), 4.29 – 4.24 (m, 2H), 4.10 – 4.01 (m, 6H), 3.94 (d, *J* = 12.2 Hz, 1H), 3.87 – 3.78 (m, 2H), 3.68 – 3.50 (m, 3H), 3.46 (t, *J* = 9.4 Hz, 1H), 3.36 – 3.18 (m, 4H), 3.06 (q, *J* = 7.3 Hz, 12H), 1.73 (s, 3H), 1.57 – 1.43 (m, 3H), 1.30 – 1.29 (m, 2H), 1.28 (d, *J* = 2.2 Hz,3H), 1.26 (t, *J* = 7.3 Hz, 18H), 0.93 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (176 MHz, Acetone-d_6) δ 179.9, 175.0, 172.3, 170.48, 170.40, 157.2, 156.6, 141.0, 140.7, 140.5, 140.4, 140.3, 140.1, 140.0, 139.9, 139.6, 138.4, 129.6, 129.4, 129.38, 129.35, 129.28, 129.25, 129.24, 129.1, 129.0, 128.99, 128.93, 128.90, 128.87, 128.84, 128.81, 128.77, 128.77, 128.74, 128.73, 128.49, 128.44, 128.40, 128.3, 128.2, 128.19, 128.17, 128.0, 127.9, 127.89, 127.81, 127.6, 127.3, 105.2, 101.9, 101.3, 98.3, 97.6, 94.2, 80.7, 80.6, 77.6, 76.4, 76.0, 75.9, 75.83, 75.80, 75.5, 74.9, 74.7, 74.4, 73.2, 72.66, 72.61, 72.0, 71.0, 70.1, 69.8, 68.8, 68.0, 67.86, 67.83, 67.5, 67.1, 67.0, 63.7, 58.5, 51.0, 50.8, 50.0, 49.9, 47.8, 47.0, 46.4, 46.3, 34.6, 32.7, 30.7, 30.59, 30.57, 30.51, 30.50, 30.48, 30.46, 30.43, 30.39, 30.35, 30.32, 30.2, 29.58, 29.51, 28.8, 28.2, 27.7, 25.9, 24.2, 23.8, 23.7, 23.4, 21.0, 18.6, 17.1, 14.4, 8.9, 8.3; ^{31}P NMR (162 MHz, Acetone-d_6) δ -2.03; HRMS (ESI) calculated for $C_{108}H_{128}N_3O_{24}P$ [M-Et_3N-H]$^-$: 1779.7348, found: 1779.7379.

Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*-D-galactopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-(4-*O*-benzyl-butanyl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4-*O*-benzyl-*α*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-77)

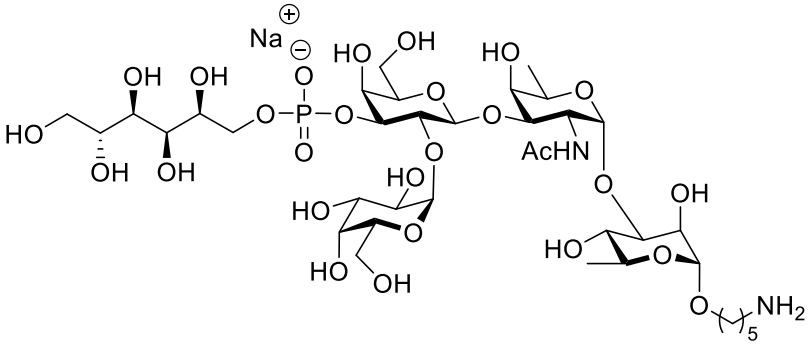


Tetrasaccharide alcohol **2-74** (20 mg, 12.42 μmol) and *H*-phosphonate **2-15** (8.6 mg, 24.83 μmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (7.6 μL, 62.08 μmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine (15.8 mg, 62.08 μmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with CH_2Cl_2, washed with saturated $Na_2S_2O_3$ solution and TEAB buffer, and then dried over Na_2SO_4. The crude product obtained after solvent removal under reduced pressure was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 30/2/1) to give **2-77** as a triethylammonium salt (15 mg, 62%).

^{1}H NMR (700 MHz, Acetone-d_6) δ 11.85 (s, 1H), 7.54 – 7.11 (m, 50H), 6.86 (d, *J* = 7.5 Hz, 1H), 5.80 (d, *J* = 3.3 Hz, 1H), 5.62 (s, 1H), 5.56 (s, 1H), 5.24 (d, *J* = 11.3 Hz, 1H), 5.20 – 5.08 (m, 3H), 4.92 (dd, *J* = 41.2, 10.0 Hz, 2H), 4.83 – 4.66 (m, 6H), 4.65 – 4.54 (m, 5H), 4.52 (s, 2H), 4.48 – 4.33 (m, 5H), 4.30 – 4.21 (m, 2H), 4.15 – 3.92 (m, 8H), 3.84 (d, *J* = 10.2 Hz, 1H), 3.80 (s, 1H), 3.69 – 3.51 (m, 4H), 3.46 (t, *J* = 9.4 Hz, 1H), 3.42 – 3.20 (m, 6H), 3.08 (q, *J* = 7.3 Hz, 11H), 1.73 (s, 3H), 1.70 – 1.57 (m, 4H), 1.57 – 1.42 (m, 4H), 1.30 – 1.28 (m, 2H), 1.28 (t, 19H), 1.17 (d, *J* = 2.7 Hz, 3H), 0.93 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (176 MHz, Acetone-d_6) δ 179.9, 172.3, 170.4, 170.4, 157.2, 156.6, 141.1, 140.7, 140.5, 140.3, 140.18, 140.13, 140.0, 139.9, 139.5, 138.4, 129.6, 129.5, 129.4, 129.37, 129.34, 129.27, 129.23, 129.19, 129.16, 129.0, 128.97, 128.91, 128.85,

128.80, 128.75, 128.71, 128.6, 128.48, 128.43, 128.35, 128.30, 128.2, 128.1, 128.0, 127.9, 127.87, 127.80, 127.65, 127.61, 127.4, 127.3, 105.1, 101.8, 101.4, 101.3, 98.3, 97.5, 94.1, 80.7, 80.6, 77.5, 76.4, 76.0, 75.8, 75.7, 75.5, 74.9, 74.8, 74.4, 73.28, 73.20, 72.5, 72.0, 71.0, 70.8, 70.1, 69.8, 68.8, 68.0, 67.5, 67.1, 67.0, 66.2, 63.7, 58.5, 51.0, 50.8, 50.0, 49.9, 47.9, 47.8, 47.0, 46.5, 46.4, 38.8, 32.7, 30.7, 30.5, 30.56, 30.50, 30.48, 30.47, 30.45, 30.42, 30.38, 30.34, 30.34, 30.31, 29.5, 28.8, 28.75, 28.70, 28.2, 27.73, 27.71, 27.1, 25.9, 24.2, 23.8, 23.7, 23.4, 21.0, 18.68, 18.63, 17.1, 14.4, 9.0, 8.9, 8.8, 8.3; ^{31}P NMR (162 MHz, Acetone-d_6) δ -2.24; HRMS (ESI) calculated for $C_{112}H_{136}N_3O_{25}P$ [M-Et$_3$N-H]$^-$: 1851.7923, found: 1851.7943.

5-Aminopentyl *α*-D-galactopyranosyl-(1→2)-3-*O*-(D-glucityl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*α*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside sodium salt (2-54)

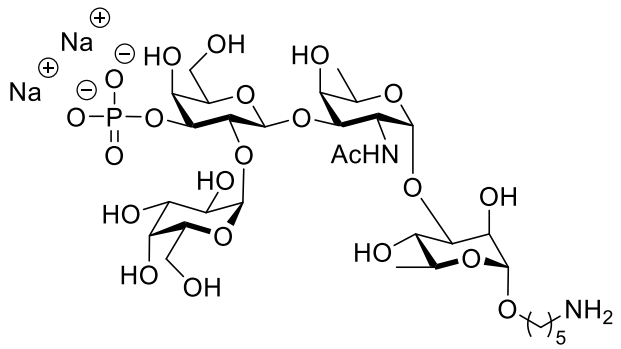


In a 20 mL vial, phosphate **2-75** (13 mg, 5.4 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and then passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-54** (3.0 mg, 54%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 5.35 (d, *J* = 3.9 Hz, 1H), 5.00 (d, *J* = 3.8 Hz, 1H), 4.81 (d, *J* = 1.8 Hz, 1H), 4.77 (d, *J* = 7.8 Hz, 1H), 4.38 (q, *J* = 6.6 Hz, 1H), 4.35 – 4.28 (m, 2H), 4.28 – 4.22 (m, 2H), 4.20 (d, *J* = 3.3 Hz, 1H), 4.15 (d, *J* = 3.0 Hz, 1H), 4.09 – 4.04 (m, 1H), 4.04 – 3.93 (m, 5H), 3.92

– 3.82 (m, 3H), 3.82 – 3.64 (m, 12H), 3.58 – 3.50 (m, 2H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.09 (s, 3H), 1.74 – 1.61 (m, 4H), 1.54 – 1.41 (m, 2H), 1.33 (d, *J* = 6.2 Hz, 3H), 1.24 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.3, 103.5, 99.4, 96.8, 94.4, 76.6, 76.0, 75.9, 75.6, 74.4, 71.9, 71.9, 71.78, 71.72, 71.0, 70.9, 70.8, 70.6, 70.2, 69.3, 69.2, 69.1, 68.55, 68.53, 67.5, 67.4, 66.9, 66.48, 66.44, 66.2, 62.7, 61.2, 60.6, 48.2, 39.3, 27.9, 26.5, 22.4, 22.1, 16.6, 15.3; ^{31}P NMR (243 MHz, D_2O) δ -0.48; HRMS (ESI) calculated for $C_{37}H_{68}N_2NaO_{27}P$ $[M\text{-}Na]^-$: 1003.3753, found: 1003.3751.

5-Aminopentyl *α*-D-galactopyranosyl-(1→2)-3-*O*-phospho-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*α*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside disodium salt (2-56)

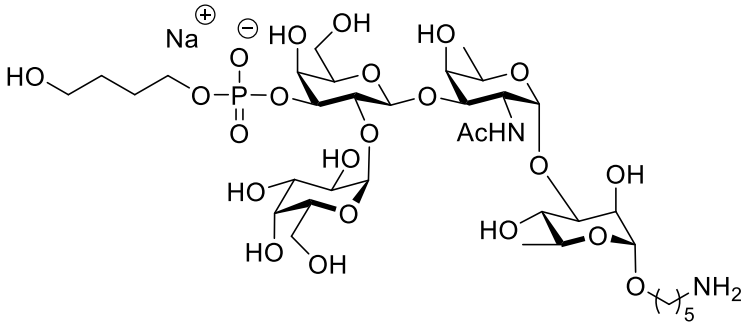


In a 20 mL vial, phosphate **2-76** (10 mg, 5.3 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, reaction mixture was filtered and then passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-56** (2.1 mg, 45%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 8.46 (s, 1H), 5.23 (d, *J* = 4.0 Hz, 1H), 5.03 (d, *J* = 3.7 Hz, 1H), 4.75 (d, *J* = 8.0 Hz, 1H), 4.58 (dd, *J* = 8.2, 4.2 Hz, 1H), 4.39 – 4.34 (m, 1H), 4.31 (dd, *J* = 11.3, 3.6 Hz, 1H), 4.28 (d, *J* = 3.3 Hz, 1H), 4.24 (dd, *J* = 11.2, 2.8 Hz, 1H), 4.18 – 4.14 (m, 1H), 4.08 (ddd, *J* = 9.8, 7.5, 3.3 Hz, 1H), 4.05 – 4.02 (m, 2H), 4.01 (dd, *J* = 3.4, 1.9 Hz, 1H), 3.81 – 3.77 (m, 3H), 3.77 – 3.69 (m, 5H), 3.63 (dd, *J* = 12.0, 8.2 Hz, 1H), 3.57 – 3.51 (m, 2H), 3.00 (dd, *J* = 8.2, 7.0 Hz, 2H),

2.08 (s, 3H), 1.74 – 1.60 (m, 4H), 1.53 – 1.38 (m, 2H), 1.33 (d, *J* = 6.2 Hz, 3H), 1.24 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.1, 103.2, 99.4, 97.9, 94.2, 76.7, 75.5, 74.6, 74.48, 74.43, 74.1, 70.8, 70.1, 70.0, 69.3, 69.2, 68.8, 68.5, 67.7, 67.4, 66.9, 66.3, 61.3, 61.0, 48.3, 39.3, 27.9, 26.5, 22.4, 22.0, 16.6, 15.4; ^{31}P NMR (243 MHz, D_2O) δ 2.58; HRMS (ESI) calculated for $C_{31}H_{55}N_2Na_2O_{22}P$ $[M-2Na+H]^-$: 839.3068, found: 839.3083.

5-Aminopentyl *α*-D-galactopyranosyl-(1→2)-3-*O*-(butanyl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*α*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside sodium salt (2-57)

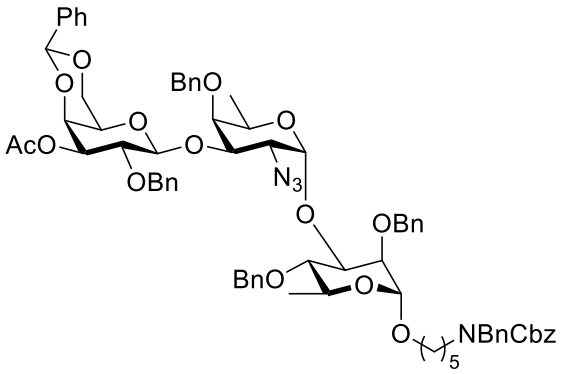


In a 20 mL vial, phosphate **2-77** (15 mg, 7.7 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and then passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-57** (1.7 mg, 24%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 5.34 (d, *J* = 4.0 Hz, 1H), 5.00 (d, *J* = 3.8 Hz, 1H), 4.82 – 4.81 (m, 1H), 4.78 – 4.76 (m, 1H), 4.38 (q, *J* = 6.6 Hz, 1H), 4.35 – 4.29 (m, 2H), 4.24 (dd, *J* = 11.1, 3.0 Hz, 1H), 4.22 – 4.19 (m, 1H), 4.15 (d, *J* = 3.2 Hz, 2H), 4.03 – 3.97 (m, 3H), 3.97 – 3.91 (m, 2H), 3.87 (dd, *J* = 9.8, 7.8 Hz, 1H), 3.81 – 3.69 (m, 9H), 3.65 (t, *J* = 6.4 Hz, 2H), 3.58 – 3.50 (m, 2H), 3.01 (t, 2H), 2.09 (s, 3H), 1.74 – 1.60 (m, 8H), 1.54 – 1.40 (m, 2H), 1.33 (d, *J* = 6.2 Hz, 3H), 1.24 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.3, 103.5, 99.4, 96.8, 94.4, 76.6, 75.85, 75.81, 75.6,

74.4, 71.9, 71.8, 70.8, 70.6, 70.1, 69.22, 69.20, 68.58, 68.52, 67.6, 67.4, 66.9, 66.2, 65.83, 65.80, 61.29, 61.23, 60.6, 48.2, 39.3, 27.9, 27.7, 26.49, 26.48, 26.43, 22.3, 22.1, 16.6, 15.3; ^{31}P NMR (243 MHz, D_2O) δ -0.41; HRMS (ESI) calculated for $C_{35}H_{64}N_2NaO_{23}P$ $[M\text{-}Na]^-$: 911.3643, found: 911.3648.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-_O_-benzylidene-2-_O_-benzyl-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-78)

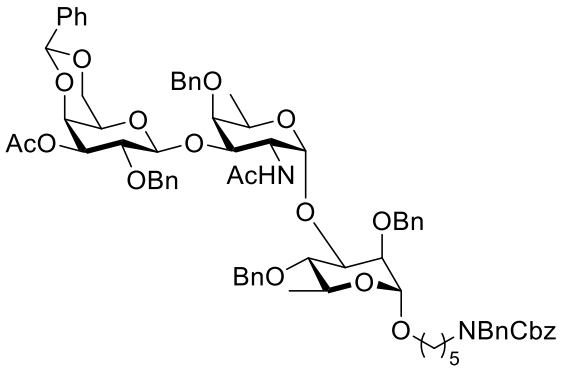


Trisaccharide **2-71** (100 mg, 0.083 mmol) was dissolved in anhydrous DCM (2 mL), then BnBr (29.6 μL, 0.25 mmol) and Ag_2O (96.2 mg, 0.42 mmol) and 4 Å molecular sieves were added. The reaction mixture was stirred at room temperature for 30 h. After filtration and concentration, the residue was purified by flash silica column chromatography (toluene/ethyl acetate = 9/1) to give **2-78** as a colorless syrup (51 mg, 47%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.43 – 7.05 (m, 35H), 5.44 (s, 1H), 5.16 – 5.09 (m, 3H), 5.04 (d, *J* = 3.1 Hz, 1H), 4.99 (d, *J* = 11.4 Hz, 1H), 4.86 (d, *J* = 10.3, 3.0 Hz, 1H), 4.80 (d, *J* = 11.4 Hz, 1H), 4.74 – 4.63 (m, 6H), 4.59 (d, *J* = 11.4 Hz, 1H), 4.45 (d, *J* = 14.4 Hz, 2H), 4.30 (t, *J* = 3.0 Hz, 1H), 4.07 (ddd, *J* = 42.7, 27.6, 10.5 Hz, 4H), 3.95 (d, *J* = 12.6 Hz, 1H), 3.83 (t, *J* = 9.0 Hz, 1H), 3.78 (s, 2H), 3.73 (d, *J* = 10.7 Hz, 1H), 3.69 – 3.62 (m, 1H), 3.61 – 3.44 (m, 2H), 3.33 (s, 1H), 3.30 – 3.08 (m, 3H), 1.98 (s, 3H), 1.53 – 1.38 (m, 4H), 1.28 (d, *J* = 6.3 Hz, 3H), 1.21 – 1.13 (m, 2H), 0.96 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 192.4, 170.8, 169.5, 156.8, 156.2, 138.9, 138.78, 138.71, 138.6, 138.16, 138.13, 138.0, 137.9, 137.8, 137.6, 137.0, 136.9, 136.5, 134.5, 129.8, 129.6,

129.2, 129.15, 129.11, 129.0, 128.9, 128.8, 128.73, 128.71, 128.6, 128.58, 128.54, 128.51, 128.49, 128.44, 128.32, 128.30, 128.2, 128.18, 128.13, 128.11, 128.0, 127.97, 127.94, 127.92, 127.90, 127.87, 127.80, 127.77, 127.72, 127.71, 127.6, 127.5, 127.4, 127.37, 127.30, 127.1, 126.5, 126.4, 126.1, 125.8, 125.7, 125.4, 105.1, 101.3, 97.5, 94.5, 79.9, 79.7, 77.8, 76.3, 75.3, 75.0, 74.3, 73.6, 73.5, 73.3, 72.7, 69.1, 68.0, 67.58, 67.55, 67.2, 66.4, 66.0, 59.4, 50.6, 50.3, 47.2, 46.2, 29.83, 29.81, 29.1, 28.0, 27.5, 23.4, 21.0, 18.1, 16.3, 14.2, 14.1; HRMS (ESI) calculated for $C_{75}H_{84}N_4O_{16}$ $[M+Na]^+$: 1319.5774, found: 1319.5787.

_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-_O_-benzylidene-2-_O_-benzyl-3-_O_-acetyl-_β_-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4-_O_-benzyl-_α_-D-fucopyranosyl-(1→3)-2,4-di-_O_-benzyl-_α_-L-rhamnopyranoside (2-79)

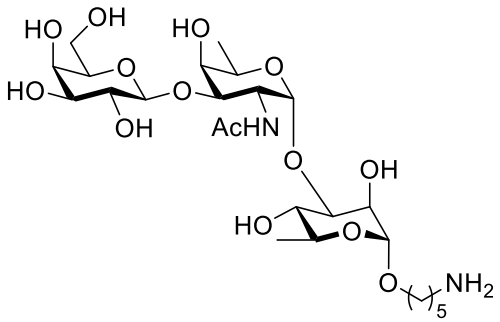


Zinc dust (100 mg) was added to a stirred solution of **2-78** (17 mg, 0.014 mol) in acetic anhydride (1 mL). The mixture was stirred for 16 h at room temperature. The solution was filtered and washed with sat. $NaHCO_3$ (20 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/ethyl acetate = 2/1) to give **2-79** as a colorless syrup (12 mg, 69%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 – 7.14 (m, 35H), 5.66 (d, *J* = 9.5 Hz, 1H), 5.48 (s, 1H), 5.22 – 5.13 (m, 3H), 5.03 (d, *J* = 3.7 Hz, 1H), 4.91 (dd, *J* = 10.3, 3.5 Hz, 1H), 4.88 (d, *J* = 12.1 Hz, 1H), 4.78 (d, *J* = 11.3 Hz, 1H), 4.75 – 4.69 (m, 2H), 4.66 – 4.59 (m, 2H), 4.59 – 4.53 (m, 3H), 4.50 (d, *J* = 9.8 Hz, 2H), 4.30 (d, *J* = 3.5 Hz, 1H), 4.15 – 3.97 (m, 4H), 3.87 (dd, *J* = 10.8, 2.8 Hz, 1H), 3.84 – 3.74 (m, 3H), 3.74 – 3.66 (m, 1H), 3.63 – 3.50 (m, 2H), 3.37 (s, 1H), 3.34 – 3.14 (m, 3H),

1.97 (s, 3H), 1.58 – 1.47 (m, 4H), 1.45 (s, 3H), 1.33 (d, *J* = 6.2 Hz, 3H), 1.27 – 1.17 (m, 2H), 0.98 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (151 MHz, cdcl$_3$) δ 170.9, 170.1, 156.8, 156.3, 139.2, 138.8, 138.4, 137.9, 137.7, 137.0, 129.2, 129.1, 128.69, 128.66, 128.59, 128.52, 128.4, 128.39, 128.37, 128.30, 128.2, 128.1, 128.06, 128.01, 127.9, 127.8, 127.4, 127.36, 127.32, 127.2, 127.1, 126.47, 126.41, 125.42, 105.8, 101.3, 97.5, 80.3, 79.9, 79.5, 76.3, 75.0, 74.92, 74.90, 73.6, 73.2, 72.4, 69.1, 67.7, 67.2, 66.6, 66.1, 50.6, 50.3, 48.5, 47.2, 46.2, 33.7, 32.0, 29.82, 29.80, 29.77, 29.74, 29.5, 29.48, 29.41, 29.25, 29.24, 28.0, 27.6, 24.9, 23.5, 23.1, 22.8, 21.5, 21.0, 18.3, 16.4, 14.2; HRMS (ESI) calculated for $C_{77}H_{88}N_2O_{17}$ $[M+Na]^+$: 1335.5975, found: 1335.5984.

5-Amino-pentyl *β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*α*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside (2-58)

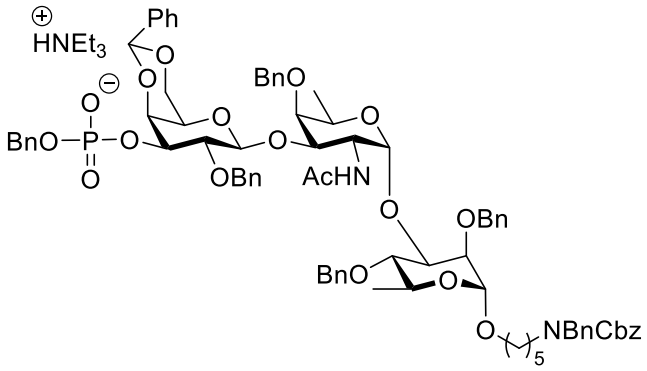


NaOMe was added slowly to a solution of **2-79** (10 mg, 7.6 µmol) in MeOH/DCM = 1/1 (1 mL) until the solution reached pH 11. The reaction mixture was stirred for 1 h, neutralized with Amberlite, filtered and concentrated under reduced pressure. The residue was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL), palladium on activated charcoal (10% Pd, 30 mg) was added and the reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and the crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-58** (2.1 mg, 46%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 4.97 (d, *J* = 3.9 Hz, 1H), 4.82 (d, *J* = 1.8 Hz, 1H), 4.49 (d, *J* = 7.7, 0.7 Hz, 1H), 4.40 (q, *J* = 6.6 Hz, 1H), 4.34 (dd, *J* = 11.2, 3.9 Hz, 1H), 4.13 (dd, *J* = 11.1, 3.1 Hz,

1H), 4.09 (d, *J* = 3.1 Hz, 1H), 4.06 – 4.02 (m, 1H), 3.93 (d, *J* = 3.4 Hz, 1H), 3.82 – 3.70 (m, 5H), 3.69 – 3.61 (m, 2H), 3.57 – 3.50 (m, 3H), 2.99 (t, 2H), 2.04 (s, 3H), 1.73 – 1.61 (m, 4H), 1.52 – 1.40 (m, 2H), 1.33 (d, *J* = 6.2 Hz, 3H), 1.22 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.4, 170.9, 104.6, 99.3, 94.8, 77.0, 75.7, 74.8, 72.4, 71.2, 70.5, 70.2, 68.5, 68.5, 67.4, 67.0, 66.5, 60.8, 48.1, 39.3, 27.9, 26.6, 22.4, 21.9, 16.6, 15.2; HRMS (ESI) calculated for $C_{25}H_{46}N_2O_{14}$ $[M+H]^+$: 599.3022, found: 599.3032.

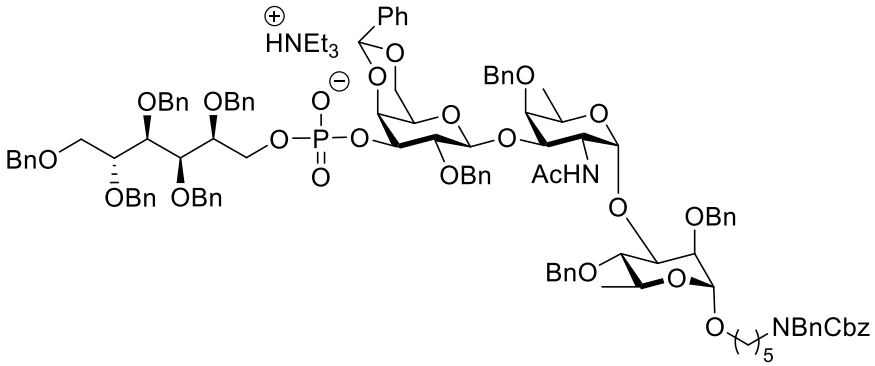
Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-*O*-benzylidene-3-*O*-(benzyl phospho)-*β*-D-galactopyranosyl-(1→3)-2- acetamido-2-deoxy-4-*O*-benzyl-*α*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-80)



NaOMe was added slowly to a solution of **2-79** (17 mg, 12.78 µmol) in MeOH/DCM = 1/1 (1 mL) until the solution reached pH 11. The reaction mixture was stirred for 1 h, neutralized with Amberlite, filtered and concentrated under reduced pressure. The residue and the *H*-phosphonate **2-14** (7.0 mg, 25.56 µmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (7.8 µL, 63.90 µmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine (16.2 mg, 63.90 µmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with CH_2Cl_2, washed with saturated $Na_2S_2O_3$ solution and TEAB buffer, and then dried over Na_2SO_4. The crude product obtained after solvent removal under reduced pressure was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 50/2/1) to give **2-80** as a triethylammonium salt (13 mg, 66%).

^{1}H NMR (600 MHz, Acetone-d_6) δ 12.20 (s, 1H), 7.49 – 7.13 (m, 40H), 7.06 (d, J = 8.9 Hz, 1H), 5.51 (s, 1H), 5.24 – 5.08 (m, 4H), 5.01 (d, J = 11.7 Hz, 1H), 4.96 – 4.82 (m, 4H), 4.81 – 4.78 (m, 1H), 4.77 – 4.70 (m, 2H), 4.70 – 4.61 (m, 5H), 4.52 (s, 2H), 4.40 – 4.31 (m, 1H), 4.22 – 4.12 (m, 2H), 4.11 – 3.97 (m, 3H), 3.92 – 3.81 (m, 2H), 3.76 – 3.64 (m, 2H), 3.63 – 3.51 (m, 3H), 3.40 – 3.28 (m, 1H), 3.24 (s, 2H), 2.99 (q, J = 7.3 Hz, 10H), 1.63 – 1.47 (m, 4H), 1.45 (s, 3H), 1.29 – 1.26 (m, 2H), 1.20 (td, J = 7.3, 1.3 Hz, 15H), 1.18 (s, 3H), 0.95 (d, J = 6.4 Hz, 3H); ^{13}C NMR (151 MHz, Acetone-d_6) δ 179.9, 175.0, 172.4, 170.4, 170.3, 140.8, 140.59, 140.52, 140.2, 140.08, 140.02, 139.5, 138.4, 129.6, 129.4, 129.3, 129.23, 129.21, 129.1, 129.0, 128.96, 128.92, 128.89, 128.85, 128.80, 128.77, 128.75, 128.73, 128.72, 128.70, 128.6, 128.44, 128.43, 128.3, 128.2, 128.20, 128.0, 127.94, 127.91, 127.86, 127.82, 127.7, 127.69, 127.65, 106.0, 102.0, 98.5, 94.2, 80.78, 80.70, 78.97, 78.93, 78.86, 78.81, 76.09, 76.04, 75.9, 75.6, 74.9, 74.8, 74.6, 73.2, 69.9, 68.9, 67.9, 67.55, 67.52, 67.0, 66.7, 58.4, 51.0, 50.8, 49.8, 49.7, 47.8, 47.0, 46.2, 38.8, 34.7, 32.7, 30.7, 30.58, 30.50, 30.48, 30.46, 30.44, 30.3, 28.8, 28.2, 27.78, 27.75, 25.9, 24.2, 23.4, 23.3, 23.2, 21.1, 18.6, 17.0, 14.4, 8.9, 8.8, 8.2; ^{31}P NMR (243 MHz, Acetone-d_6) δ -2.30; HRMS (ESI) calculated for $C_{88}H_{108}N_3O_{19}P$ [M-Et_3N-H]$^-$: 1439.6037, found: 1439.6079.

Triethylammonium *N*-(benzyl)benzyloxycarbonyl-5-aminopentyl 4,6-*O*-benzylidene-3-*O*-(2,3,4,5,6-penta-*O*-benzyl-D-glucityl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4-*O*-benzyl-*α*-D-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-*α*-L-rhamnopyranoside (2-81)

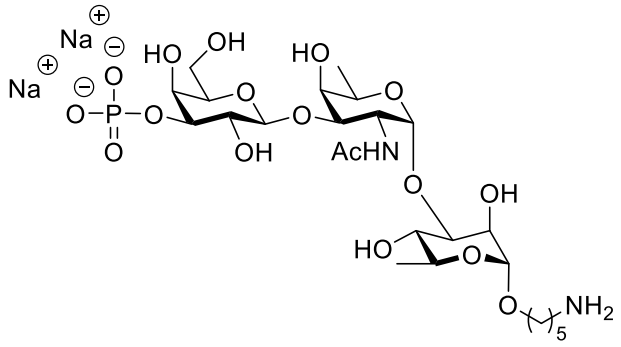


NaOMe was added slowly to a solution of **2-79** (10 mg, 7.62 μmol) in MeOH/DCM = 1/1 (1 mL) until the solution reached pH 11. The reaction mixture was stirred for 1 h, neutralized with Amberlite, filtered and concentrated under reduced pressure. The residue and the *H*-phosphonate

2-13 (12.2 mg, 15.24 µmol) were coevaporated three times with pyridine and the resulting mixture was dried under vacuum for 2 h before dissolving in anhydrous pyridine (1 mL). Pivaloyl chloride (4.7 µL, 38.09 µmol) was then added. After 5 h of stirring at room temperature, a freshly prepared solution of iodine (9.7 mg, 38.09 µmol) in pyridine/water (10/1, 0.3 mL) was added. After 3 h, the reaction was diluted with CH_2Cl_2, washed with saturated $Na_2S_2O_3$ solution and TEAB buffer, and then dried over Na_2SO_4. The crude product obtained after solvent removal under reduced pressure was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 50/2/1) to give **2-81** as a triethylammonium salt (11 mg, 70%).

^{1}H NMR (700 MHz, Acetone-d_6) δ 7.53 – 7.05 (m, 60H), 6.91 – 6.81 (m, 1H), 5.53 (s, 1H), 5.20 – 5.09 (m, 4H), 4.96 (d, *J* = 11.5 Hz, 1H), 4.87 – 4.49 (m, 21H), 4.43 – 4.34 (m, 2H), 4.32 (dt, *J* = 10.3, 4.8 Hz, 1H), 4.21 – 4.01 (m, 5H), 3.99 – 3.84 (m, 6H), 3.82 – 3.72 (m, 3H), 3.73 – 3.64 (m, 2H), 3.63 – 3.51 (m, 2H), 3.39 – 3.28 (m, 2H), 3.24 (s, 2H), 2.76 (q, *J* = 7.3 Hz, 3H), 1.59 – 1.44 (m, 4H), 1.42 (s, 3H), 1.31 – 1.28 (m, 3H), 1.28 – 1.24 (m, 2H), 1.00 (t, *J* = 7.3 Hz, 5H), 0.92 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (176 MHz, Acetone-d_6) δ 170.4, 140.8, 140.6, 140.37, 140.32, 140.3, 140.2, 140.05, 140.02, 139.9, 139.6, 138.4, 129.49, 129.41, 129.3, 129.26, 129.24, 129.1, 129.08, 129.07, 129.03, 128.9, 128.84, 128.80, 128.73, 128.72, 128.69, 128.68, 128.60, 128.5, 128.4, 128.3, 128.29, 128.24, 128.15, 128.12, 128.11, 128.0, 127.89, 127.80, 127.7, 105.8, 101.6, 98.5, 94.3, 80.75, 80.72, 80.54, 80.50, 80.4, 78.8, 78.7, 76.2, 75.9, 75.8, 75.6, 75.3, 74.9, 74.89, 74.80, 74.6, 73.9, 73.3, 73.2, 72.6, 71.1, 69.7, 68.9, 68.0, 67.5, 67.1, 66.7, 65.4, 60.6, 51.0, 50.8, 49.8, 47.8, 47.0, 46.0, 32.7, 31.8, 30.73, 30.70, 30.56, 30.51, 30.44, 30.42, 30.3, 29.5, 28.8, 28.2, 26.6, 24.2, 23.45, 23.41, 21.2, 20.9, 18.6, 17.0, 14.6, 14.4, 8.8; ^{31}P NMR (162 MHz, Acetone-d_6) δ -1.08; HRMS (ESI) calculated for $C_{122}H_{144}N_3O_{24}P$ [M-Et_3N-H]$^-$: 1963.8600, found: 1963.8827.

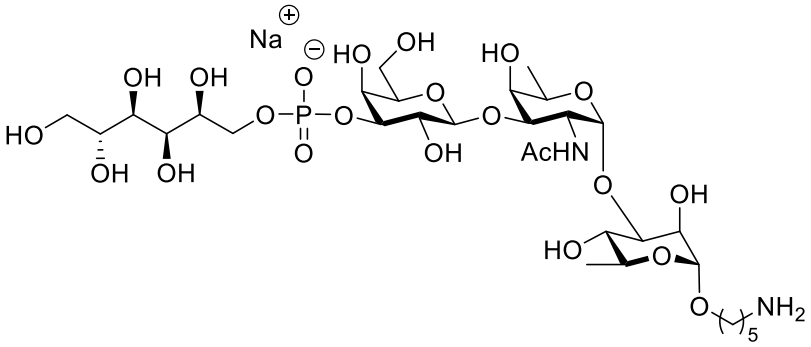
5-Aminopentyl 3-*O*-phospho-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-*α*-D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside disodium salt (2-59)



In a 20 mL vial, phosphate **2-80** (12 mg, 7.8 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and then passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-59** (2.7 mg, 48%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 4.99 (d, *J* = 3.9 Hz, 1H), 4.82 (d, *J* = 1.8 Hz, 1H), 4.58 (d, *J* = 7.9 Hz, 1H), 4.39 (q, *J* = 6.7 Hz, 1H), 4.33 (dd, *J* = 11.1, 3.8 Hz, 1H), 4.18 – 4.12 (m, 2H), 4.11 (d, *J* = 3.2 Hz, 1H), 4.11 – 4.04 (m, 1H), 4.05 – 4.01 (m, 1H), 3.81 – 3.76 (m, 2H), 3.78 – 3.69 (m, 4H), 3.66 (dd, *J* = 9.7, 7.9 Hz, 1H), 3.58 – 3.51 (m, 2H), 3.02 (t, *J* = 7.6 Hz, 2H), 2.04 (s, 1H), 1.75 – 1.60 (m, 4H), 1.54 – 1.39 (m, 2H), 1.33 (d, *J* = 6.2 Hz, 3H), 1.23 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.5, 104.3, 99.3, 94.8, 77.2, 76.7, 76.6, 75.9, 74.6, 71.0, 70.1, 69.94, 69.91, 68.5, 67.7, 67.4, 67.0, 66.5, 60.8, 48.2, 39.2, 27.9, 26.4, 22.3, 21.9, 16.6, 15.2; ^{31}P NMR (243 MHz, D_2O) δ 0.81; HRMS (ESI) calculated for $C_{25}H_{45}N_2Na_2O_{17}P$ $[M-2Na+H]^-$: 677.2540, found: 677.2550.

5-Aminopentyl 3-*O*-(D-glucityl phospho)-*β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy- -D-fucopyranosyl-(1→3)-*α*-L-rhamnopyranoside sodium salt (2-60)



In a 20 mL vial, phosphate **2-81** (11 mg, 5.3 µmol) was dissolved in Ethyl acetate/*t*-BuOH/H_2O (2 mL/2 mL/1 mL) and palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered and then passed through a Na^+ exchange column (Dowex® 50WX4 resin, Na^+ form). The crude product obtained after solvent removal was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **2-60** (2.8 mg, 61%) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 4.99 (d, *J* = 3.8 Hz, 1H), 4.82 (d, *J* = 2.1 Hz, 1H), 4.57 (d, *J* = 7.9 Hz, 1H), 4.39 (q, *J* = 6.7 Hz, 1H), 4.34 (dd, *J* = 10.9, 3.5 Hz, 1H), 4.19 – 4.05 (m, 5H), 4.06 – 4.02 (m, 1H), 4.02 – 3.95 (m, 2H), 3.94 – 3.89 (m, 1H), 3.85 (dd, *J* = 11.8, 2.9 Hz, 1H), 3.82 – 3.64 (m, 10H), 3.57 – 3.51 (m, 2H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.04 (s, 3H), 1.74 – 1.62 (m, 4H), 1.54 – 1.40 (m, 2H), 1.33 (d, *J* = 6.2 Hz, 3H), 1.23 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.4, 104.3, 99.3, 94.8, 77.5, 77.48, 77.43, 75.8, 74.4, 71.65, 71.60, 71.0, 70.9, 70.1, 69.6, 69.5, 69.4, 68.5, 67.6, 67.4, 67.0, 66.54, 66.50, 62.7, 60.8, 48.1, 39.3, 27.9, 26.56, 26.54, 22.4, 22.0, 16.6, 15.2; ^{31}P NMR (243 MHz, D_2O) δ -0.25; HRMS (ESI) calculated for $C_{31}H_{58}N_2NaO_{22}P$ $[M\text{-}Na]^-$: 841.3224, found: 841.3231.

2.4.3 Biochemistry experiments

2.4.3.1 Native CPS isolation

Following stablished protocols,[83,84,154,155] native CPS of *S. suis* serotype 9 were isolated. *S. suis* serotype 9 strain 421 donated by Simone Scherrer from Institute of Veterinary Bacteriology of

University of Zurich, were cultured in 1 L of THB overnight. At an OD of 0.8, the suspension was centrifuged at 10000 g for 30 min. The pelleted cells were resuspended in PBS and autoclaved at 121 °C for 70 min. The crude CPS was recovered by centrifugation at 10000 g for 40 min. Extraction with chloroform eliminated lipids and nucleic acids were removed by precipitation after adding $CaCl_2$ to reach a molarity of 0.1 M and ethanol to 25% v/v and then centrifuged at 9000 g for 30 min. The obtained solution was diluted with EtOH up to 80% v/v and left overnight at 4 °C to precipitate the CPS. The next day, the solution was centrifuged at 4 °C for 30 min at 9000 g. The precipitate was dissolved in 10 mL of NH_4HCO_3 50 mM and dialyzed against water for 3 days and lyophilized. The crude CPS was determined by NMR and then again dissolved in NH_4HCO_3 50 mM. The CPS was further purified by gel filtration chromatography on G25 column at a flow rate of 1 ml/min in ÄKTA or on Superdex S200 10/300 on FPLC. Fractions were collected and checked by TLC staining. The ones showing positive signals were lyophilized and stored and characterized by NMR. As well, the percentage of proteins and nucleic acids content was checked by BCA assay and Nanodrop respectively. Dot-blot ELISA assays were performed against CPS serotype 9 (using S9 rabbit antisera from SSI Diagnostica) to confirm that the native CPS was recognized by the specific polyclonal antibodies present in the sera.

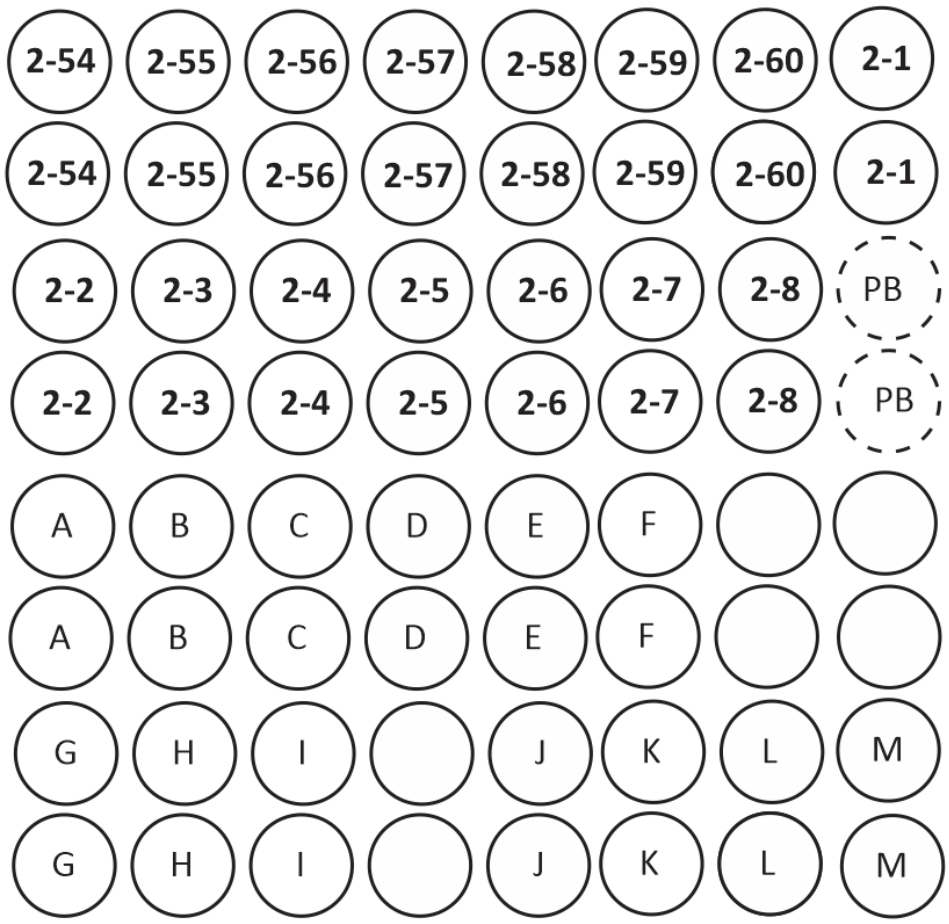
2.4.3.2 Preparation of microarrays

Amine-terminated oligosaccharides and native CPS were immobilized on commercial *N*-hydroxysuccinimide (NHS) ester-activated microarray slides (CodeLink Activated Slides; SurModics) using a piezoelectric microarray spotting device (S3; Scienion) so that 64 identical subarrays can be contained on each slide. Before the spotting, samples were diluted in 50 mM sodium phosphate buffer, pH 8.5. Slides were incubated in a humid chamber for 24 h at room temperature to complete coupling reactions. The next day, slides were quenched in 100 mM ethanolamine in 50 mM sodium phosphate buffer, pH 9, for 1 h at room temperature. Then, the slides were washed three times with deionized water, dried by centrifugation (300 x g, 5 min) and stored at 4 °C in a black box until use.

2.4.3.3 Glycan microarrays

The microarray slides with immobilized oligosaccharides previously quenched were blocked with 1% BSA-PBS for 1 hour at room temperature. Then, the slides were washed three times with PBS and dried by centrifugation. A FlexWell 64 grid was applied and slides were incubated with

dilutions of pig or rabbit or human serum in duplicate in a humid chamber for 1 h at 36 °C. The pig sera were donated by Prof. Dr. Leif Sander (Charité, Universitäts Medizin Berlin), Prof. Dr. Marcus Fulde (Department of Veterinary Medicine, Freie Universitat Berlin) and/or Dr. Ulrike Blohm (Friedrich Loeffler Institute, Greifswald). In the case of rabbit serum, it is commercial serum purchased from SSI Diagnostica. The serum samples were diluted in 1% BSA-PBS. Wells were washed three times with PBS-Tween. After the washing, slides were incubated with fluorescence-labeled secondary antibodies diluted in PBS-BSA (FITC anti-pig IgG or unlabeled pig IgG or AlexaFluor 647 anti-rabbit IgG or human IgG at 1:400 and unlabeled rabbit anti-pig IgM or Alexa Fluor 488 anti-rabbit IgM) in a light-protected humidity chamber for 45-60 minutes at 36 °C. The slides were washed three times with PBS-Tween and the grids were removed. In a Petri dish, the slide was washed with PBS-Tween during 10 minutes and rinsed with deionized water, repeating the procedure twice. For eliminating the remaining liquid, the slides were dried by centrifugation (300 x g, 5 min). The slides were scanned with a GenePix 4300A microarray scanner (Molecular Devices, Sunnyvale, CA, USA). Finally, Image analysis was carried out using GenePix Pro 7 software (Molecular Devices).



Synthetic oligosaccharides related to *Streptoccocus suis* serotype 9, 0.2 mM:

2-54- Glcol-1-P-3-[D-Gal(a1-2)]D-Gal(b1-3)D-FucNAc(a1-3)L-Rha(a1-1)aminopentanol
2-55 - [D-Gal(a1-2)]D-Gal(b1-3)D-FucNAc(a1-3)L-Rha(a1-1)aminopentanol
2-56 - P-3-[D-Gal(a1-2)]D-Gal(b1-3)D-FucNAc(a1-3)L-Rha(a1-1)aminopentanol
2-57- Butanol-P-3-[D-Gal(a1-2)]D-Gal(b1-3)D-FucNAc(a1-3)L-Rha(a1-1)aminopentanol
2-58 - D-Gal(b1-3)D-FucNAc(a1-3)L-Rha(a1-1)aminopentanol
2-59 - P-3-D-Gal(b1-3)D-FucNAc(a1-3)L-Rha(a1-1)aminopentanol
2-60 - Glcol-1-P-3-D-Gal(b1-3)D-FucNAc(a1-3)L-Rha(a1-1)aminopentanol
2-1- Glcol-1-P-3-[D-Gal(a1-2)]D-Gal(b1-3)D-FucNAc(b1-3)L-Rha(a1-1)aminopentanol
2-2 - [D-Gal(a1-2)]D-Gal(b1-3)D-FucNAc(b1-3)L-Rha(a1-1)aminopentanol
2-3 - P-3-[D-Gal(a1-2)]D-Gal(b1-3)D-FucNAc(b1-3)L-Rha(a1-1)aminopentanol
2-4 - Butanol-P-3-[D-Gal(a1-2)]D-Gal(b1-3)D-FucNAc(b1-3)L-Rha(a1-1)aminopentanol
2-5 - D-Gal(b1-3)D-FucNAc(b1-3)L-Rha(a1-1)aminopentanol
2-6- P-3-D-Gal(b1-3)D-FucNAc(b1-3)L-Rha(a1-1)aminopentanol
2-7 - Glcol-1-P-3-D-Gal(b1-3)D-FucNAc(b1-3)L-Rha(a1-1)aminopentanol
2-8 - D-FucNAc(b1-3)L-Rha(a1-1)aminopentanol

Unrelated synthetic oligosaccharides, 0.2 mM:
A - Rha(a1-1)aminopentanol
B- Rha(a1-3)Rha(a1-1)aminopentanol
C - FucNAc(a1-3)D-FucNAc(b1-1)aminopentanol
D - Glc(b1-4)FucNAc(a1-3)D-FucNAc(b1-1)aminopentanol
E- Gal(b1-3)Gal(b1-4)Glc(b1-1)aminopentanol
F - Man(a1-1)aminopentanol

Native capsular polysaccharides:
G-CPS S2, capsular polysaccharide *Streptococcus suis* serotype 2, 0.2 mg/ml
H- CWPS, cell wall polysaccharide *Streptococcus pneumoniae,* 0.2 mM
I-CPS SP5, capsular polysaccharide *Streptococcus pneumoniae* serotype 5, 0.2 mg/ml
J- CPS S9-1, capsular polysaccharide *Streptococcus suis* serotype 9, 0.2 mg/ml
K- CPS S9-2, capsular polysaccharide *Streptococcus suis* serotype 9, 0.1 mg/ml
L- CPS S9-3, capsular polysaccharide *Streptococcus suis* serotype 9, 0.3 mg/ml
M -CPS S9-4, capsular polysaccharide *Streptococcus suis* serotype 9, 0.4 mg/ml
PB-Printing buffer

Figure 2.10 Microarray printing pattern of *S. suis* serotype 9 oligosaccharides with used printing concentrations

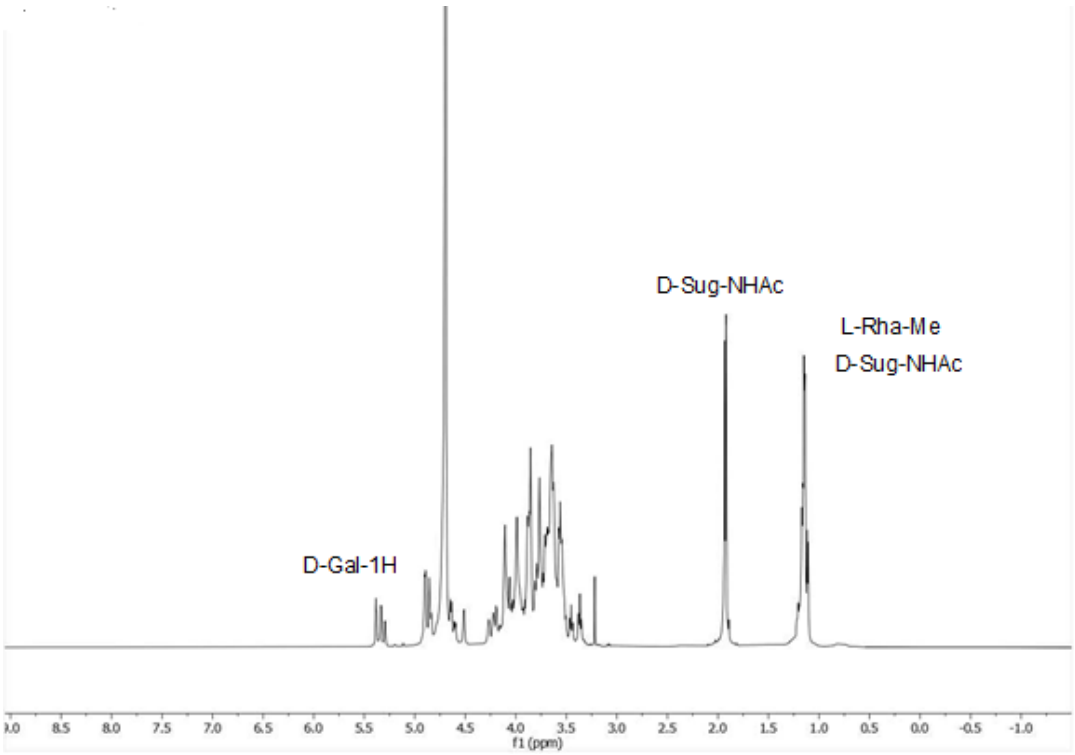


Figure 2.11 ^{1}H-NMR of isolated CPS from *S. suis* serotype 9

Chapter 3

Total syntheses of conjugation-ready repeating units of *Acinetobacter baumannii* AB5075 for glycoconjugate vaccine development

This chapter is based on the following article:

Zhang, S.; Seeberger, P. H. Total Syntheses of Conjugation-ready Repeating Units of *Acinetobacter baumannii* AB5075 for Glycoconjugate Vaccine Development. Submitted to *Chemistry - A European Journal*. The final publication *Chem. Eur. J.* **2021**, 27 (69), 17444–17451 is currently available at https://doi.org/10.1002/chem.202103234.

3.1 Introduction

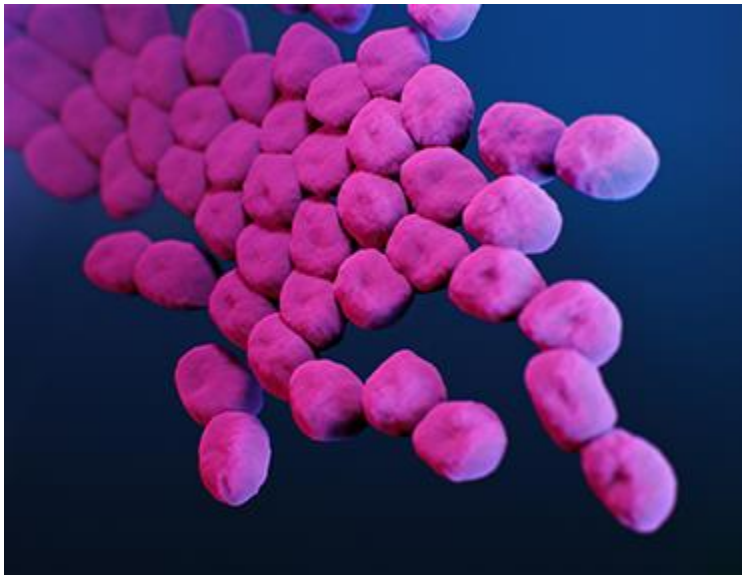


Figure 3.1 *Acinetobacter baumannii* bacterium[*]

Infections caused by multidrug-resistant bacteria are increasing in frequency and result in high morbidity and mortality.[156–159] *Acinetobacter baumannii* is a Gram-negative coccobacillus that is strictly aerobic, pleomorphic and non-motile.[160–162] It is widely distributed in the environments, including many health care facilities. As one of the six most important multidrug-resistant

[*] CDC's 2019 Antibiotic Resistance Threats Report. https://www.cdc.gov/DrugResistance/Biggest-Threats.html

microorganisms in hospitals worldwide, *A. baumannii* is considered to be responsible for around 10% of acquired Gram-negative infections in hospitals.[163–166]

Acinetobacter baumannii is capable of colonizing the skin as well as respiratory and oropharynx system,[160,167] causing severe urinary tract infections, bacteremia and pneumonia[168–172]. *A. baumannii* can survive in the environment for months that makes it difficult to control and prevent its transmission.[173] Furthermore, due to the high adaptability, *A. baumannii* can acquire resistance to antibiotics rapidly which renders multiple strains of *A. baumannii* resistant to almost all antimicrobials.[174–177] The combination of its environmental resilience and wide range of resistance makes it a serious and emerging nosocomial pathogen,[178–180]such that the World Health Organization lists this bacterium in the highest category of pathogens posing an imminent threat to human health.

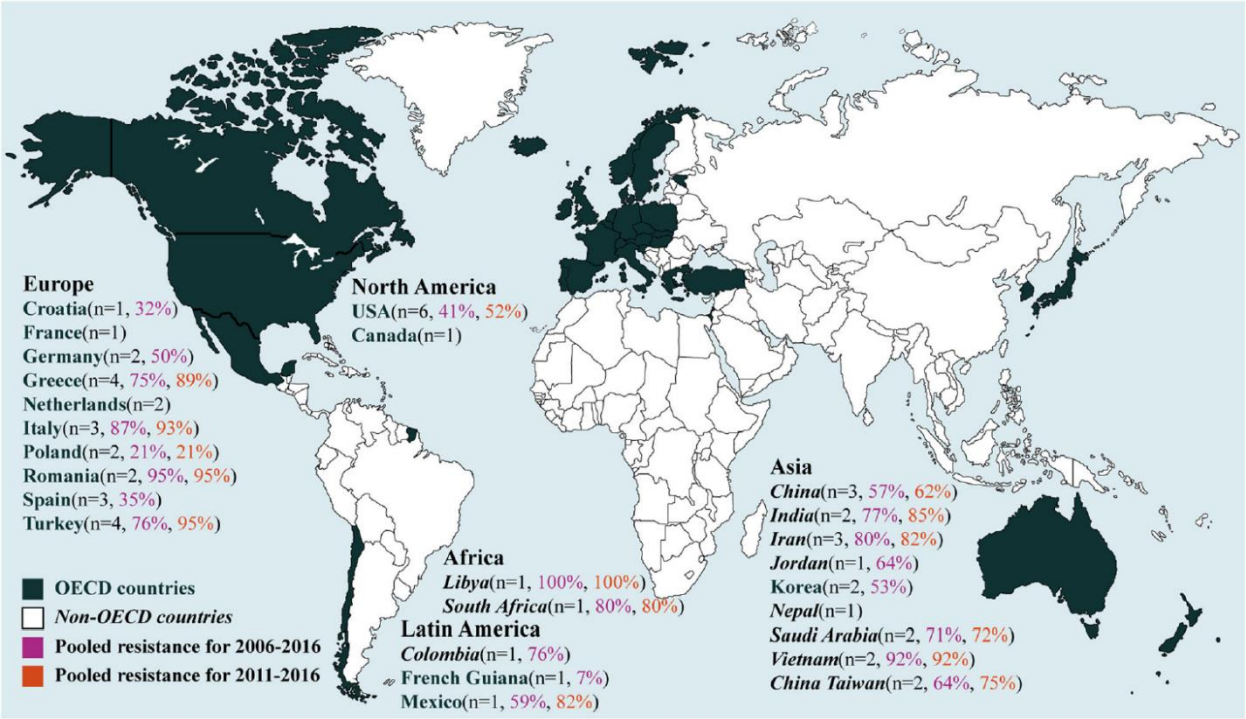


Figure 3.2 Geographical distribution of percentage of imipenem resistance to *A. baumannii* infections in OECD and non-OECD countries (Reprinted from: *Emerg. Microbes Infect.* 7, (2018))

Outbreaks of carbapenem-resistant *A. baumannii* with high mortality have been reported worldwide, especially in ICUs.[181–186] The high frequency of outbreaks can be attributed to the extreme difficulty of treating infections. Carbapenem antibiotics have been considered as the first choice to treat bacterial infections, imipenem is one member of these first line agents for empirical

therapy in areas with high rates of susceptibility.[187,188] However, significant resistance to this antibiotic has been observed in many countries (Figure 3.2).[189] In both Organisation for Economic Co-operation and Development (OECD) and non-OECD countries, the prevalence of imipenem resistance is very high, most rates are over 70% while some are higher than 90%, suggesting that stronger antimicrobial or efficient vaccines are urgently needed to fight against *A. baumannii* infections.

AB5075, a recent clinical isolate of *A. baumannii*, has been established as a model strain for studying emerging pathogenicity and antimicrobial treatments of *A. baumannii* due to its high virulence, multidrug resistance and ability to be genetically manipulated.[174–176] The outer membrane of *A. baumannii* AB5075 is surrounded by high molecular weight capsular polysaccharides [190] that form a discrete layer on the bacterial surface, that assists in evasion of the host immune defenses and increases antibiotic tolerance.[27,39,40] CPS can trigger a specific immune response,[41,191] that renders structurally unique glycans on the surface as attractive targets for development of glycoconjugate vaccines.[192,193]

3.2 Results and discussion

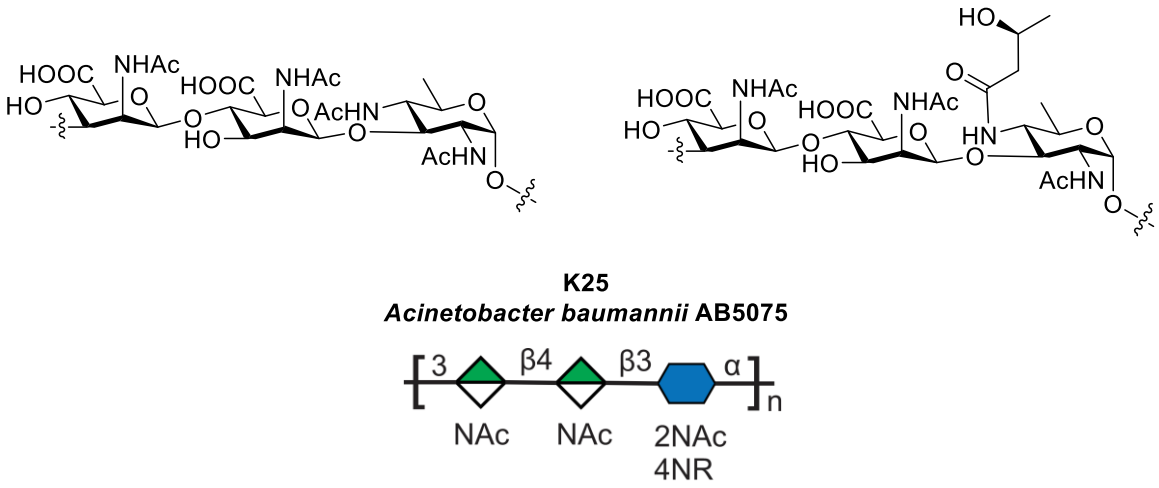


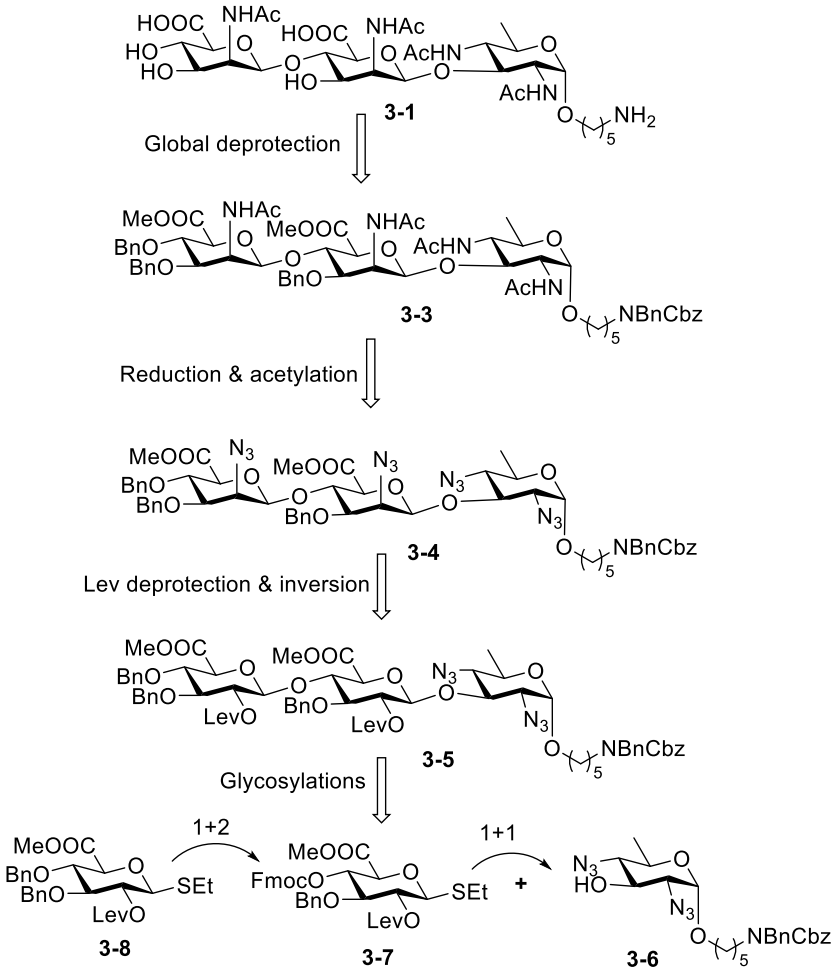
Figure 3.3 Structure of *A. baumannii* AB5075 CPS repeating units

The CPS of *A. baumannii* AB5075 is K25 type that consists of two linear trisaccharide repeating units [→3)-*β*-D-Man*p*NAcA-(1→4)-*β*-D-Man*p*NAcA-(1→3)-*α*-D-Qui*p*NAc4NR-(1→] where R indicates acetyl or (*S*)-3-hydroxybutanoyl in a ratio of approximately 1:2.5 (Figure 3.3).[194] The repeating units bear *N*-acetyl groups on D-mannuronic acid and a (*S*)-3-hydroxybutanoyl group on

D-bacillosamine. Three 1,2-*cis* linkages including two challenging β-mannosides and a terminal α-glycosidic linkage, together with the presence of a (*S*)-3-hydroxybutanoyl group and dense *N*-acetyl groups make the trisaccharides challenging targets to synthesize.

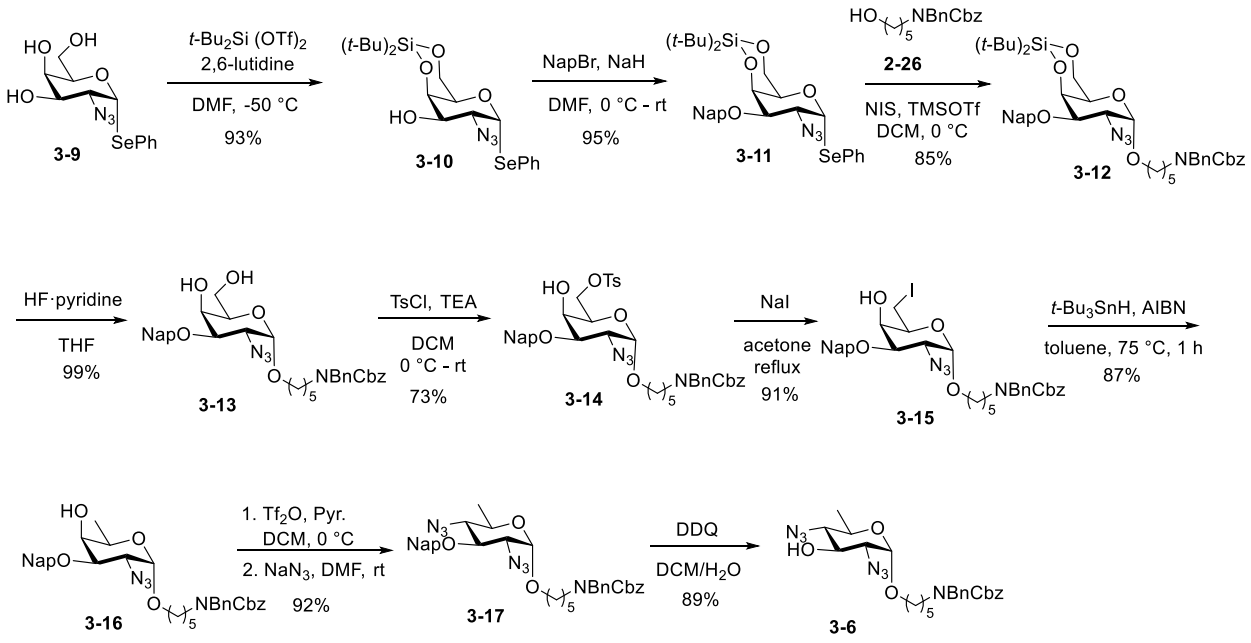
3.2.1 Synthesis of *A. baumannii* AB5075 CPS repeating unit 3-1

The retrosynthesis of first target repeating unit **3-1** reveals that **3-1** can be obtained from fully protected trisaccharide **3-3** by global deprotection (Scheme 3.1). Conversion of **3-4** to **3-3** can be achieved via reduction and acetylation of azide groups. The transformation of **3-5** to **3-4** involves the key reaction in this work: Lev esters are removed followed by double-serial inversion to create the two 1,2-*cis* mannosidic linkages. Trisaccharide skeleton **3-5** can be obtained by [1+1] and [1+2] glycosylations from three differently protected building blocks **3-6**, **3-7** and **3-8**, the β-selectivity can be ascertained by the neighboring participation of 2-OLev groups.[94,195]



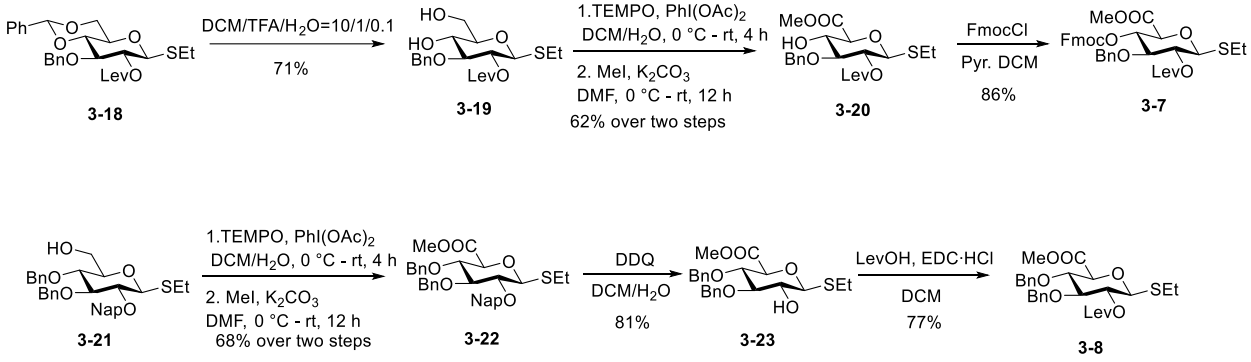
Scheme 3.1 Retrosynthetic analysis of target molecule 3-1

The total synthesis of repeating unit **3-1** commenced with the preparation of the orthogonally protected rare sugar building blocks (Scheme 3.2). D-Bacillosamine derivative **3-6** was synthesized starting from the protection of C4 and C6 positions of commercial compound **3-9**[196] with the 4,6-silyldene group and C3-OH was protected with Nap ether to give **3-11** in high yields for both steps. α-Selective glycosylation of selenoglycoside **3-11**[197] with aminopropyl linker **2-26** using NIS/TMSOTf as a promoter provided α-linked glycoside **3-12** in 85% yield.[198] The exclusive selectivity relied on the hindrance of the bulky silyldene group. The linker was designed in anticipation of the conjugation to carrier protein or a microarray surface.[199] Removal of the 4,6-silyldene group employing HF·pyridine afforded diol **3-13**, then the C6 hydroxyl in **3-13** was selectively protected with tosyl group using 4-toluenesulfonyl chloride to form **3-14** in 73% yield, followed by treatment with sodium iodide in refluxing acetone to obtain iodide **3-15**. Subsequently, reduction with tributyltin hydride and azobisisobutyronitrile (AIBN) at 75 °C gave **3-16** in 87% yield as the azide group on C2 was stable during this step. The transformation of D-fucosamine derivative **3-16** to D-bacillosamine derivative **3-17** was carried out using nucleophilic displacement of the triflate. First, the C4 hydroxyl in **3-16** was triflated using triflic anhydride (Tf_2O) and pyridine to form a 4-*O*-triflate intermediate. After a brief extraction, the triflate was treated with sodium azide in DMF to yield the desired **3-17**.[200] Removal of the Nap protecting group provided building block **3-6** in 89% yield.



Scheme 3.2 Synthesis of D-bacillosamine derivative building block 3-6

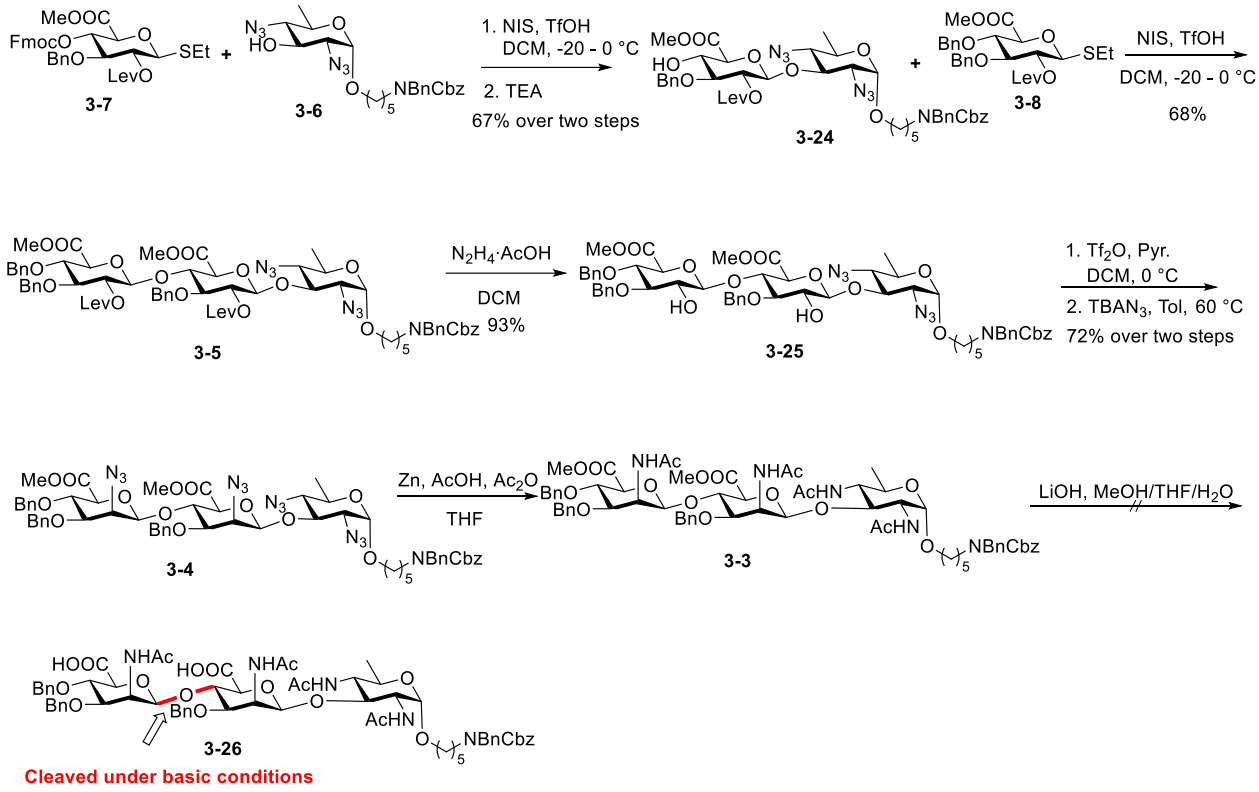
For the synthesis of glucuronate building blocks **3-7** and **3-8**, 4.6-benzylidene group of known thioglycoside **3-18**[201] was cleaved to form diol **3-19** (Scheme 3.3). Regioselective oxidation of C6-OH of **3-19** using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and bis(acetoxy)iodobenzene (BAIB) afforded the C6 carboxylic acid that was esterified with methyl iodide and K_2CO_3 to give glucuronate **3-20** in 62% yield over two steps.[202] Introduction of Fmoc ester on C4-OH furnished the desired building block **3-7** in good yield. As for the synthesis of building block **3-8**, oxidation and esterification of **3-21**[123] gave D-glucuronate **3-22** as a methyl ester in 68% yield. Then the cleavage of Nap group in **3-22** and subsequent replacement by Lev ester furnished **3-8** in good yield.



Scheme 3.3 Synthesis of D-glucuronate building blocks 3-7 and 3-8

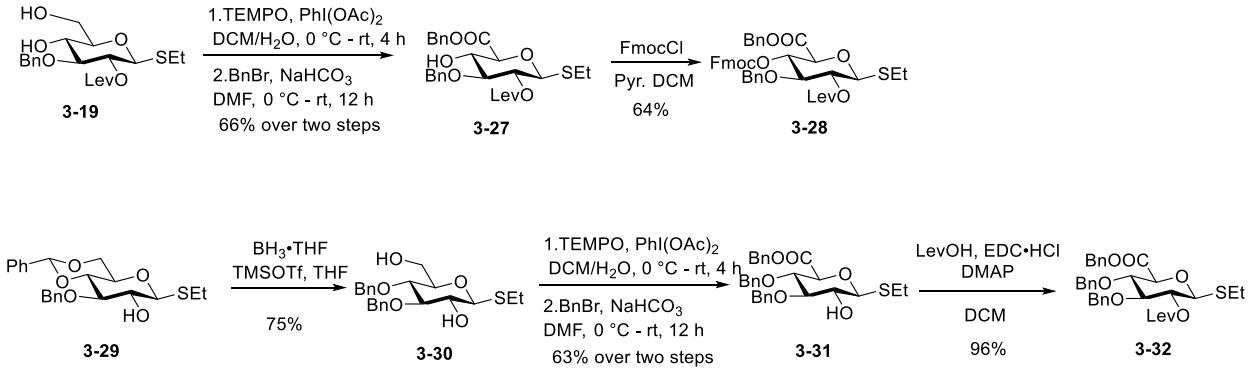
After all building blocks were obtained, the initial attempt to synthesize trisaccharide started with the union of monosaccharide **3-6** with **3-7** using NIS/TfOH as promoter to form the desired disaccharide (Scheme 3.4). Triethylamine was added to quench the reaction and cleave the Fmoc group in one pot to furnish β-linked disaccharide **3-24** in 67% yield while none of the α-isomer was observed. The stereoselectivity is the result of the participating Lev ester in **3-7**.[94] Similarly, the [1+2] glycosylation of disaccharide **3-24** with building block **3-8** catalyzed by NIS and TfOH yielded the trisaccharide backbone **3-5** with complete β-selectivity. The cleavage of Lev esters was followed by the conversion of the hydroxyl groups in **3-25** into triflates using Tf_2O and pyridine. Initially, based on the inversion conditions used in the synthesis of **3-17**, concomitant S_N2 substitution in the axial positions with sodium azide failed to provide the desired azide product **3-4**. Replacement of sodium azide with tetrabutylammonium azide ($TBAN_3$) and an increase of the temperature successfully yielded **3-4** in 72% yield. The four azide groups were then reduced with zinc followed by acetylation with acetic anhydride (Ac_2O) in THF to obtain trisaccharide **3-3**.

However, the attempted hydrolysis of the methyl ester with lithium hydroxide did not produce the desired compound **3-26** as the very fragile glycosidic bond between two D-mannuronic acids was cleaved in the aqueous basic environment to generate mono- and disaccharide fragments.



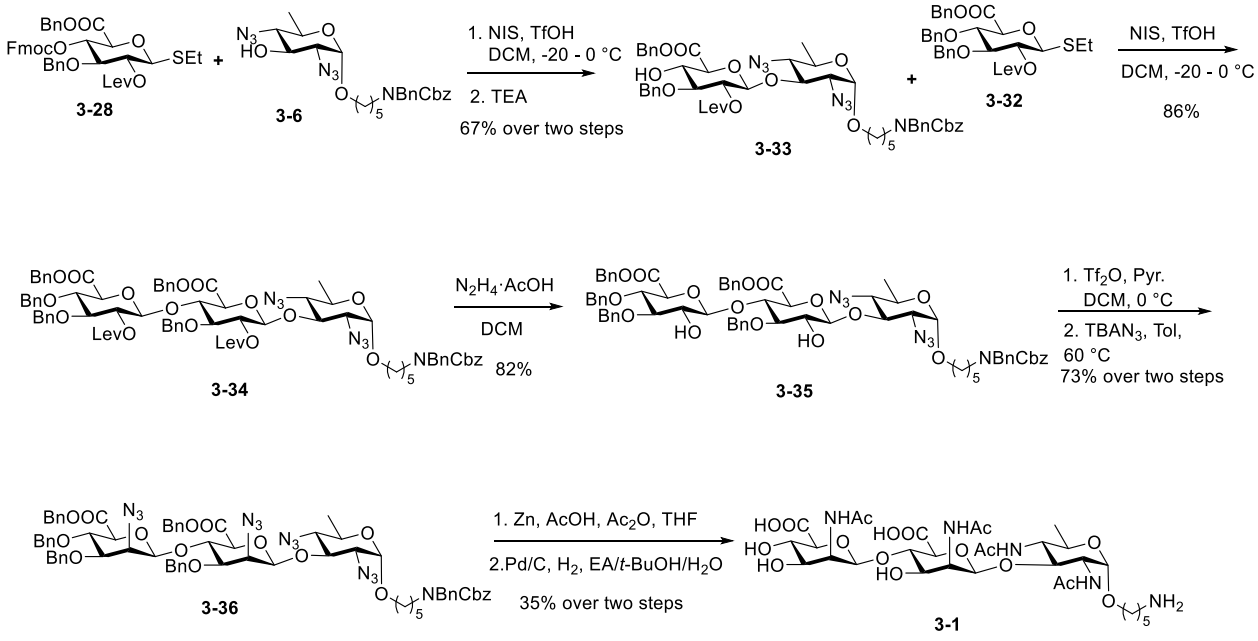
Scheme 3.4 Attempted assembly for trisaccharide 3-26

The lability of glycosidic bond between two mannuronic acids required to avoid treating the trisaccharide with strong base. Therefore, the protecting groups on C6 carboxylic acids in D-glucuronate building blocks were replacement with benzyl esters that could be cleaved during final hydrogenolysis.[203] Similar to the synthesis of compound **3-7**, efficient oxidation of C6-OH in **3-19** and subsequent esterification with benzyl bromide and $NaHCO_3$ led to the D-glucuronate **3-27** in 66% yield as a benzyl ester (Scheme 3.5). Fmoc protection of the C4 hydroxyl group completed the synthesis of building block **3-28**. Regioselective cleavage of 4,6-benzylidene in thioglycoside **3-29**[204] formed **3-30** in 75% yield. Diol **3-30** was then converted to benzyl glucuronate **3-31** by oxidation of the C6-OH and esterification. Levulinoylation of the C2 hydroxyl group in **3-31** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and DMAP produced **3-32**.



Scheme 3.5 Synthesis of benzyl glucuronate building blocks 3-28 and 3-32

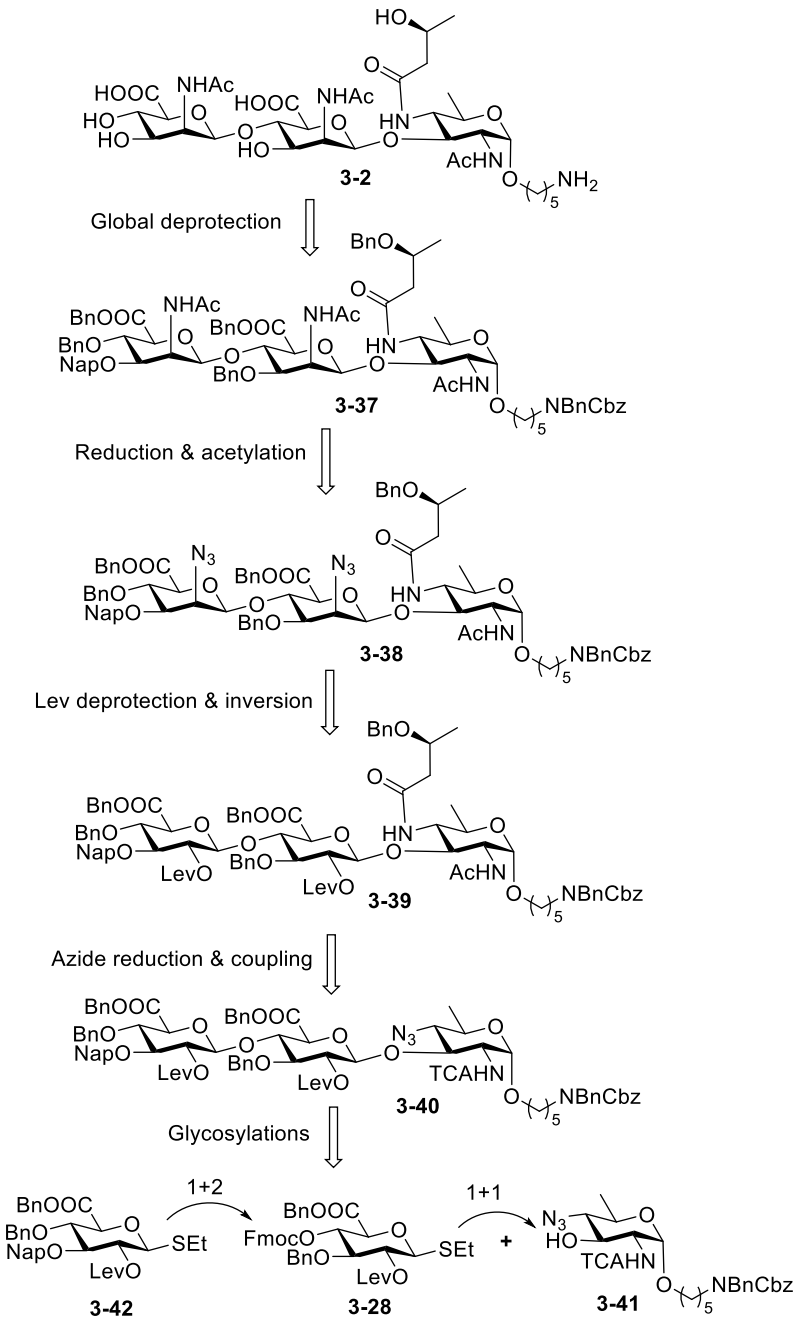
Based on the initial synthetic route to **3-26**, two sequential glycosylations in presence of NIS/TfOH promoter produced trisaccharide backbone **3-34** with exclusive *β*-selectivity (Scheme 3.6). Cleavage of the Lev esters with hydrazine acetate freed two hydroxyl groups to generate diol **3-35** that was then converted to 2′,2″-bis triflates followed by replacement with azide nucleophiles in the axial positions to provide **3-36** in good yield. The four azide groups were reduced with zinc in the presence of acetic acid followed by acetylation with acetic anhydride in THF. Due to the polarity of the intermediate containing four acetamide groups and great difficulties purifying the compound, the crude *N*-acetyl sugar was directly subjected to hydrogenolysis after extraction to furnish the repeating unit **3-1** in 35% yield over two steps.



Scheme 3.6 Assembly of trisaccharide repeating unit 3-1

3.2.2 Synthesis of *A. baumannii* AB5075 CPS repeating unit 3-2

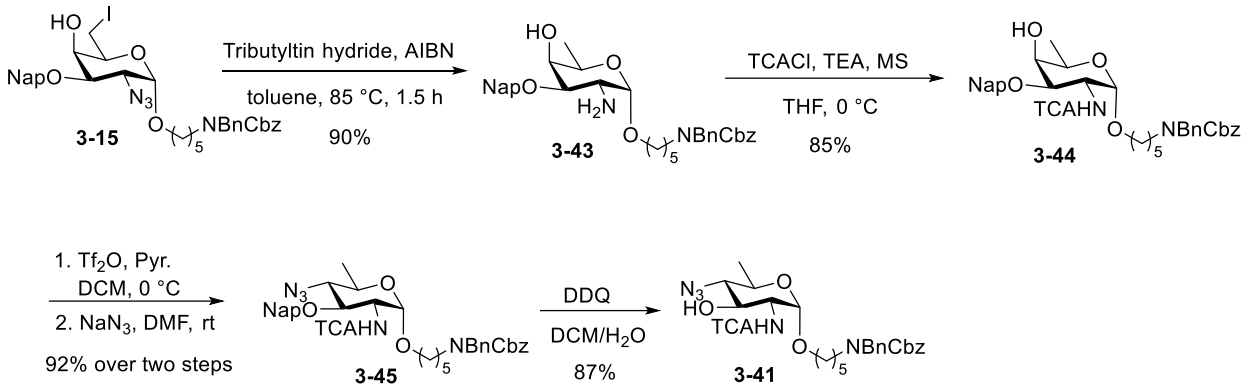
With **3-1** in hand, the other repeating unit **3-2** of AB5075 CPS containing a (*S*)-3-hydroxybutanoyl chain served as the next target.



Scheme 3.7 Retrosynthetic analysis of target molecule 3-2

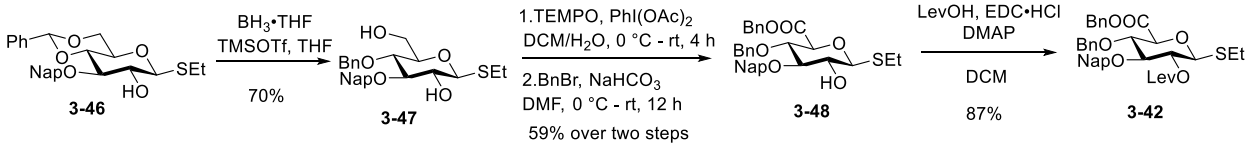
According to the retrosynthetic analysis of **3-2**, global deprotection by hydrogenolysis of **3-37** will give the target repeating unit **3-2** (Scheme 3.7). Conversion of azide group into *N*-acetyl can finish

the transformation from **3-38** to **3-37**. The (*S*)-3-hydroxybutanoyl chain must be installed prior to the inversion as the high density of azides after inversion will make it impossible to perform the coupling regioselectively. Thus, an orthogonal azide group has to be included in building block **3-41** for subsequent reduction and coupling. A trichloroacetamido (TCA) group was chosen to mask the amino group of C2 in **3-41** to ensure the exclusive azide exits in trisaccharide backbone **3-40**.[126] Glycosylations of building blocks **3-41**, **3-28** and **3-42** will give access to **3-40**, the azide group in **3-40** will be reduced and (*S*)-3-hydroxybutanoyl chain can be introduced of this stage. If successful, the following inversion and global deprotection will produce the desired target molecule **3-2**.



Scheme 3.8 Synthesis of building block 3-41

The synthesis of building block **3-41** commenced with the reduction of iodide **3-15** with tributyltin hydride and AIBN at 85 °C, to reduce the C2-azide in **3-15** to the corresponding amino group and give **3-43** in 90% yield (Scheme 3.8). Compared to the reduction of **3-15** to get **3-16**, the regioselective dehalogenation of C6-iodide and reduction of C2-azide were perfectly achieved by controlling the equivalence of tributyltin hydride, temperature and reaction time. Installation of a TCA group to protect the amine afforded **3-44** followed by inversion at the C4 position, D-bacillosamine derivative **3-45** was obtained in high yield. Removal of Nap ether with DDQ prepared **3-41** for glycosylation.

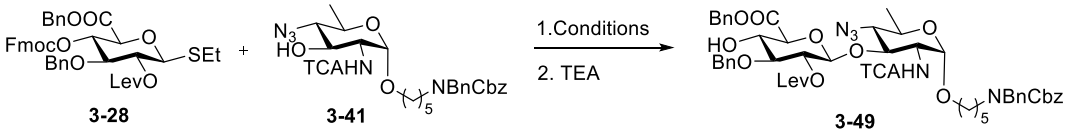


Scheme 3.9 Synthesis of building block 3-42

For the building block **3-42**, selective benzylidene opening of **3-46**[205] using BH_3·THF and TMSOTf afforded diol **3-47** in 70% yield (Scheme 3.9). Subsequent regioselective oxidation of the C6 hydroxyl group to the corresponding carboxylic acid was performed efficiently using TEMPO/BAIB, followed by treatment with benzyl bromide and $NaHCO_3$ to furnish glucuronate **3-48** as a benzyl ester in 59% overall yield. The synthesis of **3-42** was completed after Lev protection of the C2 hydroxyl in 87% yield.

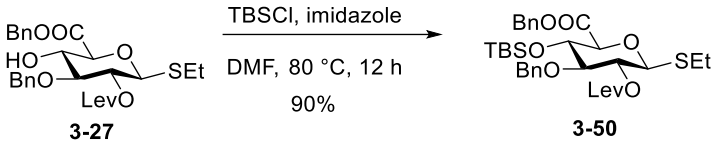
With all building blocks in hand, the coupling of **3-28** with **3-41** to form disaccharide **45** was explored (Table 3.1). The conditions established for the synthesis of **3-1**, a NIS/TfOH catalyzed glycosylation, afforded disaccharide **3-49** in low yield (24%, Entry 1). Higher temperatures (0 °C and 25 °C) or a NIS/TMSOTf promoter system (Entry 2, 3 and 4) did not significantly increase the yield. Possibly, introduction of the TCA group may be responsible for the decrease in the nucleophilicity of acceptor **3-41**.[206]

Table 3.1 Glycosylation to synthesize disaccharide 3-49



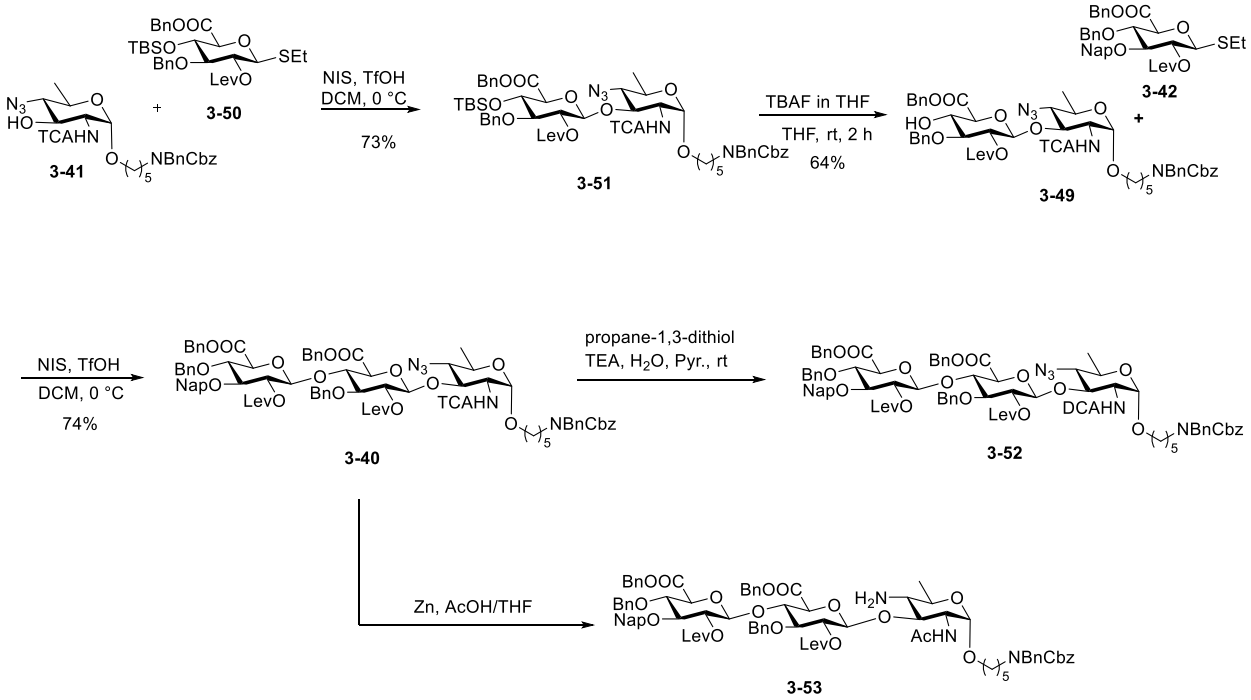
Entry	Promoter	Temperature	Result
1	NIS/TfOH	-20 °C to 0 °C	24% product formed, acceptor recovered
2	NIS/TfOH	0 °C	25% product formed, acceptor recovered
3	NIS/TMSOTf	0 °C	19% product formed, acceptor recovered
4	NIS/TfOH	0 °C to 25 °C, overnight	33% product formed, acceptor recovered

In order to improve the efficiency of the glycosylation to obtain disaccharide **3-49**, different glycosyl donors including phosphate[207] and *N*-phenyl trifluoroacetimidate[208] were also tested, however, the reactions of these donors with **3-41** gave the desired disaccharide **3-49** in even lower yields (4% and 5%). Finally, this issue was solved by introduction of an electron-donating group in donor at C4 position. The Fmoc protecting group in **3-28** was replaced with *tert*-butyldimethylsilyl ether (TBS) group. Glucuronate building block **3-50** was obtained after protection of the C4-OH in **3-27** with a TBS ether in 90% yield (Scheme 3.10).



Scheme 3.10 synthesis of building block 3-50

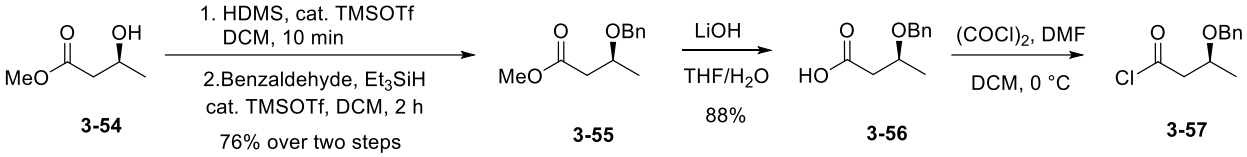
The glycosylation of **3-41** using glycosylating agent **3-50** and NIS/TfOH as promoter at 0 °C was very efficient and yielded 73% β-linked disaccharide **3-51** (Scheme 3.11). Disaccharide acceptor **3-49** was furnished after TBS cleavage by TBAF and the subsequent [1+2] glycosylation of **3-49** with **3-42** yielded trisaccharide **3-40** with complete β selectively. Chain elongation started from azide reduction of **3-40**. The Staudinger reaction did not work for this trisaccharide,[209] while treatment with 1,3-propanedithiol that was commonly used for azide reduction of oligosaccharides was too mild to reduce the azide, instead, one chlorine of the TCA group was cleaved to give a 2-dichloroacetamido-4-azido product **3-52**.[126,210] Finally, treatment of **3-40** with excess zinc and acetic acid at 40 °C in THF successfully converted the azide to the amine **3-53**, meanwhile, the TCA group on C2 position of bacillosamine was reduced to an acetamide.



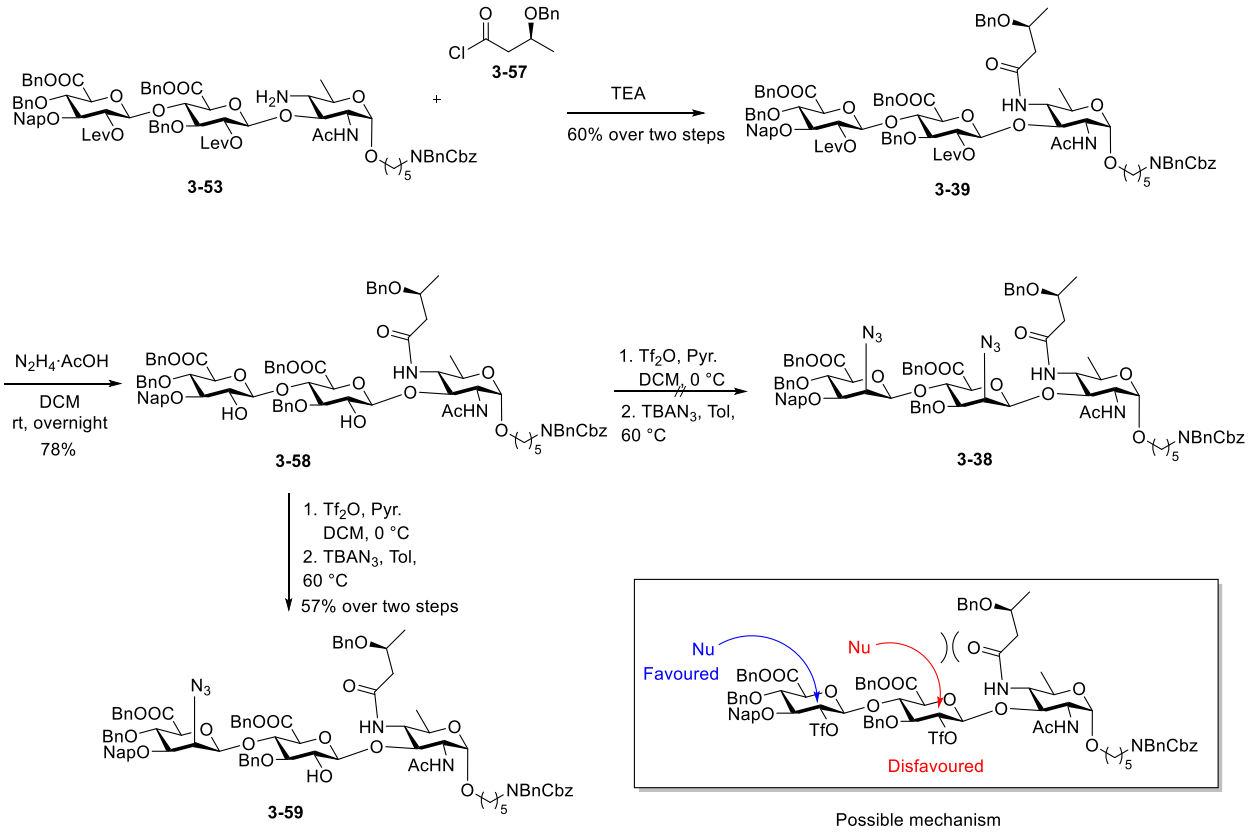
Scheme 3.11 Synthesis of trisaccharide 3-40 and reduction attempt

After the amine was obtained, the stage was set for coupling of **3-53** with (*S*)-3-hydroxybutanoyl chain. The hydroxyl group of commercial methyl (*S*)-3-hydroxybutyrate **3-54** was protected with

benzyl ether to give **3-55** under acidic conditions (Scheme 3.12). Methyl ester was then cleaved by lithium hydroxide to give (*S*)-3-*O*-benzylbutyryl acid **3-56**, without further purification, treatment of **3-56** with oxalyl chloride afforded the desired (*S*)-3-*O*-benzylbutyryl chloride **3-57**.[211,212]



Scheme 3.12 Synthesis of (*S*)-3-*O*-benzylbutyryl chloride 3-57

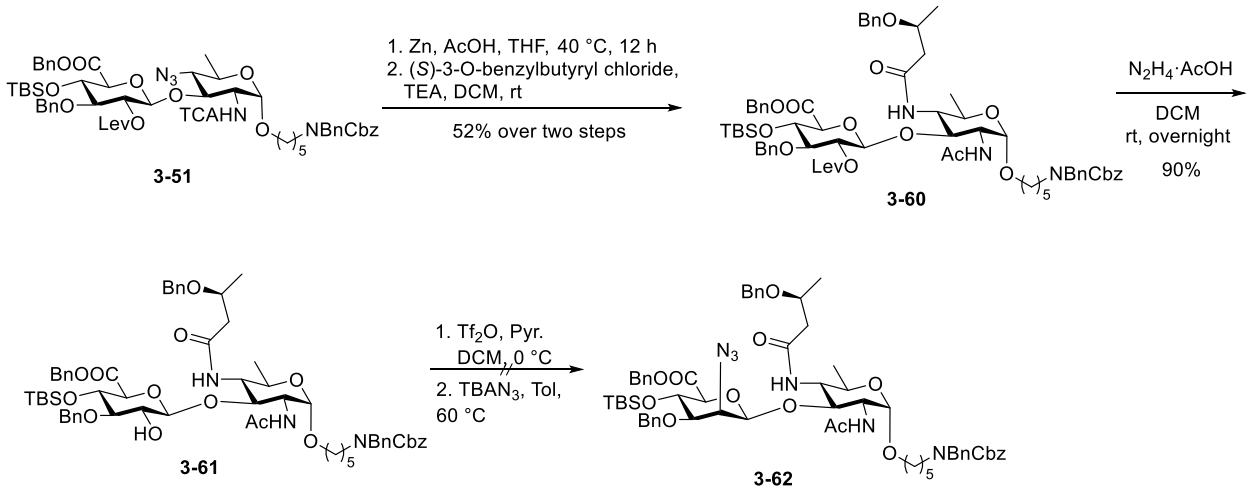


Scheme 3.13 Introduction of (*S*)-3-hydroxybutanoyl chain and double-serial inversion

Coupling of amine **3-53** with freshly made (*S*)-3-*O*-benzylbutyryl chloride gave **3-39** in 60% yield over two steps (Scheme 3.13). The Lev esters were cleaved to get diol **3-58** with two hydroxyl groups ready for inversion. However, triflation of the equatorial hydroxyl groups in **3-58** and its concomitant displacement with $TBAN_3$ failed to form the desired 2′,2″-bis azide product **3-38**. The

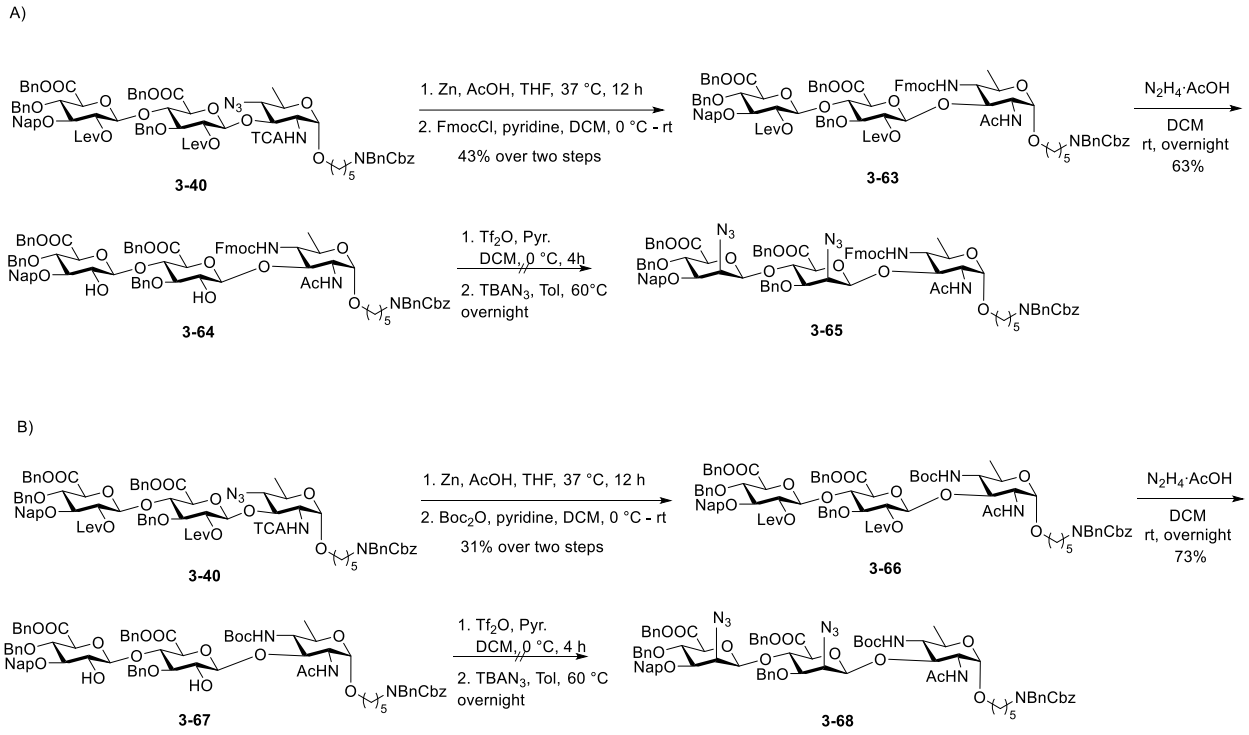
inversion succeeded only for the 2″-triflate, while the 2′-triflate was not substituted by azide but was hydrolyzed to give product **3-59** in 57% yield. The configuration and inversion sites were determined with the assistance of HMBC and HSQC. Increasing the temperature to 80 °C resulted in glycosidic bond cleavage between the two uronates.

In order to access the desired trisaccharide **3-38**, we decided to introduce the two axial azide groups one by one with two separated inversions. The first azide will be installed after inversion on disaccharide and second inversion should be performed after trisaccharide is assembled. Reduction of disaccharide **3-51** and subsequent coupling with (*S*)-3-*O*-benzylbutyryl chloride afforded **3-60** in 52% yield over two steps (Scheme 3.14). Lev was then removed with hydrazine acetate to give **3-61** followed by inversion, unfortunately, none of desired product **3-62** was observed, the alcohol starting material **3-61** was recovered.



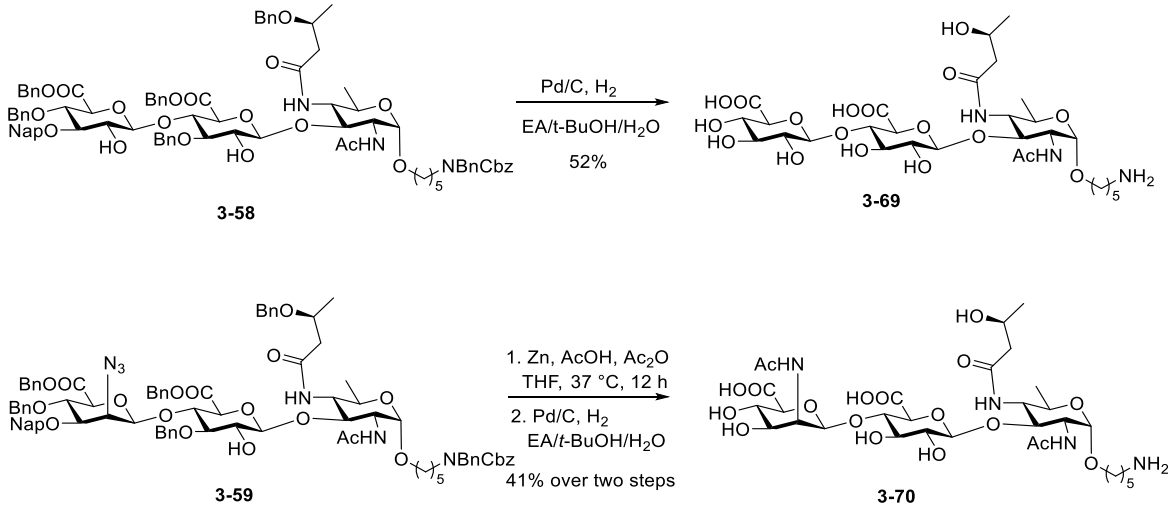
Scheme 3.14 Synthesis of disaccharide 3-61 and separated inversion strategy attempt

Since the steric hindrance effect caused by the (*S*)-3-hydroxybutanoyl chain was so strong that the separated inversion strategy did not give access to the desired product, I tried to temporarily protect the amine with Fmoc and *tert*-butyloxycarbonyl protecting group (Boc) prior to chain elongation and inversion. Trisaccharide **3-40** was reduced efficiently to an amine by zinc and protected with Fmoc group to give **3-63** in 43% overall yield (Scheme 3.15A). Delevulinoylation of **3-63** using hydrazine acetate furnished diol **3-64** that was subjected to triflation by Tf_2O and pyridine. The subsequent inversion failed to produce any azide product. The protection of amine with Boc protecting group was also attempted but the result was same as Fmoc protection: desired trisaccharide product **3-68** with two axial azide groups was failed to produce (Scheme 3.15B).



Scheme 3.15 Attempt of double-serial inversion with amine protection strategy. A) Fmoc group; B) Boc group

Since attempts to synthesize the natural repeating unit **3-2** did not meet with success, as the functionalization of amine within the D-bacillosamine derivative hinders the inversion process by disfavoring the substitution from the axial position. However, with the trisaccharide intermediates **3-58** and **3-59** we obtained, it is still a significant move to investigate the indispensability of 2′-acetamide and 2″-acetamide by glycan array screening.



Scheme 3.16 Synthesis of the analogues 3-69 and 3-70 related to 3-2

Finally, to better understand how important the *N*-acetyl groups are in antibody recognition, two analogues related to **3-2** were synthesized (Scheme 3.16). **3-69** was obtained after the global deprotection of **3-58** in 52% yield. Synthesis of analogue **3-70** involved conversion of azide in **3-59** to the corresponding NHAc by treatment with zinc and Ac_2O, due to the polarity of acetamide intermediate, purification became a super tough task. Thus, the crude acetamido sugar was directly hydrogenated under H_2 with catalysis over Pd/C to afford the second analogue **3-70** in 41% yield over two steps. Analogues **3-69** and **3-70**, together with natural repeating unit **3-1** will be employed in glycan microarray study to identify the key epitope that elicit specific immune responses against native AB5075 CPS.

3.3 Conclusion and outlook

In this chapter, the first total synthesis of a densely functionalized aminoglycoside trisaccharide repeating unit of *A. baumannii* AB5075 as well as two analogues containing a (*S*)-3-hydroxybutanoyl chain is described.

A number of synthetic challenges associated with the complicated trisaccharides were overcome including *β*-mannoside synthesis, introduction of (*S*)-3-hydroxybutanoyl and the incorporation of labile glycosidic bonds. Orthogonally protected rare sugar building blocks provided efficient and stereoselective synthetic access to the trisaccharide, S_N2 substitution of 2′,2″-bis triflate allowed for the construction of multiple 1,2-*cis* linkages.

Although the double-serial inversion on the trisaccharide containing the (*S*)-3-hydroxybutanoyl chain failed to deliver the desired 2′,2″-bis azide product, subsequent strategic adjustments did not succeed, either. The analogues based on **3-2** provide an opportunity to investigate key epitopes that induce an antibody response against the native CPS of *A. baumannii*. Double-serial inversion in the context of complex oligosaccharides is a novel approach to the synthesis of complex aminoglycosides. Conjugation-ready sugars carrying an aminopropyl linker allows for easy access to glycan microarrays and *in vivo* immunological evaluation, *en route* to the development of a synthetic glycoconjugate vaccine against *A. baumannii*.

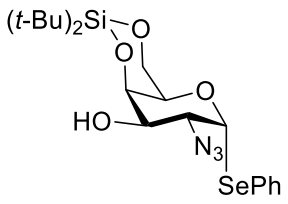
3.4 Experimental section

3.4.1 General information

Commercial grade solvents and reagents were used without further purification. Sugar building blocks indicated as commercially available were purchased from GlycoUniverse GmbH. Anhydrous solvents were obtained from a solvent drying system (JCMeyer) or dried according to reported procedures. Analytical TLC was performed on Kieselgel 60 F254 glass (Macherey-Nagel). Spots were visualized with UV light, sulphuric acid stain [1 mL of 3-methoxyphenol in 1 L of EtOH and 30 mL H_2SO_4] or ceric ammonium molybdate stain [0.5 g $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$, 12 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and 15 mL H_2SO_4 in 235 mL H_2O]. Flash chromatography was performed on Kieselgel 60 230-400 mesh (Sigma-Aldrich). Preparative HPLC purifications were performed with an Agilent 1200 Series or Agilent 1260 Infinity II. NMR spectra were recorded on a Varian 400 MHz spectrometer (Agilent), Ascend 400 MHz (cryoprobe, Bruker), Varian 600 MHz (Agilent) and Ascend 700 MHz (cryoprobe, Bruker) at 25 °C unless indicated otherwise. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks ($CHCl_3$: δ 7.26 in ^1H and 77.16 in ^{13}C; HDO δ 4.79 in ^1H). Bidimensional and non-decoupled experiments were performed to assign identities of peaks showing relevant structural features. The following abbreviations are used to indicate peak multiplicities: *s* (singlet), *d* (doublet) *dd* (doublet of doublets), *t* (triplet), *dt* (doublet of triplets), *td* (triplet of doublets), *q* (quartet), *p* (pentet), *m* (multiplet).. Coupling constants (*J*) are reported in Hertz (Hz). NMR spectra were processed using MestreNova 14.1 (MestreLab Research). High-resolution mass spectra (ESI-HRMS) were recorded with a Xevo G2-XS Q-Tof (Waters).

3.4.2 Experimental procedures

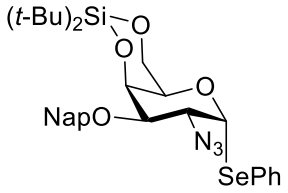
Phenyl 2-azido-2-deoxy-4,6-*O*-di-tert-butylsilyldene-1-seleno-α-D-galactopyranoside (3-10)



Compound **3-9**[196] (1.00 g, 2.90 mmol) was co-evaporated with dry toluene and dissolved in dry DMF (30 mL). The resulting solution was cooled to -50 °C and di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (1.13 mL, 3.48 mmol) and 2,6-lutidine (1.0 mL, 8.70 mmol) were added. The reaction was stirred at -50 °C for 30 minutes and subsequently quenched with brine (40 mL). The aqueous layer was washed with DCM. The combined organic phase was dried over Na_2SO_4. The solvents were removed under reduced pressure and crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 10/1) to give **3-10** as a colorless syrup (1.3 g, 93%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.45 (m, 2H), 7.24 – 7.16 (m, 3H), 5.86 (d, *J* = 5.3 Hz, 1H), 4.41 (dd, *J* = 3.5, 1.3 Hz, 1H), 4.22 (dd, *J* = 12.7, 2.3 Hz, 1H), 4.12 (dt, *J* = 2.8, 1.8 Hz, 1H), 4.01 – 3.89 (m, 2H), 3.72 (td, *J* = 10.4, 3.4 Hz, 1H), 2.71 (d, *J* = 10.8 Hz, 1H), 0.99 (s, 9H), 0.95 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.48, 129.29, 128.44, 128.01, 85.44, 72.32, 71.88, 69.84, 66.76, 62.18, 27.63, 27.32, 23.43, 20.83; HRMS (ESI) calculated for $C_{20}H_{31}N_3O_4SeSi$ $[M+Na]^+$: 508.1141, found: 508.1147.

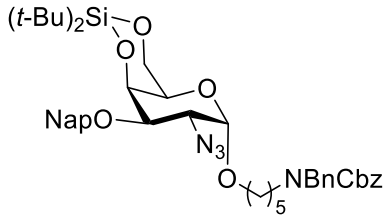
Phenyl 2-azido-2-deoxy-4,6-O-di-tert-butylsilyldene-3-O-(2-naphthylmethyl-1-seleno-α-d-galactopyranoside (3-11)



Sodium hydride was added to a solution of thiogalactoside **3-10** (1.3 g, 2.68 mmol) in DMF (20 mL) at 0 °C and stirred for 15 min at room temperature. 2-Naphthylmethyl bromide (1.18 g, 5.36 mmol) was added and the reaction mixture was stirred overnight before quenching with saturated aqueous NH_4Cl solution (10 mL) and extracting the aqueous layer with ethyl acetate (30 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 50/1) afforded **3-11** (1.6 g, 97%) as a light yellow syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 – 7.73 (m, 4H), 7.53 – 7.44 (m, 3H), 7.43 – 7.37 (m, 2H), 7.22 – 7.14 (m, 3H), 5.87 (d, *J* = 5.3 Hz, 1H), 4.87 – 4.74 (m, 2H), 4.51 (dd, *J* = 3.1, 1.0 Hz, 1H), 4.27 (dd, *J* = 10.3, 5.3 Hz, 1H), 4.13 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.96 – 3.86 (m, 2H), 3.60 (dd, *J* = 10.1, 2.9 Hz, 1H), 0.98 (s, 9H), 0.96 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.21, 134.58, 133.34, 133.21, 129.25, 128.54, 128.52, 128.06, 127.95, 127.85, 126.71, 126.31, 126.15, 125.90, 85.96, 78.86, 70.85, 70.05, 69.42, 67.07, 59.78, 27.73, 27.41, 23.51, 20.86; HRMS (ESI) calculated for $C_{31}H_{39}N_3O_4SeSi$ $[M+Na]^+$: 648.1767, found: 648.1767.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-2-deoxy-4,6-_O_-di-tert-butylsilyldene-3-_O_-(2-naphthylmethyl)-α-D-galactopyranoside (3-12)

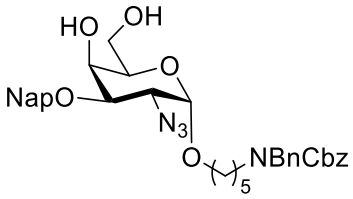


To a solution of selenoglycoside **3-11**[197] (1.6 g, 2.56 mmol), *N*-benzyloxycarbonyl-*N*-benzyl-5-aminopentanol **7** (1.26 g, 3.84 mmol) in DCM (30 mL, 0.2 M) were added 4Å molecular sieves. After 0.5 h, the mixture was cooled to 0 °C, NIS (691 mg, 3.07 mmol) and TMSOTf (46 µL, 0.256 mmol) were added. After TLC analysis indicated complete consumption of the starting material, the reaction was quenched with trimethylamine (1 mL) and diluted with DCM. After filtration, the mixture was washed with saturated aqueous $Na_2S_2O_3$, $NaHCO_3$ and brine, the combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 5/1) afforded **3-12** (1.7 g, 85%) as a colorless syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.79 (m, 4H), 7.60 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.53 – 7.44 (m, 2H), 7.42 – 7.27 (m, 9H), 7.18 (d, *J* = 7.2 Hz, 1H), 5.19 (d, *J* = 14.3 Hz, 2H), 4.97 – 4.82 (m, 3H), 4.66 – 4.59 (m, 1H), 4.51 (d, *J* = 11.1 Hz, 2H), 4.25 (t, *J* = 11.3 Hz, 1H), 4.19 – 4.10 (m, 1H), 3.97

– 3.87 (m, 1H), 3.82 (dd, *J* = 10.5, 3.5 Hz, 1H), 3.70 – 3.54 (m, 2H), 3.50 – 3.33 (m, 1H), 3.33 – 3.14 (m, 2H), 1.67 – 1.42 (m, 4H), 1.41 – 1.23 (m, 2H), 1.09 (d, *J* = 4.9 Hz, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.80, 156.26, 137.97, 136.93, 136.79, 135.46, 133.33, 133.17, 128.63, 128.57, 128.53, 128.44, 128.11, 128.01, 127.89, 127.79, 127.43, 127.36, 127.27, 126.66, 126.20, 126.03, 125.97, 98.35, 75.49, 75.40, 70.55, 69.93, 68.20, 67.42, 67.25, 58.43, 50.56, 50.27, 47.16, 46.17, 29.10, 27.74, 27.41, 23.53, 20.82; HRMS (ESI) calculated for $C_{45}H_{58}N_4O_7Si$ $[M+Na]^+$: 817.3967, found: 817.3960.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-2-deoxy-3-_O_-(2-naphthylmethyl)-_α_-D-galactopyranoside (3-13)

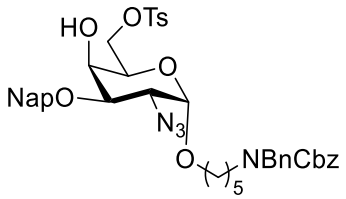


To a solution of **3-12** (940 mg, 1.18 mmol) in THF (23 mL, 0.1 M) was added HF·pyridine (70% HF, 0.25 mL, 9.47 mmol). After TLC analysis indicated complete conversion of the starting material, the reaction was quenched with triethylamine. The mixture was concentrated, resulting residue was dissolved in ethyl acetate and subsequently washed with saturated aqueous $NaHCO_3$ and brine. The aqueous layers were then extracted with ethyl acetate, combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 1/2) afforded **3-13** (780 mg, 99%) as a colorless syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.79 (m, 4H), 7.56 – 7.45 (m, 3H), 7.41 – 7.13 (m, 10H), 5.18 (d, *J* = 17.0 Hz, 2H), 4.95 – 4.77 (m, 3H), 4.57 – 4.37 (m, 2H), 4.27 – 4.09 (m, 1H), 4.00 – 3.85 (m, 2H), 3.85 – 3.54 (m, 4H), 3.49 – 3.08 (m, 3H), 2.56 (s, 2H), 1.73 – 1.41 (m, 4H), 1.41 – 1.30 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.87, 156.46, 137.89, 136.94, 136.64, 134.66, 133.30, 133.25, 128.69, 128.67, 128.56, 128.08, 128.06, 127.93, 127.85, 127.44, 127.29, 127.04, 126.43, 126.33, 125.79, 98.02, 76.15, 75.92, 72.13, 69.81, 69.46, 68.25, 67.78, 67.62, 67.38, 67.33,

67.22, 62.95, 62.55, 59.18, 50.55, 50.36, 47.30, 46.15, 32.04, 29.81, 29.49, 29.09, 28.93, 27.93, 27.17, 23.38, 23.28, 22.81, 14.27; HRMS (ESI) calculated for $C_{37}H_{42}N_4O_7$ $[M+Na]^+$: 677.2946, found: 677.2944.

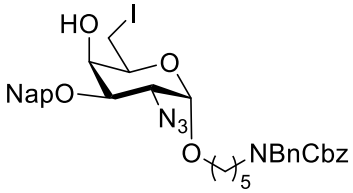
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-2-deoxy-3-_O_-(2-naphthylmethyl)-6-_O_-_p_-toluenesulfonyl-_α_-D-galactopyranoside (3-14)



To a solution of **3-13** (740 mg, 1.13 mmol) in DCM (12 mL) was added *p*-toluenesulfonyl chloride (259 mg, 1.36 mmol) and trimethylamine (0.394 mL, 2.83 mmol) at 0 °C. The reaction was stirred at room temperature overnight. After TLC analysis indicated complete conversion of the starting material, the reaction was diluted with DCM and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-14** as a colorless syrup (670 mg, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.80 (m, 4H), 7.80 – 7.73 (m, 2H), 7.54 – 7.45 (m, 3H), 7.41 – 7.14 (m, 12H), 5.19 (d, *J* = 13.9 Hz, 2H), 4.84 (q, *J* = 11.5 Hz, 3H), 4.51 (d, *J* = 9.0 Hz, 2H), 4.25 – 4.10 (m, 2H), 4.06 (s, 1H), 4.03 – 3.88 (m, 2H), 3.67 – 3.52 (m, 2H), 3.43 – 3.15 (m, 2H), 2.42 (s, 3H), 1.64 – 1.44 (m, 4H), 1.40 – 1.21 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.84, 156.30, 145.10, 138.06, 136.99, 136.86, 134.48, 133.29, 133.28, 132.70, 129.97, 128.76, 128.70, 128.65, 128.60, 128.53, 128.08, 128.01, 127.93, 127.87, 127.32, 127.09, 126.51, 126.42, 125.73, 97.89, 75.75, 75.72, 72.39, 68.85, 68.40, 67.87, 67.25, 65.99, 58.95, 50.58, 50.29, 47.19, 46.21, 29.06, 27.95, 27.51, 23.42, 21.77; HRMS (ESI) calculated for $C_{44}H_{48}N_4O_9S$ $[M+Na]^+$: 831.3034, found: 831.3029.

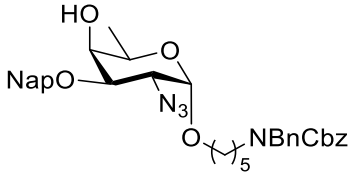
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-6-iodo-2,6-di-deoxy-3-_O_-(2-naphthylmethyl)-_α_-D-galactopyranoside (3-15)



To a solution of **3-14** (640 mg, 0.79 mmol) in acetone (20 mL) was added sodium iodide (593 mg, 3.96 mmol) and the reaction was stirred and refluxed at 80 °C for 72 h under N_2. After cooling to room temperature, ethyl acetate (20 mL) was added and the mixture was washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-15** as a colorless syrup (549 mg, 91%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.80 (m, 4H), 7.56 – 7.45 (m, 3H), 7.42 – 7.13 (m, 10H), 5.18 (d, *J* = 14.5 Hz, 2H), 4.88 (q, *J* = 11.5 Hz, 3H), 4.51 (d, *J* = 10.3 Hz, 2H), 4.20 (d, *J* = 7.0 Hz, 1H), 4.00 – 3.83 (m, 2H), 3.82 – 3.67 (m, 1H), 3.64 (dd, *J* = 10.4, 3.7 Hz, 1H), 3.51 – 3.15 (m, 5H), 2.41 (s, 1H), 1.69 – 1.46 (m, 4H), 1.41 – 1.27 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.85, 156.31, 138.04, 136.99, 136.84, 134.54, 133.31, 128.79, 128.67, 128.62, 128.56, 128.09, 128.05, 127.94, 127.89, 127.39, 127.30, 127.13, 126.51, 126.43, 125.77, 97.93, 76.18, 72.48, 70.72, 68.40, 67.28, 67.18, 58.84, 50.61, 50.33, 47.23, 46.22, 29.83, 29.10, 28.00, 27.58, 23.50; HRMS (ESI) calculated for $C_{37}H_{41}IN_4O_6$ $[M+Na]^+$: 787.1963, found: 787.1968.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-2-deoxy-3-_O_-(2-naphthylmethyl)-_α_-D-fucopyranoside (3-16)

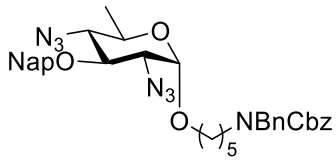


To a solution of a mixture of iodide **3-15** (1.0 g, 1.31 mmol) and AIBN (21.4 mg, 0.13 mmol) in degassed toluene (15 mL) at 75 °C, was added Bu_3SnH (422.4 µL, 1.57 mmol) under an Ar

atmosphere. After 1 h of stirring at this temperature, the mixture was cooled to room temperature, after which time the reaction mixture was diluted with ethyl acetate (80 mL), the organic layer was washed with water and brine, dried over Na_2SO4, filtered and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to afford **3-16** (730 mg, 87%) as a colorless syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.81 (m, 4H), 7.59 – 7.47 (m, 3H), 7.45 – 7.15 (m, 10H), 5.20 (d, *J* = 13.2 Hz, 2H), 4.96 – 4.81 (m, 3H), 4.52 (d, *J* = 10.0 Hz, 2H), 4.03 – 3.83 (m, 3H), 3.72 – 3.55 (m, 2H), 3.50 – 3.34 (m, 1H), 3.33 – 3.16 (m, 2H), 2.15 (s, 1H), 1.58 (qd, *J* = 11.6, 4.6 Hz, 4H), 1.31 (d, *J* = 6.6 Hz, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.85, 156.31, 138.02, 136.98, 136.84, 134.77, 133.32, 133.27, 128.72, 128.67, 128.56, 128.08, 128.05, 127.94, 127.87, 127.40, 127.30, 127.05, 126.44, 126.34, 125.83, 98.02, 72.16, 69.02, 68.18, 67.28, 65.57, 59.03, 50.59, 50.30, 47.20, 46.20, 29.20, 27.98, 27.57, 23.50, 23.43, 16.36; HRMS (ESI) calculated for $C_{37}H_{42}N_4O_6$ $[M+Na]^+$: 661.2997, found: 661.2998.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,4-di-azido-2,4-di-deoxy-3-_O_-(2-naphthylmethyl)-_α_-D-quinovopyranoside (3-17)



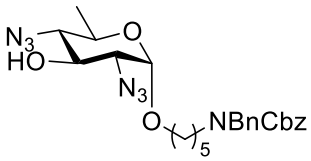
Monosaccharide **3-16** (141 mg, 0.22 mmol) was dissolved in anhydrous DCM (4 mL) and pyridine (90 µL). The solution was cooled to 0 °C and triflic anhydride (75 µL, 0.44 mmol) was added. The solution was stirred at 0 °C for 1 h and then quenched by adding cold water. The organic layer was washed twice with cold water and brine, combined organic phase was dried over Na_2SO_4, filtered and concentrated to give the triflate as a yellow solid that was used without further purification.

The solid was dissolved in anhydrous DMF (4 mL) and sodium azide (29 mg, 0.44 mmol) was added. The reaction mixture was stirred at room temperature for 5 h. After that, DMF was evaporated under reduced pressure, the residue was dissolved in ethyl acetate and washed with

water and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 3/1) to give **3-17** (1.44 g, 92%) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.71 (m, 4H), 7.49 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.43 – 7.36 (m, 2H), 7.34 – 7.04 (m, 10H), 5.11 (d, *J* = 14.8 Hz, 2H), 5.00 – 4.89 (m, 2H), 4.79 – 4.69 (m, 1H), 4.43 (d, *J* = 10.3 Hz, 2H), 3.83 – 3.73 (m, 1H), 3.54 (tq, *J* = 15.8, 6.8 Hz, 2H), 3.40 – 3.11 (m, 4H), 3.10 – 3.01 (m, 1H), 1.68 – 1.39 (m, 4H), 1.35 – 1.15 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.84, 156.31, 138.01, 137.00, 136.84, 134.83, 133.40, 133.24, 128.67, 128.61, 128.55, 128.40, 128.22, 128.17, 128.05, 127.95, 127.92, 127.79, 127.48, 127.43, 127.30, 126.28, 126.21, 126.17, 97.71, 78.56, 75.48, 68.73, 68.29, 67.29, 66.53, 63.53, 50.63, 50.33, 47.17, 46.20, 29.17, 29.15, 27.98, 27.57, 23.46, 18.50; HRMS (ESI) calculated for $C_{37}H_{41}N_7O_5$ $[M+Na]^+$: 686.3061, found: 686.3060.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,4-di-azido-2,4-di-deoxy-*α*-D-quinovopyranoside (3-6)

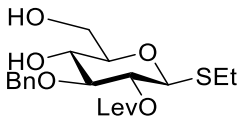


To a solution of **3-17** (150 mg, 0.23 mmol) in 20:1 (v/v) DCM-H_2O (4.2 mL), DDQ (77 mg, 0.34 mmol) was added and the reaction mixture was stirred for 5 h. The reaction was diluted with DCM (10 mL) and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-6** as a colorless syrup (106 mg, 89%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.15 (m, 10H), 5.21 (d, *J* = 13.7 Hz, 2H), 4.87 – 4.79 (m, 1H), 4.53 (d, *J* = 9.8 Hz, 2H), 4.00 (q, *J* = 9.2 Hz, 1H), 3.63 (dq, *J* = 16.0, 8.0 Hz, 2H), 3.47 – 3.15 (m, 4H), 3.08 (t, *J* = 9.7 Hz, 1H), 3.02 (s, 1H), 1.71 – 1.47 (m, 4H), 1.43 – 1.24 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.91, 156.37, 137.97, 136.81, 128.67, 128.56, 128.05, 127.94, 127.40,

127.31, 97.63, 70.83, 68.95, 68.31, 67.35, 66.40, 63.40, 50.62, 50.36, 47.19, 46.20, 29.12, 27.95, 27.56, 23.42, 18.40; HRMS (ESI) calculated for $C_{26}H_{33}N_7O_5$ $[M+Na]^+$: 523.2543, found: 523.2539.

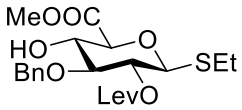
Ethyl 3-*O*-benzyl-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranoside (3-19)



Thioglycoside **3-18**[201] (6.0 g) was dissolved in a mixed solution of DCM/TFA/H_2O (10/1/0.1, 120 mL/12 mL/1.2mL) at 0 °C. The reaction mixture was stirred at room temperature for 30 min, quenched with triethylamine (20 mL) and washed with saturated aqueous $NaHCO_3$ and brine, the organic layer was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 2/3) to afford **3-19** (3.5 g, 71%) as a colorless syrup.

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.43 – 7.30 (m, 5H), 5.01 (t, 1H), 4.85 (d, *J* = 11.7 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 1H), 4.45 (dd, *J* = 10.0, 1.0 Hz, 1H), 3.90 (dd, *J* = 12.0, 1.2 Hz, 1H), 3.79 (dd, *J* = 12.0, 1.2 Hz, 1H), 3.70 – 3.64 (m, 1H), 3.61 – 3.55 (m, 1H), 3.43 – 3.38 (m, 1H), 2.85 – 2.77 (m, 1H), 2.76 – 2.64 (m, 3H), 2.64 – 2.55 (m, 2H), 2.46 (s, 3H), 2.19 (s, 3H), 1.29 – 1.26 (m, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 206.35, 171.72, 138.29, 128.75, 128.13, 128.07, 83.95, 83.82, 79.55, 74.80, 72.12, 70.53, 62.70, 37.95, 29.95, 28.23, 24.26, 15.01; HRMS (ESI) calculated for $C_{20}H_{28}O_7S$ $[M+Na]^+$: 435.1448, found: 435.1454.

Methyl (ethyl 3-*O*-benzyl-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranosyl) uronate (3-20)

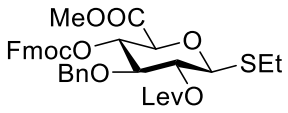


Thioglycoside **3-19** (1.5 g, 3.64 mmol) was dissolved in DCM (30 mL) and H_2O (6 mL). 2,2,6,6-Tetramethylpiperidine 1-oxyl (56.8 mg, 0.36 mmol) was added followed by $PhI(OAc)_2$ (1.87 g,

5.82 mmol) at 0 °C. The reaction mixture was warmed to room temperature after 15 min. Additional $PhI(OAc)_2$ (586 mg, 1.82 mmol) and 2,2,6,6-tetramethylpiperidine 1-oxyl (56.8 mg, 0.36 mmol) were added. After 4 h, the reaction mixture was quenched by the addition of saturated aqueous $Na_2S_2O_3$, and the organic layer was washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was dissolved in DMF (30 mL), K_2CO_3 (754 mg, 5.46 mmol) and MeI (340 µL, 5.46 mmol) were added at 0 °C. Then the reaction was stirred overnight at room temperature. After complete consumption of the starting material, the mixture was diluted with ethyl acetate (20 mL) and washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 3/2) to obtain **3-20** (1.0 g, 62%) as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 – 7.21 (m, 5H), 5.02 (t, *J* = 9.6 Hz, 1H), 4.83 – 4.80 (m, 2H), 4.46 (d, *J* = 10.1 Hz, 1H), 3.99 – 3.94 (m, 1H), 3.90 – 3.86 (m, 1H), 3.83 (s, 3H), 3.60 (t, *J* = 9.0 Hz, 1H), 3.03 (s, 1H), 2.82 – 2.65 (m, 4H), 2.56 (t, *J* = 6.9 Hz, 2H), 2.19 (s, 3H), 1.30 – 1.24 (m, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.34, 171.58, 169.43, 138.27, 128.62, 128.60, 128.58, 128.12, 128.10, 127.97, 84.36, 82.52, 77.94, 74.86, 71.97, 71.20, 52.96, 37.94, 29.98, 28.11, 24.31, 14.90; HRMS (ESI) calculated for $C_{21}H_{28}O_8S$ $[M+Na]^+$: 463.1397, found:463.1405.

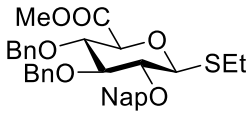
Methyl (ethyl 3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranosyl) uronate (3-7)



To a solution of **3-20** (940 mg, 2.14 mmol) in anhydrous DCM (20 mL), pyridine (688 µL, 8.56 mmol) and fluorenylmethyloxycarbonyl chloride (1.1 g, 4.27 mmol) were added and the mixture was stirred for 5 h. After the starting material was consumed, diluted with DCM (10 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine. Then, organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-7** as a white solid (1.2 g, 86%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (t, *J* = 7.6 Hz, 2H), 7.69 – 7.58 (m, 2H), 7.42 (q, *J* = 7.4 Hz, 2H), 7.35 – 7.21 (m, 7H), 5.17 – 5.06 (m, 2H), 4.76 – 4.64 (m, 2H), 4.50 – 4.43 (m, 2H), 4.36 (dd, *J* = 10.5, 7.6 Hz, 1H), 4.25 (t, *J* = 7.3 Hz, 1H), 4.07 (d, *J* = 10.0 Hz, 1H), 3.83 (t, *J* = 9.2 Hz, 1H), 3.71 (s, 3H), 2.82 – 2.64 (m, 4H), 2.55 (td, *J* = 6.9, 5.5 Hz, 2H), 2.20 (s, 3H), 1.28 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.25, 171.39, 167.37, 154.09, 143.42, 143.13, 141.43, 141.39, 137.70, 128.46, 128.44, 128.20, 128.09, 128.07, 128.02, 127.92, 127.34, 125.28, 125.19, 120.22, 83.92, 80.50, 76.39, 75.13, 74.67, 71.09, 70.52, 52.99, 46.72, 37.92, 29.98, 28.04, 24.10, 14.84; HRMS (ESI) calculated for $C_{36}H_{38}O_{10}S$ $[M+Na]^+$: 685.2078, found: 685.2080.

Methyl (ethyl 3,4-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-*β*-D-glucopyranosyl) uronate (3-22)

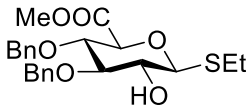


Thioglycoside **3-21**[123] (2.13 g, 3.91 mmol) was dissolved in DCM (30 mL) and H_2O (6 mL). 2,2,6,6-Tetramethylpiperidine 1-oxyl (61.2 mg, 0.39 mmol) was added followed by $PhI(OAc)_2$ (2.0 g, 6.26 mmol) at 0 °C. The reaction mixture was warmed to room temperature after 15 min. Additional $PhI(OAc)_2$ (630 mg, 1.96 mmol) and 2,2,6,6-tetramethylpiperidine 1-oxyl (61.2 mg, 0.39 mmol) were added. After 4 h, the reaction mixture was quenched by the addition of saturated aqueous $Na_2S_2O_3$, and the organic layer was washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was dissolved in DMF (40 mL), K_2CO_3 (648 mg, 4.69 mmol) and MeI (365 µL, 5.87 mmol) were added at 0 °C. Then, the reaction was stirred overnight at room temperature. After complete consumption of the starting material, the mixture was diluted with ethyl acetate (20 mL) and washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 8/1) to obtain **3-22** (1.0 g, 62%) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.79 (m, 4H), 7.61 – 7.47 (m, 3H), 7.43 – 7.26 (m, 10H), 5.14 (d, *J* = 10.5 Hz, 1H), 5.03 – 4.90 (m, 3H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.69 (d, *J* = 10.8 Hz,

1H), 4.61 (d, *J* = 9.7 Hz, 1H), 4.05 – 3.87 (m, 2H), 3.78 (d, *J* = 3.0 Hz, 4H), 3.60 (dd, *J* = 9.7, 8.8 Hz, 1H), 2.95 – 2.74 (m, 2H), 1.38 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.81, 138.35, 137.83, 135.36, 133.33, 133.16, 128.54, 128.51, 128.24, 128.17, 128.09, 128.04, 127.98, 127.82, 127.81, 127.76, 127.13, 126.37, 126.12, 126.03, 85.94, 85.90, 81.27, 79.38, 78.17, 75.89, 75.74, 75.19, 52.57, 25.24, 15.08; HRMS (ESI) calculated for $C_{34}H_{35}O_6S$ $[M+Na]^+$: 595.2125, found: 595.2131.

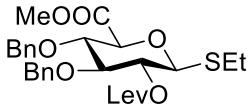
Methyl (ethyl 3,4-di-*O*-benzyl-1-thio-*β*-D-glucopyranosyl) uronate (3-23)



To a solution of **3-22** (200 mg, 0.35 mmol) in 20:1 (v/v) DCM-H_2O (5.25 mL), DDQ (119 mg, 0.52 mmol) was added and the reaction mixture was stirred for 5 h. The reaction was diluted with DCM (10 mL) and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 4/1) to give **3-23** as a white solid (123 mg, 81%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 – 7.17 (m, 10H), 4.96 (dd, *J* = 11.3, 3.2 Hz, 1H), 4.89 (dd, *J* = 11.3, 3.3 Hz, 1H), 4.84 (dd, *J* = 10.8, 3.3 Hz, 1H), 4.63 (dd, *J* = 10.9, 3.2 Hz, 1H), 4.40 – 4.35 (m, 1H), 3.94 (dd, *J* = 9.8, 3.0 Hz, 1H), 3.88 – 3.83 (m, 1H), 3.74 (s, 3H), 3.65 – 3.56 (m, 2H), 2.81 – 2.70 (m, 2H), 2.44 (s, 1H), 1.32 (td, *J* = 7.4, 3.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.73, 138.44, 137.86, 128.66, 128.54, 128.14, 128.10, 128.06, 128.02, 127.99, 86.90, 85.19, 78.87, 78.44, 75.41, 75.30, 72.92, 52.65, 24.56, 15.31; HRMS (ESI) calculated for $C_{23}H_{28}O_6S$ $[M+Na]^+$: 455.1499, found: 455.1508.

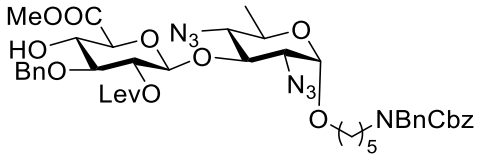
Methyl (ethyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranosyl) uronate (3-8)



Levulinic acid (66 mg, 0.57 mmol), EDC·HCl (117.6 mg, 0.57 mmol) and 4-dimethylaminopyridine (7 mg, 0.06 mmol) were added to a solution of thioglycoside **3-23** (123 mg, 0.29 mmol) in DCM (30 mL). The mixture was stirred for 4 h at room temperature. Then, the reaction mixture was washed with saturated aqueous $NaHCO_3$ and brine, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-8** (116 mg, 77%) as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.15 (m, 10H), 5.05 (dd, *J* = 10.0, 9.1 Hz, 1H), 4.84 – 4.69 (m, 3H), 4.60 (d, *J* = 10.8 Hz, 1H), 4.41 (d, *J* = 10.0 Hz, 1H), 3.97 – 3.84 (m, 2H), 3.77 – 3.66 (m, 4H), 2.80 – 2.61 (m, 4H), 2.58 – 2.44 (m, 2H), 2.17 (s, 3H), 1.24 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.25, 171.58, 168.49, 138.06, 137.68, 128.56, 128.53, 128.17, 128.10, 128.08, 127.92, 84.19, 83.44, 79.28, 78.36, 75.32, 75.28, 71.65, 52.68, 37.93, 29.98, 28.11, 24.06, 14.81; HRMS (ESI) calculated for $C_{28}H_{34}O_8S$ $[M+Na]^+$: 553.1867, found: 553.1871.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl methyl-3-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyluronate-(1→3)-2,4-di-azido-2,4-di-deoxy-*α*-D-quinovopyranoside (3-24)**

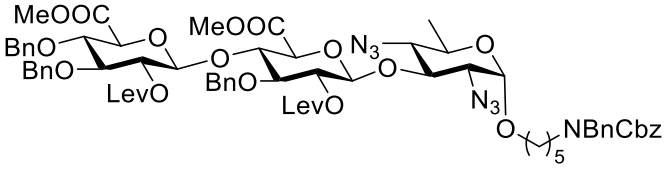


Thioglycoside **3-7** (150 mg, 0.226 mmol) and compound **3-6** (86 mg, 0.164 mmol) were coevaporated three times with toluene, the resulting mixture was dried under high vacuum for 2 h. Then, the mixture was dissolved in anhydrous DCM (1 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to -20 °C, NIS (55 mg, 0.246 mmol) and TfOH (2.9 µL, 0.03 mmol) were added. The reaction mixture was stirred at 0 °C

for 4 h. After complete consumption of the staring material, Et_3N (0.1 mL) was added and the mixture was stirred for 1 h. Then, the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 3/2) to give **3-24** (70 mg, 67%) as a colorless syrup.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 – 7.16 (m, 15H), 5.25 – 5.15 (m, 2H), 5.05 (t, *J* = 1.9 Hz, 1H), 4.86 (dd, *J* = 11.8, 1.9 Hz, 2H), 4.79 (dd, *J* = 11.9, 1.9 Hz, 1H), 4.73 (dd, *J* = 8.0, 1.6 Hz, 1H), 4.52 (d, *J* = 13.6 Hz, 2H), 4.00 (t, *J* = 1.8 Hz, 1H), 3.97 – 3.90 (m, 2H), 3.81 (s, 3H), 3.68 – 3.53 (m, 3H), 3.46 – 3.19 (m, 4H), 3.14 (s, 1H), 3.11 – 3.04 (m, 1H), 2.81 – 2.73 (m, 1H), 2.73 – 2.62 (m, 2H), 2.52 – 2.42 (m, 1H), 2.18 (s, 3H), 1.71 – 1.49 (m, 4H), 1.41 – 1.33 (m, 2H), 1.31 (d, *J* = 1.8 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.38, 171.67, 169.63, 156.82, 156.29, 138.40, 138.00, 136.99, 136.83, 128.66, 128.60, 128.53, 128.37, 128.07, 128.04, 127.95, 127.92, 127.89, 127.84, 127.47, 127.40, 127.30, 101.50, 97.57, 81.37, 77.99, 74.76, 74.07, 72.64, 72.04, 68.29, 68.20, 67.28, 67.27, 66.88, 66.17, 62.85, 52.89, 50.63, 50.34, 47.16, 46.16, 37.82, 29.99, 29.14, 27.95, 27.80, 27.54, 23.41, 18.52; HRMS (ESI) calculated for $C_{45}H_{55}N_7O_{13}$ $[M+Na]^+$: 924.3750, found: 924.3629.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl methyl-3,4-di-_O_-benzyl-2-_O_-levulinoyl-_β_-D-glucopyranosyluronate-(1→4)-methyl-3-_O_-benzyl-2-_O_-levulinoyl-_β_-D-glucopyranosyluronate-(1→3)-2,4-di-azido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-5)

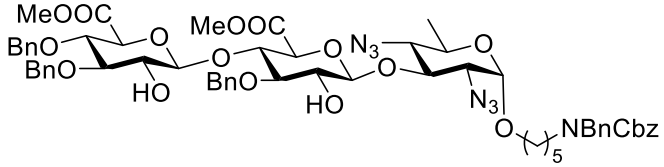


Thioglycoside **3-8** (70.6 mg, 0.133 mmol) and disaccharide **3-24** (60 mg, 0.067 mmol) were coevaporated three times with toluene, the resulting mixture was dried under high vacuum for 2 h. Then, the mixture was dissolved in anhydrous DCM (1 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to -20 °C, NIS (30 mg, 0.133 mmol) and TfOH (1.2 µL, 0.013 mmol) were added. The reaction mixture was stirred at 0

°C for 4 h. After complete consumption of the staring material, Et_3N (0.1 mL) was added to quench the reaction. Then, the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-5** (56 mg, 68%) as a colorless syrup.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 – 7.12 (m, 25H), 5.24 – 5.15 (m, 2H), 5.04 – 4.96 (m, 3H), 4.88 – 4.68 (m, 6H), 4.64 (dd, *J* = 8.0, 1.5 Hz, 1H), 4.58 (t, *J* = 11.9 Hz, 2H), 4.52 (d, *J* = 13.1 Hz, 2H), 4.19 (ddd, *J* = 10.1, 8.6, 1.5 Hz, 1H), 3.98 (dd, *J* = 9.6, 1.5 Hz, 1H), 3.93 – 3.87 (m, 3H), 3.78 (s, 3H), 3.71 – 3.61 (m, 3H), 3.54 (s, 3H), 3.46 – 3.19 (m, 5H), 3.07 – 3.00 (m, 1H), 2.81 – 2.66 (m, 4H), 2.65 – 2.56 (m, 2H), 2.56 – 2.41 (m, 2H), 2.30 – 2.23 (m, 1H), 2.19 (s, 3H), 2.14 (s, 3H), 1.65 – 1.50 (m, 4H), 1.39 – 1.31 (m, 2H), 1.28 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.39, 206.34, 171.67, 171.56, 168.40, 168.22, 156.82, 156.30, 138.84, 138.07, 138.02, 137.68, 137.01, 136.85, 128.67, 128.61, 128.56, 128.52, 128.48, 128.29, 128.11, 128.08, 128.03, 128.01, 127.97, 127.93, 127.91, 127.86, 127.84, 127.74, 127.47, 127.38, 127.32, 101.45, 100.83, 97.53, 81.83, 80.31, 79.44, 78.62, 77.77, 75.08, 74.96, 74.54, 74.25, 73.13, 72.65, 72.62, 68.31, 67.29, 67.27, 66.76, 66.12, 62.82, 52.80, 52.52, 50.66, 50.37, 47.19, 46.21, 37.89, 37.83, 30.01, 29.96, 29.11, 27.97, 27.82, 27.78, 27.70, 27.55, 23.38, 18.50; HRMS (ESI) calculated for $C_{71}H_{83}N_7O_{21}$ $[M+Na]^+$: 1392.5534, found: 1392.5532.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl methyl-3,4-di-_O_-benzyl-_β_-D-glucopyranosyluronate-(1→4)-methyl-3-_O_-benzyl-_β_-D-glucopyranosyluronate-(1→3)-2,4-di-azido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-25)

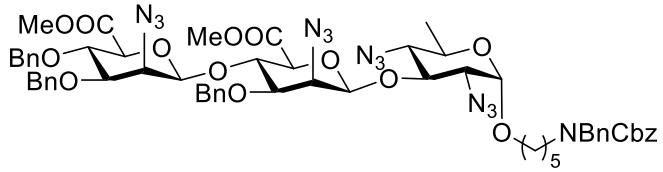


Trisaccharide **3-5** (36 mg, 26.2 μmol) was dissolved in DCM (2.0 mL), N_2H_4·AcOH (48 mg, 525 μmol) was added and the mixture was stirred at room temperature for 12 h. Then, the reaction was quenched with acetone (0.5 mL) and concentrated under reduced pressure. The residue was

purified by flash silica column chromatography (toluene/ethyl acetate = 2/1) to give **3-25** (29 mg, 93%) as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 – 7.12 (m, 25H), 5.25 – 5.17 (m, 2H), 4.97 (dd, *J* = 11.4, 8.0 Hz, 2H), 4.92 – 4.76 (m, 4H), 4.64 – 4.48 (m, 5H), 4.11 (td, *J* = 9.0, 1.5 Hz, 1H), 4.05 (dd, *J* = 9.8, 1.5 Hz, 1H), 3.99 – 3.90 (m, 1H), 3.86 – 3.75 (m, 5H), 3.71 – 3.59 (m, 4H), 3.59 – 3.56 (m, 4H), 3.47 – 3.40 (m, 1H), 3.37 (d, *J* = 9.3 Hz, 1H), 3.34 – 3.20 (m, 4H), 3.06 (t, *J* = 9.6 Hz, 1H), 2.79 (s, 1H), 1.67 – 1.49 (m, 4H), 1.45 – 1.32 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.19, 168.77, 156.85, 156.32, 138.77, 138.62, 137.99, 137.89, 137.00, 136.84, 129.16, 128.84, 128.68, 128.62, 128.53, 128.49, 128.35, 128.20, 128.11, 128.10, 128.06, 127.98, 127.95, 127.89, 127.81, 127.62, 127.56, 127.47, 127.32, 125.42, 104.20, 104.04, 97.26, 83.54, 82.63, 79.01, 78.84, 78.49, 75.31, 75.20, 74.97, 74.84, 74.65, 74.59, 74.11, 68.41, 68.32, 67.78, 67.31, 67.29, 66.45, 62.76, 53.15, 52.44, 50.65, 50.37, 47.15, 46.16, 37.21, 32.05, 30.16, 29.83, 29.79, 29.62, 29.49, 29.12, 27.95, 27.55, 23.43, 23.35, 22.82, 18.47, 14.27; HRMS (ESI) calculated for $C_{61}H_{71}N_7O_{17}$ $[M+Na]^+$: 1196.4799, found: 1196.4983.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl methyl-2-azido-2-deoxy-3,4-di-_O_-benzyl-_β_-D-mannopyranosyluronate-(1→4)-methyl-2-azido-2-deoxy-3-_O_-benzyl-_β_-D-mannopyranosyluronate-(1→3)-2,4-di-azido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-4)

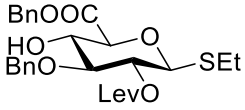


Trisaccharide **3-25** (17 mg, 0.011 mmol) was dissolved in anhydrous DCM (1.0 mL) and pyridine (18 µL, 0.22 mmol). The solution was cooled to 0 °C and triflic anhydride (12 µL, 0.066 mmol) was added. The solution was stirred at 0 °C for 4 h and the reaction was quenched by adding cold water. The organic layer was washed twice with cold water and cold brine, organic layer was dried over Na_2SO_4. The solvent was evaporated giving the triflate compound as a yellow solid that was used without further purification.

The solid was dissolved in anhydrous toluene (1.0 mL) and tetrabutylammonium azide (31 mg, 0.11 mmol) was added. The reaction mixture was stirred overnight at 60 °C. The reaction was diluted with ethyl acetate and wash with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated and the resulting residue was purified by flash silica column chromatography (toluene/ethyl acetate = 1/1) give **3-4** (12.3 mg, 72%) as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.04 (m, 25H), 5.17 – 5.04 (m, 2H), 4.80 – 4.62 (m, 7H), 4.57 (dd, *J* = 12.0, 2.2 Hz, 1H), 4.52 (dd, *J* = 10.8, 2.0 Hz, 1H), 4.43 (d, *J* = 13.4 Hz, 2H), 4.12 (td, *J* = 9.4, 2.1 Hz, 1H), 3.96 – 3.87 (m, 2H), 3.84 – 3.76 (m, 2H), 3.76 – 3.73 (m, 1H), 3.68 (dd, *J* = 9.8, 2.2 Hz, 1H), 3.64 (s, 3H), 3.58 (dt, *J* = 9.2, 2.5 Hz, 1H), 3.55 (s, 3H), 3.53 – 3.43 (m, 3H), 3.35 – 3.09 (m, 4H), 2.96 (td, *J* = 9.8, 2.2 Hz, 1H), 1.58 – 1.40 (m, 4H), 1.30 – 1.20 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.23, 168.14, 156.84, 156.31, 137.99, 137.93, 137.70, 137.45, 128.75, 128.70, 128.62, 128.57, 128.51, 128.29, 128.23, 128.21, 128.19, 128.11, 128.03, 127.98, 127.52, 127.45, 127.31, 101.50, 101.03, 96.99, 79.91, 79.45, 78.62, 76.17, 75.46, 75.32, 75.10, 73.97, 73.45, 72.50, 68.36, 67.31, 66.44, 63.16, 62.48, 62.03, 52.91, 52.54, 50.63, 50.37, 47.17, 46.11, 32.06, 29.83, 29.50, 29.10, 27.92, 27.56, 23.40, 23.30, 22.83, 18.50, 14.27; HRMS (ESI) calculated for $C_{61}H_{69}N_{13}O_{15}$ $[M+Na]^+$: 1246.4928, found: 1246.4882.

Benzyl (ethyl 3-*O*-benzyl-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranosyl) uronate (3-27)

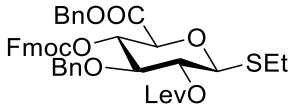


Compound **3-19** (3.5 g, 8.5 mmol) was dissolved in DCM (70 mL) and H_2O (14 mL). 2,2,6,6-Tetramethylpiperidine 1-oxyl (132.7 mg, 0.85 mmol) was added followed by $PhI(OAc)_2$ (4.4 g, 13.6 mmol) at 0 °C. The reaction mixture was warmed to room temperature after 15 min. Additional $PhI(OAc)_2$ (1.4 g, 4.3 mmol) and 2,2,6,6-tetramethylpiperidine 1-oxyl (132.7 mg, 0.85 mmol) were added. After 4 h, the reaction mixture was quenched by the addition of saturated aq. $Na_2S_2O_3$, and the organic layer was washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was dissolved in DMF (80

mL), $NaHCO_3$ (1.1 g, 12.75 mmol) and BnBr (1.52 mL, 12.75 mmol) were added at 0 °C. Then, the reaction was stirred overnight at room temperature. After complete consumption of the starting material, the mixture was diluted with ethyl acetate (50 mL) and washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to obtain **3-27** (2.9 g, 66%) as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34 – 7.16 (m, 10H), 5.20 – 5.12 (m, 2H), 4.97 – 4.91 (m, 1H), 4.71 (s, 2H), 4.36 (d, *J* = 10.0 Hz, 1H), 3.89 (t, *J* = 9.4 Hz, 1H), 3.81 (d, *J* = 9.8 Hz, 1H), 3.50 (t, *J* = 9.0 Hz, 1H), 2.87 (s, 1H), 2.72 – 2.52 (m, 4H), 2.46 (t, *J* = 6.4 Hz, 2H), 2.09 (s, 3H), 1.16 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.32, 171.56, 168.60, 138.23, 135.06, 128.73, 128.62, 128.57, 128.31, 128.07, 127.94, 84.38, 82.54, 78.10, 74.86, 71.90, 71.24, 67.52, 37.92, 29.95, 28.09, 24.32, 15.08; HRMS (ESI) calculated for $C_{27}H_{32}O_8S$ $[M+Na]^+$: 539.1710, found: 539.1714.

Benzyl (ethyl 3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranosyl) uronate (3-28)

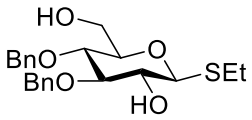


To a solution of **3-27** (2.1 g, 4.07 mmol) in anhydrous DCM (2.5 mL), pyridine (2.0 mL, 40.7 mmol) and fluorenylmethyloxycarbonyl chloride (2.1 g, 8.14 mmol) were added and the mixture was stirred for 5 h. After the starting material was consumed, diluted with DCM (10 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine. Then, organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (toluene/acetone = 30/1) to give **3-28** as a white solid (1.9 g, 64%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (dd, *J* = 7.6, 3.9 Hz, 2H), 7.59 (dd, *J* = 14.5, 7.5 Hz, 2H), 7.43 (tt, *J* = 7.5, 1.8 Hz, 2H), 7.39 – 7.20 (m, 12H), 5.26 – 5.09 (m, 4H), 4.69 (s, 2H), 4.49 (d, *J* = 10.0 Hz, 1H), 4.29 (dd, *J* = 10.3, 7.6 Hz, 1H), 4.20 (dd, *J* = 10.3, 6.9 Hz, 1H), 4.17 – 4.10 (m, 2H), 3.84

(t, *J* = 9.1 Hz, 1H), 2.85 – 2.64 (m, 4H), 2.61 – 2.52 (m, 2H), 2.20 (s, 3H), 1.29 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.16, 171.31, 166.72, 153.88, 143.35, 143.06, 141.35, 141.28, 137.62, 134.86, 128.56, 128.50, 128.47, 128.42, 128.37, 128.35, 128.00, 127.97, 127.94, 127.83, 127.25, 125.20, 125.14, 120.12, 83.82, 80.53, 76.29, 74.98, 74.56, 71.03, 70.27, 67.68, 46.59, 37.83, 29.90, 27.98, 23.98, 14.91; HRMS (ESI) calculated for $C_{42}H_{42}O_{10}S$ $[M+Na]^+$: 761.2391, found: 761.2396.

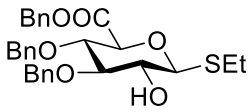
Ethyl 3,4-di-*O*-benzyl-1-thio-*β*-D-glucopyranoside (3-30)



To a solution of thioglucoside **3-29**[204] (1.0 g, 2.49 mmol) in THF (10 mL) were added BH_3·THF (16.2 mL, 16.2 mmol) and TMSOTf (90 μL, 0.50 mmol) at 0 °C. Reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction as monitored by TLC. The reaction mixture was diluted with ethyl acetate and quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate (2 × 10 mL) and combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-30** (750 mg, 75%) as a colorless syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.27 (m, 10H), 4.99 (d, *J* = 11.2 Hz, 1H), 4.95 – 4.87 (m, 2H), 4.69 (d, *J* = 11.0 Hz, 1H), 4.39 (d, *J* = 9.7 Hz, 1H), 3.91 (dd, *J* = 12.1, 2.7 Hz, 1H), 3.73 (dd, *J* = 12.1, 4.8 Hz, 1H), 3.68 – 3.58 (m, 2H), 3.58 – 3.50 (m, 1H), 3.48 – 3.39 (m, 1H), 2.76 (qd, *J* = 7.5, 1.2 Hz, 2H), 2.57 (s, 1H), 2.10 (s, 1H), 1.34 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.56, 137.96, 128.61, 128.18, 128.05, 127.91, 86.35, 85.86, 79.68, 75.34, 75.24, 73.42, 62.13, 24.71, 15.49; HRMS (ESI) calculated for $C_{22}H_{28}O_5S$ $[M+Na]^+$: 427.1550, found: 427.1558.

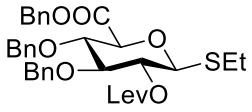
Benzyl (ethyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranosyl) uronate (3-31)



Thioglycoside **3-30** (2.08 g, 5.15 mmol) was dissolved in DCM (50 mL) and H_2O (10 mL). 2,2,6,6-Tetramethylpiperidine 1-oxyl (80 mg, 0.52 mmol) was added followed by $PhI(OAc)_2$ (2.65 g, 8.23 mmol) at 0 °C. The reaction mixture was warmed to room temperature after 15 min. Additional $PhI(OAc)_2$ (828 mg, 2.57 mmol) and 2,2,6,6-tetramethylpiperidine 1-oxyl (80 mg, 0.52 mmol) were added. After 4 h, the reaction mixture was quenched by the addition of saturated aqueous $Na_2S_2O_3$, and the organic layer was washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was dissolved in DMF (50 mL), $NaHCO_3$ (648 mg, 7.72 mmol) and BnBr (0.9 mL, 7.72 mmol) were added at 0 °C. Then, the reaction was stirred overnight at room temperature. After complete consumption of the starting material, the mixture was diluted with ethyl acetate (20 mL) and washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 5/1) to obtain **3-31** (1.65 g, 63%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.26 (m, 13H), 7.21 – 7.13 (m, 2H), 5.21 (d, *J* = 2.2 Hz, 2H), 5.01 – 4.84 (m, 2H), 4.79 (d, *J* = 10.8 Hz, 1H), 4.52 (d, *J* = 10.8 Hz, 1H), 4.45 – 4.34 (m, 1H), 4.00 (d, *J* = 9.8 Hz, 1H), 3.95 – 3.83 (m, 1H), 3.68 – 3.57 (m, 2H), 2.86 – 2.68 (m, 2H), 2.49 (s, 1H), 1.33 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.15, 138.38, 137.81, 135.09, 128.67, 128.64, 128.62, 128.59, 128.44, 128.07, 127.96, 127.88, 86.87, 85.13, 78.91, 78.53, 75.39, 75.20, 72.86, 67.46, 24.51, 15.37; HRMS (ESI) calculated for $C_{29}H_{32}O_6S$ $[M+Na]^+$: 531.1812, found: 531.1818.

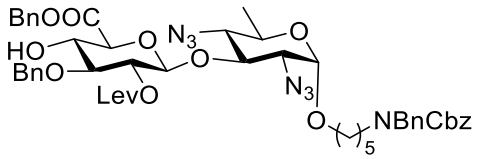
Benzyl (ethyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranosyl) uronate (3-32)



Levulinic acid (639.8 mg, 5.51 mmol), EDC·HCl (1.06 g, 5.51 mmol) and 4-dimethylaminopyridine (67 mg, 0.55 mmol) were added to a solution of compound **3-31** (1.4 g) in CH_2Cl_2 (30 mL). The mixture was stirred for 4 h at room temperature. Then, the reaction mixture was washed with saturated aqueous $NaHCO_3$ and brine, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 3/1) to give **3-32** (1.6 g, 96%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.25 (m, 13H), 7.18 – 7.11 (m, 2H), 5.20 (s, 2H), 5.09 (dd, *J* = 10.0, 9.1 Hz, 1H), 4.84 – 4.69 (m, 3H), 4.53 – 4.41 (m, 2H), 4.03 – 3.88 (m, 2H), 3.73 (t, *J* = 8.9 Hz, 1H), 2.83 – 2.62 (m, 4H), 2.62 – 2.44 (m, 2H), 2.19 (s, 3H), 1.26 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.20, 171.55, 167.90, 138.04, 137.66, 135.07, 128.68, 128.62, 128.60, 128.50, 128.44, 128.04, 127.99, 127.92, 127.88, 84.17, 83.40, 79.34, 78.48, 75.30, 75.18, 71.61, 67.48, 37.91, 29.94, 28.09, 24.01, 14.90; HRMS (ESI) calculated for $C_{34}H_{38}O_8S$ [M+Na]$^+$: 629.2180, found: 629.2181.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-3-_O_-benzyl-2-_O_-levulinoyl-_β_-D-glucopyranosyluronate-(1→3)-2,4-di-azido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-33)

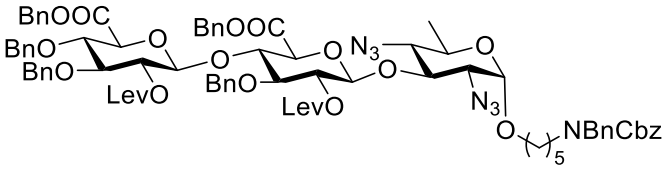


Thioglycoside **3-28** (158 mg, 0.21 mmol) and compound **3-6** (56 mg, 0.11 mmol) were coevaporated three times with toluene, the resulting mixture was dried under high vacuum for 2 h. Then the mixture was dissolved in anhydrous DCM (2 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to 0 °C, NIS (48 mg, 0.21 mmol) and TfOH (2.8 µL, 0.03 mmol) were added. The reaction mixture was stirred at 0 °C for 3 h. After complete consumption of the staring material, triethylamine (0.1 mL) was added and the mixture was stirred for 1 h. Then, the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and

concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 3/2) to give **3-33** as a colorless syrup (70 mg, 67%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 – 7.14 (m, 20H), 5.32 – 5.15 (m, 4H), 5.05 (dd, *J* = 9.6, 8.0 Hz, 1H), 4.85 (d, *J* = 11.8 Hz, 2H), 4.81 – 4.73 (m, 2H), 4.52 (d, *J* = 13.7 Hz, 2H), 4.05 – 3.93 (m, 3H), 3.68 – 3.50 (m, 3H), 3.45 – 3.31 (m, 2H), 3.31 – 3.18 (m, 2H), 3.08 (t, *J* = 9.6 Hz, 2H), 2.83 – 2.60 (m, 3H), 2.52 – 2.41 (m, 1H), 2.19 (s, 3H), 1.66 – 1.50 (m, 4H), 1.32 – 1.25 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.39, 171.68, 168.90, 156.84, 156.30, 138.37, 138.02, 137.00, 136.85, 135.05, 129.55, 128.69, 128.67, 128.61, 128.57, 128.55, 128.41, 128.37, 128.26, 128.07, 127.97, 127.93, 127.89, 127.86, 127.49, 127.41, 127.31, 124.54, 120.27, 101.32, 97.54, 81.44, 74.83, 74.22, 72.69, 72.05, 68.28, 67.58, 67.31, 66.72, 66.22, 63.00, 50.63, 50.35, 47.18, 46.18, 37.83, 32.06, 30.01, 29.83, 29.81, 29.79, 29.50, 29.38, 29.12, 27.95, 27.82, 27.55, 23.40, 22.83, 18.53, 14.28; HRMS (ESI) calculated for $C_{51}H_{59}N_7O_{13}$ $[M+Na]^+$: 100.4063, found: 1000.4036.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-3,4-di-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyluronate-(1→4)-benzyl-3-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyluronate-(1→3)-2,4-di-azido-2,4-di-deoxy-*α*-D-quinovopyranoside (3-34)**

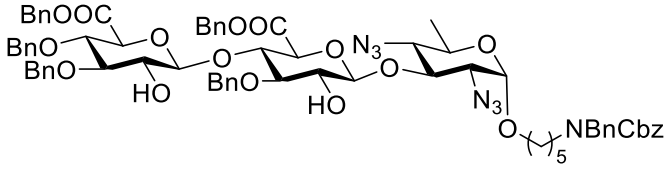


Thioglycoside **3-32** (68 mg, 0.113 mmol) and disaccharide **3-33** (55 mg, 0.056 mmol) were coevaporated three times with toluene, the resulting mixture was dried under high vacuum for 2 h. Then, the mixture was dissolved in anhydrous DCM (2 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to 0 °C, NIS (25.3 mg, 0.113 mmol) and TfOH (1.5 µL, 0.017 mmol) were added. The reaction mixture was stirred at 0 °C for 3 h. After complete consumption of the staring material, triethylamine (0.1 mL) was added and the mixture was stirred for 1 h. Then, the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4,

filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 3/2) to give **3-34** as a colorless syrup (74 mg, 86%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 – 7.09 (m, 35H), 5.36 (dd, *J* = 12.2, 7.4 Hz, 1H), 5.20 (d, *J* = 16.4 Hz, 2H), 5.13 – 4.90 (m, 6H), 4.84 (dt, *J* = 17.6, 5.0 Hz, 1H), 4.75 – 4.61 (m, 4H), 4.57 – 4.49 (m, 3H), 4.41 (dd, *J* = 11.1, 7.9 Hz, 1H), 4.31 (t, *J* = 8.0 Hz, 1H), 4.17 (q, *J* = 8.7 Hz, 1H), 4.01 (t, *J* = 8.9 Hz, 1H), 3.92 (s, 1H), 3.82 (q, *J* = 8.9 Hz, 1H), 3.70 – 3.47 (m, 4H), 3.46 – 3.16 (m, 5H), 3.11 – 3.02 (m, 1H), 2.82 – 2.41 (m, 7H), 2.27 – 2.12 (m, 7H), 1.72 – 1.48 (m, 4H), 1.37 – 1.24 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.45, 206.37, 171.79, 171.54, 167.77, 167.16, 156.82, 156.28, 146.97, 138.84, 138.07, 138.01, 137.83, 136.99, 136.85, 135.04, 129.78, 129.18, 129.07, 129.02, 128.91, 128.83, 128.76, 128.67, 128.65, 128.61, 128.56, 128.53, 128.49, 128.44, 128.40, 128.26, 128.07, 128.04, 127.96, 127.92, 127.88, 127.82, 127.79, 127.44, 127.37, 127.31, 101.35, 100.53, 97.50, 81.50, 80.25, 79.41, 78.43, 77.70, 75.10, 74.99, 74.88, 74.85, 74.43, 73.10, 72.54, 68.29, 68.21, 67.57, 67.36, 67.29, 66.66, 66.13, 62.87, 50.65, 50.35, 47.19, 46.21, 37.91, 37.83, 32.05, 30.01, 29.97, 29.86, 29.82, 29.77, 29.49, 29.08, 27.91, 27.68, 27.53, 23.36, 22.82, 18.52, 14.27; HRMS (ESI) calculated for $C_{83}H_{91}N_7O_{21}$ $[M+Na]^+$: 1544.6160, found: 1544.6113.

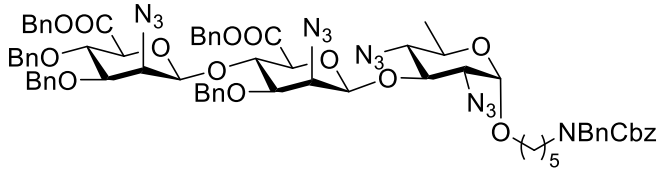
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-3,4-di-_O_-benzyl-_β_-D-glucopyranosyluronate-(1→4)-benzyl-3-_O_-benzyl-_β_-D-glucopyranosyluronate-(1→3)-2,4-di-azido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-35)



Trisaccharide **3-34** (74 mg, 0.0486 mmol) was dissolved in DCM (2.0 mL), N_2H_4·AcOH (89.6 mg, 0.972 mmol) was added and the mixture was stirred at room temperature for 12 h. Then, the reaction was quenched with acetone (0.5 mL) and concentrated under vacuum. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-35** (52 mg, 82%) as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66 – 6.98 (m, 35H), 5.34 – 5.14 (m, 4H), 5.11 – 5.00 (m, 2H), 4.96 (dd, *J* = 11.3, 4.8 Hz, 2H), 4.91 – 4.78 (m, 3H), 4.74 (d, *J* = 10.7 Hz, 1H), 4.63 (d, *J* = 7.3 Hz, 1H), 4.53 (d, *J* = 14.9 Hz, 2H), 4.43 (dd, *J* = 12.2, 8.9 Hz, 2H), 4.15 – 4.04 (m, 2H), 3.99 (q, *J* = 9.0 Hz, 1H), 3.82 – 3.72 (m, 2H), 3.70 – 3.53 (m, 4H), 3.53 – 3.45 (m, 2H), 3.45 – 3.19 (m, 4H), 3.07 (t, *J* = 9.6 Hz, 1H), 2.71 (s, 1H), 1.71 – 1.49 (m, 4H), 1.41 – 1.25 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.47, 168.20, 156.82, 156.29, 138.67, 138.60, 137.99, 137.94, 136.98, 136.83, 135.04, 134.92, 128.88, 128.83, 128.76, 128.67, 128.60, 128.55, 128.50, 128.45, 128.38, 128.31, 128.10, 128.04, 127.96, 127.92, 127.88, 127.81, 127.77, 127.72, 127.67, 127.48, 127.40, 127.30, 103.85, 103.69, 97.27, 83.39, 82.51, 78.87, 78.58, 78.34, 75.22, 75.05, 74.97, 74.95, 74.73, 74.45, 74.39, 68.38, 68.29, 67.94, 67.54, 67.34, 67.27, 66.40, 62.86, 50.63, 50.34, 47.14, 46.15, 29.81, 29.08, 27.92, 27.52, 23.38, 23.32, 18.48; HRMS (ESI) calculated for $C_{73}H_{79}N_7O_{17}$ $[M+Na]^+$: 1348.5425, found: 1348.5392.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-2-azido-2-deoxy-3,4-di-_O_-benzyl-_β_-D-mannopyranosyluronate-(1→4)-benzyl-2-azido-2-deoxy-3-_O_-benzyl-_β_-D-mannopyranosyluronate-(1→3)-2,4-di-azido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-36)



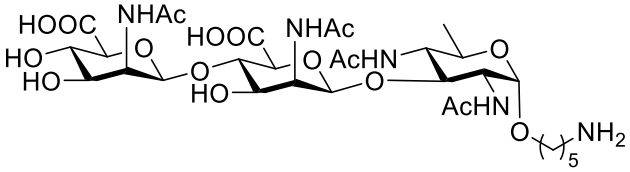
Trisaccharide **3-35** (19 mg, 0.014 mmol) was dissolved in anhydrous DCM (1.0 mL) and pyridine (23.1 µL, 0.287 mmol). The solution was cooled to 0 °C and triflic anhydride (14.5 µL, 0.086 mmol) was added. The solution was stirred at 0 °C for 4 h and then quenched by adding cold water. The organic layer was washed twice with cold water and cold brine, organic layer was dried over Na_2SO_4. The solvent was evaporated giving the triflate compound as a yellow solid that was used without further purification.

The solid was dissolved in anhydrous toluene (1.0 mL) and tetrabutylammonium azide (40.7 mg, 0.143 mmol) was added. The reaction mixture was stirred overnight at 60°C. The reaction was diluted with ethyl acetate and washed with saturated aqueous $NaHCO_3$ and brine. The organic

layer was dried over Na_2SO_4, filtered and evaporated, resulting residue was purified by flash silica column chromatography (toluene/ethyl acetate = 15/1) give **3-36** (14 mg, 73%) as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 – 6.99 (m, 35H), 5.32 – 5.01 (m, 6H), 4.86 – 4.71 (m, 4H), 4.71 – 4.64 (m, 1H), 4.60 (dt, *J* = 12.3, 3.9 Hz, 2H), 4.55 – 4.40 (m, 4H), 4.19 (td, *J* = 9.3, 4.3 Hz, 1H), 4.01 – 3.83 (m, 4H), 3.79 (s, 1H), 3.68 – 3.48 (m, 4H), 3.45 – 3.15 (m, 5H), 3.06 (td, *J* = 9.6, 4.2 Hz, 1H), 1.67 – 1.46 (m, 4H), 1.39 – 1.16 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.70, 167.47, 156.82, 156.31, 138.06, 137.82, 137.46, 135.25, 135.12, 128.83, 128.76, 128.73, 128.69, 128.68, 128.62, 128.60, 128.57, 128.52, 128.46, 128.40, 128.24, 128.18, 128.11, 128.02, 127.98, 127.92, 127.91, 127.79, 127.44, 127.31, 101.25, 100.83, 96.97, 79.69, 79.23, 78.18, 76.36, 75.36, 75.26, 75.07, 74.48, 73.70, 72.11, 68.35, 67.57, 67.39, 67.30, 67.23, 66.44, 63.18, 62.82, 61.63, 50.63, 50.36, 47.17, 46.12, 29.83, 29.07, 27.91, 27.53, 23.37, 18.51; HRMS (ESI) calculated for $C_{61}H_{77}N_{13}O_{15}$ $[M+Na]^+$: 1398.5554, found: 1398.5521.

5-Aminopentyl 2-*N*-acetyl-2-deoxy-*β*-D-mannopyranosyluronate-(1→4)-2-*N*-acetyl-2-deoxy-*β*-D-mannopyranosyluronate-(1→3)-2,4-di-*N*-acetyl-2,4-di-deoxy-*α*-D-quinovopyranoside (3-1)



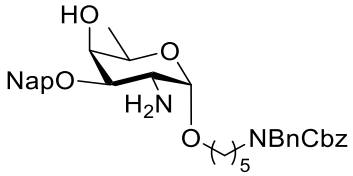
To a stirred solution of trisaccharide **3-36** (14 mg, 0.0102 mmol) in acetic acid/acetic anhydride/THF (0.2/0.4/0.6, 1.2 mL) was added zinc (100 mg) under nitrogen. The mixture was stirred for 12 h at 37 °C. The solution was filtered and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to give a colorless solid that was used without further purification.

In a 20 mL vial, the crude product was dissolved in EA/*t*-BuOH/H_2O (2 mL/2 mL/1 mL), palladium on activated charcoal (10% Pd, 30 mg) was added. The reaction mixture was stirred

under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered. The crude product obtained after removal of the solvent was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **3-1** (2.7 mg, 35% over two steps) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 4.60 – 4.48 (m, 3H), 4.34 – 4.13 (m, 2H), 3.98 – 3.28 (m, 12H), 2.97 – 2.80 (m, 2H), 2.00 – 1.74 (m, 12H), 1.62 – 1.46 (m, 4H), 1.40 – 1.23 (m, 2H), 1.03 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (176 MHz, D_2O) δ 175.36, 175.02, 174.95, 174.76, 174.33, 174.00, 100.26, 98.56, 96.93, 78.07, 78.02, 76.70, 76.40, 73.36, 71.83, 70.62, 68.85, 67.75, 66.73, 55.93, 53.31, 53.23, 53.02, 39.39, 28.10, 26.52, 22.37, 22.27, 22.07, 21.96, 21.89, 16.71; HRMS (ESI) calculated for $C_{31}H_{51}N_5O_{17}$ $[M+H]^+$: 766.3353, found: 766.3349.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-amino-2-deoxy-3-_O_-(2-naphthylmethyl)-_α_-D-fucopyranoside (3-43)

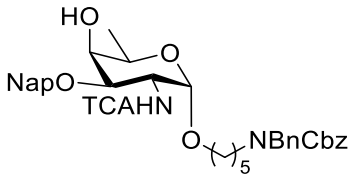


To a solution of a mixture of iodide **3-15** (1.2 g, 1.57 mmol) and AIBN (25.8 g, 0.16 mmol) in degassed toluene (20 mL) at 85 °C, was added Bu_3SnH (2.1 mL, 7.85 mmol) under an Ar atmosphere. After 1.5 h of stirring at this temperature, the mixture was cooled to room temperature, the reaction mixture was diluted with ethyl acetate (50 mL), the organic layer was washed with water (80 mL) and brine (80 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (ethyl acetate/MeOH/H_2O = 40/1/1) to afford **3-43** (865 mg, 90%) as a colorless syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.80 (m, 4H), 7.55 – 7.48 (m, 3H), 7.43 – 7.16 (m, 10H), 5.20 (d, *J* = 13.8 Hz, 2H), 4.90 (d, *J* = 11.6 Hz, 1H), 4.86 – 4.79 (m, 1H), 4.73 (d, *J* = 11.6 Hz, 1H), 3.96 – 3.84 (m, 2H), 3.72 – 3.44 (m, 1H), 3.44 – 3.17 (m, 2H), 3.11 (dd, *J* = 10.2, 3.9 Hz, 1H), 2.19 – 1.95 (m, 1H), 1.64 – 1.43 (m, 5H), 1.44 – 1.20 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$)

δ 156.81, 156.28, 137.96, 136.94, 136.79, 135.25, 133.29, 133.15, 128.63, 128.57, 128.53, 128.03, 127.98, 127.89, 127.85, 127.36, 127.24, 126.83, 126.43, 126.27, 125.79, 99.55, 80.54, 71.44, 68.14, 67.91, 67.25, 65.72, 53.55, 50.54, 50.28, 47.19, 46.18, 29.28, 27.99, 27.93, 27.58, 23.60, 23.50, 16.64; HRMS (ESI) calculated for $C_{37}H_{44}N_2O_6$ $[M+H]^+$: 613.3272, found: 613.3277.

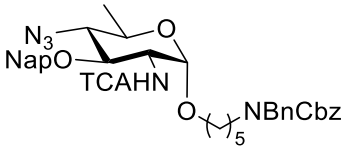
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-trichloroacetamido-2-deoxy-3-_O_-(2-naphthylmethyl)-_α_-D-fucopyranoside (3-44)



The mixture of **3-43** (1.45 g, 2.37 mmol) with 4 Å molecular sieves was dissolved in anhydrous THF (30 mL) under nitrogen atmosphere. Triethylamine (661 µL, 4.74 mmol) and trichloroacetyl chloride (396 µL, 3.55 mmol) were added at 0 °C. The mixture was stirred for 1 h at 0 °C, and diluted with ethyl acetate (50 mL), washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), the combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 1/1) to give **3-44** (1.52 g, 85%) as a colorless syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.73 (m, 4H), 7.56 – 7.44 (m, 3H), 7.44 – 7.14 (m, 10H), 6.94 – 6.68 (m, 1H), 5.25 – 5.12 (m, 2H), 4.94 – 4.68 (m, 3H), 4.56 – 4.42 (m, 3H), 3.95 (d, *J* = 3.1 Hz, 1H), 3.92 – 3.84 (m, 1H), 3.81 – 3.54 (m, 2H), 3.45 – 3.11 (m, 3H), 2.54 (s, 1H), 1.62 – 1.43 (m, 4H), 1.37 – 1.33 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.91, 156.81, 156.33, 137.89, 136.92, 136.71, 134.83, 133.26, 133.20, 130.73, 128.70, 128.56, 128.07, 128.03, 127.90, 127.87, 127.47, 127.29, 126.65, 126.45, 126.28, 125.58, 97.02, 92.85, 71.44, 71.32, 68.26, 68.14, 67.97, 67.32, 65.80, 50.65, 50.36, 47.17, 46.16, 37.22, 32.06, 30.17, 29.84, 29.80, 29.61, 29.51, 29.06, 28.97, 27.94, 27.44, 27.22, 23.73, 23.40, 22.84, 22.81, 16.51, 14.28; HRMS (ESI) calculated for $C_{39}H_{43}Cl_3N_2O_7$ $[M+Na]^+$: 779.2028, found: 779.2032.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 4-azido-2-trichloroacetamido-2,4-di-deoxy-3-_O_-(2-naphthylmethyl)-_α_-D-quinovopyranoside (3-45)

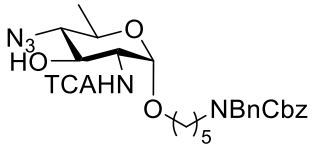


Compound **3-44** (1.52 g, 2.01 mmol) was dissolved in anhydrous DCM (30 mL) and pyridine (0.8 mL). The solution was cooled to 0 °C and triflic anhydride (676 µL, 4.02 mmol) was added. The solution was stirred at 0 °C for 1 h and the reaction was quenched by adding cold water. The organic layer was washed twice with cold water and cold brine, organic layer was dried over Na_2SO_4. The solvent was evaporated giving the triflate compound as a yellow solid that was used without further purification.

The solid was dissolved in anhydrous DMF (30 mL) and sodium azide (261 mg, 4.02 mmol) was added. The reaction mixture was stirred at room temperature for 5 h. The solvent was evaporated under reduced pressure, the residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4. The solvent was evaporated and resulting residue was purified by flash silica column chromatography (hexane/ethyl acetate = 3/1) to give **3-45** (1.44 g, 92%) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.75 – 7.61 (m, 4H), 7.42 – 7.02 (m, 12H), 6.83 – 6.53 (m, 1H), 5.48 (d, *J* = 6.4 Hz, 1H), 5.14 – 4.97 (m, 2H), 4.88 – 4.73 (m, 2H), 4.52 (t, *J* = 13.2 Hz, 1H), 4.43 – 4.27 (m, 3H), 4.00 – 3.86 (m, 1H), 3.81 – 3.62 (m, 1H), 3.59 – 3.38 (m, 1H), 3.35 – 3.18 (m, 1H), 3.18 – 2.99 (m, 2H), 1.52 – 1.27 (m, 3H), 1.24 – 1.01 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.81, 160.61, 156.70, 156.22, 137.83, 136.85, 136.63, 134.71, 134.60, 133.20, 133.08, 128.62, 128.47, 128.42, 127.96, 127.89, 127.79, 127.75, 127.40, 127.21, 126.80, 126.69, 126.24, 126.07, 125.71, 96.92, 92.67, 73.79, 73.66, 71.02, 68.45, 68.35, 68.14, 67.21, 64.74, 50.90, 50.77, 50.57, 50.30, 47.09, 46.06, 28.92, 28.75, 27.84, 27.28, 23.65, 23.24, 16.44; HRMS (ESI) calculated for $C_{39}H_{42}Cl_3N_5O_6$ $[M+Na]^+$: 804.2093, found: 804.2092.

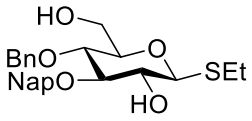
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 4-azido-2-trichloroacetamido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-41)



To a solution of **3-45** (720 mg, 0.92 mmol) in 20:1 (v/v) DCM-H_2O (21 mL), DDQ (313 mg, 1.38 mmol) was added and the reaction mixture was stirred for 5 h. The reaction was diluted with DCM (10 mL) and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 3/1) to give **3-41** as a colorless syrup (514 mg, 87%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 – 7.11 (m, 10H), 6.93 (d, *J* = 8.9 Hz, 1H), 5.25 – 5.14 (m, 2H), 4.87 – 4.74 (m, 1H), 4.52 (q, *J* = 6.1 Hz, 2H), 4.11 – 4.03 (m, 1H), 4.00 – 3.78 (m, 1H), 3.75 – 3.54 (m, 2H), 3.45 – 3.12 (m, 4H), 2.86 (s, 1H), 1.69 – 1.47 (m, 4H), 1.43 – 1.32 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.99, 156.84, 156.45, 137.80, 136.91, 136.61, 128.74, 128.63, 128.16, 128.01, 127.92, 127.85, 127.54, 127.31, 96.60, 96.46, 92.50, 72.64, 71.94, 68.78, 68.59, 68.04, 67.47, 66.75, 66.64, 56.04, 55.64, 50.70, 50.50, 47.32, 46.17, 29.83, 29.07, 28.49, 27.95, 27.39, 24.18, 23.43, 22.83, 18.42; HRMS (ESI) calculated for $C_{28}H_{34}Cl_3N_5O_6$ $[M+Na]^+$: 664.1467, found: 664.1465.

Ethyl 4-_O_-benzyl-3-_O_-(2-naphthylmethyl)-1-thio-_β_-D-glucopyranoside (3-47)

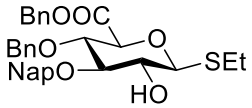


To a solution of thioglucoside **3-46**[205] (4.0 g, 8.85 mmol) in THF (50 mL) were added BH_3·THF (57 mL, 57.5 mmol) and TMSOTf (320 μL, 1.77 mmol) at 0 °C. Reaction mixture was stirred at room temperature for 4 h. Upon completion of reaction as monitored by TLC. The reaction mixture was diluted with ethyl acetate (50 mL) and quenched with saturated aqueous $NaHCO_3$. The aqueous layer was washed with ethyl acetate (2 × 50 mL) and combined organic layer was dried

over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-47** (2.8 g, 70%) as a colorless syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.78 (m, 4H), 7.60 – 7.45 (m, 3H), 7.41 – 7.29 (m, 5H), 5.19 – 5.02 (m, 2H), 4.94 (d, *J* = 10.8 Hz, 1H), 4.72 (d, *J* = 11.0 Hz, 1H), 4.40 (d, *J* = 9.8 Hz, 1H), 3.92 (dd, *J* = 12.1, 2.7 Hz, 1H), 3.79 – 3.54 (m, 4H), 3.54 – 3.41 (m, 1H), 2.77 (qd, *J* = 7.5, 1.0 Hz, 2H), 2.33 (s, 2H), 1.35 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.01, 136.04, 133.43, 133.12, 128.62, 128.41, 128.16, 128.06, 127.81, 126.81, 126.21, 126.09, 126.03, 86.43, 85.82, 79.73, 75.39, 75.28, 73.51, 62.19, 29.82, 24.74, 15.51; HRMS (ESI) calculated for $C_{26}H_{30}O_5S$ $[M+Na]^+$: 477.1706, found: 477.1716.

Benzyl (ethyl 4-*O*-benzyl-3-*O*-(2-naphthylmethyl)-1-thio-*β*-D-glucopyranosyl) uronate (3-48)

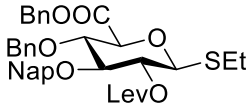


Thioglycoside **3-47** (1.8 g, 3.96 mmol) was dissolved in DCM (35 mL) and H_2O (7 mL). 2,2,6,6-Tetramethylpiperidine 1-oxyl (62 mg, 0.40 mmol) was added followed by $PhI(OAc)_2$ (2.1 g, 6.34 mmol) at 0 °C. The reaction mixture was warmed to room temperature after 15 min. Additional $PhI(OAc)_2$ (638 mg, 1.98 mmol) and 2,2,6,6-tetramethylpiperidine 1-oxyl (62 mg, 0.40 mmol) were added. After 4 h, the reaction mixture was quenched by the addition of saturated aqueous $Na_2S_2O_3$, and the organic layer was washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was dissolved in DMF (40 mL), $NaHCO_3$ (499 mg, 5.94 mmol) and BnBr (0.7 mL, 5.94 mmol) were added at 0 °C. Then, the reaction was stirred overnight at room temperature. After complete consumption of the starting material, the mixture was diluted with ethyl acetate (20 mL) and washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 4/1) to obtain **3-48** (1.3 g, 59%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.77 (m, 4H), 7.56 – 7.46 (m, 3H), 7.39 – 7.24 (m, 8H), 7.21 – 7.11 (m, 2H), 5.22 (d, *J* = 1.9 Hz, 2H), 5.16 – 4.99 (m, 2H), 4.81 (d, *J* = 10.7 Hz, 1H), 4.54 (d, *J* = 10.7 Hz, 1H), 4.45 – 4.35 (m, 1H), 4.01 (d, *J* = 9.8 Hz, 1H), 3.97 – 3.87 (m, 1H), 3.73 – 3.61 (m,

2H), 2.86 – 2.68 (m, 2H), 2.49 (s, 1H), 1.34 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.15, 137.84, 135.82, 135.10, 133.38, 133.11, 128.67, 128.63, 128.58, 128.43, 128.40, 128.04, 127.92, 127.84, 127.78, 126.84, 126.20, 126.05, 126.03, 78.93, 78.57, 75.41, 75.20, 72.92, 67.45, 24.51, 15.36; HRMS (ESI) calculated for $C_{33}H_{34}O_6S$ $[M+Na]^+$: 581.1968, found: 581.1973.

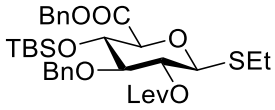
Benzyl (ethyl 4-*O*-benzyl-3-*O*-(2-naphthylmethyl)-2-*O*-levulinoyl-1-thio-*β*-D-glucopyranosyl) uronate (3-42)



Levulinic acid (333 mg, 2.86 mmol), EDC·HCl (548 mg, 2.86 mmol) and 4-dimethylaminopyridine (35 mg, 0.29 mmol) were added to a solution of compound **3-48** (800 mg) in CH_2Cl_2 (15 mL). The mixture was stirred for 4 h at room temperature. Then, the reaction mixture was washed with saturated aqueous $NaHCO_3$ and brine, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 3/1) to give **3-42** (820 mg, 87%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.78 (m, 3H), 7.77 – 7.73 (m, 1H), 7.53 – 7.46 (m, 2H), 7.44 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.39 – 7.31 (m, 5H), 7.31 – 7.25 (m, 3H), 7.19 – 7.09 (m, 2H), 5.21 (s, 2H), 5.12 (dd, *J* = 10.0, 9.1 Hz, 1H), 5.01 – 4.87 (m, 2H), 4.75 (d, *J* = 10.7 Hz, 1H), 4.52 (d, *J* = 10.7 Hz, 1H), 4.44 (d, *J* = 10.0 Hz, 1H), 4.05 – 3.90 (m, 2H), 3.78 (t, *J* = 8.7 Hz, 1H), 2.81 – 2.64 (m, 2H), 2.64 – 2.58 (m, 2H), 2.55 – 2.37 (m, 2H), 2.11 (s, 3H), 1.26 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.19, 171.57, 167.93, 137.69, 135.57, 135.09, 133.32, 133.08, 128.70, 128.65, 128.63, 128.47, 128.25, 128.07, 127.98, 127.94, 127.78, 126.81, 126.21, 126.12, 126.07, 84.21, 83.46, 79.42, 78.51, 75.40, 75.22, 71.62, 67.51, 37.79, 29.85, 28.08, 24.04, 14.91; HRMS (ESI) calculated for $C_{38}H_{40}O_8S$ $[M+Na]^+$: 679.2336, found: 379.2336.

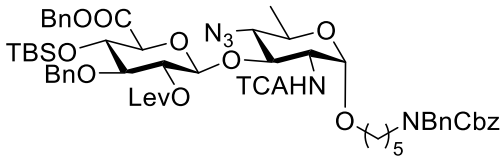
Benzyl (ethyl 3-*O*-benzyl-2-*O*-levulinoyl-4-*O*-*tert*-butyldimethylsilyl-1-thio-*β*-D-glucopyranosyl) uronate (3-50)



To a solution of glycuronate **3-27** (300 mg, 0.58 mmol) in anhydrous DMF (6 mL), *tert*-butyldimethylsilyl chloride (438 mg, 2.9 mmol), imidazole (198 mg, 2.9 mmol) and 4-dimethylaminopyridine (14.2 mg, 0.12 mmol) were added. The reaction mixture was stirred overnight at 80 °C. Then, the reaction was quenched by addition of methanol (3 mL). Ethyl acetate (20 mL) was added and the mixture was washed with saturated aqueous $NaHCO_3$ and brine. The combined organic phases were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 10/1) to give **3-50** (328 mg, 90%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.23 (m, 10H), 5.28 – 5.08 (m, 3H), 4.79 – 4.67 (m, 2H), 4.45 (d, *J* = 1.7 Hz, 1H), 4.06 (t, *J* = 1.7 Hz, 1H), 3.96 (d, *J* = 1.7 Hz, 1H), 3.57 (t, *J* = 1.7 Hz, 1H), 2.81 – 2.46 (m, 5H), 2.44 – 2.28 (m, 1H), 2.13 (s, 3H), 1.27 – 1.21 (m, 3H), 0.86 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.32, 176.73, 173.10, 143.30, 140.12, 133.80, 133.67, 133.53, 133.48, 132.63, 132.41, 89.13, 88.90, 85.55, 80.08, 77.27, 76.97, 72.51, 43.06, 35.01, 34.94, 33.21, 30.98, 28.82, 23.12, 20.01, 1.15, 0.10; HRMS (ESI) calculated for $C_{33}H_{46}O_8S_{Si}$ $[M+Na]^+$: 653.2575, found: 653.2579.

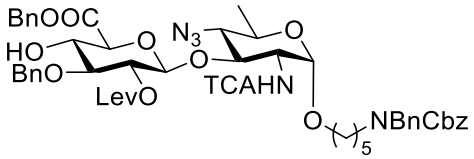
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-3-*O*-benzyl-2-*O*-levulinoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate-(1→3)-4-azido-2-trichloroacetamido-2,4-di-deoxy-*α*-D-quinovopyranoside (3-51)**



Thioglycoside **3-50** (328 mg, 0.52 mmol) and compound **3-41** (278 mg, 0.43 mmol) were coevaporated three times with toluene, the resulting mixture was dried under high vacuum for 2 h. Then, the mixture was dissolved in anhydrous DCM (5 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to 0 °C, NIS (117 mg, 0.52 mmol) and TfOH (11.5 µL, 0.13 mmol) were added. The reaction mixture was stirred at 0 ^{o}C for 3 h. After complete consumption of the staring material, triethylamine (0.1 mL) was added. Then, the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-51** (385 mg, 73%) as a light yellow syrup.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 – 7.16 (m, 20H), 6.86 – 6.69 (m, 1H), 5.28 – 5.13 (m, 4H), 5.08 (t, *J* = 1.1 Hz, 1H), 4.71 – 4.60 (m, 4H), 4.57 – 4.46 (m, 2H), 4.16 (dq, *J* = 11.6, 5.3 Hz, 1H), 4.06 (t, *J* = 8.8 Hz, 1H), 3.95 (p, *J* = 10.6 Hz, 2H), 3.69 – 3.46 (m, 2H), 3.42 – 3.18 (m, 4H), 3.15 (t, *J* = 9.7 Hz, 1H), 2.76 – 2.61 (m, 3H), 2.41 (ddd, *J* = 17.5, 10.8, 4.2 Hz, 1H), 2.13 (s, 3H), 1.64 – 1.48 (m, 4H), 1.34 – 1.30 (m, 5H), 0.86 (s, 9H), -0.02 (d, *J* = 2.8 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.81, 177.21, 172.86, 166.42, 166.30, 161.89, 161.39, 142.96, 142.93, 141.99, 141.80, 140.05, 133.78, 133.75, 133.70, 133.65, 133.62, 133.41, 133.35, 133.25, 133.17, 133.08, 132.98, 132.66, 132.63, 132.54, 132.37, 105.70, 101.82, 97.82, 87.25, 81.41, 79.99, 78.11, 77.22, 73.29, 73.19, 72.51, 72.45, 71.39, 70.97, 59.74, 55.75, 55.45, 52.19, 51.19, 43.16, 42.29, 37.13, 35.24, 34.96, 34.94, 34.90, 34.86, 34.68, 34.57, 34.03, 32.95, 32.54, 30.97, 30.93, 28.63, 28.44, 27.93, 27.90, 27.88, 24.93, 23.59, 23.10, 19.35, 1.13, 0.01; HRMS (ESI) calculated for $C_{59}H_{74}Cl_3N_5O_{14}Si$ $[M+Na]^+$: 1232.3959, found: 1232.3944.

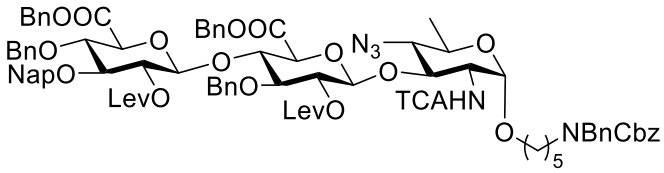
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-3-_O_-benzyl-2-_O_-levulinoyl-_β_-D-glucopyranosyluronate-(1→3)-4-azido-2-trichloroacetamido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-49)



Disaccharide **3-51** (260 mg, 0.215 mmol) was dissolved in anhydrous THF (5 mL), TBAF (322 µL, 0.322 mmol) was added dropwise at 0 °C. Then, the reaction was warmed to room temperature and stirred for 2 h. After all starting material was consumed, the reaction was extracted with ethyl acetate, washed with saturated aqueous $NaHCO_3$ and brine. Combined organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to offer **3-49** (150 mg, 64%) as a light yellow syrup.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 – 7.15 (m, 20H), 6.85 – 6.69 (m, 1H), 5.34 – 5.12 (m, 4H), 5.02 (dd, *J* = 9.6, 7.9 Hz, 1H), 4.82 – 4.72 (m, 2H), 4.69 – 4.59 (m, 2H), 4.51 (d, *J* = 15.6 Hz, 2H), 4.21 – 4.12 (m, 1H), 4.00 (t, *J* = 9.3 Hz, 2H), 3.96 – 3.89 (m, 1H), 3.69 – 3.47 (m, 2H), 3.47 – 3.41 (m, 1H), 3.39 – 3.22 (m, 2H), 3.18 (td, *J* = 10.5, 7.9 Hz, 2H), 3.00 – 2.82 (m, 2H), 2.80 – 2.66 (m, 2H), 2.64 – 2.56 (m, 1H), 2.20 (s, 3H), 1.62 – 1.46 (m, 4H), 1.35 – 1.30 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.95, 172.19, 168.76, 161.35, 156.89, 156.37, 137.97, 137.82, 136.86, 136.66, 135.04, 134.58, 130.76, 128.71, 128.64, 128.58, 128.40, 128.33, 128.29, 128.18, 128.14, 128.01, 127.91, 127.50, 127.30, 125.26, 118.71, 100.74, 96.72, 92.77, 80.67, 74.62, 74.48, 72.20, 71.90, 68.17, 67.57, 67.45, 66.29, 65.87, 54.66, 50.66, 50.38, 47.12, 46.03, 38.07, 37.22, 34.43, 32.86, 32.06, 30.16, 30.01, 29.87, 29.83, 29.79, 29.61, 29.50, 28.94, 27.87, 27.46, 27.21, 23.54, 23.32, 22.83, 19.84, 18.51, 14.28; HRMS (ESI) calculated for $C_{53}H_{60}Cl_3N_5O_{14}$ $[M+Na]^+$: 1118.3095, found: 1118.3062.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-4-_O_-benzyl-2-_O_-levulinoyl-3-_O_-(2-naphthylmethyl)-_β_-D-glucopyranosyluronate-(1→4)-benzyl-3-_O_-benzyl-2-_O_-levulinoyl-_β_-D-glucopyranosyluronate-(1→3)-4-azido-2-trichloroacetamido-2,4-di-deoxy-_α_-D-quinovopyranoside (3-40)

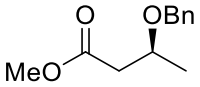


Thioglycoside **3-42** (264 mg, 0.402 mmol) and disaccharide **3-49** (220 mg, 0.201 mmol) were coevaporated three times with toluene, the resulting mixture was dried under high vacuum for 2 h. Then, the mixture was dissolved in anhydrous DCM (4 mL) and 4 Å molecular sieves were added. The solution was stirred at room temperature for 30 min and then cooled to 0 °C, NIS (90 mg, 0.402 mmol) and TfOH (5.3 µL, 0.06 mmol) were added. The reaction mixture was stirred at 0 °C for 3 h. After complete consumption of the staring material, triethylamine (0.1 mL) was added and the mixture was stirred for 1 h. Then, the reaction was diluted with DCM (15 mL), filtered and washed with saturated aqueous $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to give **3-40** as a colorless syrup (250 mg, 74%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 – 7.78 (m, 3H), 7.72 (s, 1H), 7.53 – 7.47 (m, 2H), 7.43 – 7.10 (m, 31H), 6.86 – 6.65 (m, 1H), 5.34 (t, *J* = 10.0 Hz, 1H), 5.25 – 5.15 (m, 2H), 5.08 (d, *J* = 12.1 Hz, 1H), 5.03 – 4.94 (m, 4H), 4.90 (d, *J* = 11.8 Hz, 1H), 4.82 (dd, *J* = 16.7, 11.6 Hz, 2H), 4.69 – 4.58 (m, 3H), 4.57 – 4.47 (m, 3H), 4.44 (dd, *J* = 11.0, 1.4 Hz, 1H), 4.30 (dd, *J* = 7.9, 1.6 Hz, 1H), 4.20 – 4.09 (m, 2H), 4.02 (d, *J* = 9.4 Hz, 1H), 3.94 (t, *J* = 9.9 Hz, 1H), 3.86 (td, *J* = 9.4, 1.6 Hz, 1H), 3.67 – 3.41 (m, 5H), 3.39 – 3.17 (m, 3H), 3.17 – 3.08 (m, 1H), 2.77 – 2.59 (m, 5H), 2.54 (dq, *J* = 17.9, 10.0 Hz, 1H), 2.39 (dt, *J* = 12.2, 4.1 Hz, 1H), 2.35 – 2.28 (m, 1H), 2.17 (s, 3H), 2.13 (s, 3H), 1.65 – 1.47 (m, 4H), 1.31 (dd, *J* = 6.1, 4.0 Hz, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.82, 206.66, 172.02, 171.77, 167.74, 167.31, 161.39, 156.82, 156.34, 138.32, 137.89, 137.82, 136.74, 135.64, 135.06, 135.00, 133.33, 133.06, 129.09, 129.02, 128.95, 128.90, 128.70, 128.67, 128.58, 128.56, 128.52, 128.42, 128.38, 128.28, 128.21, 128.11, 128.04, 128.02, 127.91, 127.84, 127.79, 127.66, 127.55, 127.30, 126.58, 126.26, 126.21, 126.08, 125.91, 100.68, 100.57, 96.72, 92.70, 81.66, 79.50,

79.14, 78.49, 76.69, 75.08, 74.93, 74.88, 74.76, 74.48, 73.12, 72.15, 72.00, 68.22, 67.57, 67.48, 67.42, 67.38, 66.22, 65.82, 54.63, 50.71, 50.40, 47.13, 46.15, 38.15, 37.68, 37.22, 32.87, 32.05, 30.16, 30.01, 29.97, 29.93, 29.86, 29.83, 29.79, 29.63, 29.49, 28.95, 27.85, 27.47, 27.21, 23.51, 23.34, 22.82, 19.86, 19.83, 18.51, 14.26; HRMS (ESI) calculated for $C_{89}H_{94}Cl_3N_5O_{22}$ $[M+Na]^+$: 1712.5348, found: 1712.5311.

Methyl (*S*)-3-(benzyloxy)butanoate (3-55)



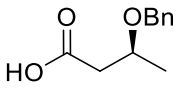
Under a nitrogen atmosphere, to a solution of **3-54** (800 mg, 6.8 mmol) in DCM (50 mL) were added 1,1,1,3,3,3-hexamethyldisilazane (0.78 mL, 3.7 mmol) and TMSOTf (123 µL, 0.68 mmol) at room temperature. The reaction was stirred for 10 min; then, the reaction mixture was evaporated in vacuo. The residue was used to the next step without further purification.

To a solution of the crude compound in DCM (50 mL) were added molecular sieves, the resulting mixture was stirred for 20 min before benzaldehyde (1.03 mL, 10.2 mmol) and TMSOTf (123 µL, 0.68 mmol) were added. After 10 min, triethylsilane (1.62 mL, 10.2 mmol) was added and the reaction was stirred for 2 h at room temperature. Upon completion, the reaction was quenched by triethylamine and washed with saturated aqueous $NaHCO_3$ and brine. Combined organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 2/1) to offer **3-55** (1.07 g, 64%) as a light yellow syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.10 (m, 5H), 4.65 – 4.48 (m, 2H), 4.11 – 4.02 (m, 1H), 3.71 (s, 3H), 2.69 (dd, *J* = 15.1, 7.4 Hz, 1H), 2.47 (dd, *J* = 15.0, 5.7 Hz, 1H), 1.30 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 129.89, 128.99, 128.69, 128.47, 127.79, 127.70, 72.01, 70.96, 51.77, 41.98, 41.45, 19.93.

NMR data was in accordance with previously reported values.[213]

(*S*)-3-(Benzyloxy)butanoic acid (3-56)

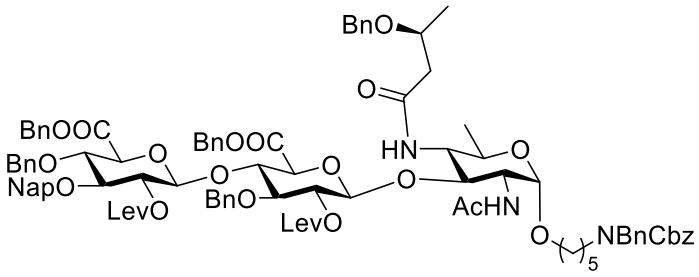


Methyl (*S*)-benzyloxybutanoate **3-55** (100 mg, 0.48 mmol) was dissolved in THF/H_2O (4/1 mL), LiOH (34.5 mg, 1.44 mmol) was added. The mixture was stirred at room temperature for 8 h. After acidification with 1 M HCl (aq.), the mixture was extracted with ethyl acetate (20 mL) and dried over Na_2SO_4. After concentration, **3-56** was obtained as a colourless oil (82mg, 88%). The product was pure enough for characterization and further application.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.45 – 6.82 (m, 5H), 4.55 – 4.33 (m, 2H), 3.99 – 3.89 (m, 1H), 2.59 (dd, *J* = 15.4, 7.3 Hz, 1H), 2.40 (dd, *J* = 15.4, 5.5 Hz, 1H), 1.20 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.32, 138.22, 128.88, 128.51, 128.48, 127.81, 127.77, 71.68, 70.96, 41.85, 19.79.

NMR data was in accordance with previously reported values.[214]

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-4-*O*-benzyl-2-*O*-levulinoyl-3-*O*-(2-naphthylmethyl)-*β*-D-glucopyranosyluronate-(1→4)-benzyl-3-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyluronate-(1→3)-2-*N*-acetyl-4-*N*-((*S*)-3-*O*-benzylbutyryl)-2,4-di-deoxy-*α*-D-quinovopyranoside (3-39)**



To a stirred solution of trisaccharide **3-53** (230 mg, 0.136 mmol) in acetic acid/THF (1.0 mL/4.0 mL) was added zinc (890 mg) under nitrogen. The mixture was stirred for 8 h at 40 °C. The solution was filtered and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried

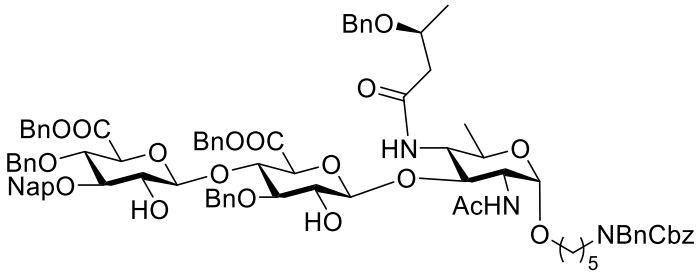
over Na_2SO_4, filtered and concentrated to give a colorless solid that was used without further purification.

A solution of (*S*)-3-*O*-benzylbutyric acid **3-56**[212,214] (132 mg, 0.68 mmol) in anhydrous DCM (6.0 mL) was treated with oxalyl chloride (117 µL, 1.36 mmol) and a catalytic amount of DMF (5.2 µL) at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was concentrated under reduced pressure to afford crude (*S*)-3-*O*-benzylbutyryl chloride.

Crude Amine was dissolved in anhydrous DCM (4 mL) under nitrogen, triethylamine (95 µL, 0.68 mmol) and a solution of (*S*)-3-*O*-benzylbutyryl chloride in anhydrous DCM (2 mL) were added. The reaction mixture was stirred at room temperature overnight. After that, the reaction mixture was quenched with MeOH (0.3 mL) at 0 °C and concentrated. The residue was purified by flash silica column chromatography (toluene/ ethyl acetate = 1/2) to give **3-39** as a colorless syrup (140 mg, 60% over two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.65 (m, 3H), 7.61 (s, 1H), 7.40 (dt, *J* = 6.4, 3.7 Hz, 2H), 7.34 – 6.93 (m, 36H), 6.16 – 5.60 (m, 2H), 5.24 – 4.88 (m, 6H), 4.88 – 4.61 (m, 5H), 4.60 – 4.49 (m, 3H), 4.49 – 4.31 (m, 6H), 4.18 (d, *J* = 7.8 Hz, 2H), 4.04 – 3.89 (m, 3H), 3.76 (t, *J* = 9.3 Hz, 1H), 3.66 – 3.31 (m, 7H), 3.19 (s, 3H), 2.63 (q, *J* = 7.7 Hz, 2H), 2.56 – 2.28 (m, 6H), 2.18 (q, *J* = 8.6 Hz, 2H), 2.07 (s, 3H), 1.98 (s, 3H), 1.88 (s, 3H), 1.56 – 1.38 (m, 4H), 1.29 – 1.22 (m, 2H), 1.13 (d, *J* = 6.1 Hz, 3H), 1.07 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 207.15, 206.69, 171.90, 171.76, 171.67, 167.72, 138.71, 138.26, 137.88, 135.61, 135.02, 134.94, 133.34, 133.08, 129.41, 129.13, 128.95, 128.73, 128.65, 128.46, 128.28, 128.24, 128.19, 128.09, 128.04, 127.98, 127.89, 127.81, 127.65, 127.53, 127.33, 126.65, 126.32, 126.16, 125.95, 100.70, 100.53, 97.53, 81.57, 79.48, 78.22, 75.73, 75.13, 74.96, 74.46, 74.32, 73.24, 72.96, 72.37, 71.07, 67.92, 67.71, 67.51, 67.39, 53.06, 50.37, 47.25, 44.05, 38.21, 37.63, 32.08, 29.99, 29.85, 29.51, 28.76, 27.92, 27.77, 27.36, 23.82, 23.41, 22.84, 19.95, 18.34, 14.28. HRMS (ESI) calculated for $C_{100}H_{111}N_3O_{24}$ $[M+Na]^+$: 1760.7450, found: 1760.7382.

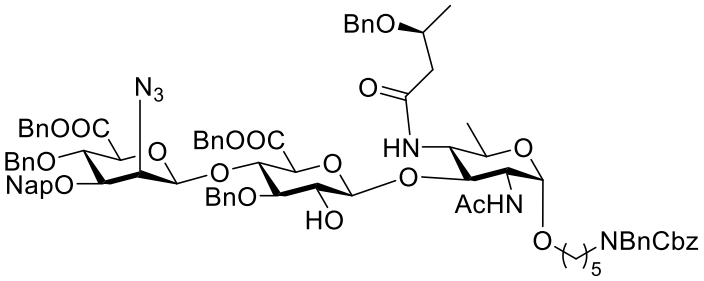
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-4-_O_-benzyl-3-_O_-(2-naphthylmethyl)-_β_-D-glucopyranosyluronate-(1→4)-benzyl-3-_O_-benzyl-_β_-D-glucopyranosyluronate-(1→3)-2-_N_-acetyl-4-_N_-((_S_)-3-_O_-benzylbutyryl)-2,4-di-deoxy-_α_-D-quinovopyranoside (3-58)



Trisaccharide **3-39** (140 mg, 0.0806 mmol) was dissolved in DCM (5.0 mL), N_2H_4·AcOH (74 mg, 0.806 mmol) was added and the mixture was stirred at room temperature for 12 h. Then, the reaction was quenched with acetone (0.5 mL) and concentrated under vacuum. The residue was purified by flash silica column chromatography (toluene/ethyl acetate = 1/1) to give **3-58** (97 mg, 78%) as a light yellow solid.

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.79 – 7.62 (m, 4H), 7.44 – 7.34 (m, 4H), 7.34 – 7.06 (m, 32H), 6.99 (d, *J* = 7.4 Hz, 2H), 6.07 – 5.61 (m, 2H), 5.11 (d, *J* = 17.9 Hz, 4H), 5.03 – 4.91 (m, 3H), 4.82 (dd, *J* = 11.6, 6.8 Hz, 2H), 4.72 – 4.56 (m, 3H), 4.54 – 4.31 (m, 5H), 4.29 – 4.11 (m, 2H), 4.05 (p, *J* = 6.3 Hz, 1H), 3.99 – 3.82 (m, 3H), 3.72 – 3.62 (m, 2H), 3.62 – 3.37 (m, 5H), 3.34 (t, *J* = 8.4 Hz, 1zH), 3.31 – 3.12 (m, 3H), 2.99 – 2.85 (m, 3H), 2.69 (dd, *J* = 14.7, 6.9 Hz, 0H), 2.35 (dd, *J* = 14.8, 5.9 Hz, 0H), 2.24 (dd, *J* = 14.5, 7.7 Hz, 1H), 2.14 (dd, *J* = 14.6, 5.3 Hz, 1H), 1.91 (d, *J* = 15.2 Hz, 3H), 1.61 – 1.37 (m, 6H), 1.29 – 1.22 (m, 4H), 1.14 (d, *J* = 6.2 Hz, 2H), 1.09 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 171.96, 171.79, 171.54, 168.53, 168.32, 156.88, 156.43, 138.94, 138.76, 138.65, 137.99, 137.88, 136.68, 136.14, 135.05, 134.75, 133.44, 133.12, 129.08, 129.06, 129.01, 128.92, 128.74, 128.65, 128.63, 128.56, 128.45, 128.40, 128.37, 128.24, 128.18, 128.05, 127.95, 127.91, 127.88, 127.84, 127.80, 127.77, 127.64, 127.62, 127.55, 127.35, 126.74, 126.18, 126.15, 126.02, 125.97, 104.31, 103.37, 97.42, 83.30, 82.20, 78.95, 78.89, 75.27, 75.07, 74.96, 74.80, 74.46, 74.27, 74.12, 73.34, 72.99, 71.27, 71.11, 68.28, 68.02, 67.82, 67.42, 55.55, 53.20, 50.45, 47.22, 46.02, 44.29, 29.83, 29.10, 28.88, 27.88, 27.41, 23.80, 23.68, 23.37, 20.40, 20.02, 18.18. HRMS (ESI) calculated for $C_{90}H_{99}N_3O_{20}$ $[M+Na]^+$: 1564.6714, found: 1564.6676.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-2-azido-2-deoxy-4-_O_-benzyl-3-_O_-(2-naphthylmethyl)-_β_-D-mannopyranosyluronate-(1→4)-benzyl-3-_O_-benzyl-_β_-D-glucopyranosyluronate-(1→3)-2-_N_-acetyl-4-_N_-((_S_)-3-_O_-benzylbutyryl)-2,4-di-deoxy-_α_-D-quinovopyranoside (3-59)



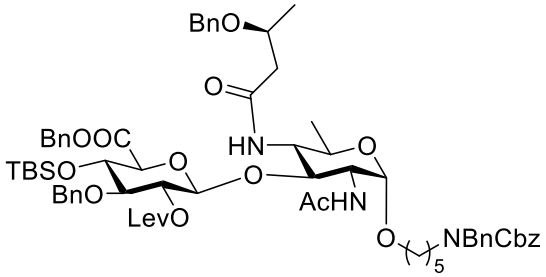
Trisaccharide **3-58** (17 mg, 0.011 mmol) was dissolved in anhydrous DCM (1.0 mL) and pyridine (18 µL, 0.22 mmol). The solution was cooled to 0 °C and triflic anhydride (12 µL, 0.066 mmol) was added. The mixture was stirred at 0 °C for 4 h and the reaction was quenched by adding cold water. The organic layer was washed twice with cold water and cold brine, organic layer was dried over Na_2SO_4. The solvent was evaporated giving the triflate as a yellow solid that was used without further purification.

The solid was dissolved in anhydrous toluene (1.0 mL) and tetrabutylammonium azide (31 mg, 0.11 mmol) was added. The reaction mixture was stirred overnight at 60 °C. The reaction was diluted with ethyl acetate and wash with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated, resulting residue was purified by flash silica column chromatography (toluene/ethyl acetate = 1/1) give **3-59** (9.8 mg, 57%) as a light yellow solid.

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.82 – 7.61 (m, 3H), 7.47 – 6.93 (m, 39H), 5.94 – 5.67 (m, 2H), 5.17 – 5.02 (m, 3H), 4.96 (t, *J* = 10.1 Hz, 2H), 4.74 (s, 2H), 4.71 – 4.56 (m, 4H), 4.53 – 4.32 (m, 5H), 4.29 (s, 1H), 4.26 – 4.08 (m, 2H), 3.99 – 3.91 (m, 1H), 3.87 (q, *J* = 13.1 Hz, 2H), 3.80 (t, *J* = 9.1 Hz, 1H), 3.72 (s, 1H), 3.65 – 3.33 (m, 7H), 3.32 – 3.10 (m, 4H), 3.01 – 2.84 (m, 1H), 2.34 – 2.23 (m, 1H), 2.19 – 2.07 (m, 1H), 1.90 (s, 3H), 1.57 – 1.43 (m, 4H), 1.30 – 1.23 (m, 2H), 1.13 (d, *J* = 6.1 Hz, 3H), 1.09 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 171.87, 167.86, 167.72, 138.72, 138.13, 137.90, 135.17, 134.95, 134.86, 133.40, 133.25, 129.19, 129.00, 128.96, 128.90, 128.81, 128.75, 128.73, 128.65, 128.63, 128.56, 128.46, 128.41, 128.37, 128.28, 128.19, 128.08,

128.03, 127.97, 127.91, 127.85, 127.83, 127.78, 127.67, 127.59, 127.35, 126.74, 126.41, 126.26, 125.68, 104.40, 100.58, 97.44, 81.88, 79.50, 75.48, 75.24, 74.99, 74.86, 74.19, 73.88, 73.00, 72.17, 71.14, 68.44, 67.83, 67.60, 67.43, 67.40, 61.74, 55.47, 53.56, 53.22, 50.45, 47.22, 44.25, 32.07, 29.85, 29.51, 27.46, 23.70, 22.84, 20.05, 18.22, 14.26; HRMS (ESI) calculated for $C_{90}H_{98}N_6O_{19}$ $[M+Na]^+$: 1589.6779, found: 1589.6774.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-3-_O_-benzyl-2-_O_-levulinoyl-4-_O_-_tert_-butyldimethylsilyl-_β_-D-glucopyranosyluronate-(1→3)-2-_N_-acetyl-4-_N_-((_S_)-3-_O_-benzylbutyryl)-2,4-di-deoxy-_α_-D-quinovopyranoside (3-60)



To a stirred solution of disaccharide **3-51** (52 mg, 0.043 mmol) in acetic acid/THF (0.4 mL/1.2 mL) was added zinc (280 mg) under nitrogen. The mixture was stirred for 12 h at 40 °C. The solution was filtered and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to give an amine that was used without further purification.

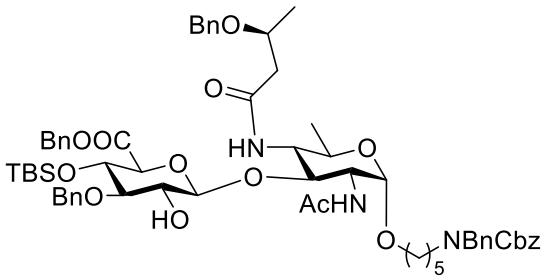
A solution of (*S*)-3-*O*-benzylbutyric acid **3-56** (39 mg, 0.203 mmol) in DCM (2.0 mL) was treated with oxalyl chloride (35 µL, 0.406 mmol) and a catalytic amount of DMF (1.6 µL) at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was concentrated to afford crude (*S*)-3-*O*-benzylbutyryl chloride.

The amine was dissolved in anhydrous DCM (2 mL) under nitrogen, Et_3N (28 µL, 0.203 mmol) and a solution of (*S*)-3-*O*-benzylbutyryl chloride in anhydrous DCM (1 mL) were added. The reaction mixture was stirred at room temperature overnight. After that, the reaction mixture was quenched with MeOH (0.3 mL) at 0 °C and concentrated. The residue was purified by flash silica

column chromatography (toluene/ ethyl acetate = 2/1) to give **3-60** as a colorless syrup (28 mg, 52% over two steps).

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.56 – 7.11 (m, 25H), 6.29 – 6.12 (m, 1H), 5.99 – 5.57 (m, 1H), 5.35 – 5.14 (m, 4H), 4.97 (t, *J* = 8.6 Hz, 1H), 4.80 – 4.46 (m, 8H), 4.31 (t, *J* = 11.3 Hz, 1H), 4.17 – 4.04 (m, 3H), 3.84 – 3.49 (m, 5H), 3.43 – 3.22 (m, 3H), 2.85 – 2.77 (m, 1H), 2.76 – 2.63 (m, 2H), 2.60 – 2.45 (m, 2H), 2.44 – 2.35 (m, 1H), 2.17 (s, 3H), 2.03 (s, 3H), 1.70 – 1.53 (m, 5H), 1.43 – 1.36 (m, 2H), 1.31 (d, *J* = 6.1 Hz, 3H), 1.23 (d, *J* = 6.3 Hz, 3H), 0.88 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 212.07, 178.65, 177.20, 176.90, 174.72, 174.50, 173.20, 161.95, 161.48, 143.96, 143.17, 142.94, 142.91, 141.65, 140.10, 134.21, 134.08, 133.88, 133.83, 133.76, 133.69, 133.64, 133.62, 133.59, 133.55, 133.47, 133.44, 133.40, 133.35, 133.25, 133.23, 133.18, 133.03, 132.98, 132.93, 132.90, 132.84, 132.62, 132.60, 132.56, 132.37, 132.22, 132.15, 131.19, 105.67, 102.54, 86.91, 80.38, 79.23, 78.56, 78.12, 77.13, 76.88, 76.05, 76.03, 73.12, 72.91, 72.74, 72.51, 72.47, 72.40, 60.91, 58.19, 55.50, 52.38, 51.04, 49.02, 46.83, 46.59, 43.16, 37.10, 34.94, 34.90, 34.87, 34.83, 34.53, 34.06, 33.63, 32.89, 32.85, 32.32, 30.91, 30.86, 29.05, 28.53, 28.32, 27.86, 26.68, 25.03, 24.85, 24.83, 23.38, 23.04, 19.30, 6.19, 0.90, 0.01; HRMS (ESI) calculated for $C_{70}H_{91}N_3O_{16}Si$ $[M+Na]^+$: 1280.6061, found: 1280.6033.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-3-_O_-benzyl-4-_O_-_tert_-butyldimethylsilyl-_β_-D-glucopyranosyluronate-(1→3)-2-_N_-acetyl-4-_N_-((_S_)-3-_O_-benzylbutyryl)-2,4-di-deoxy-_α_-D-quinovopyranoside (3-61)

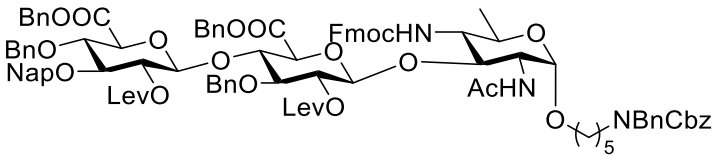


Disaccharide **3-60** (18 mg, 14.3 μmol) was dissolved in DCM (1.0 mL), N_2H_4·AcOH (26.3 mg, 28.6 μmol) was added and the mixture was stirred at room temperature for 12 h. Then, the reaction was quenched with acetone (0.5 mL) and concentrated under vacuum. The residue was purified by

flash silica column chromatography (toluene/ethyl acetate = 1/1) to give **3-61** (15 mg, 90%) as a light yellow solid.

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.54 – 7.20 (m, 25H), 6.12 – 5.75 (m, 2H), 5.33 – 5.21 (m, 3H), 5.18 (dd, *J* = 11.7, 4.8 Hz, 2H), 4.79 – 4.49 (m, 6H), 4.45 – 4.27 (m, 2H), 4.14 – 4.06 (m, 1H), 3.97 – 3.93 (m, 2H), 3.80 – 3.60 (m, 4H), 3.58 (t, *J* = 8.2 Hz, 1H), 3.47 – 3.25 (m, 4H), 3.09 (s, 1H), 2.47 – 2.33 (m, 2H), 2.12 – 2.02 (m, 3H), 1.75 – 1.56 (m, 4H), 1.45 – 1.35 (m, 2H), 1.31 (d, *J* = 6.2 Hz, 3H), 1.24 (d, *J* = 6.0 Hz, 3H), 0.86 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 178.08, 177.44, 176.99, 173.61, 162.06, 161.62, 144.03, 143.14, 143.08, 142.15, 141.85, 140.18, 134.40, 134.24, 134.21, 134.03, 133.98, 133.92, 133.88, 133.83, 133.81, 133.76, 133.66, 133.62, 133.46, 133.39, 133.37, 133.34, 133.16, 133.15, 133.10, 133.04, 132.77, 132.71, 132.58, 132.53, 131.37, 109.53, 102.67, 88.76, 83.65, 81.66, 79.86, 79.40, 78.29, 77.01, 76.96, 76.24, 76.19, 73.49, 73.01, 72.64, 72.62, 60.78, 58.75, 58.41, 55.83, 55.63, 52.43, 51.28, 49.35, 46.97, 46.60, 37.26, 35.03, 34.98, 34.69, 34.28, 34.01, 33.13, 32.59, 31.09, 31.06, 29.03, 28.88, 28.58, 28.02, 25.13, 24.93, 23.42, 23.29, 19.45, 1.34, 0.01; HRMS (ESI) calculated for $C_{65}H_{85}N_3O_{14}Si$ $[M+Na]^+$: 1182.5693, found: 1182.5663.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-4-_O_-benzyl-2-_O_-levulinoyl-3-_O_-(2-naphthylmethyl)-_β_-D-glucopyranosyluronate-(1→4)-benzyl-3-_O_-benzyl-2-_O_-levulinoyl-_β_-D-glucopyranosyluronate-(1→3)-2-_N_-acetyl-4-_N_-fluorenylmethoxycarbonyl-2,4-di-deoxy-_α_-D-quinovopyranoside (3-63)

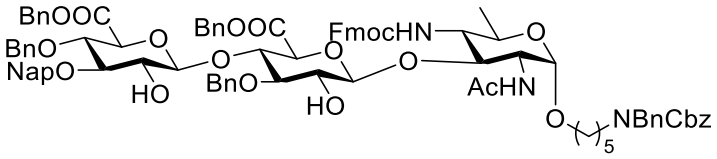


To a stirred solution of trisaccharide **3-40** (50 mg, 0.030 mmol) in acetic acid/THF (0.5 mL/1.5 mL) was added zinc (190 mg) under nitrogen. The mixture was stirred for 8 h at 40 °C. The solution was filtered and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to form a colorless solid that was used without further purification.

The crude amine was dissolved in anhydrous DCM (1.0 mL), pyridine (22 µL, 0.275 mmol) and FmocCl (14.2 mg, 0.055 mmol) were added, the mixture was stirred overnight. After the starting material was consumed, diluted with DCM (10 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine. Then, organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 1/1) to give **3-63** as a white solid (22 mg, 43% over two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 – 7.57 (m, 6H), 7.57 – 7.46 (m, 2H), 7.39 (dt, *J* = 5.9, 3.5 Hz, 2H), 7.35 – 6.91 (m, 35H), 6.04 – 5.60 (m, 1H), 5.43 – 5.22 (m, 1H), 5.10 (q, *J* = 7.8 Hz, 2H), 5.05 – 4.94 (m, 1H), 4.92 – 4.63 (m, 8H), 4.61 – 4.53 (m, 2H), 4.48 – 4.32 (m, 5H), 4.26 (d, *J* = 7.8 Hz, 2H), 4.14 (d, *J* = 6.5 Hz, 2H), 4.06 (t, *J* = 8.4 Hz, 1H), 3.93 (d, *J* = 8.8 Hz, 1H), 3.77 (t, *J* = 9.3 Hz, 1H), 3.64 – 3.39 (m, 5H), 3.34 (t, *J* = 9.2 Hz, 1H), 3.30 – 3.06 (m, 4H), 2.58 (d, *J* = 6.8 Hz, 2H), 2.54 – 2.30 (m, 4H), 2.29 – 2.10 (m, 2H), 2.05 (s, 3H), 1.96 (s, 3H), 1.85 (s, 3H), 1.55 – 1.37 (m, 4H), 1.31 – 1.19 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 207.31, 206.80, 171.65, 167.75, 167.36, 156.86, 156.39, 144.06, 141.39, 138.49, 137.84, 135.65, 135.00, 133.33, 133.05, 129.23, 128.92, 128.77, 128.73, 128.71, 128.69, 128.64, 128.60, 128.46, 128.44, 128.26, 128.23, 128.21, 128.19, 128.07, 128.05, 128.00, 127.98, 127.88, 127.83, 127.81, 127.79, 127.44, 127.32, 127.19, 126.56, 126.28, 126.10, 125.91, 125.34, 125.21, 120.04, 100.53, 97.44, 81.77, 79.49, 78.25, 75.16, 74.93, 74.47, 73.14, 72.25, 67.94, 67.60, 67.47, 67.38, 66.63, 56.86, 53.00, 50.58, 50.36, 47.41, 47.20, 46.08, 38.15, 37.58, 37.23, 34.54, 32.88, 32.06, 30.19, 29.96, 29.84, 29.79, 29.50, 28.74, 27.81, 27.59, 27.32, 23.87, 23.38, 22.85, 22.83, 20.27, 19.85, 18.23, 14.58, 14.27, 11.57; HRMS (ESI) calculated for $C_{104}H_{109}N_3O_{24}$ $[M+Na]^+$: 1806.7293, found: 1806.7228.

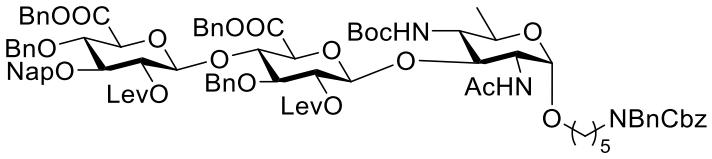
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-4-_O_-benzyl-3-_O_-(2-naphthylmethyl)-_β_-D-glucopyranosyluronate-(1→4)-benzyl-3-_O_-benzyl-_β_-D-glucopyranosyluronate-(1→3)-2-_N_-acetyl-4-_N_-fluorenylmethoxycarbonyl-2,4-di-deoxy-_α_-D-quinovopyranoside (3-64)



Trisaccharide **3-63** (20 mg, 11.2 μmol) was dissolved in DCM (1.0 mL), N_2H_4·AcOH (10.3 mg, 112 μmol) was added and the mixture was stirred at room temperature for 12 h. Then, the reaction was quenched with acetone (0.5 mL) and concentrated under vacuum. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 3/2) to give **3-64** (11.2 mg, 63%) as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 – 7.69 (m, 6H), 7.67 – 7.03 (m, 39H), 6.08 – 5.75 (m, 1H), 5.28 – 5.11 (m, 4H), 5.07 (d, *J* = 12.1 Hz, 1H), 5.00 (d, *J* = 12.2 Hz, 2H), 4.95 (d, *J* = 11.5 Hz, 2H), 4.75 (d, *J* = 10.7 Hz, 2H), 4.67 (d, *J* = 14.4 Hz, 2H), 4.61 (s, 1H), 4.53 (d, 4H), 4.42 (d, *J* = 10.8 Hz, 1H), 4.31 (s, 1H), 4.26 – 4.14 (m, 2H), 4.01 (t, *J* = 9.0 Hz, 1H), 3.92 (d, *J* = 9.5 Hz, 1H), 3.78 (q, *J* = 11.5 Hz, 3H), 3.69 – 3.50 (m, 5H), 3.49 – 3.18 (m, 6H), 1.84 – 1.61 (m, 4H), 1.50 – 1.42 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.46, 157.22, 156.42, 144.01, 141.49, 138.91, 137.94, 137.84, 136.18, 134.97, 134.64, 133.42, 133.08, 129.07, 128.86, 128.76, 128.66, 128.62, 128.56, 128.39, 128.37, 128.20, 128.07, 127.96, 127.89, 127.81, 127.78, 127.57, 127.46, 127.33, 127.22, 127.15, 126.79, 126.29, 126.10, 125.92, 125.07, 124.85, 120.24, 104.02, 97.24, 83.36, 82.80, 78.89, 78.10, 75.31, 75.16, 74.74, 74.19, 68.75, 68.07, 67.87, 67.44, 66.05, 53.02, 50.37, 47.52, 47.07, 45.91, 39.55, 39.20, 37.23, 36.92, 36.78, 34.54, 33.60, 32.88, 32.61, 32.40, 32.07, 31.57, 30.44, 30.31, 30.18, 30.10, 29.89, 29.85, 29.81, 29.64, 29.51, 28.78, 28.12, 27.57, 27.23, 26.90, 26.61, 26.55, 26.00, 23.72, 23.20, 22.87, 22.85, 22.81, 20.28, 19.87, 19.37, 18.00, 14.71, 14.57, 14.34, 14.28, 11.57, 11.06; HRMS (ESI) calculated for $C_{94}H_{97}N_3O_{20}$ $[M+Na]^+$: 1610.6558, found: 1610.6506.

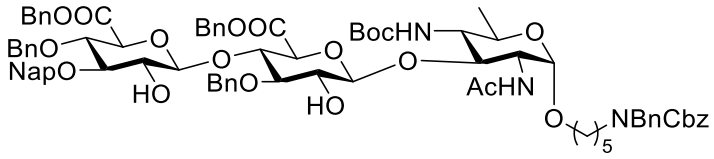
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-4-_O_-benzyl-2-_O_-levulinoyl-3-_O_-(2-naphthylmethyl)-_β_-D-glucopyranosyluronate-(1→4)-benzyl-3-_O_-benzyl-2-_O_-levulinoyl-_β_-D-glucopyranosyluronate-(1→3)-2-_N_-acetyl-4-_N_-_tert_-butyloxycarbonyl-2,4-di-deoxy-_α_-D-quinovopyranoside (3-66)



To a stirred solution of trisaccharide **3-40** (50 mg, 0.030 mmol) in acetic acid/THF (0.5 mL/1.5 mL) was added zinc (190 mg) under nitrogen. The mixture was stirred for 8 h at 40 °C. The solution was filtered and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to give an amine that was used without further purification. Crude amine was dissolved in anhydrous DCM (1.0 mL), pyridine (200 µL, 0.275 mmol), Boc_2O (12 mg, 0.055 mmol) and DMAP (0.4 mg) were added, the mixture was stirred overnight. After the starting material was consumed, diluted with DCM (10 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine. Then organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash silica column chromatography (hexane/ethyl acetate = 1/1) to give **3-66** as a white solid (15 mg, 31% over two steps).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 – 7.65 (m, 4H), 7.52 – 7.05 (m, 33H), 6.09 – 5.62 (m, 2H), 5.32 (s, 1H), 5.23 – 5.05 (m, 4H), 4.97 (s, 3H), 4.92 – 4.77 (m, 4H), 4.72 – 4.59 (m, 2H), 4.58 – 4.39 (m, 5H), 4.23 (s, 1H), 4.14 (td, *J* = 8.6, 3.3 Hz, 1H), 4.00 (s, 1H), 3.88 (s, 1H), 3.75 (s, 1H), 3.64 – 3.44 (m, 4H), 3.41 – 3.16 (m, 5H), 2.80 – 2.62 (m, 3H), 2.62 – 2.45 (m, 3H), 2.36 – 2.20 (m, 2H), 2.15 (d, *J* = 3.7 Hz, 3H), 2.09 (d, *J* = 11.8 Hz, 3H), 1.93 (d, *J* = 15.9 Hz, 3H), 1.64 – 1.48 (m, 4H), 1.48 –1.40 (m, 5H) 1.28 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.64, 171.68, 167.73, 167.28, 138.40, 137.76, 137.69, 135.54, 134.90, 133.23, 132.95, 129.21, 128.87, 128.74, 128.63, 128.61, 128.59, 128.53, 128.50, 128.34, 128.16, 128.12, 128.08, 127.97, 127.95, 127.89, 127.77, 127.72, 127.69, 127.35, 127.22, 126.52, 126.17, 126.00, 125.85, 100.55, 97.34, 79.47, 75.08, 74.85, 74.41, 73.04, 68.63, 67.70, 67.35, 67.27, 50.23, 47.09, 38.03, 37.54, 31.95, 29.88, 29.73, 29.40, 28.42, 27.70, 27.60, 23.27, 22.73, 22.70, 18.02, 14.17; HRMS (ESI) calculated for $C_{94}H_{107}N_3O_{24}$ $[M+Na]^+$: 1684.7137, found: 1684.7086.

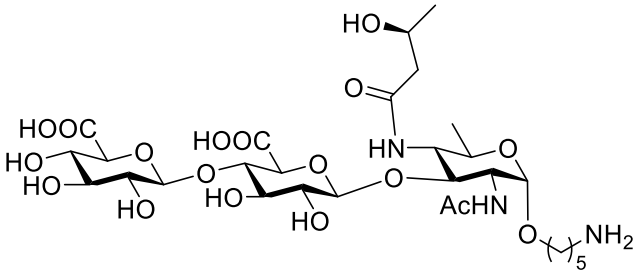
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl benzyl-4-_O_-benzyl-3-_O_-(2-naphthylmethyl)-_β_-D-glucopyranosyluronate-(1→4)-benzyl-3-_O_-benzyl-_β_-D-glucopyranosyluronate-(1→3)-2-_N_-acetyl-4-_N_-_tert_-butyloxycarbonyl-2,4-di-deoxy-_α_-D-quinovopyranoside (3-67)



Trisaccharide **3-66** (15 mg, 9.0 μmol) was dissolved in DCM (1.0 mL), N_2H_4·AcOH (8.3 mg, 90 μmol) was added and the mixture was stirred at room temperature for 12 h. Then, the reaction was quenched with acetone (0.5 mL) and concentrated under vacuum. The residue was purified by flash silica column chromatography (hexane/ethyl acetate = 3/2) to give **3-67** (9.6 mg, 73%) as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4H), 7.55 – 7.15 (m, 31H), 7.07 (d, *J* = 7.0 Hz, 2H), 6.07 – 5.75 (m, 1H), 5.27 – 5.18 (m, 4H), 5.18 – 5.09 (m, 2H), 5.08 – 5.00 (m, 2H), 4.94 (d, *J* = 11.4 Hz, 1H), 4.74 (qd, *J* = 16.9, 5.8 Hz, 5H), 4.58 – 4.48 (m, 2H), 4.43 (d, *J* = 10.7 Hz, 1H), 4.31 (s, 1H), 4.28 – 4.20 (m, 1H), 4.08 (t, *J* = 9.0 Hz, 1H), 3.97 (d, *J* = 10.4 Hz, 1H), 3.86 (d, *J* = 9.5 Hz, 1H), 3.80 (t, *J* = 9.1 Hz, 1H), 3.72 – 3.58 (m, 3H), 3.54 (t, *J* = 8.3 Hz, 2H), 3.46 – 3.35 (m, 3H), 3.34 – 3.20 (m, 2H), 2.01 (d, *J* = 15.5 Hz, 3H), 1.68 – 1.49 (m, 7H), 1.28 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.52, 156.41, 138.74, 137.86, 136.65, 136.18, 134.93, 134.63, 133.42, 133.09, 129.14, 128.83, 128.80, 128.76, 128.66, 128.63, 128.60, 128.42, 128.21, 128.08, 127.96, 127.82, 127.78, 127.63, 127.33, 126.77, 126.27, 126.10, 125.93, 104.05, 97.31, 83.39, 82.90, 79.64, 78.93, 75.61, 75.41, 75.26, 74.78, 74.39, 73.08, 68.76, 68.40, 67.80, 67.44, 53.02, 50.36, 47.07, 37.23, 34.53, 32.88, 32.07, 30.30, 30.18, 29.88, 29.84, 29.80, 29.55, 29.51, 28.58, 27.74, 27.34, 27.23, 23.73, 23.20, 22.84, 22.81, 19.87, 18.00, 14.57, 14.28, 11.57; HRMS (ESI) calculated for $C_{84}H_{95}N_3O_{20}$ $[M+Na]^+$: 1488.6401, found: 1488.6349.

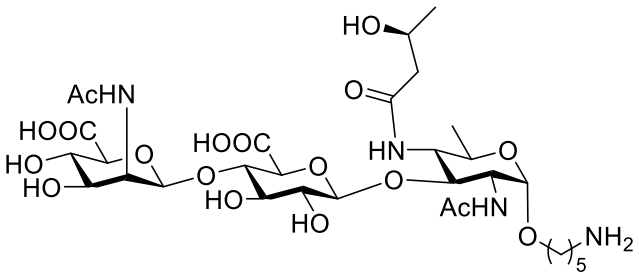
5-Aminopentyl *β*-D-glucopyranosyluronate-(1→4)-*β*-D-glucopyranosyluronate-(1→3)-2-*N*-acetyl-4-*N*-((*S*)-3-hydroxybutyryl)-2,4-di-deoxy-*α*-D-quinovopyranoside (3-69)



In a 20 mL vial trisaccharide **3-58** (11 mg, 7.1 µmol) was dissolved in EA/*t*-BuOH/H_2O (2 mL/2 mL/1 mL), palladium on activated charcoal (10% Pd, 40 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered. The crude product, obtained after removal of the solvent, was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **3-69** (2.7 mg, 52%) as a white solid.

^{1}H NMR (700 MHz, D_2O) δ 4.49 (d, *J* = 7.9 Hz, 1H), 4.35 (d, *J* = 8.0 Hz, 1H), 4.14 – 4.03 (m, 2H), 3.88 (t, *J* = 10.2 Hz, 1H), 3.85 – 3.81 (m, 1H), 3.80 – 3.77 (m, 1H), 3.72 (d, *J* = 9.8 Hz, 1H), 3.67 (t, *J* = 9.2 Hz, 1H), 3.63 – 3.55 (m, 2H), 3.53 (t, *J* = 9.0 Hz, 1H), 3.48 – 3.43 (m, 2H), 3.42 – 3.38 (m, 1H), 3.26 – 3.21 (m, 1H), 3.17 (t, *J* = 8.7 Hz, 1H), 2.93 (t, *J* = 7.7 Hz, 2H), 2.33 (dd, *J* = 14.5, 5.0 Hz, 1H), 2.27 (dd, *J* = 14.4, 8.4 Hz, 1H), 1.94 (s, 3H), 1.67 – 1.53 (m, 4H), 1.39 (tq, *J* = 16.0, 7.6 Hz, 2H), 1.15 (d, *J* = 6.5 Hz, 3H), 1.13 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (176 MHz, D_2O) δ 174.52, 174.38, 174.24, 173.79, 167.25, 103.43, 102.14, 97.04, 80.58, 76.38, 75.61, 75.22, 75.18, 73.91, 72.96, 72.44, 71.49, 67.80, 66.99, 65.01, 55.54, 53.59, 45.07, 39.39, 28.11, 26.51, 22.35, 22.25, 21.98, 16.91; HRMS (ESI) calculated for $C_{29}H_{49}N_3O_{18}$ $[M+H]^+$: 728.3084, found: 728.3088.

5-Aminopentyl 2-*N*-acetyl-2-deoxy-*β*-D-mannopyranosyluronate-(1→4)-*β*-D-glucopyranosyluronate-(1→3)-2-*N*-acetyl-4-*N*-((*S*)-3-hydroxybutyryl)-2,4-di-deoxy-*α*-D-quinovopyranoside (3-70)

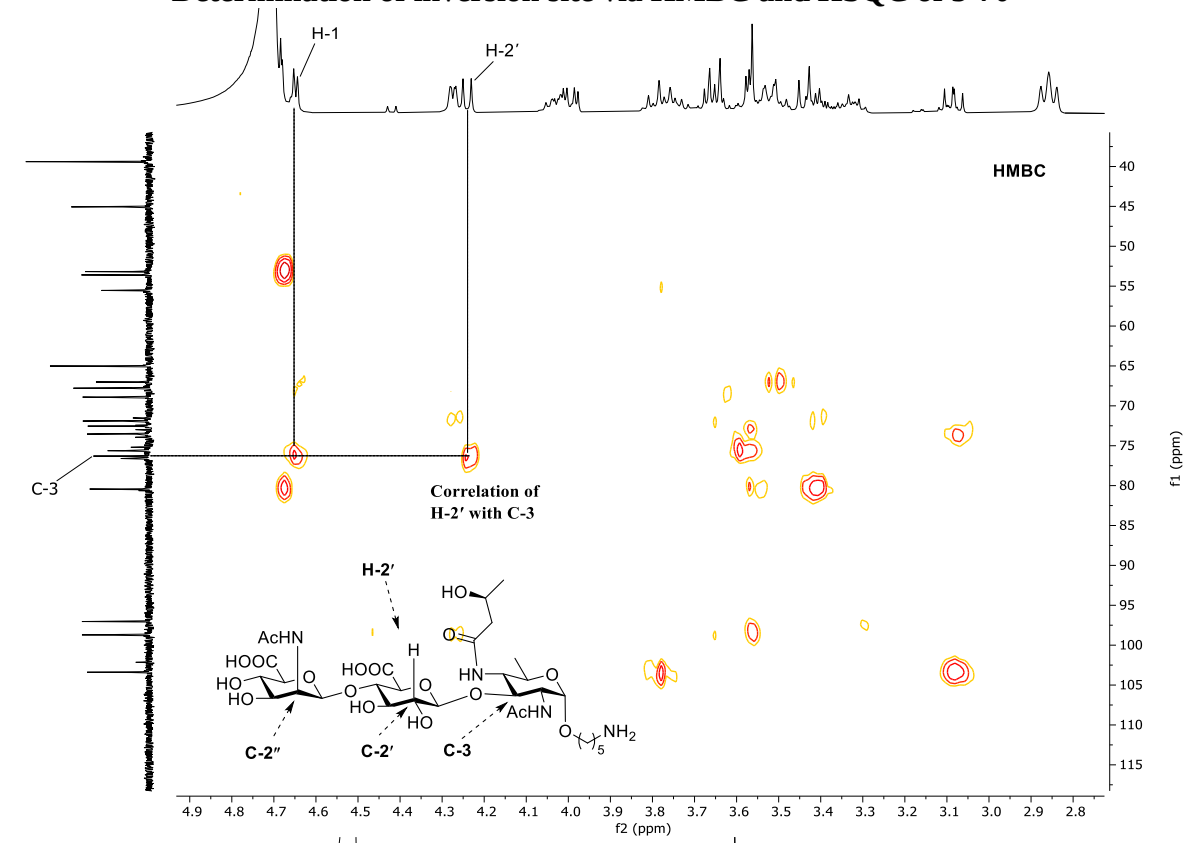


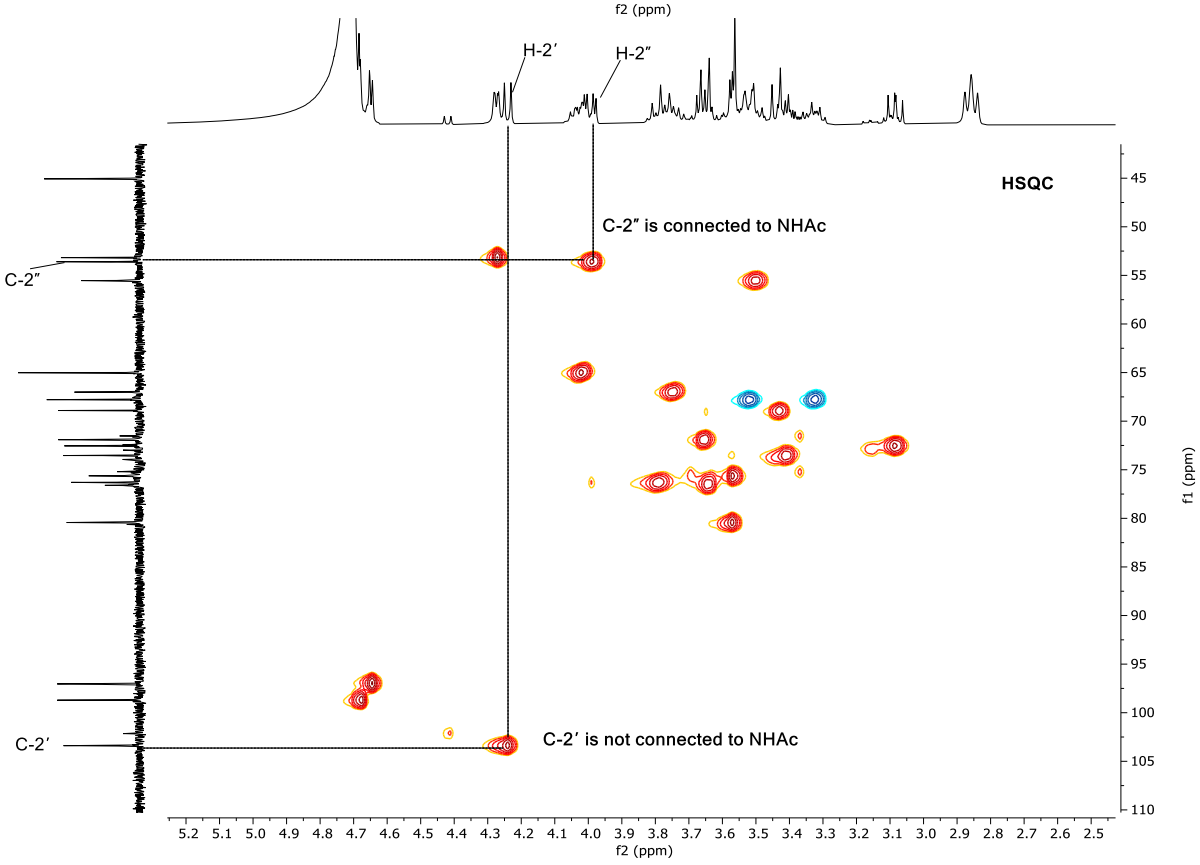
To a stirred solution of trisaccharide **3-59** (13 mg, 8.3 μmol) in acetic acid/acetic anhydride/THF (0.2/0.4/0.6, 1.2 mL) was added zinc (40 mg) under nitrogen. The mixture was stirred for 12 h at 37 °C. The solution was filtered and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to give a colorless solid that was used without further purification.

In a 20 mL vial, the crude product in the last step was dissolved in EA/*t*-BuOH/H_2O (2 mL/2 mL/1 mL), palladium on activated charcoal (10% Pd, 40 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere for 8 h. After complete consumption of the starting material, the reaction mixture was filtered. The crude product obtained after removal of the solvent was purified by reversed phase HPLC using a preparative Hypercarb column (From 0% to 30% MeCN in H_2O in 30 min, flow rate 3 mL/min) to afford **3-70** (2.6 mg, 41% over two steps) as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.68 (d, *J* = 1.5 Hz, 1H), 4.65 (d, *J* = 3.5 Hz, 1H), 4.29 – 4.26 (m, 1H), 4.24 (d, *J* = 7.8 Hz, 1H), 4.08 – 3.96 (m, 2H), 3.84 – 3.71 (m, 2H), 3.70 – 3.61 (m, 2H), 3.61 – 3.47 (m, 4H), 3.46 – 3.37 (m, 2H), 3.37 – 3.26 (m, 1H), 3.12 – 3.05 (m, 1H), 2.86 (t, *J* = 7.6 Hz, 2H), 2.33 – 2.15 (m, 2H), 1.91 (s, 3H), 1.86 (s, 3H), 1.61 – 1.43 (m, 4H), 1.39 – 1.21 (m, 2H), 1.13 – 1.01 (m, 6H); ^{13}C NMR (176 MHz, D_2O) δ 175.34, 175.00, 174.52, 174.38, 173.90, 103.39, 102.15, 98.71, 97.04, 80.63, 80.43, 76.60, 76.30, 75.62, 75.20, 73.94, 73.54, 72.98, 72.54, 72.44, 71.91, 71.52, 68.90, 67.79, 67.01, 65.01, 55.53, 53.59, 53.17, 45.06, 39.39, 28.12, 26.51, 22.37, 22.33, 22.24, 22.10, 22.04, 22.01, 21.97, 16.90; HRMS (ESI) calculated for $C_{31}H_{52}N_4O_{18}$ $[M+H]^+$: 769.3349, found: 769.3336.

Determination of inversion site via HMBC and HSQC of 3-70





Chapter 4

Conclusion and perspectives

Bacteria are microscopic, single-cell organisms that live almost everywhere in the world, they are responsible for the deaths of millions of people in human history, the terrible pandemics caused by pathogenic bacteria are well known as bubonic plague, cholera, diphtheria, tetanus, typhus, sepsis, syphilis, tuberculosis and many more. The discovery and development of antibiotics allowed human to treat bacterial infections effectively and have saved many lives. However, despite the undeniable impact antibiotics have had on curing bacterial diseases, antibiotics suffer from a number of drawbacks that must be overcome, particularly the resistance development caused by overuse of antimicrobials. In this situation, vaccines have become attractive alternatives against bacterial infections.

Glycans play major metabolic, structural and physical roles in biological systems. All living cells from humans to virus, plants and bacteria are covered by glycans. In bacteria, the capsular polysaccharides, major components of cell surface, are not only important virulence factors for bacteria to infect the hosts, but also act as pathogenic antigens to induce specific immune responses. Due to their diversity and specificity, capsular polysaccharides become attractive targets for the development of antibacterial vaccines. This dissertation focused on the development of semisynthetic glycoconjugate vaccines that comprise synthetic oligosaccharide antigens derived from capsular polysaccharides and a carrier protein.

Streptococcus suis bacteria are one of the most serious health problems for pigs and an emerging zoonotic agent in humans working in the swine industry. Based on the repeating unit of capsular polysaccharides on *Streptococcus suis* serotype 9, tools of organic chemistry were used to construct a collection of 15 synthetic oligosaccharide antigens. The syntheses tackled challenges associated with complex glycan targets, including the introduction of 1,2-*cis* glycosidic bonds and labile phosphodiesters. A glycan microarray study was then performed, sera collected from pigs infected with *S. suis* serotype 9, immunized rabbits and humans vaccinated against *S. pneumoniae* (007sp WHO reference sera) were screened for antibodies binding to synthetic oligosaccharides and isolated *S. suis* serotype 9 CPS. A phosphorylated trisaccharide showed strong binding to rabbit sera and was identified as an attractive lead for the development of a glycoconjugate vaccine against *S. suis* serotype 9.

The same concept was also applied to the *Acinetobacter baumannii*. *A. baumannii* is a critical pathogen that is responsible for around 10% hospital and community acquired Gram-negative infections. Furthermore, some strains of *A. baumannii* like AB5075 are resistant to most of exiting antibiotics, posing an imminent threat to human health. The CPS of *A. baumannii* AB5075 consists of two linear trisaccharide repeating units bear *N*-acetyl groups on D-mannuronic acid and a (*S*)-3-hydroxybutanoyl group on D-bacillosamine. In this thesis, the synthesis of AB5075 repeating units overcame the challenges associated with the complicated trisaccharide including *β*-mannoside synthesis, introduction of (*S*)-3-hydroxybutanoyl and the incorporation of labile glycosidic bonds. Multiple 1,2-*cis* linkages in the densely functionalized aminoglycoside trisaccharides were constructed using a double-serial inversion strategy. The synthetic oligosaccharides carrying an aminopropyl linker allows for easy access to glycan microarrays and *in vivo* immunological evaluations.

As described in this dissertation, in the syntheses of complex oligosaccharides, although most challenges were overcome, dealing with very fragile group such as ketone on rare sugar is still challenging. Inversion strategy was employed to synthesize *β*-mannosides as there is no effective and general method to provide highly *cis*-selective glycosylation between mannoses. Better understanding of glycosylation mechanism and more novel stereoselective glycosylation strategies are needed.

In summary, a series of conjugation-ready oligosaccharides resembling the capsular polysaccharides of *Streptococcus suis* serotype 9 and *Acinetobacter baumannii* AB5075 were synthesized. The glycan epitope of lead antigens related to *S. suis* serotype 9 was identified. Future conjugation and *in vivo* evaluations will contribute to the development of novel semisynthetic glycoconjugate vaccines against *S. suis* serotype 9 and *A. baumannii* AB5075.

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