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SYNTHESIS OF NATIVE AND FLUORINATED LIPOPHOSPHOGLYCAN CAPPING STRUCTURES OF *LEISHMANIA DONOVANI* AND PREPARATION OF A FULLY SYNTHETIC VACCINE CANDIDATE

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Erklärung

Diese Dissertation wurde im Sinne von § 7 der Promotionsordnung vom 28. November 2011 von Frau Prof. Dr. Anja Hoffmann-Röder betreut.

Eidesstattliche Versicherung

Diese Dissertation wurde eigenständig und ohne unerlaubte Hilfe erarbeitet.

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Freiwillige Einverständniserklärung:

Die Betreuerin dieser Arbeit darf – unter Angabe der Co-Autorenschaft des Verfassers der Doktorarbeit – hieraus Inhalte und Daten zur Veröffentlichung in wissenschaftlichen Zeitschriften und Präsentationen verwenden.

München, ...

Andreas Baumann

-TO MY FAMILY

AND TO ANJA-

„Es ist nicht genug, zu wissen, man muß auch anwenden; es ist nicht genug, zu wollen, man muß auch tun.“

JOHANN WOLFGANG VON GOETHE

ABSTRACT

Owing to their structural uniqueness and dense distribution on the surface of pathogenic organisms, carbohydrate antigens are interesting target compounds for vaccine development. However, a major drawback of glycoconjugate vaccines lies within the reduced metabolic stability of their carbohydrate epitopes in biological systems. This often leads to a limited bioavailability and thus to poor immune responses. In that regard, glycomimetic incorporation has evolved as a powerful tool to overcome this limitation by increasing the biostability of crucial glycotopes *in vivo*. For instance, strategic fluorination of glycans has become a promising approach to meet this challenge, as fluorosugars are thought to sustain the properties of their natural counterparts while enhancing the stability of the glycosidic linkage against e.g. enzymatic degradation. In this work we applied this concept to a well-known leishmanial antigenic structure and developed a synthetic access to diverse fluorinated derivatives of the lipophosphoglycan terminating neutral cap structures of *Leishmania donovani* to set the stage for their future use in immunological studies

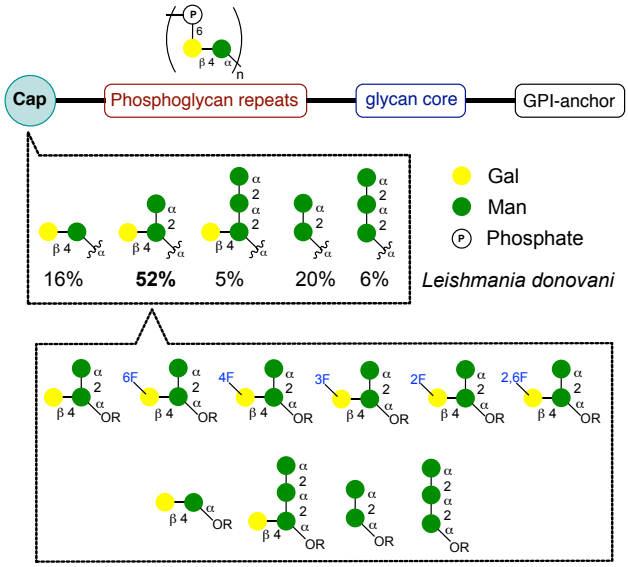

FIGURE A. Depiction of the LPG of Leishmania donovani, the percental distribution of the terminating neutral cap structures (mole%) as well as the synthesized native and fluorinated haptens equipped with an amine-handle for conjugation reactions (R = $-(CH_2)_5-NH_2$).

The species of the protozoan parasite *Leishmania* affect people in more than 88 countries of the world and lead to approximately 70.000 deaths per year. Several synthetic approaches and immunological studies have already targeted structural characteristics of the leishmanial cell surface lipophosphoglycan and confirmed the diagnostic and immunogenic potential of these structures. Inspired by these preliminary works, we decided to focus on the β-Gal-(1→4)-[α-Man-(1→2)]-α-Man trisaccharide epitope derived from the lipophosphoglycan of *Leishmania donovani*. This structure represents the most abundant terminating neutral cap structure and contains essential lipophosphoglycan elements (see FIGURE A), in particular the β-Gal-(1→4)-α-Man motif, which is besides a unique glycosylation pattern among eukaryotic organisms. In that regard, fluorine incorporation was envisaged for the galactose moiety in order to stabilize and maintain this important β-Gal-(1→4)-α-Man glycosidic linkage *in vivo*. Thus, distinct fluorination at the 2-, 3-,[1] 4-, 6- and 2,6-position of the

[1] The 3F-β-Gal-(1→4)-[α-Man-(1→2)]-α-Man trisaccharide was prepared by *Stefan Marchner* in the course of his Master thesis in the *Hoffmann-Röder* research group.

galactose moiety was accomplished and could help to address the question of how fluorine incorporation affects binding affinity and antibody selectivity on a molecular level. Moreover, as the work-flow of rational vaccine design requires comparison of all terminating neutral cap structures in biological evaluations, the syntheses of the α-D-Man-(1→2)-α-D-Man, β-Gal-(1→4)-α-Man, α-Man-(1→2)-α-Man-(1→2)-α-Man and α-Man-(1→2)-α-Man-(1→2)-[β-Gal-(1→4)]-α-Man motifs were also accomplished.

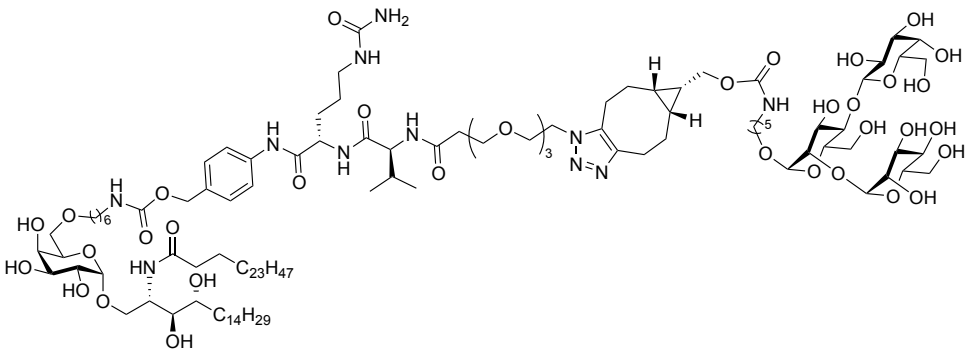

FIGURE B. Synthesized monovalent fully synthetic vaccine candidate comprised of the native trisaccharide, an enzymatically cleavable dipeptide linker and an α-GalCer derivative as T helper epitope.

In a second project, we provided access to a fully synthetic vaccine candidate against *Leishmania donovani* by using a strain-promoted azide alkyne cycloaddition (SPAAC) as the key step. Fully synthetic constructs are of particular interest due to their superior reproducibility and their high structural homogeneity. They usually comprise defined and fully characterized chemical units, whereby each part has to fulfill its specific role during the immunization event. In this regard, we prepared a monovalent fully synthetic vaccine construct containing the native β-Gal-(1→4)-[α-Man-(1→2)]-α-Man terminating neutral cap structure of *Leishmania donovani* as the B cell epitope and an α-GalCer derivative as the T helper-like epitope (see FIGURE B). The latter, a NKT cell glycolipid agonist, has been proven by several research groups to exhibit excellent T helper-like characteristics, including a rapid IgM to IgG class switch, which is highly desirable in vaccine developement. The α-GalCer moiety was linked to the carbohydrate B cell epitope *via* a self-immolative valine-citrulline linker in accordance to literature precedent. This construct enables liberation of the α-GalCer derivative within B cells. Future vaccination studies should help to evaluate the efficacy of covalently-attached NKT cell glycolipid agonists in synthetic vaccines and further confirm the potential of this compound class as effective T helper epitopes.

Additionally, studies toward the preparation of a multivalent fully synthetic vaccine construct containing a Regioselectively Addressable Functionalized Template (RAFT) as the link between B cell epitopes and the α-GalCer derivative were attempted. In that regard, a cyclopeptide scaffold developed by *Dumy and co-workers* was synthesized, which should enable the formation of a glycocluster imitating the dense surface distribution of pathogenic surface glycans. Preliminary synthetic studies targeting the assembly of the three components were executed but require further optimization to allow for isolation of the vaccine constructs in sufficient purity. Nevertheless, this work lies the foundation for the strategic construction of potential multivalent vaccine candidates benefitting from the cluster glycoside effect.

ACKNOWLEDGEMENTS

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ABBREVIATION

[α]	optical rotation
Å	Ångström
AA	amino acid
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
ADC	antibody-drug conjugate
AgOTf	silver triflate
Ala	L-Alanine
Alloc	allyloxycarbonyl
APC	antigen-presenting cell
Ar	undefined aryl substiuent
BCR	B cell receptor
$BF_3{\cdot}Et_2O$	boron trifluoride diethyl etherate
Bn	benzyl
BnBr	benzyl bromide
Boc	*tert*-butyloxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
br	broad (NMR)
Bu_2SnO	dibutyltin oxide
Bz	benzoyl
BzCl	benzoyl chloride
c	concentration
°C	degree Celsius
calc.	calculated
Cat	cathepsin
Cbz	carboxybenzyl
CD	Cluster of Differentiation
^{c}Hex	cyclohexane
Cit	L-Citrulline
CL	cutaneous leishmaniasis
Con A	Concanavalin A
COSY	^{1}H correlation spectroscopy (NMR)
CPS	capsular polysaccharide
CuAAC	copper(I)-catalyzed azide-alkyne cycloaddition
δ	chemical shift (NMR)
Δ	heating (under reflux)
d	day(s), doublet (NMR)
Da	Dalton
DAST	diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DC	dendritic cell
DCC	*N*,*N*'-dicyclohexycybodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DT	diphteria toxoid
ECF	ethyl chloroformate
EEDQ	*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
ESI	electronspray ionisation (HRMS)
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethylacetate
EtOH	ethanol
FA	formic acid
FCA	Freund's Complete Adjuvant
Fmoc	fluorenylmethyloxycarbonyl
FmocCl	fluorenylmethyloxycarbonyl chloride
g	gram(s)
Gal	D-Galactose
Gly	L-Glycine
GIPL	glycoinositolphospholipid
h	hour(s)
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium-hexafluorphosphat
HCV	hepatitis C virus
Hib	Haemophilus influenza B
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond connectivity (NMR)
HOAt	1-Hydroxy-7-azabenzotriazole
HOSu	*N*-hydroxysuccinimide
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence (NMR)
Hz	Hertz (frequency)
IBCF	isobutyl chloroformate
Ig	immunoglobulin
IL	interleukin
iNKT cell	invariant natural killer T cell
J	coupling constant (NMR)
KLH	keyhole limpet haemocyanin
l	wave lenght unit
LG	leaving group
LPG	lipophosphoglycan
Lys	L-Lysine
M	molar
m	meter(s), multiplet (NMR)
Man	D-Mannose
MCL	muco-cutaneous leishmaniasis
Me	methyl
MeCN	acetonitrile
MenC	meningococcal group C

MeOH	methanol
MHC	Major Histocompatibility Complex
min	minute(s)
mL	milliliter(s)
mmol	millimole(s)
MRSA	meticillin-resistant *Staphylococcus aureus*
MS	molecular sieves, mass spectrometry
NaOAc	sodium acetate
NaOMe	sodium methoxide
NEt_3	triethylamine
NIS	*N*-iodosuccinimide
NK cell	natural killer cell
NLR	Nod-like receptor
NMR	nuclear magnetic resonance
Nu	undefined nucleophile
OTf	triflate
OTos	tosylate
p	para
p-ABOH	4-Aminobenzyl alcohol
PAMP	pathogen-associated molecular pattern
PEG	polyethylenglycol
PG	protecting group
Ph	phenyl
PivCl	pivaloyl chloride
PKDL	post-kala-azar dermal leishmaniasis
PNA	peanut agglutinin
PPG	proteophosphoglycan
ppm	parts per million (NMR)
Pro	L-Proline
PRR	pattern-recognition receptor
PSA	polysaccharide A of *Bacteroides fragilis*
PTFAI	*N*-phenyltrifluoroacetimidate
p-TsOH	*para*-toluenesulfonic acid
PyBop	benzotriazol-1-yl-oxytripyrrolidino-phosphonium hexafluorophosphate
q	quartet (NMR)
R	undefined substituent
R_f	retention factor (TLC)
rt	room temperature
RV	reverse vaccinology
s	singlet (NMR)
sAP	secreted acid phosphatase
Sp1	pneumococcal Serotype 1 polysaccharide of *Streptococcus pneumoniae*
SPAAC	strain-promoted azide alkyne cycloaddition
SPPS	solid-phase peptide synthesis
t	triplet (NMR)
TBAB	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBS	tert-butyldimethylsilyl
TBSOTf	*tert*-butyldimethylsilyl triflate
TCA	trichloroacetonitrile
TCR	T cell receptor
T_D	thymus dependent
TetC	tetracycline-resistance protein
TFA	trifluoroacetic acid
Tf_2O	triflic anhydride
TfOH	triflic acid
THF	tetrahydrofuran
T_I	thymus independent
TLC	thin-layer chromatography
TLR	Toll-like receptor
TMSOTf	trimethylsilyl triflate
t_R	retention time (HPLC)
Trt	trityl
TrtCl	trityl chloride
Val	L-Valine
VL	visceral leishmaniasis
VRE	vancomycin-resistant enterococci
W	Watt(s)
WHO	World Health Organistion
wt%	weight percent
ZPS	zwitterionic polysaccharide

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1 INTRODUCTION

The immune system is a defense mechanism protecting the body against pathogens such as bacteria, viruses, fungi, protozoa and helminths. It is also responsible for eliminating redundant and dead endogenous cells as well as for combating malignant cancer cells. Nowadays, an efficient support is provided to the immune system by active immunizations, which stimulate specific immune responses and therefore induce preventive and protective immunity to disease-causing pathogens or tumors.[1]

Since *Edward Jenner's* first active immunization against smallpox in 1796, such vaccinations have essentially contributed to the control and eradication of many infectious diseases.[2] For instance, vaccination has led to the extermination of smallpox, the almost entirely elimination of poliomyelitis, and a decrease in the occurance of diphtheria, tetanus, measles and mumps by more than 95%.[3] However, despite these great achievements in the fight against infectious diseases, there is still a need for new vaccines against pathogens, which yet remain a principal cause of death or newly emerge as a serious threat to general health care due to arising antibiotic resistances.[4] For example, every year millions of people still die of acute respiratory infections, diarrhoeal disease or from tuberculosis.[5] Besides, multidrug-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) pose a substantial danger for future society and require the development of novel and effective vaccines.[6]

However, traditional vaccine formulations derived from killed or attenuated pathogens or components thereof exhibit diverse limitations and do no longer correspond to the state of art.[7] For instance, these approaches cannot *„[...] be used to develop vaccines against microbes that do not grow in vitro [...]"*[2] and *„[...] against pathogens with antigenic hypervariability (e.g., serogroup B meningococcus, HIV, HCV) or against pathogens with an intracellular phase, causing infections that are predominantly controlled by T cells, such as tubercolosis and malaria [...]."*[2] Therefore, new technologies have been developed over the last few decades to overcome these drawbacks. New genome sequencing methods have enabled reverse vaccinology (RV) as a promising tool for identifying and creating synthetic protein vaccine candidates from scratch.[2, 8] Recombinant DNA or synthetic oligonucleotides and peptides, which represent pathogen specific structures, are becoming increasingly important for vaccine formulation.[9] Furthermore, the synthetic progress in carbohydrate chemistry has made the densly packed and often unique surface glycan structures on pathogens and malignant cells an attractive target for vaccine development.[10]

Although it is well-known since 1923 that pneumococcal antigens targeted by the immune system are surface polysaccharides,[11] it took another sixty years until the first polysaccharide vaccine PneumoVax23 (Merck) entered the market in 1983.[10] The current version of this vaccine contains unconjugated capsular polysaccharides (CPS) from 23 out of 90 known serotypes isolated from *Streptococcus pneumoniae*.[12] However, such polysaccharide-based vaccines are poorly immunogenic and induce a short-lasting T cell-independent immune response without triggering a B cell-mediated immunological memory.[7, 13] Already in 1931 *Avery* and *Goebel* suggested that the immunogenicity of polysaccharides can be effectively increased by conjugation to an immunogenic carrier protein.[14] Such glycoconjugate vaccines are able to elicit an immunological memory specifically directed against the carbohydrate antigen, due to the T cell-dependent immune response generated by the immunogenic T helper epitope.[10] For example, the introduction of a Hib

glycoconjugate vaccine[15] and a meningococcal group C (MenC) glycoconjugate vaccine[16] in the United Kingdom between 1992 and 1999 led to a fast and effective reduction of the diseases in all age groups.[7] Yet, the polysaccharides used for these glycoconjugate vaccines are not readily available, as most of the formulations are prepared from CPS extracted from bacterial cultures.[10, 17] This process includes several purification steps and a constant quality control, as the isolated polysaccharides exhibit structural heterogeneity due to their nontemplate-driven biosynthesis and are often co-isolated with toxic impurities, such as cell wall polysaccharides.[17] Furthermore, the conjugation of polysaccharides to huge carrier proteins, like keyhole limpet haemocyanin (KLH) or diphtheria toxoid (DT), is accompanied by a certain amount of inaccuracy, with deviations in composition and structure of the resulting conjugates.[10, 18, 19] Therefore, the production of fully synthetic glycoconjugate vaccines with defined structural elements is getting more and more into the focus of academia and industry.[17] However, in order to be able to rationally design a defined and fully synthetic carbohydrate vaccine, a profound knowledge of the immune response to carbohydrates and T helper epitopes is necessary.

1.1 CARBOHYDRATES AND IMMUNOLOGY

Carbohydrates occur in all living cells and their importance is constituted by their widespread application in nature. They appear as mono-, oligo- and polysaccharides and are usually part of biopolymers or other natural products.[20] For instance, carbohydrates are present in glycolipids, lipopolysaccharides, glycoproteins, peptido- and proteoglycans, which in large parts build up the cell's glycocalyx.[10, 20] Therefore, carbohydrates not only play a significant role as the most important energy storage material, but also take part in many cellular processes, such as cell recognition, differentiation, cellular transport and adhesion.[10] With regard to the latter, pathogens use their densely packed cell surface glycans for attachment and invasion. However, as these structures are highly exposed on the cell surface, they also serve as first contact points for the immune system.[1] This makes the individual glycan structures on pathogens and malignant cells attractive targets for the development of vaccine candidates. A comprehensive understanding of glycoimmunology and the immune system helps to identify guidelines for the design of effective carbohydrate-based vaccine candidates against various infectious diseases.[6]

1.1.1 The Immune System

For the protection against foreign invaders and internal threats, the immune system provides beyond several external protective mechanisms two distinct types of immune response: the innate (non-adaptive) immune response and the adaptive (antigen-specific) immune response.[10] Effective external barriers are, for example, the skin, the mucosa, reflexes such as coughing or sneezing, as well as commensal microorganisms that compete with pathogens for nutrition.[21] In addition to this, the innate immune response reacts quickly and unspecifically within a few minutes upon infection and thus represents the host's first line of immune defence. It is mediated by granulocytes and by antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, whose main task is to recognize pathogen-associated molecular patterns (PAMPs) using their numerous pattern-recognition receptors (PRRs).[10, 22] These PRRs, including the Toll-like receptor family

(TLRs),[23] are able to detect a multitude of structurally diverse but highly conserved pathogenic structures (see FIGURE 1.1).[6] Besides, members of the Nod-like receptor family (NLRs), which in contrast to TLRs are soluble proteins, screen the cytosol for foreign invaders.[6] PRR stimulation on APCs leads to initiation of the pro-inflammatory machinery, including the production of soluble cytokines and chemokines.[10, 22] Another important contributors to the innate immune system are natural killer cells (NK cells), which eliminate malignant cancer cells or viral infected cells by cytotoxic mechanisms.[21, 24] The cells of the innate immune system are additionally supported by the complement system. The complement system consists of a large number of resting proteins in the blood plasma and in the extracellular fluid, which are activated *via* signal cascades upon contact with pathogens.[21] For instance, these proteins self-assemble into complexes on pathogenic cell surfaces to form pore structures at the membranes, which ultimately lead to lysis of the infected cell.[25]

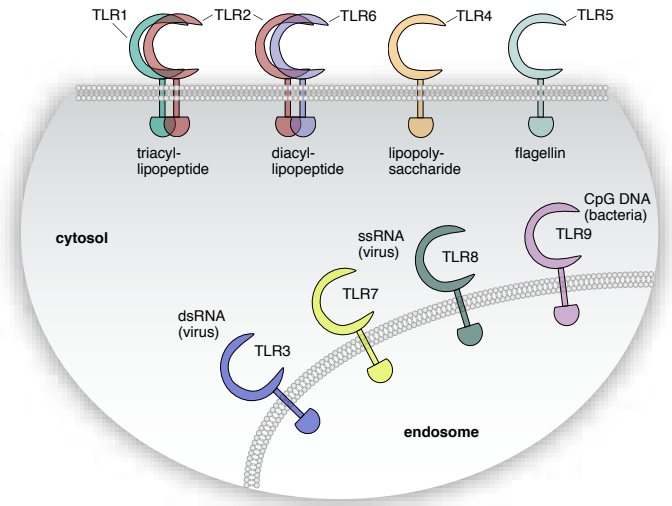


FIGURE 1.1. Cellular localization of Toll-like receptors and description of their ligands. (Adapted from ref.[6, 21, 23])

In contrast, the adaptive immune response becomes effective after days to weeks and provides an antigen-specific and long-lasting immunity.[10] It operates *via* T and B lymphocytes, as well as immunoglobulins (Ig), which are secreted by B cells. The lymphocytes are capable of recognizing very low concentrations of almost each foreign antigen *via* specific receptor sites, such as the B cell receptor (BCR) or the T cell receptor (TCR).[21] In addition, these cells are able to mediate a long-lasting B cell memory, which provides immunity to future infections after successful recovery.[10] The adaptive immune system thus plays a decisive role in the final elimination of a pathogen. Thereby, DCs act as the most important connection between the innate and the adaptive immune response. They not only inform the cells of the adaptive immune system about foreign invaders, but also give detailed information about their location, type and strength.

1.1.2 The Immune Response

Co-stimulatory molecules, which are secreted upon binding of PAMPs to PRRs, activate DCs and lead to a maturation process. Fully mature DCs are able to take up antigens *via* endocytotic pathways and process them through a series of catalytic steps in order to provide small antigen fragments.[26] These antigen fragments, in most cases small peptides, can be presented on the cell surface by forming complexes with the major

histocompatibility complex (MHC). Cytosolic or nuclear antigen fragments are presented *via* MHC class I (MHCI) and lysosomal antigen fragments are presented *via* MHC class II (MHCII) (see FIGURE 1.2).[21] Closed ends of the MHCI binding groove allows presentation of smaller peptides of 8–9 amino acids in length, whereas the open ends of the MHCII binding groove allow for the complexation of larger peptides (> 10 amino acids).[27, 28]

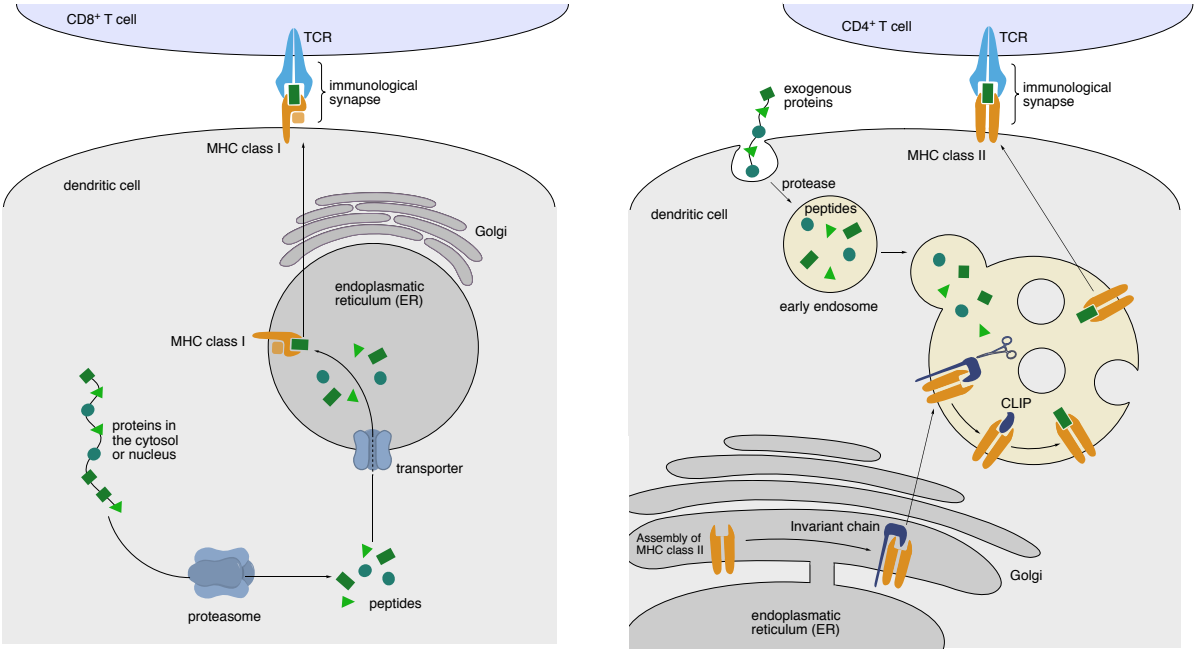


FIGURE 1.2. The basic MHCI antigen presentation pathway is schematically depicted on the left side. The basic MHCII antigen presentation pathway is schematically depicted on the right side. Thereby, the MHCII molecule is assembled in the endoplasmatic reticulum (ER) and the binding groove is blocked by an invariant chain (Ii). In the endosome, the Ii becomes degraded and only the class II-associated Ii peptide (CLIP) remains in the binding groove until it is replaced by an antigenic peptide. (Adapted from ref.[28])

After migration to the draining lymph nodes the antigen-presenting DCs stimulate naive T cells, which are specific for the displayed MHC:peptide complex and thus trigger and amplify the adaptive immune response.[10] Key feature of T cell activation is the nature of the immunological synapse, which consists of a ternary complex formed by the processed antigen, the TCR and the MHC.[10]

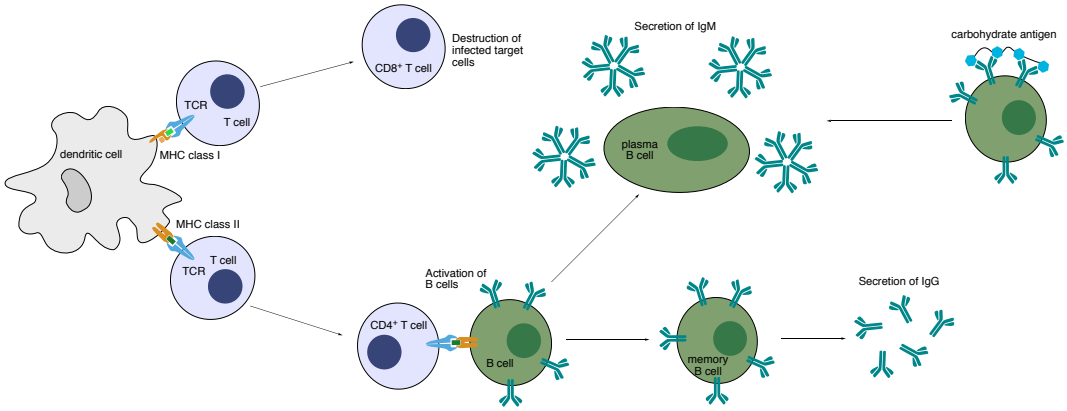


FIGURE 1.3. Schematic depiction of the adaptive immune response and direct activation of B cells by carbohydrate antigens without T cell help. (Adapted from ref.[10])

Antigen presentation *via* MHCI (e.g. viral antigens) induces activation of cytotoxic T lymphocytes ($CD8^+$ T cells, MHCI), which destroy infected target cells directly. In contrast, antigen presentation *via* MHCII induces the activation and proliferation of T helper cells ($CD4^+$ T cells, MHCII), which in turn activate B cells and promote their proliferation, affinity maturation, antibody isotype switching and differentiation into plasma cells (produce rapidly large amounts of low-affinity IgM antibodies) or memory cells (see FIGURE 1.3).[26] The latter remain in the body for a long time and are always ready to secrete high-affinity IgG antibodies to evoke a fast immune response against already known antigens.[10]

In contrast to thymus-dependent (T_D) antigens, such as peptides and proteins, which are able to induce T cell activation through MHCII-restricted pathways, thymus-independent (T_I) antigens stimulate the production of antibodies without MHCII-restricted T cell help.[29] Carbohydrates, with a few recently discovered exceptions (see below), belong to T_I antigens. They are ususally poorly immunogenic and consequently do not elicit a strong and long-lasting immune response. The mostly polymeric structure of carbohydrate antigens allows binding to several BCRs simultaneously, which in turn leads to a direct activation of B cells without T cell help (see FIGURE 1.3).[10] As a consequence these activated B cells cannot undergo affinity maturation, isotype switching or differentiation into memory B cells, and predominantly produce low-affinity IgM antibodies and a short-lived immune response.[10] However, upon covalent coupling of a T_I carbohydrate antigen to a T_D immunogenic carrier protein, it is possible to elicit a T cell-mediated activation of carbohydrate specific B cells.[30] Therefore, it is supposed, that glycoconjugates are recognized by BCRs specific to the carbohydrate antigen and are subsequently endocytosed and processed. Processed fragments of the T_D antigen are then presented *via* MHCII of the carbohydrate specific B cell. The formation of an immunological synapse with a peptide specific T cell activates the carbohydrate specific B cell to finally elicit a T_D immune response against a T_I carbohydrate antigen (see FIGURE 1.4).

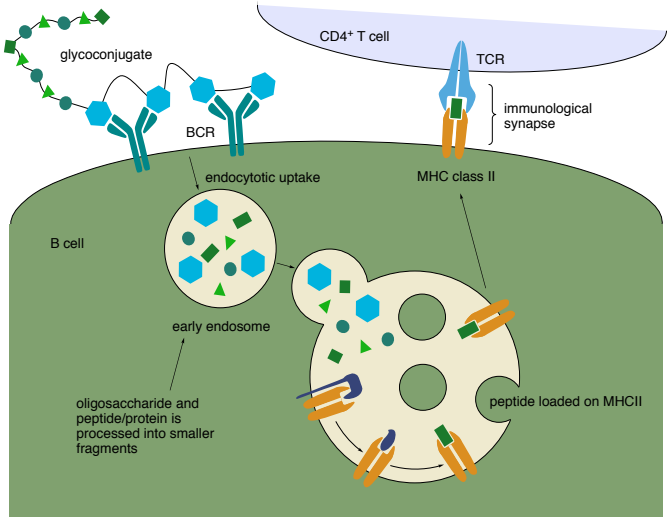


FIGURE 1.4. Schematic depiction of the activation of T cells by a glycoconjugate vaccine. (Adapted from ref.[26])

More recently, a new mechanism for the activation of the adaptive immune system by glycoconjugates was suggested. Here, it is assumed that glycoconjugates are converted by endolysosomal digestion into smaller glycopeptides (approximately 10 kDa) and are subsequently presented on MHCII molecules to TCRs of $CD4^+$ T cells.[31] Several studies were able to show, that synthetic *„[...] glycopeptides or naturally glycosylated immunogenic glycopeptides [...] can bind to MHC molecules and that glycan portions specifically influence T*

cell recognition of the peptides they are bound to."[27] Besides glycopeptides, a few other glycoantigens are also able to induce a T cell dependent immune response. For instance, the adaptive immune system can be stimulated and activated by zwitterionic polysaccharides (ZPS).[7] These are polysaccharides consisting of alternating positively and negatively charged monosaccharides (see FIGURE 1.5). They occur, for example, in the pneumococcal Serotype 1 polysaccharide (Sp1) of *Streptococcus pneumoniae* and in polysaccharide A of *Bacteroides fragilis* (PSA). After ZPSs are processed into smaller fragments, they can bind to MHCII to induce T cell activation for a robust carbohydrate specific immune response.[27, 32]

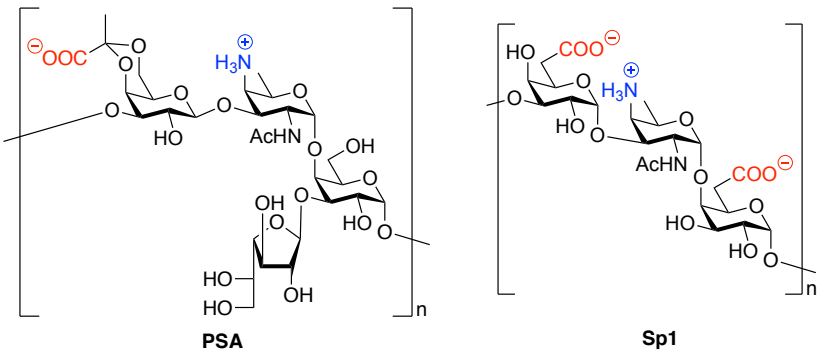


FIGURE 1.5. Chemical structures of zwitterionic PSA and Sp1.[7]

Furthermore, several glycolipids are able to activate invariant natural killer T cells (iNKT).[33] Such glycolipids, like the synthetic α-galactosylceramide[34] (α-GalCer or αGC, also known as KRN7000) (see FIGURE 1.6), are presented on the surface of APCs by the non-polymorphic MHCI-like molecule CD1d. This complex subsequently forms an immunological synapse with the TCR of an iNKT cell. The stimulation of the iNKT cells leads to fast T_H1- and T_H2-type cytokine productions, including IFNγ and IL-4, and an increased expression level of CD40 ligand (CD40L; induces DC maturation).[35, 36] Therefore, this CD1d-restricted T cell subset is capable of modulating DC and B cell activity and can increase DC-induced B and T cell responses.[37] It is important to note, that the iNKT TCRs bind to CD1d:glycosylceramide complexes with higher affinities and longer half-lives than the corresponding TCRs of $CD8^+$ and $CD4^+$ T cells to MHCI/II:peptide complexes.[27] Co-crystallization *„[...] of glycosylceramides with CD1d molecules demonstrated that the aliphatic lipid tails fit into the CD1d binding groove, whereas its monosaccharide head group extends above the surface of the lipid-binding groove and thereby is exposed for recognition by the TCR of iNKT cells.*"[27, 36] Further aspects of iNKT cell activation and the use of glycosylceramides as T helper epitopes for carbohydrate-based vaccines will be discussed later (see CHAPTER 1.2.3).

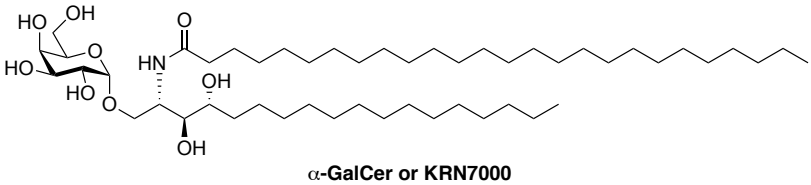


FIGURE 1.6. Synthetic iNKT cell agonist α-Galactosylceramide, which can also be isolated from the marine sponge *Agelas mauritianus*.[37]

1.2 DESIGN KIT FOR FULLY SYNTHETIC GLYCOCONJUGATE VACCINES

Traditional glycoconjugate vaccine formulations are usually composed of a mixture of diverse molecules. They include naturally extracted glycans conjugated to a carrier protein, as well as co-administered adjuvants such as Freund's Complete Adjuvant (FCA), which is a solution of heat inactivated mycobacteria emulsified in mineral oil.[38] All these elements make carbohydrate-based vaccines highly heterogeneous and impair the reproducibility of the immune response due to batch to batch variability.[39] Even semi-synthetic glycoconjugate vaccines, which at least contain synthetic carbohydrate haptens, still suffer from these disadvantages. In contrast, fully synthetic glycoconjugate vaccines consist of well-defined chemical units, which can be precisely assembled and allow for a distinct characterization of the molecule with standard analytical methods.[40] Within such a designed construct, each part has to play a specific role during the immunization process. The synthetic carbohydrate antigen should represent the minimal protective epitope derived from the CPS of interest. Moreover, it is preferentially conjugated to a non-immunogenic multivalent scaffold in order to provide a glycocluster imitating the dense distribution of pathogenic surface glycans (see FIGURE 1.7).[39]

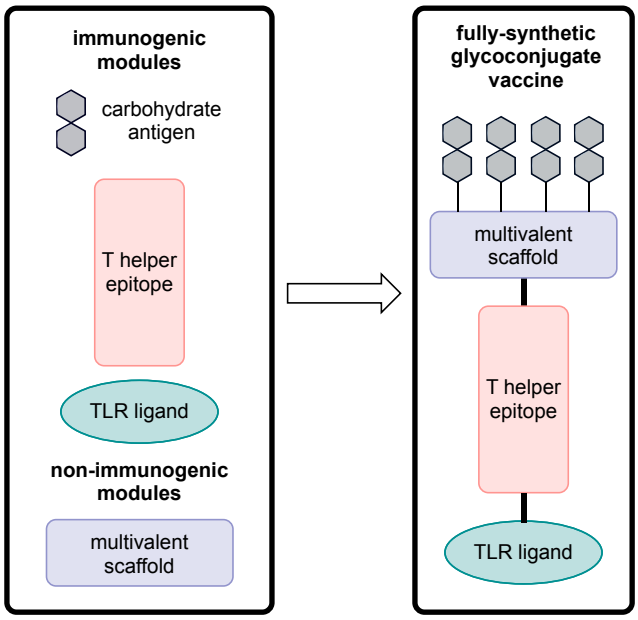


FIGURE 1.7. Building blocks for a fully synthetic glycoconjugate vaccine.

Furthermore, a T helper epitope, such as a defined immunogenic peptide, oligosaccharide or glycolipid is often covalently attached to the antigenic module. Several approaches also include an integrated TLR ligand in order to stimulate DC maturation as well as cytokine production, which allows to omit co-administering of adjuvants.[39] Additionally, the synthetic approach allows for the incorporation of carbohydrate mimetics into the antigenic determinant. This has become a promising tool to render the glycotope metabolically more stable as well as to increase the „non-self"-character and immunogenicity of the glycoconjugate.

1.2.1 Synthetic Oligosaccharide Antigens

Synthetic carbohydrate epitopes, which are structurally well-defined and free of impurities, can be used as attractive alternatives to isolated polysaccharides for the production of glycoconjugate vaccines. For instance, in 2004, *Verez-Bencomo et al.* described large-scale synthesis, pharmaceutical development, clinical evaluation and introduction of a first semi-synthetic oligosaccharide vaccine against *Haemophilus influenzae* type b in humans.[41] During the development process of such a synthetic glycoconjugate vaccine, identification of a suitable carbohydrate epitope that is able to induce protective immunity constitutes a main challenge. Over the last decades, significant efforts have been focused on the investigation of minimum epitope sizes that can provide the same immunological effects as isolated polysaccharides by eliciting functional antibodies. Already in 1960, *Elvin Kabat* showed with antidextran sera that most antibody binding sites were fitting to either a hexasaccharide or smaller determinants.[42, 43] Subsequent studies on pathogenic glycotopes have revealed minimal functional epitopes even as short as tetra-[44] or disaccharides,[43, 45] and thus much smaller in size than native CPSs. However, identifying these motifs is a time-consuming procedure and requires large amounts of synthetic oligosaccharides as *„Antigen design has traditionally been an iterative process: synthetic targets are chosen based on the chemical structure of repeating units and after conjugation to a carrier protein, evaluated in immunization experiments in animals. If the resulting antibody response does not target the pathogen, different antigenic constructs will have to be synthesized.“*[17] Nowadays, there are several novel tools, which allow for a much faster elucidation of the minimal protective epitope. In particular, the application of glycan microarrays, which require only small amounts of glycans, has made it possible to screen extensive libaries of synthetic glycotopes with sera from infected patients (see FIGURE 1.8).[46]

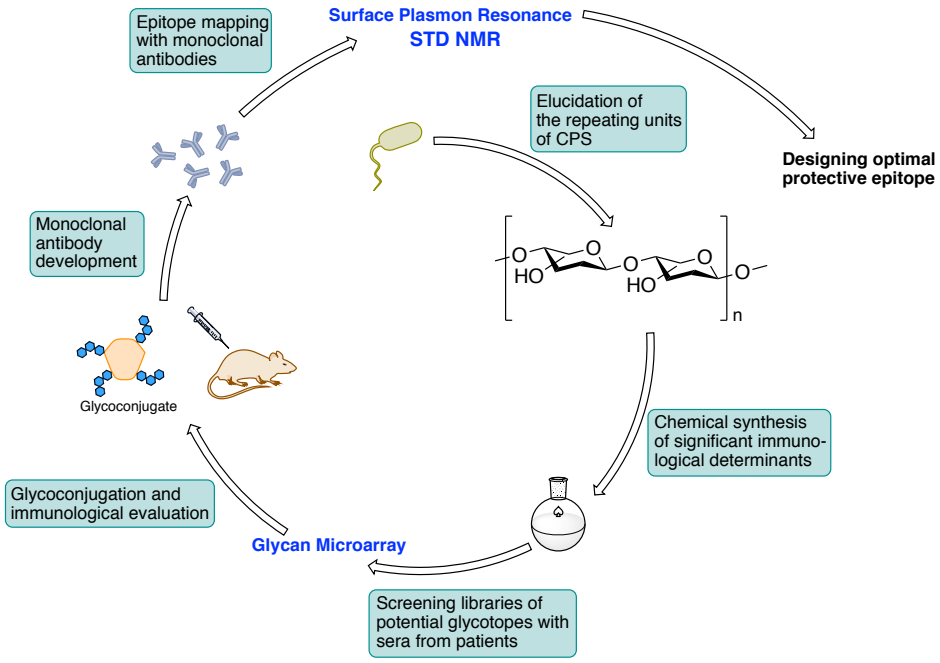


FIGURE 1.8. Work-flow of rational vaccine design process. (Adapted from ref.[17])

For instance, using this technique, all possible immunogenic determinants including various chain lengths, branching residues and side chain functionalities can be taken into account in a single experiment.[17] Thus, the outcome of glycan microarrys can rapidly reveal promising synthetic minimal glycotopes capable of eliciting highly specific antibodies against the native CPS. This approach is of particular interest for the development of synthetic glycoconjugate vaccines against pathogens like protozoan parasites, which are difficult to be cultivated *in vitro*.[17, 47] Furthermore, new methodologies such as surface plasmon resonance (SPR)[48] and saturation transfer difference (STD) NMR[49] have made it possible to further investigate the interplay between carbohydrates and antibodies by defining the recognition elements.[17, 50] If used together, these complementary techniques can lead to the design of an optimal protective epitope for the construction of a glycoconjugate vaccine candidate (see FIGURE 1.8). The success of this approach has already been proven by the development of several different vaccine candidates, as for example against *Clostridium difficile*[45, 51] or *Streptococcus pneumoniae* type 8.[52]

However, despite the efficient progress in synthetic oligosaccharide chemistry, e.g. by enzymatic formation of oligosaccharides,[53, 54] programmable one-pot glycosylation techniques,[55, 56] or recent improvements in stereoselective *O*-glycosylation[57] and automated solid-phase oligosaccharide synthesis (SPOS),[58, 59] glycan assembly still remains a difficult task and a limiting factor in vaccine development. In particular, stereoselective formation of key glycosidic bonds and incorporation of rare sugars are here main challenges. A glycosylation reaction between an electrophilic glycosyl donor and a nucleophilic hydroxyl group of the glycosyl acceptor generally provides two major types of *O*-glycosides: the 1,2-*cis* (α-glycoside) and the 1,2-*trans* (β-glycoside) glycoside (see FIGURE 1.9).

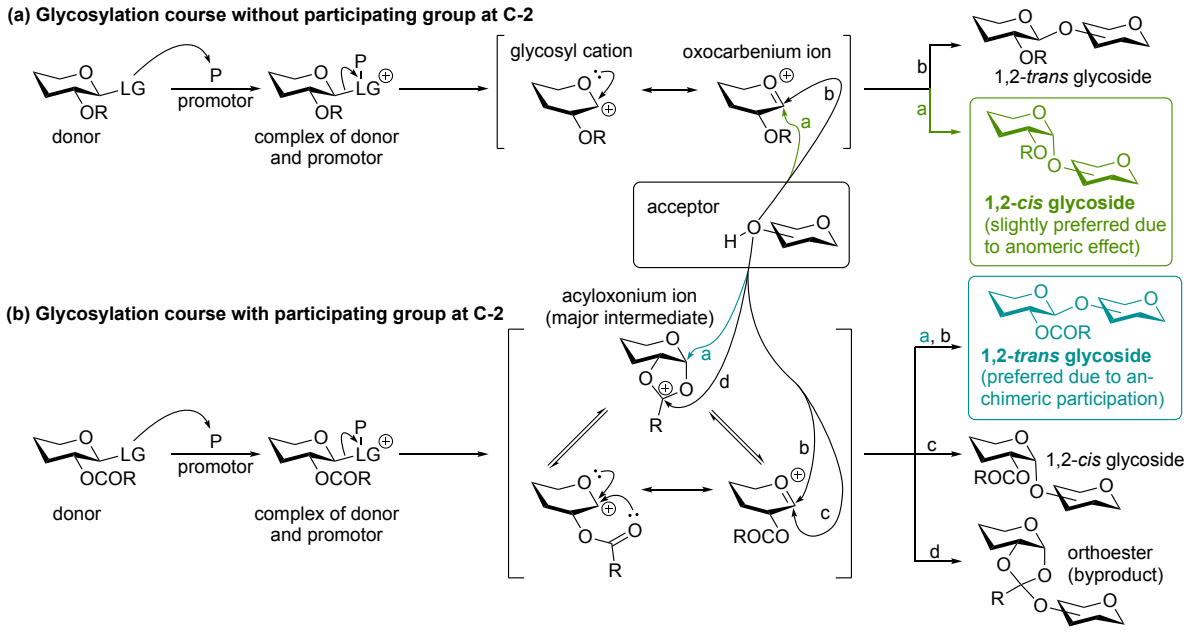


FIGURE 1.9. Glycosylation without (a) and with (b) a participating group at C-2. (Adapted from ref.[57])

Whereas formation of 1,2-*trans* glycosides can be usually obtained by exploiting the participatory effect of a neighboring group at C-2 (see FIGURE 1.9),[60, 61] formation of the corresponding 1,2-*trans* glycosides in the absence of participating neighboring groups or formation of 1,2-*cis* glycosidic linkages have been proven to be much more demanding for chemists.[57] In addition, the complexity of glycosylation reactions can be

visualized by the plethora of by-products that might occur. Side reactions, such as eliminations, migrations, cyclization and especially donor hydrolysis and rearrangement make each glycosylation a highly complex reaction that requires individual optimization.[62] Nevertheless, a large number of synthetic tools is available for influencing the stereochemical outcome and yield of glycosylation reactions including *inter alia* the choice of the protecting group pattern (neighboring, long range or steric effects), promotor, type of donor and acceptor, temperature, solvent, concentration, pressure and other additives as well as the sequence of addition (see FIGURE 1.10).[57, 62]

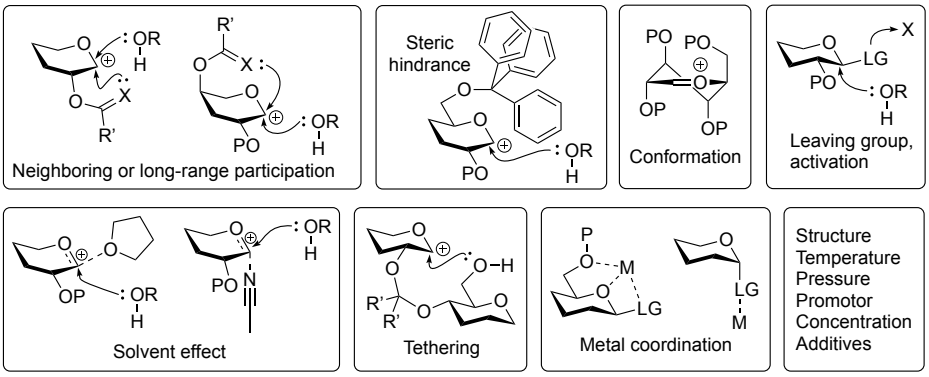


FIGURE 1.10. Methods affecting the stereochemical outcome of glycosylations. (Adapted from ref.[57])

Furthermore, the „armed-disarmed“ concept for glycosylating agents has made the synthesis of oligosaccharides more predictable and enables chemists to design chemoselective building blocks for defined reaction conditions.[55] This concept, which was first reported by *Fraser-Reid and co-workers*,[63] characterizes the relative reactivities of glycosyl donors and acceptors influenced by their protecting group pattern. In this regard, more reactive species, which predominantly bear electron donating protecting groups, such as benzyl ethers, are defined as „armed“ donors/acceptors, whereas less reactive species, which mainly bear electron withdrawing protecting groups, such as acyl esters, are defined as „disarmed“ donors/acceptors. However, not only the character of the protecting groups influences the relative reactivity, but also their positions.[64]

Thus, many factors have to be taken into account and each glycosylation reaction requires fine-tuning of the exact experimental parameters, which often hampers generation of extensive libraries of complex oligosaccharide epitopes. Therefore, providing different and diverse antigenic carbohydrate determinants represents the major obstacle towards the selection of a suitable structure for the development of a promising vaccine candidate.

1.2.2 Multivalency

Proteins usually bind carbohydrate ligands with high specificity but weak affinity.[65] For instance, the dissociation constants for protein-carbohydrate interactions of single monosaccharides or oligosaccharides lie in the mM–μM range. However, nature compensates this low affinity binding by presenting carbohydrates as clusters on the cell surface,[66] which leads to a shift of the dissociation constant into the nM range due to multiple simultaneous binding events.[39] This so called multivalency or „cluster glycoside effect“[67] has also been used beneficially for the preparation of glycoconjugate vaccines, which has recently been summarized in several reviews.[39, 40, 65, 68] In this connection, the multivalent presentation of carbohydrate antigens not only increases the affinity to a complementary BCR, but also promotes BCR clustering, which is essential for receptor-mediated internalization and downstream T cell signaling.[69]

The glyco cluster effect can be induced by usage of various multivalent scaffolds, as for example carbohydrates, linear peptides, cyclopeptides and dendrimers, just to name a few.[68, 70] In the following some selected examples are described in order to illustrate the applicability of this approach in vaccine development.

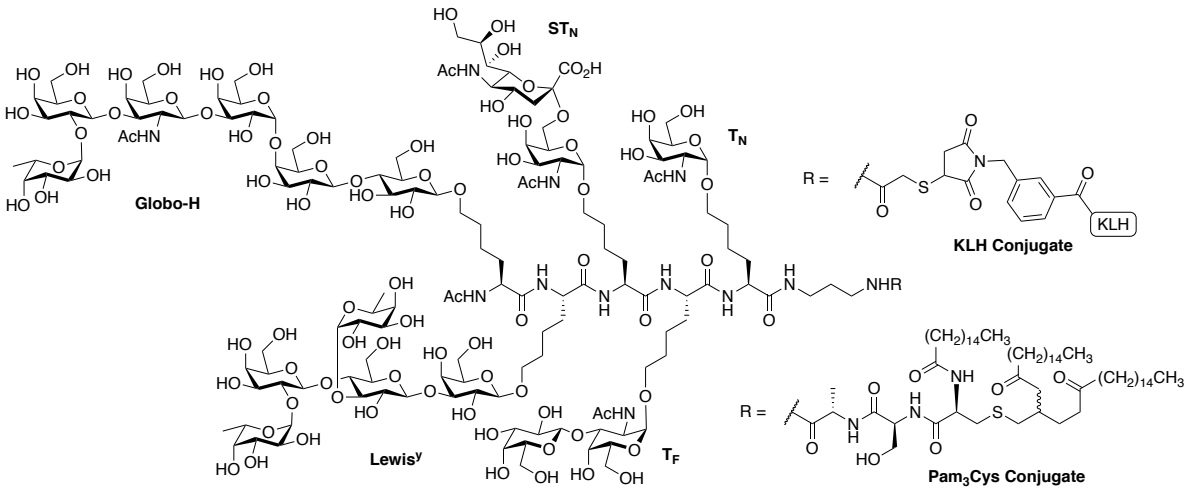


FIGURE 1.11. Synthetic unimolecular pentavalent anti-tumor vaccine candidate by *Danishefsky and co-workers*.[71]

For instance, this concept also enables the vaccination against different antigens at the same time due to a simultaneous presentation of numerous different carbohydrate haptens in one molecule. This could be of particular interest for the preparation of tumor vaccines containing tumor-associated carbohydrate antigens (TACAs), as these overexpressed aberrant glycosylation patterns, which are tumor progession markers,[72] exhibit a high degree of heterogeneity.[73] *Danishefsky and co-workers*[71] reported on a unimolecular pentavalent and hexavalent carbohydrate vaccine targeting prostate and breast cancer simultaneously (see FIGURE 1.11). For example, the pentavalent construct contained five different TACAs, namely the T_N-, ST_N, T_F-, Globo-H and Lewis^y-antigen (see FIGURE 1.11), bound to a linear peptide backbone, which was either conjugated to KLH or the TLR2-ligand Pam_3Cys. These vaccines were reported to be significantly more immunogenic than the monomeric KLH conjugates.[71] However, *Slovin et al.*[74] showed in recent clinical studies that immunization with a hexavalent vaccine including GM2, Globo H, Lewis^y, glycosylated MUC1-32mer, T_N- and T_F, elicited lower antibody titers than those obtained from individual monovalent vaccines.

Therefore, the development of multivalent anti-cancer vaccines bearing different antigens still remains a matter of debate.

An illustrating example for a multivalent carbohydrate-based scaffold was given by *Brimble and co-workers*[75] who described the synthesis of a neoglycoconjugate vaccine candidate comprised of the membrane-bound tumor-associated MUC1 glycoprotein[76] and a central triazole-linked carbohydrate scaffold bearing e.g. three copies of the T_N-antigen (see FIGURE 1.12).

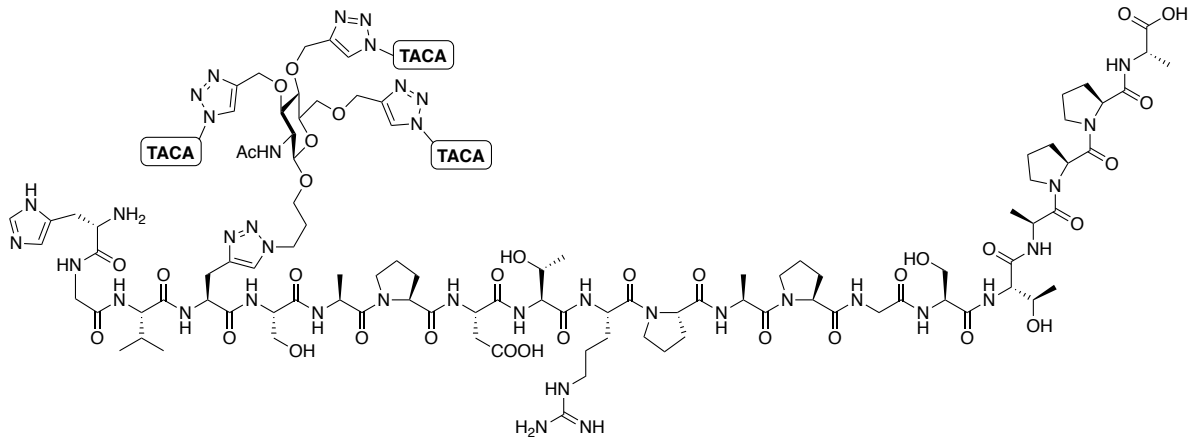


FIGURE 1.12. Synthetic multivalent neoglycoconjugate prepared by *Brimble and co-workers*.[75]

Dumy and co-workers[77, 78], *Danishefsky and co-workers*[79] and *BenMohamed and co-workers*[80] also used cyclopeptide scaffolds for the multivalent presentation of TACAs.[40] These so-called Regioselectively Addressable Functionalized Templates (RAFTs), which were first described by *Mutter and co-workers*,[81] are usually *„[...] composed of an antiparallel β-sheet constrained by two L-proline-glycine β-turns and stabilized into a locked and rigid conformation by intramolecular hydrogen bonds. Multivalent anchoring sites for carbohydrate binding units and other moieties are provided by lysine side chains that are oriented on both sides of the platform, thus preventing undesired hindrances between the assembled elements.“*[65] These non-immunogenic[77] RAFTs were also applied by *Danishefsky and co-workers*[82] and *Wang and co-workers*[83] for the preparation of multivalent carbohydrate-based anti-HIV vaccines.[40] For example, the *Wang* group assembled four α-Man_4 oligosaccharides derived from the $Man_9GlcNAc_2$ epitope, which is expressed on the HIV gp120 glycoprotein, together with T helper epitopes on a cyclic decapeptide. In addition, they synthesized a corresponding fluorinated construct (see FIGURE 1.13) and demonstrated significant affinity of these multivalent scaffolds to human HIV-neutralizing antibody 2G12 in SPR experiments.[83] For a more detailed overview, *Pifferi et al.* recently summarized the use of cyclopeptide scaffolds in carbohydrate-based vaccine development.[40]

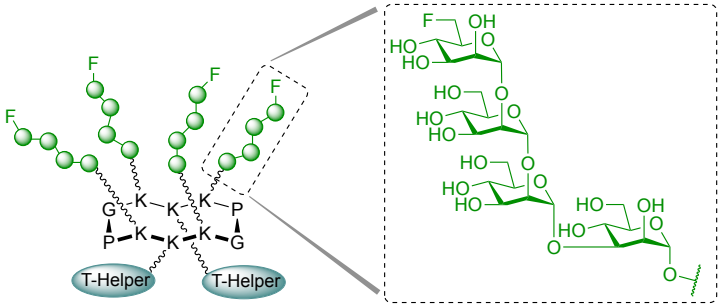


FIGURE 1.13. Synthetic multivalent HIV vaccine candidate prepared by *Wang and co-workers*.[83]

1.2.3 Synthetic T helper epitopes

Nonhomogenous T helper epitopes, such as carrier proteins, are still predominantly used for the preparation of glycoconjugate vaccines. But as mentioned before, the production and application of semi-synthetic constructs is accompanied by several drawbacks. Conjugation reactions proceed inhomogeneously and lead to a certain batch-to-batch variability. Furthermore, a strong B cell response to the carrier protein might lead to the suppression of carbohydrate-specific antibodies.[84, 85] This carrier-induced epitope suppression mostly occurs due to a pre-existing immunity against the carrier protein, which hampers the extensive use of a universal carrier for different haptens since most individuals might have been exposed to this antigen before.[86] Synthetic peptides with defined conjugation sites and designed structures fitting into the binding groove of MHCII molecules can overcome these disadvantages. For instance, *Boons and co-workers*[87] prepared a fully synthetic anti-tumor glycoconjugate vaccine composed of a MUC1-T_N-B cell epitope, a Pam_3CysSK_4 TLR-ligand and a 13 amino acid peptide sequence derived from polio virus[88] as T helper epitope (see FIGURE 1.14).

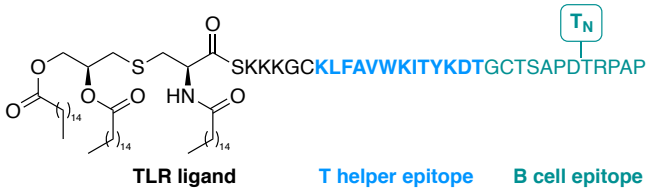


FIGURE 1.14. Fully synthetic anti-tumor glycoconjugate vaccine prepared by *Boons and co-workers*.[87]

This construct induced a robust IgG immune response and the sera of immunized mice were able to bind the native MUC1 antigen expressed by MCF7 cancer cells.[87]

Nevertheless, the use of a MHCII ligand as a T helper epitope, whether derived from a carrier protein or a synthetic peptide, is accompanied by other handicaps. For instance, MHCII molecules are polymorphic, resulting in a variable efficiency of MHCII:peptide complex formation in different individuals. In addition, there is a low frequency of T cells that are specific for a characteristic MHCII:peptide complex and the few specific naive T cells might require support by professinal APCs before becoming activated. However, all these drawbacks can be avoided by using α-GalCer (see FIGURE 1.6) or a potent derivative thereof as T helper-like epitope. This glycolipid is a ligand for the non-polymorphic CD1d molecule, *„[...] which is expressed by both lymphoid lineages (including all B cells) and nonlymphoid lineages.“*[89] A cell-surface bound CD1d:glycolipid complex targets pre-primed iNKT cells, which are able to release signalling cytokines instantaneously upon activation.[36] Besides, iNKT cells outnumber peptide-specific naive T cells, even though only 0.01% to 1% of peripheral blood T cells are iNKT cells.[89, 90] *„The large number of iNKT cells available and their pan-reactivity to αGC avoids generating competition between anti-peptide responses upon processing of the carrier protein, thereby avoiding the ‘epitopic overload’ effect that limits polysaccharide-protein vaccine efficacy [...].“*[89] Among the first examples of vaccines covalently conjugated with iNKT cell agonists were constructs reported by *Cavallari et al.*[89] and *Anderson et al.*[91] in 2014.[92, 93] *Cavallari et al.* covalently attached α-GalCer *via* a linker, that can be cleaved within B cell lysosomes, to *Streptococcus pneumoniae* serotype 4 CPS (see FIGURE 1.15). This construct led to class switching from IgM to IgG and affinity maturation, which consequently generated high-affinity carbohydrate specific protective antibodies

and induced carbohydrate-specifc memory B cells.[89] Furthermore, they observed complete protection against a lethal challenge of *Streptococcus pneumoniae* in mice even three months after the last vaccination. As *Anderson et al.* observed a rearrangement of the ceramide moiety of α-GalCer under acidic conditions and a reorganization under basic or neutral conditions (see FIGURE 1.16), they decided to attach the antigen to the free amine *via* an enzymatically cleavable linker.[91]

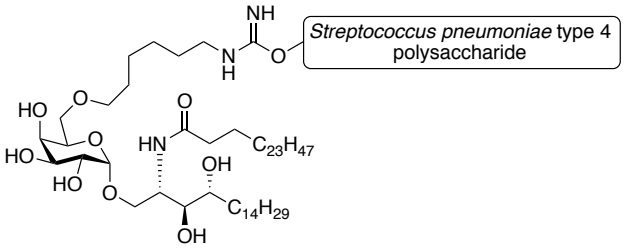


FIGURE 1.15. Semisynthetic carbohydrate-lipid vaccine against *Streptococcus pneumoniae* prepared by *Cavallari et al.*[89]

This approach would allow for the release of α-GalCer after cleavage of the linker and reorganization of the ceramide within the cell. They used short peptide sequences derived from Ovalbumin (OVA) and the lymphocytic choriomeningitis virus glycoprotein as antigens in order to generate cytotoxic T lymphocytes (CTLs) that suppress allergy. Therefore, *Anderson et al.* sensitized mice to Ovalbumin and subsequently vaccinated them with the ovalbumin peptide conjugate. The vaccine led to the generation of peptide-specific CTLs and after challenging the mice with the ovalbumin peptide again, they observed a decrease in allergic airway inflammation.[91] Co-administration of both compounds only led to weak effects.

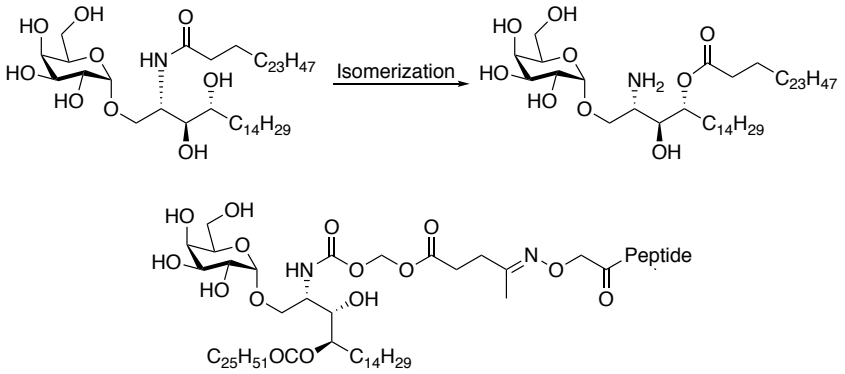


FIGURE 1.16. Isomerization of ceramide moiety and glycolipid-peptide vaccine with allergy suppression activity by *Anderson et al.*[91]

In subsequent studies, *Anderson et al.*[94] prepared NKT cell dependent glycolipid-peptide vaccines with potent antitumor activity. For instance, one of these vaccine candidates was comprised of the glycolipid derivative, a self-immolative valine-citrulline linker and the OVA_{257} epitope SIINFEKL (see FIGURE 1.17).

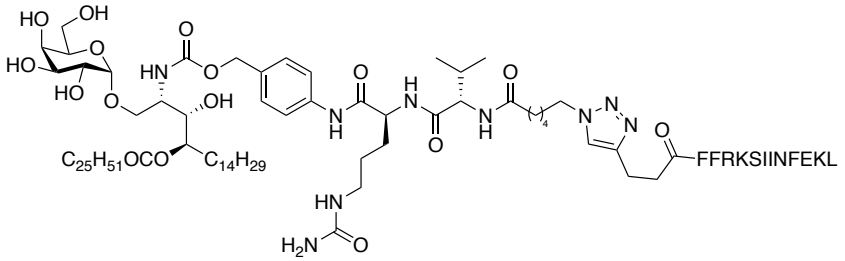


FIGURE 1.17. Glycolipid-peptide vaccine with anti-tumor activity by *Anderson et al.*[94]

This vaccine was tested against an aggressive mouse melanoma model, using a transplantable tumor cell line that has been modified to express ovalbumin. After administration of the vaccine, they observed a marked anti-tumor activity.[94] Again, coadministration of both compounds only led to weak activity.

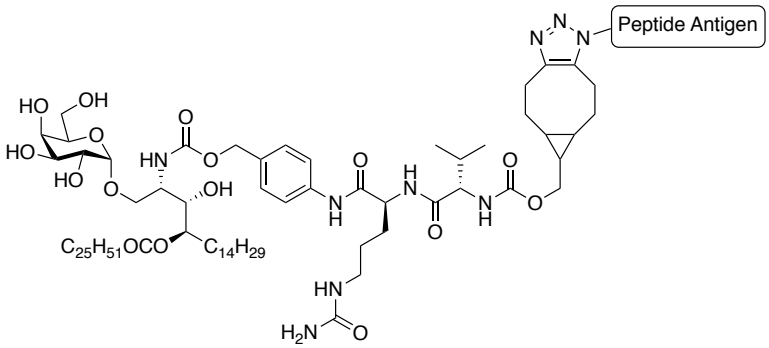


FIGURE 1.18. Glycolipid-peptide vaccine increasing influenza-specific T cell memory generation by *Anderson et al*.[95]

Recently, *Anderson et al.*[95] reported on a glycolipid-peptide vaccine against influenza A virus (IAV) using strain-promoted alkyne-azide cycloaddition (SPAAC) to assemble the α-GalCer with antigenic peptides derived fom virus-associated proteins (see FIGURE 1.18). They were able to show that the vaccine induced T cell responses providing a preventive immunity against IAV in mice. Another approach by *Yin et al.*[96] using the ST_N tumor antigen covalently linked to α-GalCer (see FIGURE 1.19) induced a robust immune response with IgM to IgG class switching and highly specific anti-ST_N IgG antibodies. Further examples of NKT-cell glycolipid agonists in synthetic vaccine development were recently summarized by *Liu et al*.[93]

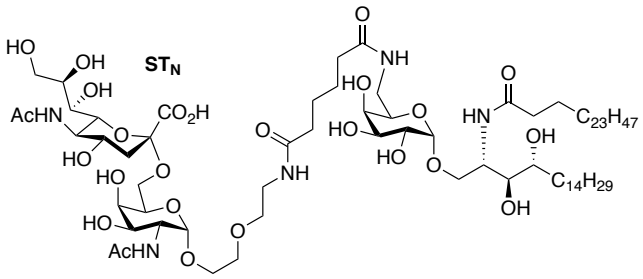


FIGURE 1.19. Anti-tumor carbohydrate-lipid vaccine prepared by *Yin et al.*[96]

1.2.4 Carbohydrate Mimetics

The low *in vivo* stability of immunogenic glycoconjugates is a further critical aspect in the design and development of effective synthetic vaccine candidates. A rapid loss of the carbohydrate hapten due to enzymatic degradation will influence the antibody specificity and thus the outcome and quality of the immune response. The use of carbohydrate mimetics represents a promising approach for stabilizing glycosidic bonds of carbohydrate-based epitopes to preserve the antigenic determinant. Thereby, the designed carbohydrate mimetic should resemble the native compound as much as possible in terms of size, sterics, conformation, hydrophobic properties and electronic configuration.[97] Common carbohydrate mimetics include *C*-glycosides[98-100] and *S*-glycosides[101-105] as well as carbasugars[106-108] and thiasugars[109] (see FIGURE 1.20).[110]

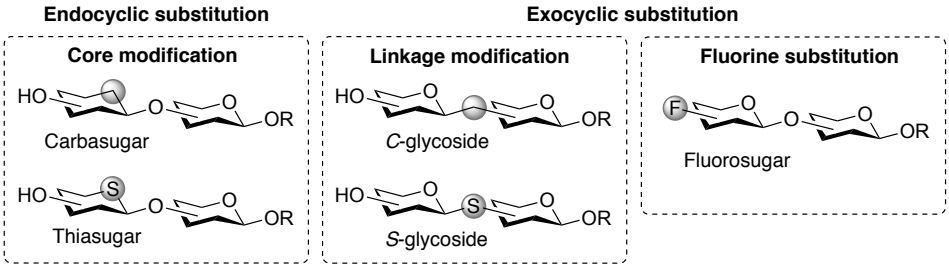


FIGURE 1.20. Schematic depiction of different classes of carbohydrate mimetics.

Furthermore, deoxy sugars and in particular strategically fluorinated carbohydrate derivatives (see FIGURE 1.20) have been applied as effective mimics of their natural counterparts.[97, 110] As the fluorine atom (1.47 Å) has a similar *van der Waals* radius to that of oxygen (1.52 Å), it is widely used as a bioisoster for hydroxyl groups. In 1987, *Withers et al.*[111] confirmed an improved metabolic stability of fluorinated carbohydrates in carbohydrate-enzyme-recognition studies, as they were able to show that 2-deoxy-2-fluoro-D-glycosyl fluorides are capable to slow down transglycosylations by destabilizing the oxocarbenium ion transition state.[111, 112] *Guo et al.*[113] also demonstrated that fluorinated sialic acid conjugates are able to act as inhibitors of influenza virus sialidases. Besides, fluorinated carbohydrates might also provide better antibody-binding affinities.[97, 114] For instance, *Glaudemans et al.*[115] described systematic studies on binding affinities of fluorinated methylglycosides to the monoclonal anti-dextran IgA W3129. They found that a 2-deoxy-2-fluoroglycoside exhibited an identical binding constant in comparison to the unfluorinated glucose, whereas the corresponding 3-deoxy-3-fluoro derivative showed a three times increased binding constant. However, the 4-fluoro and 6-fluoro analogs were not recognized by the antibody at all. In addtion, fluorinated carbohydrates are used as valuable probes to investigate biological recognition processes and enzymatic mechanisms.[114, 116-119] Fluorine bearing carbohydrate antigens could also be used to break immunotolerance, as fluorosugars exhibit an increased "non-self"-character due to the complete absence of fluorine in the body. This is of particular interest for the development of anti-cancer vaccines based on tumor associated carbohydrate antigens (TACAs), as these aberrant glycosylation patterns mostly contain self-glycans and therefore lead to poor immunogenicities. However, pathogenic epitopes that elicit poor immune responses might also profit from this concept. Diverse modified ST_N anitgens, bearing the fluorine substituents in the acyl side chain (see FIGURE 1.21), demonstrated that immunotolerance can be overcome by this strategy, as most of the fluorinated

compounds exhibited a higher immunogenicity than the corresponding native antigen.[120, 121] Some of the fluorinated glycoconjugate vaccines induced higher IgG titers and an enhanced IgM to IgG class switch in comparison to the native ST_N glycoconjugate vaccine.

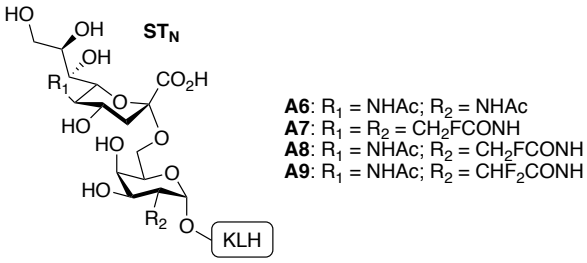


FIGURE 1.21. Fluorine bearing ST_N glycoconjugate vaccines prepared by *Yang et al*.[121]

Several antisera were also able to strongly bind to LS-C human colon cancer cells expressing the natural ST_N.[121] Furthermore, *Hoffmann-Röder and co-workers* reported on the synthesis of various fluorinated T_F antigens incorporated into the mucin-1 (MUC1) tandem repeat peptide sequence (see FIGURE 1.22).[122-125] Several fluorinated glycopeptides were conjugated to tetanus toxoid (TTox) and immunized in mice. They observed comparable immune responses for the native and the fluorinated vaccines and the induced antibody isotypes revealed that the fluorinated vaccines primarily induced IgG antibodies and a smaller amount of IgM antibodies.[122, 125] The anti-sera also exhibited cross reactivity to native MUC1 antigens exposed on the surface of MCF7 breast cancer cells.[122] In addition, degradation studies with a β-galactosidase gave first evidence for an increased metabolic stability of fluorinated TACAs.[125]

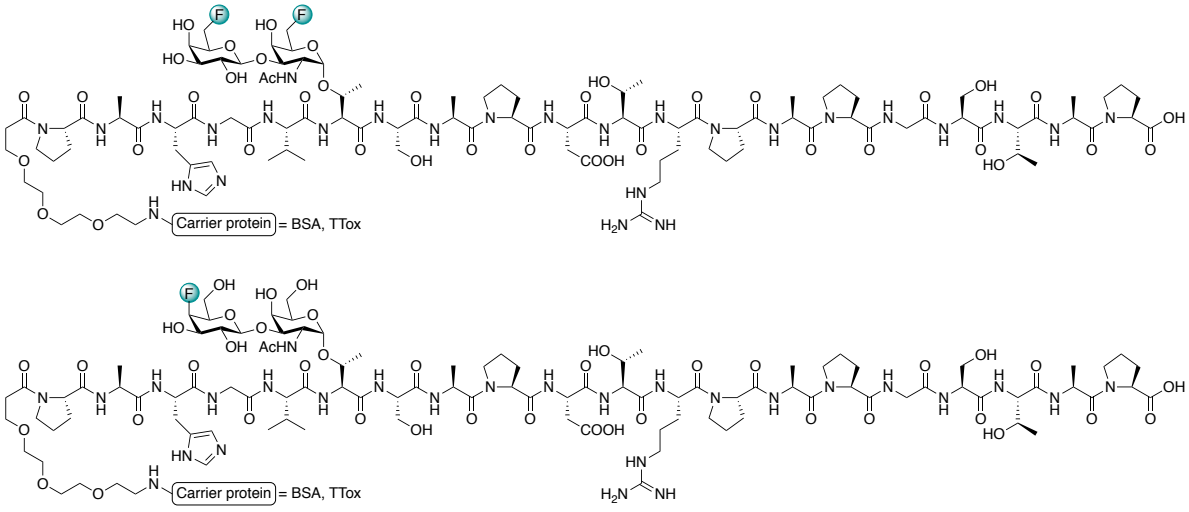


FIGURE 1.22. Fluorinated MUC1-glycopeptide vaccines prepared by *Hoffmann-Röder and co-workers*.[122, 125]

1.3 LEISHMANIASIS

Leishmaniasis is a vector-borne disease which is induced by several different protozoan parasites of the genus *Leishmania*, belonging to the family of *Trypanosomatidae* from the class of *Kinetoplastidae*. It is endemic in large areas of tropic and subtropic regions and the mediterranean basin with around 350 million people (in 88 countries) living at risk of becoming infected and 1.5 million new infections occuring each year.[126] Approximately 70.000 deaths arise annually among the permanent 12 million infected people worldwide.[126, 127] The disease is caused by approximately 20 leishmanial species, which are transmitted to humans by the bite of around 30 different phlebotomine sandfly species.[128] There are four main clinical manifestations of the disease: cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis (MCL), visceral leishmaniasis (VL; also known as kala-azar) and post-kala-azar dermal leishmaniasis (PKDL) (see TABLE 1.1). In CL, different *Leishmania* parasites (e.g. *Leishmania major*, *Leishmania mexicana*) infect macrophages in the dermis leading to ulcers and nodules in the skin, which leave the patients with disfiguring scars after spontaneous and slow healing.[128] In MCL (e.g. *Leishmania braziliensis*), the destructive ulcerations occur in the mucosa, especially in the nose and mouth region of the patients and lead to painful and non-self-healing lesions.[128] Whereas symptoms of CL and MCL are limited to the skin and mucosal surfaces, other *Leishmania* species (*Leishmania donovani*, *Leishmania infantum*) also spread to internal organs (liver, spleen, bone marrow) to cause VL.[126] This clinical form is fatal if left untreated and accounts for most of the annually reported deaths.[127] For instance, VL patients suffer from fever and weigth loss as well as from abdominal pain due to an increased splenomegaly.[128] In an advanced stadium, bacterial co-infections or massive bleeding can lead to death, if patients do not receive medical care.[128] More than 90% of VL cases occur in Bangladesh, India, Sudan, Nepal, Brazil and Ethiopia but the disease is also present in all habitats of its sandfly vector.[128, 129] Leishmaniasis was classified by the World Health Organization (WHO) as a major tropical disease, ranking second among parasitic diseases with VL featuring a death count that is only surpassed by malaria.[128, 129]

TABLE **1.1**. Main *Leishmania* species that affect humans.[a]

Main disease manifestation	Species
Old World, subgenus Leishmania	
Visceral Leishmaniasis	*L. donovani* and *L. infantum*
Cutaneous Leishmaniasis	*L. major*, *L. tropica* and *L. aethiopica*
New World, subgenus Leishmania	
Visceral Leishmaniasis	*L. infantum*
Cutaneous Leishmaniasis	*L. infantum*, *L. mexicana*, *L. pifanol* and *L. amazonensis*
New World, subgenus Viannia	
Cutaneous Leishmaniasis	*L. braziliensis*, *L. guyanesis*, *L. panamensis* and *L. peruviana*
Mucocutaneous Leishmaniasis	*L. braziliensis* and *L. panamensis*

[a] Adapted from[126] and references therein

So far, there is no vaccine against Leishmaniasis commercially available and only a few drugs have entered the market for the fight against *Leishmania* parasites.[130] However, all of them have significant drawbacks and

consequently new drugs as well as effective anti-leishmanial vaccines are urgently required due to increasing resistances against given therapeutics

1.3.1 Current antileishmanial therapy

In 1945, pentavalent antimonials were brought to market against Leishmaniasis and have been part of the WHO model list of essential medicines since 1988.[129] Sodium stibogluconate **A1** (*Pentostam*®) and meglumine antimoniate **A2** (*Glucantime*®) are prodrugs (see FIGURE 1.23), which require an intracellular reduction of their pentavalent antimonite, as the active derivative is the trivalent form.[129] These drugs exhibit a high cure rate of 85–95%, except in Bihar State in India, where more than 60% of treated patients stay unresponsive due to already existing resistances.[131, 132] Furthermore, antimonials require a long-term treatment (up to 4 weeks), are toxic and frequently exhibit diverse side-effects.[128, 129]

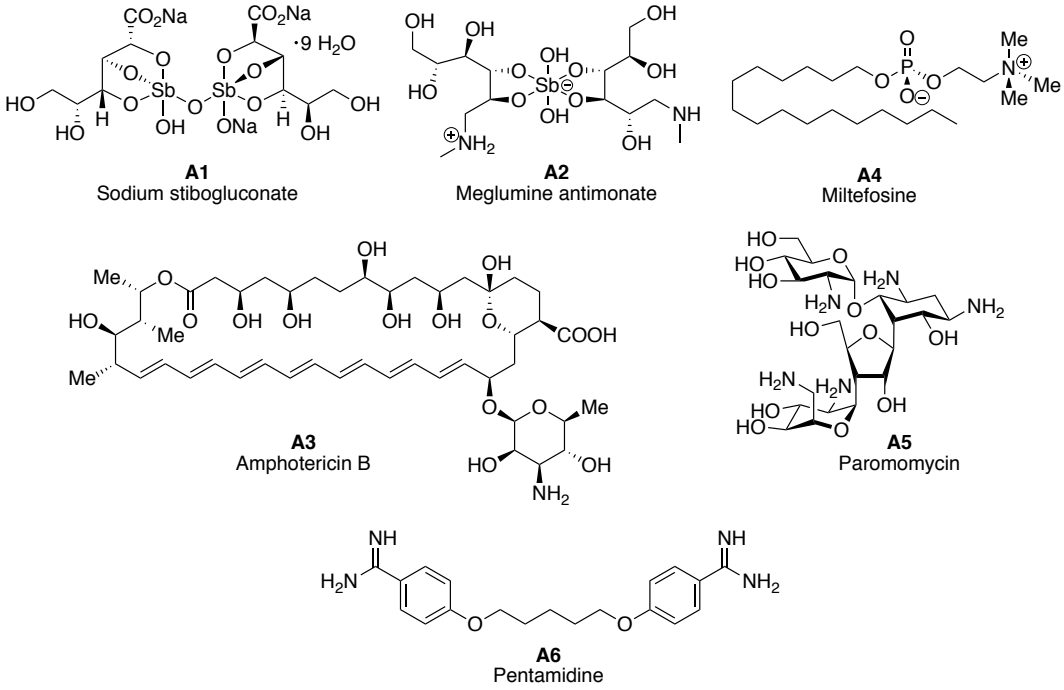


FIGURE 1.23. Structures of anti-leishmanial drugs in use.

In areas where antimonial treatment has become ineffective, conventional Amphotericin B **A3** has evolved as the first-line treatment against Leishmaniasis. Amphotericin B is a macrolide polyene antifungal antibiotic (see FIGURE 1.23), which is currently used in preparations containing amphotericin B desoxycholate (*Fungizone*®) or several different liposomal formulations (e.g. *AmBisome*®).[129, 133] These formulations have an increased bioavailability as well as improved pharmacokinetic properties.[129] With more than 95% cure efficacy, liposomal amphotericin B is a WHO recommended drug for the treatment of all clinical forms of Leishmaniasis. However, these drugs are expensive and not sufficiently stable at high temperatures (guaranteed up to 25 °C), which restrict their application in developing countries.[129] Furthermore, there is evidence that laboratory *Leishmania* strains have already developed resistances against amphotericin B.[134] Another antileishmanial drug is miltefosine **A4** (*Impavido*®) (see FIGURE 1.23), which was originally

developed as an anticancer drug[135] and is the first orally administered and latest antileishmanial drug launched on the market.[129] The drug exhibits high cure rates and is currently registered in India, Germany and Columbia.[131] However, like all other drugs mentioned, miltefosine is hallmarked by several drawbacks. Especially the teratogenic properties prohibit its use in pregnant women.[128] In addition, the drug has a long half-life, which could lead to the development of resistances as it has already been shown in laboratory *Leishmania* strains.[134] This problem has also occured for paromomycin **A5** (see FIGURE 1.23), an aminoglycoside antibiotic with good anti-parasitic activity.[136] Furthermore, an aromatic diamidine, so called pentamidine **A6** (see FIGURE 1.23) is used, when the pentavalent antimonials suffer from resistances.[129] A major side effect of this drug however is induction of irreversible insulin-dependent diabetes mellitus.[137]

Although the aforementioned drugs still allow an effective treatment of Leishmaniasis, new drugs are urgently needed in order to meet challenging problems like drug resistance, availability and toxicity. Therefore, several new synthetic anti-leishmanial drugs have been proposed with some of them being currently in clinical trials.[129] Another strategy to fight Leishmaniasis lies within the development of safe, effective and affordable protective vaccines. Although many research groups have focused on the development of new anti-leishmanial vaccine candidates, only three formulations have entered the clinical trial process until today.[130] The vaccines in developmental stage include live attenuated *Leishmania* vaccines, killed parasites and fractions thereof as well as defined vaccines containing recombinant proteins or DNA.[130]

1.3.2 The dimorphic life cyle of *Leishmania* parasites

The life cycle of *Leishmania* parasites can be divided into two main stages: a promastigote stage in the sandfly vector and an amastigote stage, which develops within the cells of its mammalian host (see FIGURE 1.24). The flagellated procyclic promastigotes are attached to the midgut of their anthropod vector and differentiate into an infective metacyclic form.[138] These non-proliferating promastigotes detach from the midgut of the sandfly and migrate to the stomodeal valve, which is part of the sandfly's foregut.[126, 139] From there, the promastigotes are transferred, together with immunomodulatory parasitic proteophosphoglycans, to the host during the vector's blood meal.[126, 128, 139] At the site of infection, the parasites attach to one of several cell types (neutrophils, macrophages, monocytes, stromal cells and dendritic cells) *via* their flagellum and become phagocytosed.[126] It is within mononuclear phagocytes that *Leishmania* parasites find best conditions for replication and long-term survival. *Leishmania* promastigotes are able to directly invade these cell types, like dermal DCs and resident dermal macrophages, which are the most abundant infected cell type after 24 h of infection.[140] Furthermore, by studying *Leishmania major*, *Lasky and co-workers* proposed that neutrophils might aid the promastigotes as 'Trojan Horses' and thus lead to the invasion into long-lived macrophages without triggering innate antimicrobial defences.[126, 141, 142] Therefore, the promastigotes are readily internalized by neutrophils and survive within their phagosomes. Then the neutrophils are induced to undergo apoptosis and become phagocytosed by macrophages. Thus, the promastigotes are efficiently cargoed into the macrophage phagosome and evade a macrophage defence response.[142] However, still little is known about the mechanisms of direct and indirect *in vivo* phagocytic uptake.

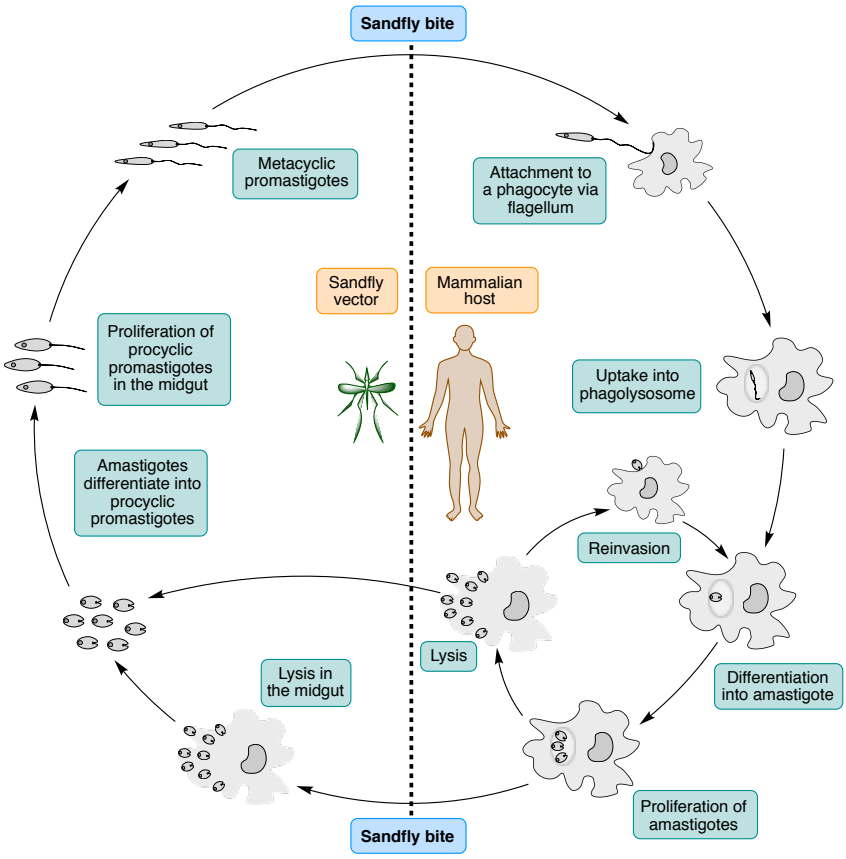


FIGURE 1.24. Life cycle of Leishmania parasites within the sandfly vector and the mammalian host.[126, 128]

Furthermore, it is yet unclear which cell type is responsible for the transport of the parasites from the primary infection site to the draining lymph nodes. It is assumed that inflammatory monocyte-derived DCs (moDCs) are attracted to the primary infection site by alarmins[143] (endogenous molecules signalling tissue and cell damage, *e.g.* IL-1β). The moDCs become infected and thereby help the parasites to migrate to the draining lymph node.[126] As soon as the promastigotes have established themselves within their host cell, they transform into non-flagellated and replicating amastigotes, which multiply and survive within the phagolysosomes through a complex parasite-host interaction.[128, 144] The number of amastigotes increases until the cells burst and other local cells can be invaded. *Leishmania donovani* amastigotes exhibit a special preference for CD11b and CD11c deficient resident tissue macrophages, which can be found in the lymph nodes, the spleen, the bone marrow and the liver.[126, 145] The transmission cycle is completed, when the infected phagocytes or parasitic amastigotes are taken up during the blood feeding of another sandfly. The amastigotes transform within 4 to 25 days to the promastigote stage, multiply in the midgut of the infected sandfly vector and subsequently participate in a new life cycle.[130]

One important survival strategy of *Leishmania* parasites in order to persist the hostile environment of sandfly vector and mammalian host is based on a dense and dynamic glycocalyx located on the cell surface. The cell-surface glycoconjugates undergo morphological and structural changes during the parasites life-cycle and help to avoid destruction in the midgut of the sandfly, the host's bloodstream and in the phagolysosomes of the macrophages.[146] One of the the most abundant surface macromolecules that can be found as part of the glycocalyx of *Leishmania* parasites is the highly complex glycoconjugate lipophosphoglycan (LPG).[147]

1.3.3 Lipophosphoglycans

The surface coat of *Leishmania* parasites is composed of different glycoconjugates, which are bound to the surface membrane by a highly conserved glycosylphosphatidylinositol anchor (GPI anchor). These GPI-anchored surface molecules include glycoinositolphospholipids (GIPLs), glycoprotein 63 (gp63), proteo-phosphoglycans (PPGs) and lipophosphoglycans (LPGs).[148] With approximately 5×10^6 copies/cell, LPG is the major leishmanial surface glycoconjugate and covers the whole cell surface as well as the flagellum.[146] It is predominantly expressed on the promastigote form, whereas it appears to a far lesser extent on an intracellular amastigote.[146, 149, 150] In contrast, GIPLs and PPGs maintain a high expression level throughout all developmental stages.[147, 151] LPGs are composed of four domains, of which three are conserved structures that can be found among all *Leishmania* species: (1) a 1-*O*-alkyl-2-lyso-phospatidyl-myo lipid anchor, (2) a phosphosaccharide core and (3) a phosphoglycan repeat consisting of [→6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3)$^-$] building blocks (see FIGURE 1.25).[146-148] The fourth domain is a terminating neutral cap structure comprised of di-, tri- or tetrasaccharides, which mostly contain galactose or mannose. These cap structures as well as substituent groups branching from the phosphoglycan repeat are polymorphic among all *Leishmania* species.[146] For instance, in *Leishmania donovani*, the most abundant cap structure terminating the non-reducing end consists of the branched trisaccharide α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* (52%) followed by the disaccharides α-D-Man*p*-(1→2)-α-D-Man*p* (20%) and β-D-Gal*p*-(1→4)-α-D-Man*p* (16%), while the trisaccharide α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p* (6%) and the tetrasaccharide α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* (5%) can be found to a lesser extent (see

FIGURE 1.26).[152] In contrast, the linear trisaccharide β-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→4)-α-D-Man*p* and the disaccharide β-D-Gal*p*-(1→4)-α-D-Man*p* were identified as major terminating cap structures in *Leishmania chagasi*.[153] Importantly, the Gal*p*-(β-1,4)-Man*p* linking unit, which is present in most of the neutral cap structures as well as in the phosphoglycan repeat, can be assumed as a unique structural element among eucaryotic glycoconjugates.[154]

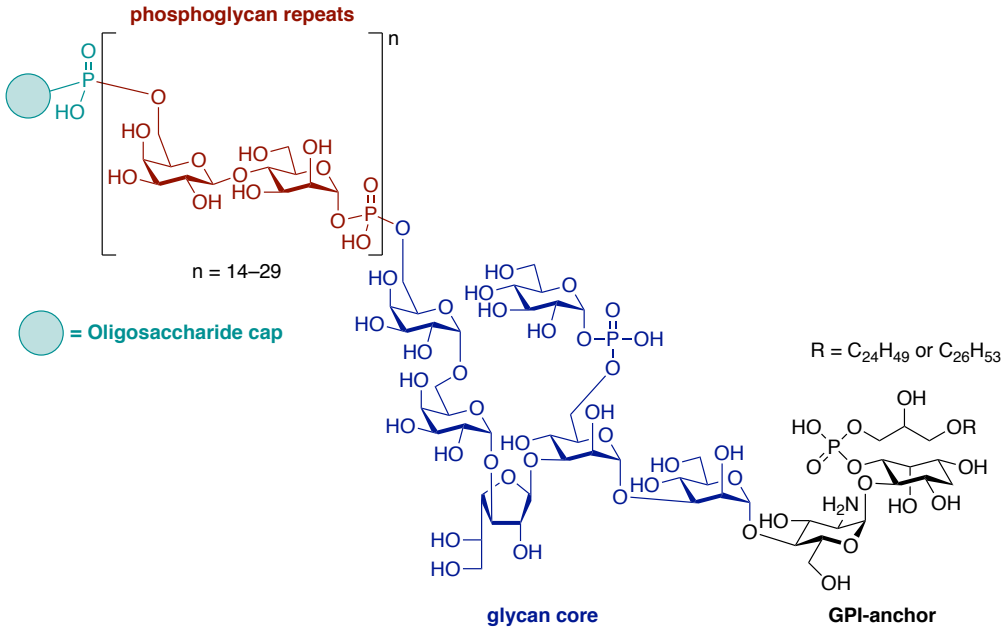


FIGURE 1.25. Conserverd structural elements of LPG.[147]

Furthermore, LPGs can be divided into two different types with regard to their side chain polymorphism.[147] Whereas *Leishmania donovani* does not express branching carbohydrate substituents at the phosphosaccharide backbone at all,[155] most other *Leishmania* strains carry different species-specific side chain residues in the phosphoglycan domain. For instance, the phosphoglycan repeats of *Leishmania major*, *Leishmania mexicana*, *Leishmania infantum* and *Leishmania tropica* are glycosylated with different carbohydrate residues (glucose, galactose, arabinose) at C3 of galactose, whereas they are mannosylated at C2 of mannose within *Leishmania aethiopica*.[147, 156, 157] However, these branching groups are modified during metacyclogenesis either by downregulation, elongation or even upregulation.[148]

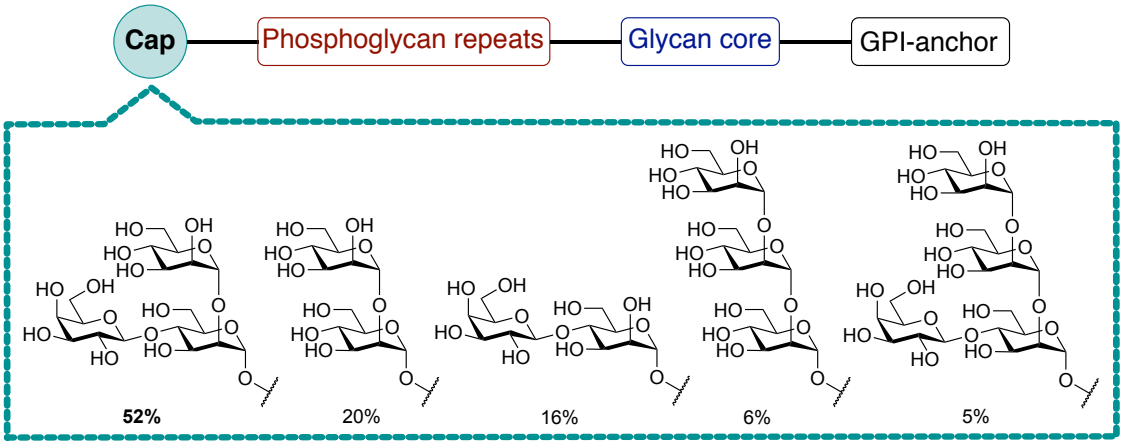


FIGURE 1.26. Terminating neutral cap structures in Leishmania donovani and their percental distribution (mole%).[152]

Metacyclogenisis additionally includes an increase in the number of phosphoglycan repeat units whereby the length of LPG is approximately doubled. This process leads to a thickening of the glycocalyx from around 6 nm in procyclic promastigotes up to 12 nm in metacyclic promastigotes.[155] These two events seem to play an important role for detachment from epithelial cells in the midgut of the sandfly. It is assumed that procyclic promastigotes bind *via* parts of their LPG structure to specific receptors on the surface of midgut epethelial cells. By downregulation or modification of side chain residues and elongation of the phosphoglycan repeat, the important binding moieties either disappear or become less accessible due to conformational changes.[155, 158] For instance, *Sacks et al.* found for *Leishmania donovani* that neutral cap structures seem to be crucial for attachment to the midgut of the sandfly.[155] They showed that binding of parasites to the midgut was inhibited in the presence of LPG-derived oligosaccharides. Furthermore, they reported that both, the procyclic and metacyclic LPGs are bearing the species-specific cap structures, but that only logarithmic phase promastigotes were fully agglutinated by β-Gal-binding peanut agglutinin (PNA), whereas 10–20% of stationary phase parasites were left free. A similar change in lectin-mediated agglutination was found using α-Man-binding Concanavalin A (Con A). These results and further experiments led them to the assumption that terminal capping oligosaccharides become less accessible due to conformational changes and are partially hidden, eventually leading to the release of the parasites from the midgut wall.[155, 159] Nevertheless, *Anish et al.*[160] and *Liu et al.*[161] were able to show that antibodies raised against the *Leishmania donovani* derived terminating cap tetrasaccharide α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* were able to cross react with parasitic promastigotes and with axenic amastigotes. The increase of surface-bound LPG is a further important feature of metacyclogenisis as it leads to the protection against complement-mediated lysis. Besides, surface-bound LPG is not only essential for preventing destruction of promastigotes in vector and host but is also required for virulence and survival of promastigotes whithin host macrophages, which further assures the differentiation into amastigotes in a hostile environment.[148]

1.3.4 Synthetic anti-leishmanial glycoconjugate vaccines

LPGs represent the principal interface for host-pathogen interactions and thus are at the front line for initial contact with the host immune system. Therefore, parasitic LPGs constitute excellent targets for vaccine development, in particular as most of the crucial LPG structures also occur as part of a number of secreted and membrane-bound *Leishmania* glycoproteins. For instance, the aforementioned mucin-like PPG (secreted and membrane-bound) and the secreted acid phosphatase (sAP) also contain [→6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3)$^-$] repeating units in their protein-bound phosphoglycans and species-specific terminating neutral cap structures.[162, 163]

Over the last three decades, several research groups have focused on the synthesis of leishmanial carbohydrate epitopes for vaccine and diagnostic tool development.[10] *Nikolaev et al.* carried out syntheses of different *Leishmania* phosphoglycans[164] not only to illuminate the biosynthetic pathways, which are responsible for the construction of the backbone and the branching substituent groups (*inter alia* fluorinated phosphodisaccharides),[165-167] but also in order to elucidate their immunological properties.[168] The group reported several phosphoglycans conjugated to TetC (see FIGURE 1.27) to be used by *Bates* and *Rogers* as glycoconjugate vaccines for immunization in BALB/c mice, which were subsequently challenged by the bite

of *Leishmania mexicana* infected *Lutzomyia longipalpis* sand flies.[168] Thereby, the *Leishmania mexicana*-TetC glycoconjugate vaccine (see FIGURE 1.27) was able to induce significant protection in comparison to control TetC immunizations.[169]

***Leishmania donovani* glycoconjugate**

[β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-$(CH_2)_9$)]$_n$-**TetC**

***Leishmania mexicana* glycoconjugate**

[β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-$(CH_2)_9$)]$_n$-**TetC**

β-D-Glc*p*-(1→3)

***Leishmania major* glycoconjugate 1**

[β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-$(CH_2)_9$)]$_n$-**TetC**

β-D-Gal*p*-(1→3) β-D-Gal*p*-(1→3)

***Leishmania major* glycoconjugate 2**

[β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3H-$(CH_2)_9$)]$_n$-**TetC**

β-D-Gal*p*-(1→3) β-D-Gal*p*-(1→3)

β-D-Ara*p*-(1→2) β-D-Ara*p*-(1→2)

FIGURE 1.27. Chemical structures of glycoconjugate vaccines used for immunological evaluation. Reported hapten loading (n) per mole of TetC: 4.85 (*Leishmania donovani*), 4.15 (*Leishmania mexicana*), 3.37 (*Leishmania major* 1), 3.80 (*Leishmania major* 2).[169]

Moreover, the same group also prepared *C*-phosphonate analogs of the [→6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-*O*-PO_3)$^-$] repeating unit *via* a blockwise chain elongation approach (see FIGURE 1.28).[170] By replacing the anomeric oxygen of the mannose residue with a CH_2-group, they assumed to obtain a phosphoglycan analog with conformationally identical characteristics but also a higher chemical stability.[10, 168] Other research groups also reported elegant synthetic approaches to obtain unmodified *Leishmania* phosphoglycan repeating units, which are summarized elsewhere.[10]

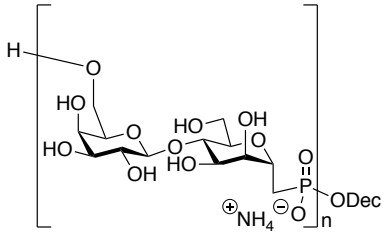
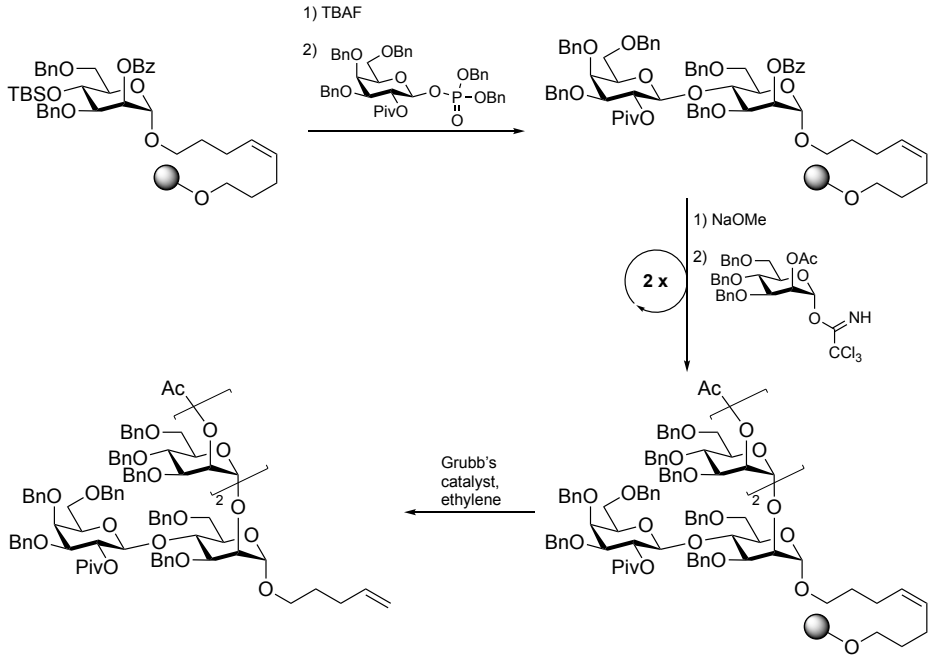


FIGURE 1.28. Chemical structures of *C*-phosphonate analog where n = 1-3. Dec = decenyl.[170]

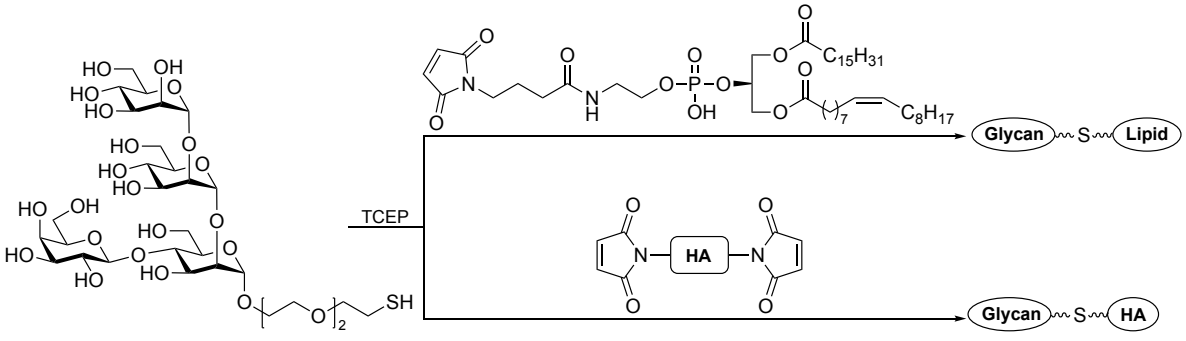
In addition to phosphoglycan units, also epitopes derived from the neutral cap structures, which are for the most part exposed to immune surveillance mechanisms (see CHAPTER 1.3.3), have evoked particular interest for vaccine development. Several research goups have conducted synthetic efforts in order to obtain the neutral cap tetrasaccharide α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* from *Leishmania donovani* (see FIGURE 1.26). The first synthesis of the neutral cap tetrasaccharide was conducted by *Fraser-Reid and co-workers* using an *n*-pentenyl glycoside protocol.[171] Amongst others,[172-174] the *Seeberger* group has carried out some notable synthetic work by preparing the LPG cap tetrasaccharide *via* solid-phase synthesis.[175, 176] Thereby, they assembled the tetrasaccharide using a mannosyl building block bound to the solid support *via* a pentenyl linker (see SCHEME 1.1). The synthesized tetrasaccharide was *inter*

alia conjugated to the TLR-ligand Pam_3Cys to give a fully synthetic vaccine.[175] In a further approach, they introduced a thiol handle at the reducing end of the synthetic leishmanial epitope and conjugated the tetrasaccharide to a phospholipid or the influenza virus coat protein hemaglutinin (HA), respectively (see SCHEME 1.2). These glycoconjugates were embedded into the lipid membrane of reconstituted influenza virus virosomes and the obtained semi-synthetic vaccines were used for immunological evaluation. Both vaccines elicited epitope-specific IgM and IgG antibodies in BALB/c mice and the antisera were able to cross-react *in vitro* with the corresponding antigens expressed in axenic *Leishmania* amastigotes.[161]



SCHEME 1.1. Solid-phase synthesis of *Leishmania donovani* cap tetrasaccharide by *Seeberger and co-workers*.[175]

In subsequent work, the *Seeberger* group also investigated the diagnostic potential of several different leishmanial neutral cap structures.[160] Therefore, they used diagnostically relevant epitopes in synthetic glycan microarrays and confirmed their diagnostic potential by screening sera from infected humans and a large pool of infected dogs. These screening results revealed the importance of the Gal*p*-(β-1,4)-Man*p* linking pattern for antibody binding. They selected the cap tetrasaccharide α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* from *Leishmania donovani* to generate anti-glycan antibodies. In a mouse model, a tetrasaccharide glycoconjugate was able to elicit IgGs specific to the capping oligosaccharides and the obtained mouse polyclonal sera detected the parasite with high specificity.[160]



SCHEME 1.2. Synthesis of glycoconjugates containing leishmanial cap tetrasaccharides. TCEP = tricarboxyethylphosphin.[161]

2 OBJECTIVE

New vaccine formulations against pathogens, which yet remain a principal cause of death or newly emerge as a serious threat due to arising antibiotic resistances, are urgently required.[4] In that regard, glycoconjugate vaccines represent effective weapons in the fight against pathogenic diseases, with fully synthetic constructs being particularly useful due to their superior reproducibility and their structural homogeneity.[17] A synthetic approach toward a glycoconjugate vaccine candidate also allows for a tailor-made epitope design, which enables the application of a minimal protective epitope as well as the use of carbohydrate mimetics. The latter are of particular interest, since glycomimetics are thought to overcome the reduced metabolic stability of carbohydrate epitopes in biological systems.[177] In this context, fluorine incorporation has become an important tool to enhance the biostability of crucial glycotopes *in vivo*. However, fluorinated carbohydrates may not only help to increase the metabolic stability of glycosidic linkages but also to break immunotolerance, as fluorosugars exhibit an increased "non-self"-character due to the absence of fluorine from most organisms. Both advantages have been proven in anti-cancer vaccine formulations containing fluorinated tumor-associated carbohydrate antigens.[105, 121, 122, 125]

The aim of this work was to establish synthetic routes toward selectively fluorinated analogs of a well-known leishmanial antigenic structure to set the stage for their future use in immunological studies. Due to the severe pathophysiology of the protozoan parasite *Leishmania*,[128] several research groups have focused on the synthesis of leishmanial carbohydrate epitopes for vaccine development over the last decades. In particular substructures of the promastigote surface lipophosphoglycans (LPG) have been prepared and used for preliminary immunological evaluations.[160, 161, 168] These studies confirmed the immunogenic and diagnostic potential of leishmanial LPG glycotopes. Inspired by these findings, we focused on the LPG neutral cap structures of *Leishmania donovani* (see FIGURE 2.1), which is the main species causing fatal anthroponotic visceral leishmaniasis.[128] Our approach should thus consider the essential LPG motifs α-D-Man*p*-(1→2)-α-D-Man*p* and in particular β-D-Gal*p*-(1→4)-α-D-Man*p*, which is also a unique glycosylation pattern among eukaryotic organisms.[154] Therefore, leishmanial glycan mimetics consisting of the α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* epitope should be synthesized, as this trisaccharide not only contains the aforementioned crucial LPG elements but also represents the most abundant terminating neutral cap structure of *Leishmania donovani*.[152] Fluorine incorporation was envisaged at the galactose moiety in order to stabilize and maintain the important β-D-Gal*p*-(1→4)-α-D-Man*p* glycosidic linkage *in vivo*. Therefore, syntheses of target glycans, including native trisaccharide **1**, as well as fluorinated derivatives **6**–**10** should be tackled (see FIGURE 2.1). Thereby, distinct fluorination at the 2-, 3-, 4-, 6- and 2,6-position of the galactose moiety should help to address the question of how fluorine incorporation affects binding affinity and antibody selectivity on a molecular level. Moreover, as the work-flow of rational vaccine design demands comparison of all terminating neutral cap structures in biological evaluations (e.g. patient sera screening, degradation studies), syntheses of the α-D-Man*p*-(1→2)-α-D-Man*p*, β-D-Gal*p*-(1→4)-α-D-Man*p*, α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p* and α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* motifs **2**–**5** (see FIGURE 2.1) should also be carried out.

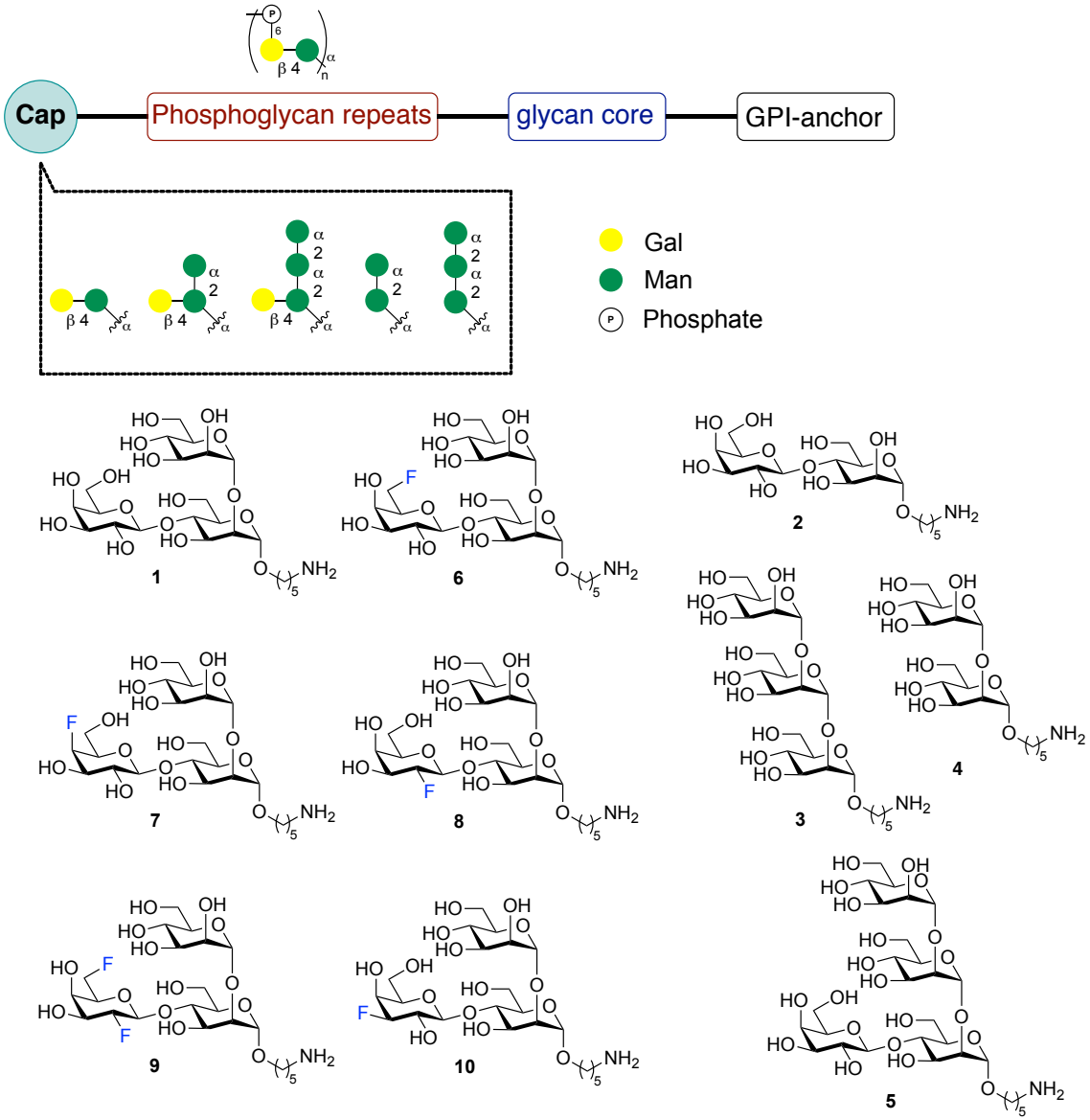


FIGURE 2.1. Targeted natural and fluorinated neutral cap structures derived from the LPG of *Leishmania donovani*.

In a second project, a synthetic route toward a monovalent fully synthetic vaccine candidate comprised of native trisaccharide **1** and α-GalCer derivative **11** as T helper-like epitope (see FIGURE 2.2) should be established on the basis of literature known protocols.[89, 91, 94] In this approach, the α-GalCer moiety should be linked to the carbohydrate B cell epitope *via* a self-immolative valine-citrulline linker in accordance to literature precedent[94] in order to allow liberation of the α-GalCer derivative within B cells. This approach should lay the foundation for future vaccination studies in order to evaluate the efficacy of covalently-attached NKT cell glycolipid agonists in synthetic vaccines and further confirm the excellent T helper-like characteristics of this class of compounds.[93]

Furthermore, synthetic studies targeting a novel multivalent fully synthetic vaccine candidate against *Leishmania donovani* should be carried out (see FIGURE 2.2). This approach should take advantage of a non-immunogenic multivalent scaffold, which enables the formation of a glycocluster imitating the dense surface distribution of pathogenic surface glycans. Therefore, we envisoned the synthesis of a cyclopeptide scaffold,

or so-called Regioselectively Addressable Functionalized Template (RAFT),[40] to link the carbohydrate epitopes with α-GalCer derivative **11** (see FIGURE 2.2). The cyclic decapeptide should allow for a regioselective multivalent attachment of the carbohydrate B cell epitopes as well as conjugation to a T helper epitope.

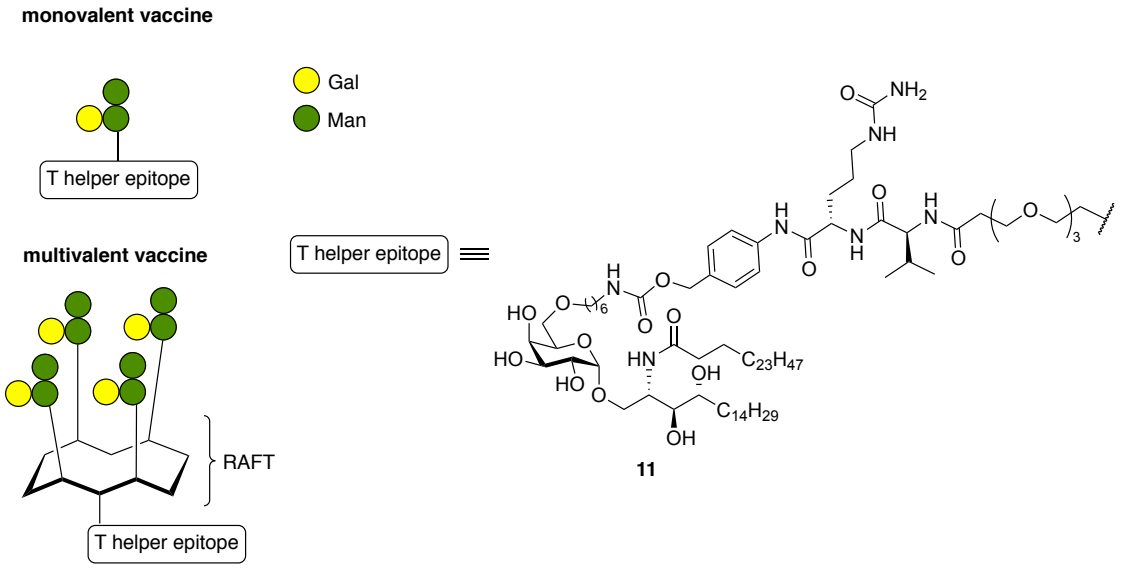


FIGURE 2.2. Schematic depiction of the targeted monovalent and multivalent fully synthetic vaccine candidates against *Leishmania donovani*.

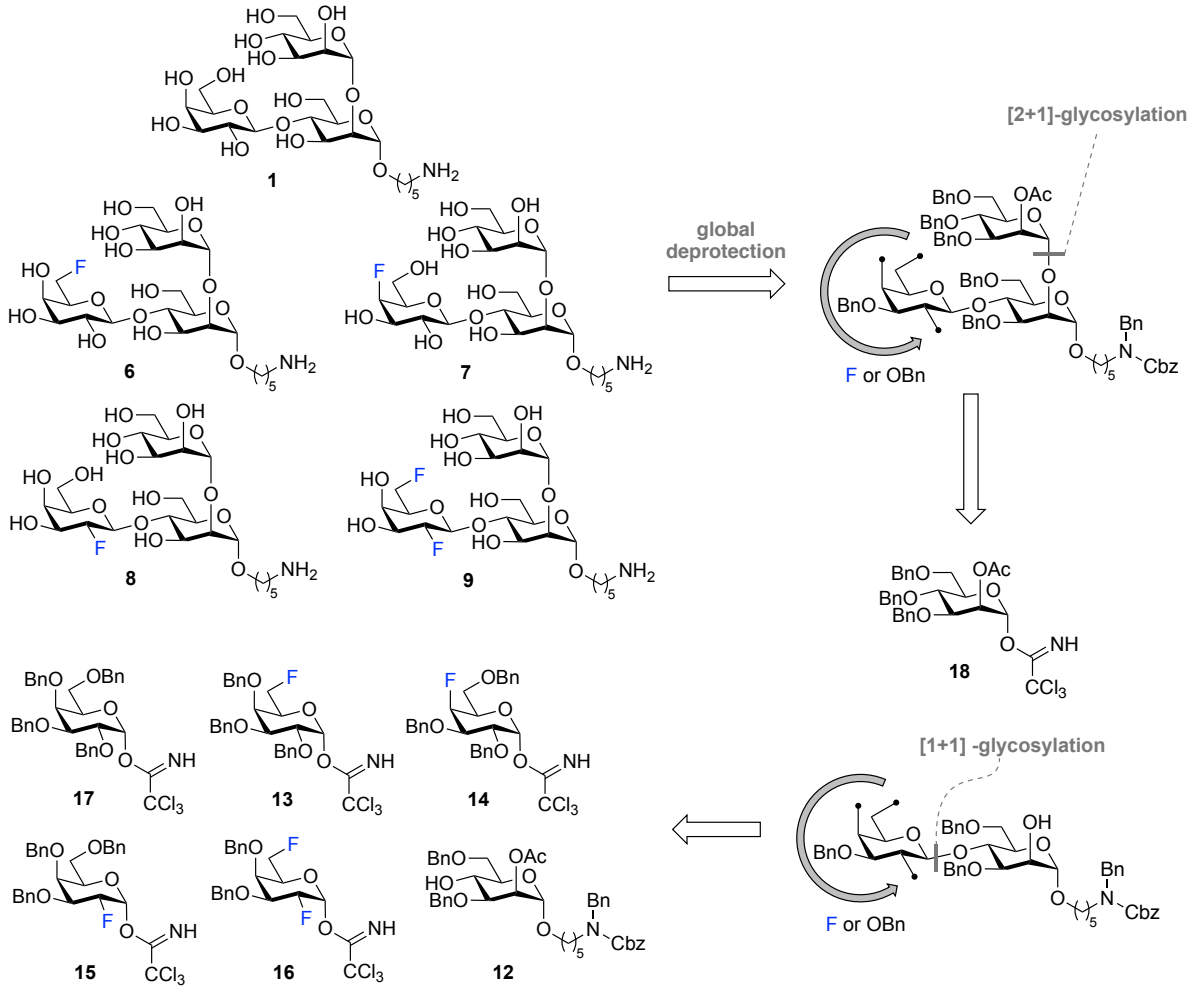
3 RESULTS AND DISCUSSION

3.1 RETROSYNTHESIS

3.1.1 Retrosynthetic analysis of the natural and fluorinated neutral cap structures

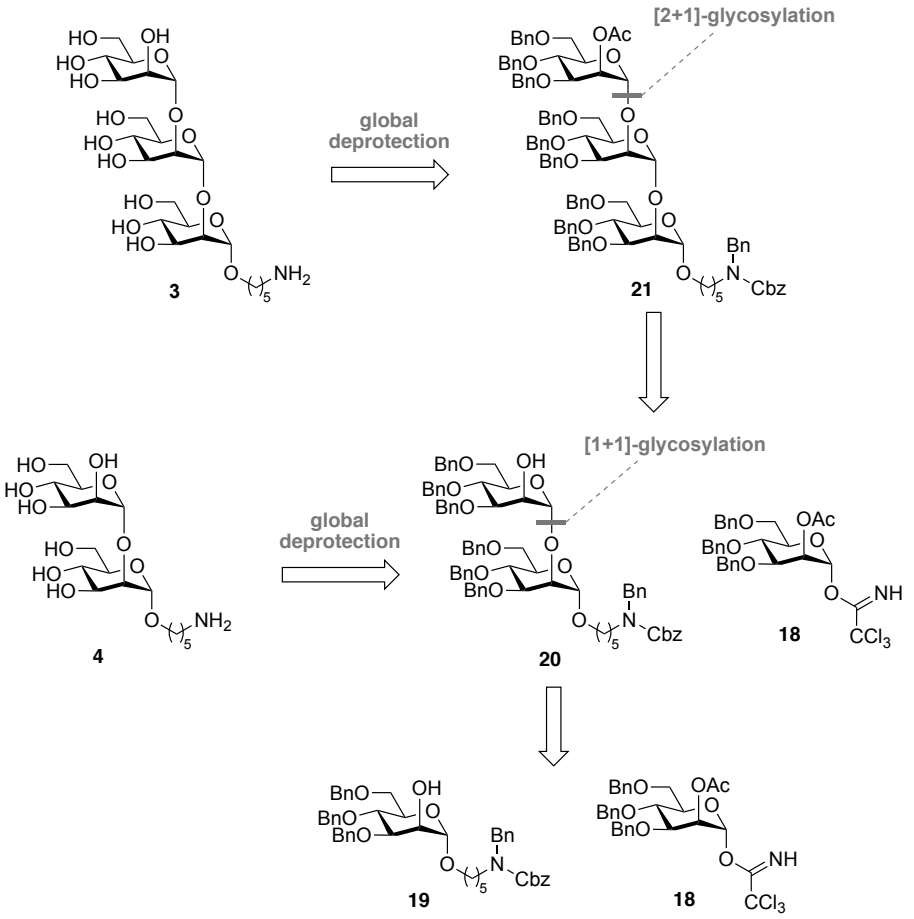
For the assembly of the LPG terminating neutral cap structures and the fluorine containing derivatives we envisioned convergent synthetic strategies in accordance to literature precedents.[160, 161]

The synthesis of the branched trisaccharide α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* **1** and its fluorinated analogs **6–9** should start from suitably protected monosaccharide building blocks and approach the corresponding target structures *via* a sequential [1+1+1]-glycosylation protocol.



SCHEME 3.1. Retrosynthetic analysis of branched trisaccharide **1** and its fluorinated derivatives **6–9**.

In this regard, retrosynthetic analysis revealed bridging mannosyl acceptor **12**[161] as key building block (see SCHEME 3.1). This core synthon should be used in a [1+1]-glycosylation with a range of suitably protected galactosyl trichloroacetimidate donors to give the corresponding disaccharide subunits. Thereby, the protecting group pattern of the donors should enhance the reactivity of the desactivated fluorinated donors and allow for a selective cleavage of the acetate protecting group at C-2 of the mannose residue after glycosylation. Therefore, we envisaged benzyl ethers as permanent and activating protecting groups for the hydroxyl groups of fluorinated donors **13**–**16**.[123, 124, 178-180] For comparison, a similar protecting group pattern without a participating group at C-2 should be installed at native galactosyl donor **17**.[180] One of the major challenges posed by this approach, however, would be to secure a β-selective glycosylation without anchimeric assistance. The final trisaccharides **1** and **6**–**9** should then be accessed *via* glycosylation of the β-(1→4) linked disaccharide acceptors with mannosyl donor **18**,[181] followed by global deprotection steps. In this second glycosylation step, the acetate group at C-2 of mannosyl donor **18** should guarantee for the required α-selectivity. The assembled and fully deprotected trisaccharides could then be used for conjugation reactions *via* the amine-linker installed at the anomeric center of the bridging mannose moiety.

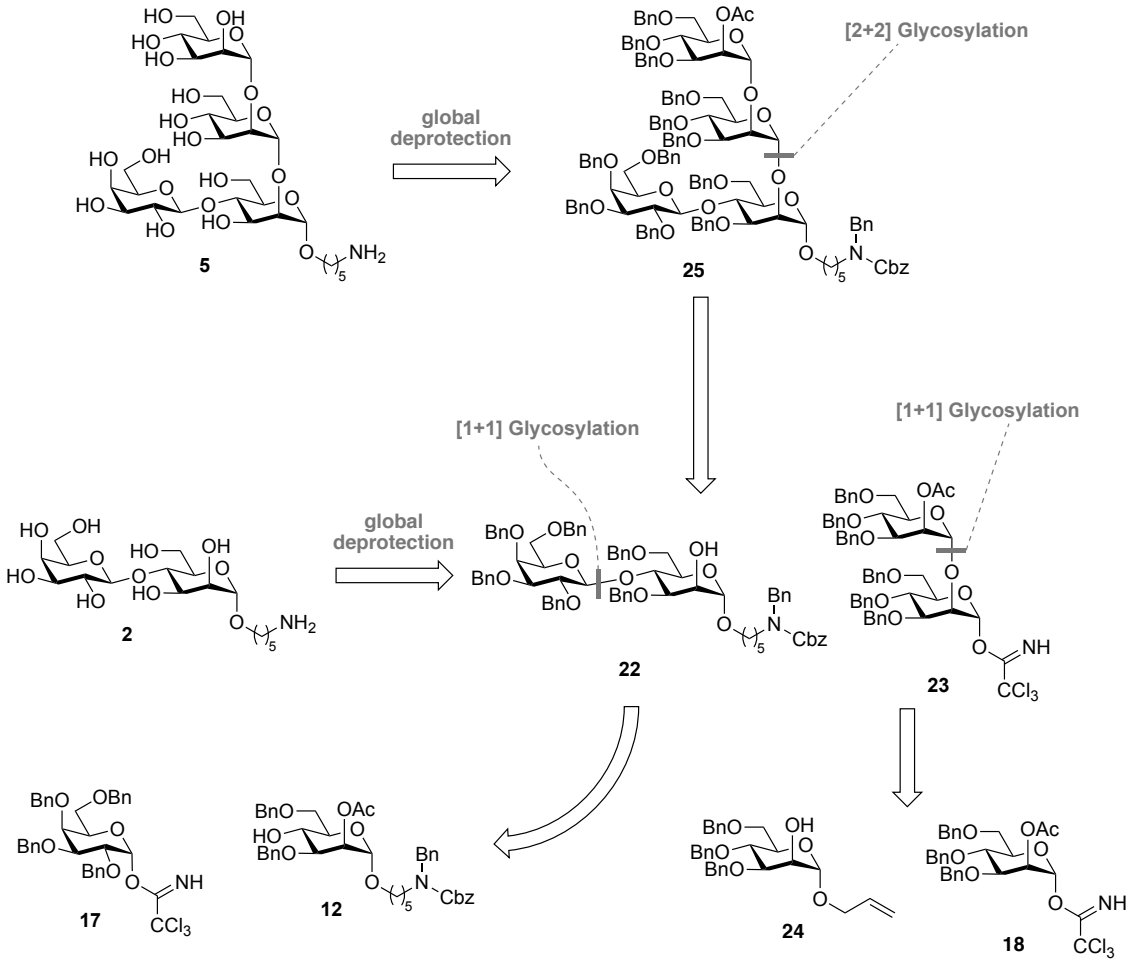


SCHEME 3.2. Retrosynthetic analysis of di- and trimannose capping structures **3** and **4**.

The di- and trimannose capping structures α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p* **3** and α-D-Man*p*-(1→2)-α-D-Man*p* **4** should be accesed *via* a [1+1]- or [1+1+1]-glycosylation protocol, respectively. Thereby, the target structures can be retrosynthetically traced back to already presented mannosyl donor **18**[181] and an

amine-functionalized mannosyl acceptor **19** (see SCHEME 3.2). Sequential α-selective assembly of these building blocks followed by global deprotection of intermediate compounds **20** and **21** should then afford the two leishmanial LPG terminating neutral cap structures for subsequent conjugation reactions.

Furthermore, disaccharide subunit β-D-Gal*p*-(1→4)-α-D-Man*p* **2** should be prepared *via* a [1+1]-glycosylation starting from galactosyl donor **17**[180] and mannosyl acceptor **12**[161] (see SCHEME 3.3). Subsequent global deprotection of **22** should provide **2** for following conjugation reactions.

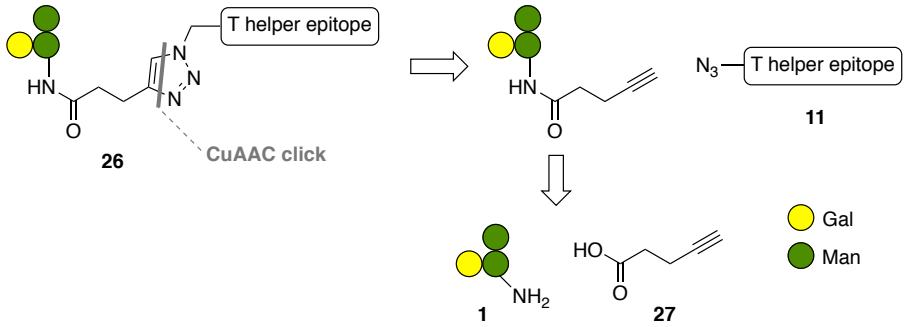


SCHEME 3.3. Retrosynthetic analysis of disaccharide **2** and tetrasaccharide **5**.

Moreover, retrosynthetic analysis of branched tetrasaccharide α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* **5** suggested a [2+2]-glycosylation strategy[174] starting from appropriate disaccharide building blocks **22** and **23** (see SCHEME 3.3). For this purpose, dimannosyl donor **23** should be prepared from mannosyl acceptor **24** and mannosyl donor **18**. Subsequent glycosylation with acceptor **22** and ensuing global deprotection of intermediate **25** should then lead to desired tetrasaccharide **5**, which could again be used in future conjugation reactions due to its amine linker.

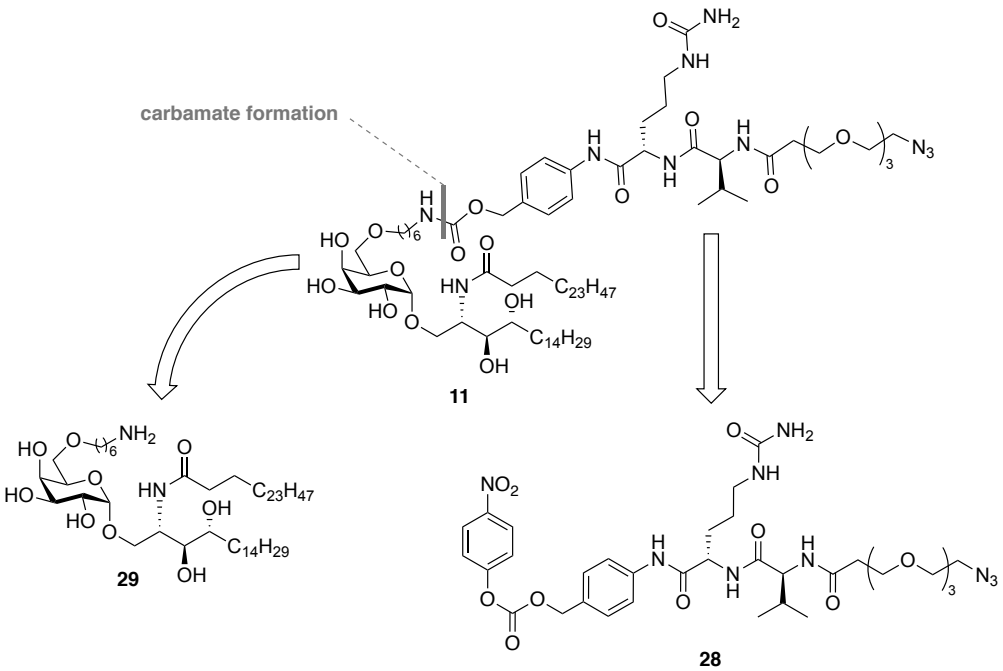
3.1.2 Retrosynthetic analysis of the fully synthetic vaccine candidates

The assembly of the fully synthetic vaccine candidate **26** (see SCHEME 3.4) should involve native trisaccharide α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* **1** and α-GalCer derivative **11** (see SCHEME 3.5) as a T helper-like epitope in accordance to literature precedents.[89, 91, 94] Therefore, B cell epitope **1** and the α-GalCer moiety should be merged together *via* a copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) (see SCHEME 3.4). For this purpose, 4-pentynoic acid **27** should be attached to **1** prior to the click reaction.



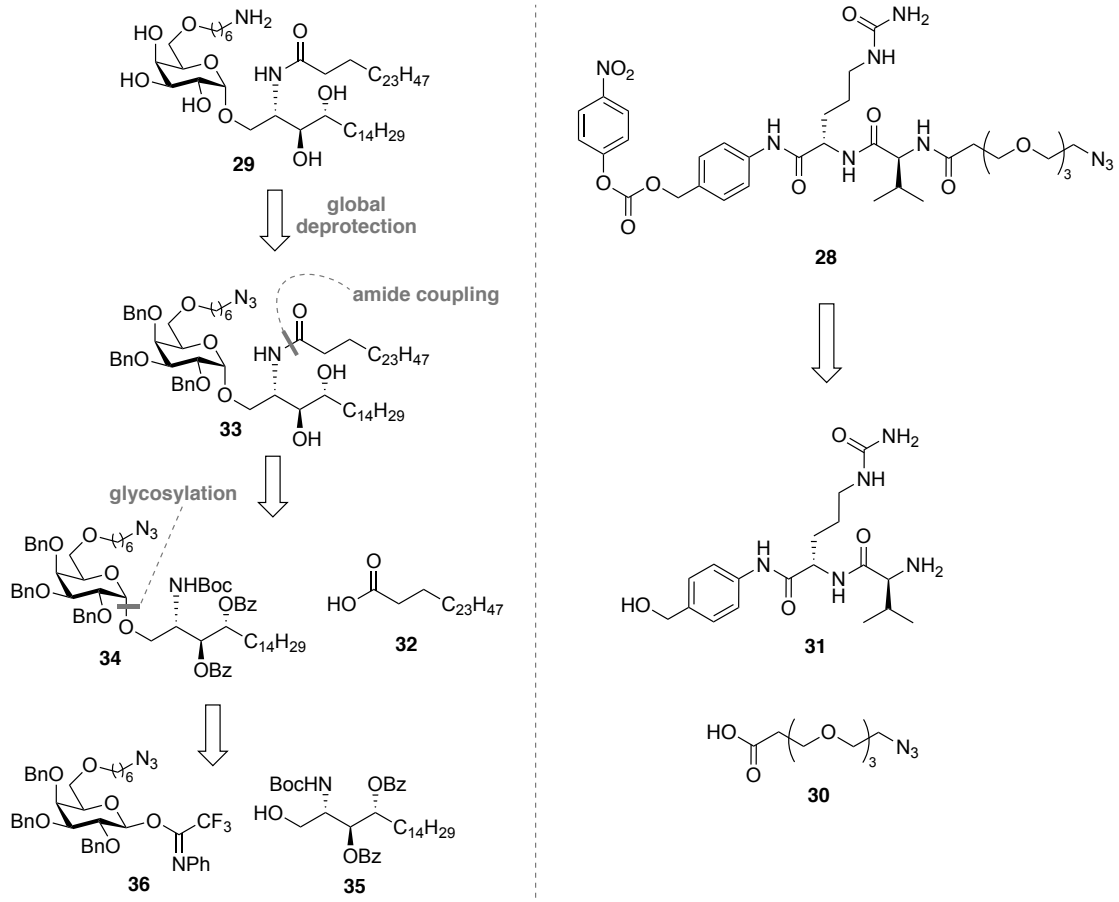
SCHEME 3.4. Retrosynthetic analysis of fully synthetic monovalent glycoconjugate vaccine candidate **26** comprised of α-GalCer derivative **11** and trisaccharide α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* **1**.

The synthesis of azide-bearing α-GalCer derivative **11** equipped with a self-immolative valine-citrulline (Val-Cit) linker should follow literature known procedures.[89, 94] Thereby, we envisioned to attach enzymatically cleavable linker **28** *via* carbamte formation to literature known amine-functionalized compound **29** (see SCHEME 3.5).



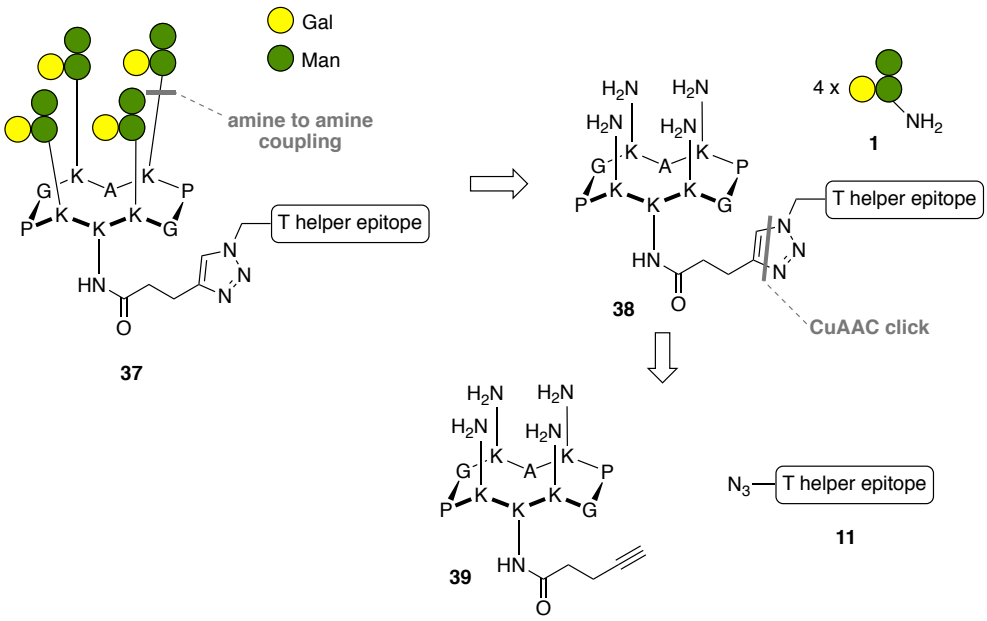
SCHEME 3.5. Retrosynthetic analysis of T helper epitope **11**.

Similarly, assembly of the Val-Cit linker **28** should follow literature known protocols[94, 182] and include installation of an azide polyethyleneglycol (PEG) spacer **30** at dipeptide **31** (see SCHEME 3.6) to improve the solubility of the construct. The preparation of α-GalCer derivative **29** was planned in accordance to literature precedent,[89] albeit with additional late-stage introduction of cerotic acid **32** to give compound **33** (see SCHEME 3.6). This should improve the handling of the intermediate compounds during work-up and purification by providing better solubility. The synthesis of precursor **34** should use readily available phytosphingosine dervative **35** and *N*-phenyltrifluoroacetimidate (PTFAI) donor **36**, based on the work of *Cavallari et al*.[89]



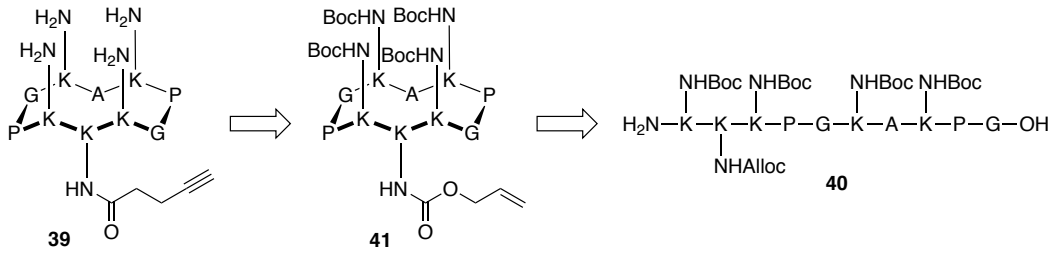
SCHEME 3.6. Retrosynthetic analysis of the α-GalCer derivative **29** and the Val-Cit linker **28**.

To allow for the preparation of a multivalent glycoconjugate vaccine candidate **37**, an additional cyclic decapeptide (RAFT)[183] should be used to enable attachment of four copies of the corresponding anti-leishmanial B cell epitope (see SCHEME 3.7). Therefore, we envisaged a synthetic approach, which would link the carbohydrate epitope to the vaccine construct *via* an amine to amine coupling in the last step (see SCHEME 3.7). This way, construct **38** comprised of RAFT and α-GalCer derivative could also be used as versatile basis for the assembly of other vaccine candidates. For the preparation of the required α-GalCer-RAFT conjugate **38** we planned a CuAAC reaction between alkyne-bearing cyclic decapeptide **39** and azide-bearing α-GalCer derivative **11** (see SCHEME 3.7).



SCHEME 3.7. Retrosynthetic analysis of a fully synthetic multivalent glycoconjugate vaccine candidate comprised of a cyclic decapeptide, an α-GalCer derivative and the trisaccharide α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* **1**.

For this purpose, the four side chain amine residues of the cyclic decapeptide need to be deprotected prior to conjugation (see SCHEME 3.8) in order to avoid undesired acyl migration of the ceramide moiety of α-GalCer under acidic deprotection conditions (see CHAPTER 1.2.3).[94]



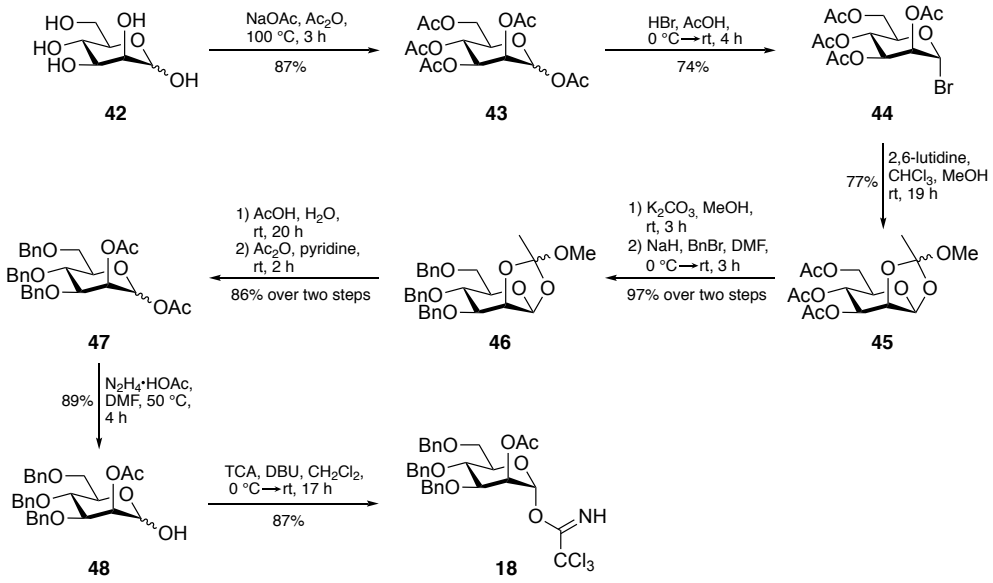
SCHEME 3.8. Retrosynthetic analysis of cyclic decapeptide **38**.

The envisioned synthetic approach toward RAFT **39** follows the work of *Dumy and co-workers*[183] and should proceed *via* solid-phase peptide synthesis (SPPS) of linear amino acid sequence **40**, ensued by intramolecular cyclization reaction with subsequent orthogonal lysine side chain deprotection at the ‚lower' side of compound **41**. Installation of an alkyne moiety for the CuAAC reaction and cleavage of the remaining boc-protecting groups (see SCHEME 3.8) would then afford the desired compound **39**.

3.2 SYNTHESIS OF THE MONOSACCHARIDE BUILDING BLOCKS

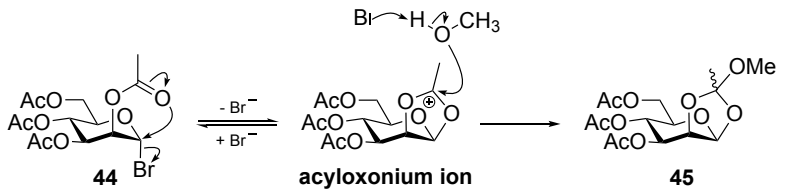
3.2.1 Synthesis of Mannosyl donor 18

The synthesis of mannosyl donor **18** was carried out over eight steps according to a literature known procedure (see SCHEME 3.9).[181, 184] The synthesis commenced with the peracetylation of commercially available D-mannose **42** in a mixture of sodium acetate and acetic anhydride[185] to furnish pentaacetate **43** in a very good yield of 87% and as a 7:3 mixture of α/β-anomers.



SCHEME 3.9. Synthesis of mannosyl donor **18** over eight steps starting from commercially available D-mannose.[181, 184]

The subsequent bromination at the anomeric center of pentaacetate **43** was carried out in a solution of hydrogen bromide (33%) in conc. acetic acid. The anomeric effect as well as the anchimeric assistance of the acetate protecting group at C-2 led to the stereoselective formation of α-bromide **44**.[186] The latter was quickly purified over a short pad of silica, as it decomposes rapidly during flash column chromatography. Bromide **44** was immediately dissolved together with 2,6-lutidine in a 1:1 mixture of methanol and chloroform in order to obtain orthoester **45**.[187] Thereby, the nucleophilic attack of methanol at the intermediate acyloxonium ion provided the diasteriomeric mixture of compound **45** (see SCHEME 3.10).[188]

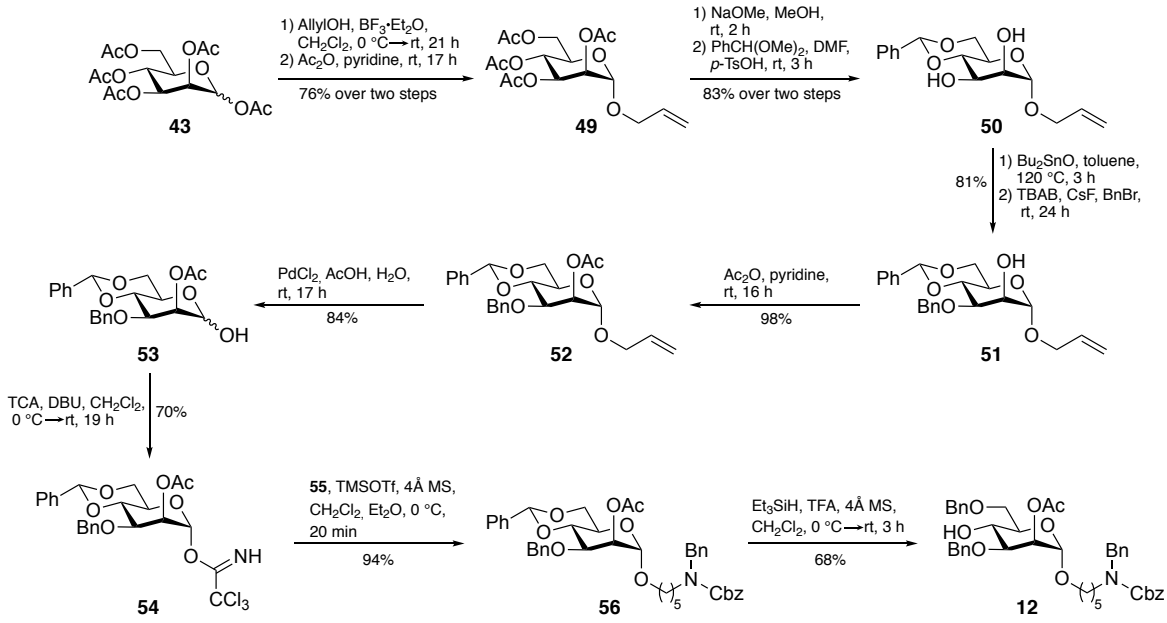


SCHEME 3.10. Mechanism of the orthoester formation.[188]

After recrystallization, the desired orthoester **45** was isolated in 56% yield (exo/endo 92:8) over two steps. Afterwards, the base-stable orthoester allowed for the selective removal of the acetate protecting groups with potassium carbonate in methanol, resulting in quantitative formation of the corresponding triol, which was benzylated with sodium hydride and benzyl bromide in DMF to provide compound **46** in an excellent yield of 97% over two steps.[184] Acidic hydrolysis of orthoester **46** with aqueous acetic acid gave a mixture of 1- and 2-acetates.[189, 190] However, reacetylation of the free hydroxyl groups in pyridine and acetic anhydride produced the desired compound **47** as a mixture of α/β anomers in 86% yield over two steps. Subsequent selective deprotection of the anomeric center with hydrazinium acetate afforded α/β-lactol **48** in a very good yield of 89%.[191, 192] Finally, the pure α-mannosyl donor **18** was obtained in 87% yield after treatment of hemiacetal **48** with trichloroacetonitrile (TCA) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[191, 193, 194] In summary, compound **18** was afforded over nine steps in 32% overall yield and was ready to use for the following glycosylation reactions.

3.2.2 Synthesis of Mannosyl acceptor 12

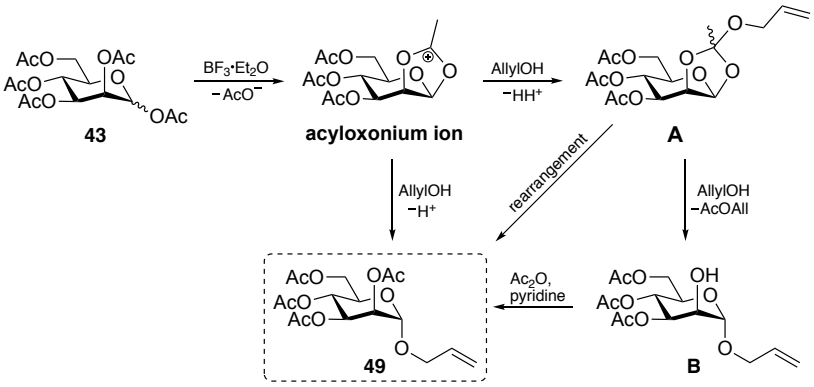
Mannosyl acceptor **12** was prepared over nine steps according to literature precedent,[160, 161, 195] applying an allyl group as an orthogonal temporary protecting group at the anomeric center (see SCHEME 3.11). Besides, a shortened seven-step synthesis,[196, 197] using a thioglycoside as glycosyl donor, was also carried out (see SCHEME 3.18). The longer literature known procedure started with readily available pentaacetate **43**, which was reacted with allylic alcohol in the presence of boron trifluoride diethyl etherate ($BF_3 \cdot Et_2O$) in order to obtain allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside **49**.[198, 199]



SCHEME 3.11. Synthesis of mannosyl acceptor **12** over eigth steps according to literature precedent.[160, 161, 195]

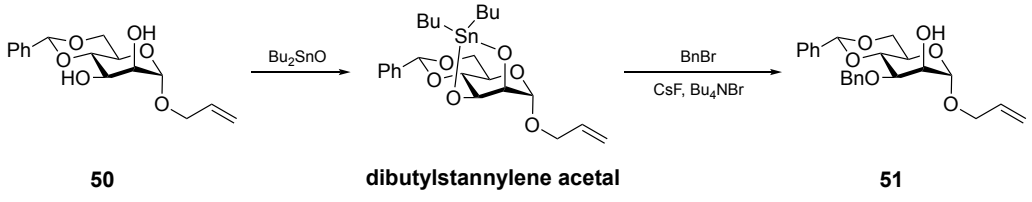
However, in the course of this reaction, the formation of several by-products was observed, including *inter alia* in accordance to *Khamsi et al.*[199] the allyl glycoside deacetylated at C-2 (see SCHEME 3.12).[200-202] Presence of the latter suggested deacetylation to be a main problem of the reaction, eventually leading to a

complex product mixture due to acyl migration. As a consequence, subsequent reacetylation of the crude product in a mixture of acetic anhydride and pyridine was necessary in order to increase the yield and to abolish almost completely the by-products.[199] Moreover, due to the participatory effect, allyl glycoside **49** was exclusively obtained as the α-product in 76% yield. Subsequent cleavage of the acetate protecting groups was carried out under mild *Zemplén* conditions[203] with catalytic amounts of sodium methanolate in methanol.[204] The resulting crude tetrol was obtained in quantitative yield and was used in the next step without further purification. Thus, transacetalization with equimolar amounts of benzaldehyde dimethylacetal and catalytic amounts of *p*-toluenesulfonic acid in DMF furnished 4,6-*O*-benzylidene derivative **50** regioselectively and in a very good yield of 83% over two steps.[205, 206] However, the use of excess amounts of benzaldehyde dimethylacetal is not recommended, as otherwise the additional 2,3-*O*-benzylidene protection might occur. The rationale for the introduction of a 4,6-*O*-benzylidene acetal is the possibility of a selective 4-deprotection in the last step of the synthesis.



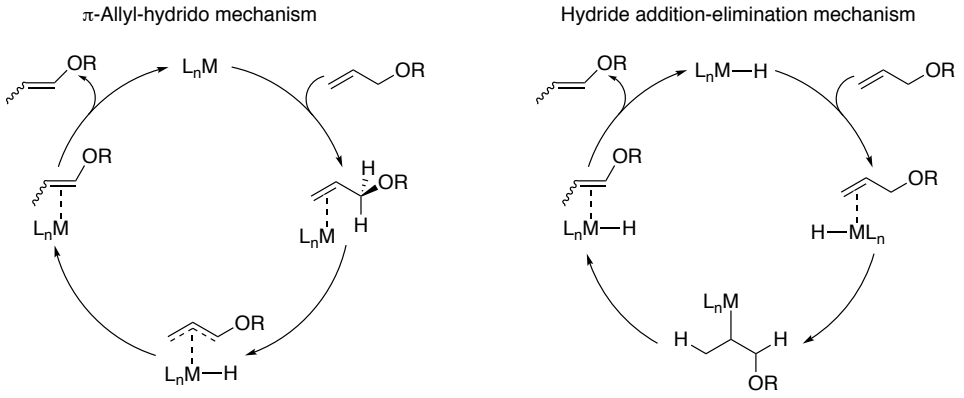
SCHEME 3.12. Nucleophilic attack of the allylic alcohol at the intermediate acyloxonium ion can either lead to the desired compound **49** or to the corresponding orthoester (**A**), which on the one hand can rearrange to afford allyl glycoside **49** or on the other hand can react with allylic alcohol to give deacetylated compound (**B**). (Adapted from ref.[199])

Afterwards, the equatorial hydroxyl group at C-3 was selectively benzylated by treatment of compound **50** with dibutyltin oxide (Bu_2SnO) in toluene and subsequent alkylation of the resulting 2,3-*O*-dibutylstannylene with benzyl bromide in the presence of tetrabutylammonium bromide (TBAB) and cesium fluoride (see SCHEME 3.13).[207, 208] Here, as proposed by *Dong et al.*,[209] the preferential cleavage of the equatorial Sn-O bond of the formed dibutylstannylene acetal might be controlled by stereoelectronic effects and the subsequent alkylation is probably influenced by the nucleophilicity of the intermediate oxygen species. The suggested mechanism proceeds *via* coordination of a halide atom to the tetracoordinated tin atom, which then presumably enhances the subsequent cleavage of the stannylene acetal ring.[210, 211] Afterwards, the obtained reactive oxygen anion could attack the electrophile in a S_N2 mode, leading to the alkylated hydroxyl group. Final cleavage of the remaining Sn-O bond would then give the 3-*O*-benzylated compound.



SCHEME 3.13. Regioselective formation of 3-*O*-benzylated compound **51** *via* a dibutylstannylene acetal.

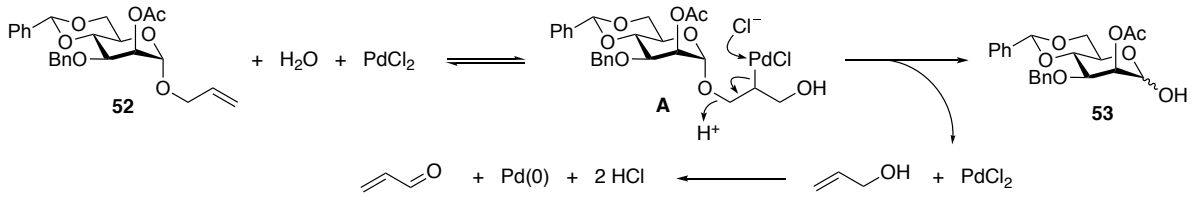
Thus, selectively protected compound **51** was obtained in 81% yield. Afterwards, the hydroxyl group at C-2 had to be protected in an orthogonal fashion in order to enable a selective deprotection at this position at a later point of the synthesis. Fully protected compound **52** was afforded by acetylation of the free hydroxyl group in a mixture of acetic anhydride and pyridine. This reaction proceeded smoothly and gave **52** in an excellent yield of 98%. Subsequent anomeric deallylation, which was necessary for the transformation into a glycosyl trichloroacetimidate, was carried out in accordance to a literature known protocol.[161] Therefore, allyl glycoside **52** was first mixed with sodium acetate and an excess amount of palladium(II) chloride, before the mixture was suspendend in aqueous acetic acid.[161, 212] This reaction mixture was stirred for 17 hours at room temperature to furnish lactol **53** in a very good yield of 84% and as a mixture of α/β anomers.



SCHEME 3.14. Proposed mechanisms for the transition metal catalyzed isomerization of allylic double bonds.[213]

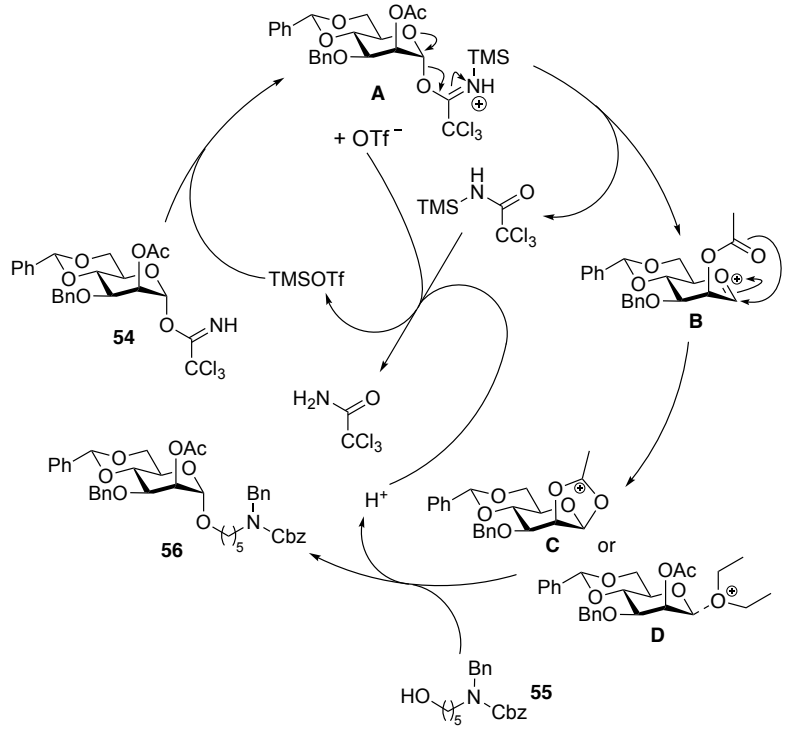
Usually, the anomeric deallylation requires catalytic amounts of a transition metal and proceeds *via* a two step reaction sequence, which is initiated by a transition metal catalyzed double-bond migration followed by acid-catalyzed hydrolysis.[213] Common catalysts for the isomerization are for example tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$)[214] or (1,5-cyclooctadien)bis(methyldiphenylphosphin)-iridium(I) hexafluorophosphate ($[Ir(COD)(PMePh_2)_2]PF_6$),[215, 216] which presumably lead to the formation of a π-allyl-hydrido intermediate and a subsequent 1,3-hydrogen shift (see SCHEME 3.14).[213] Furthermore, metal hydride complexes such as chlorohydridotris(triphenylphosphine)ruthenium(II) ($RuHCl(PPh_3)_3$)[217] or tris(triphenylphosphine)rhodium(I) chloride ($[Rh(PPh_3)]Cl$)[218] can be applied, which might lead to isomerization of the allylic residue *via* an addition-elimination mechanism. In this case, the metal hydride adds to the double bond and forms a prop-1-enyl ether by β-hydrogen elimination (see SCHEME 3.14).[213]

In contrast, the use of equimolar or excess amounts of palladium(II) chloride has been reported to directly afford the free alcohol.[212, 213] Several different possible mechanisms have been suggested for this method, which has been widely applied in carbohydrate chemistry.[213] For instance, one proposed mechanism involves an *anti*-Markovnikov hydroxypalladation (**A**) followed by β-alkoxy cleavage (see SCHEME 3.15). The released allylic alcohol would then be able to reduce palladium(II) chloride to palladium(0) under the formation of acrolein.[213, 219]



SCHEME 3.15. Possible mechanism for the deallylation of the anomeric center with an excess amount of $PdCl_2$.[213]

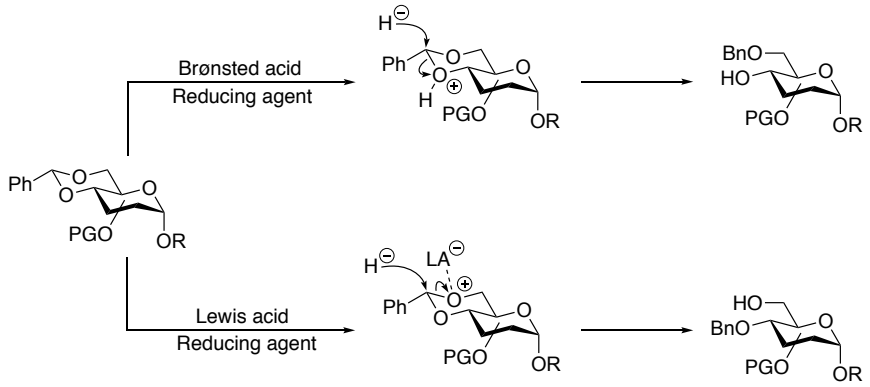
Subsequent treatment of compound **53** with TCA in the presence of DBU furnished α-mannosyl trichloroacetimidate **54** in 70% yield,[161, 193] which served to introduce protected aminopentyl linker **55** at the anomeric center *via Schmidt* glycosylation.[193] Therefore, donor **54** had to be activated by catalytic amounts of a Brønsted or Lewis acid.



SCHEME 3.16. Mechanism for glycosylation of mannosyl donor **54** with amine linker **55** under *Schmidt* conditions.

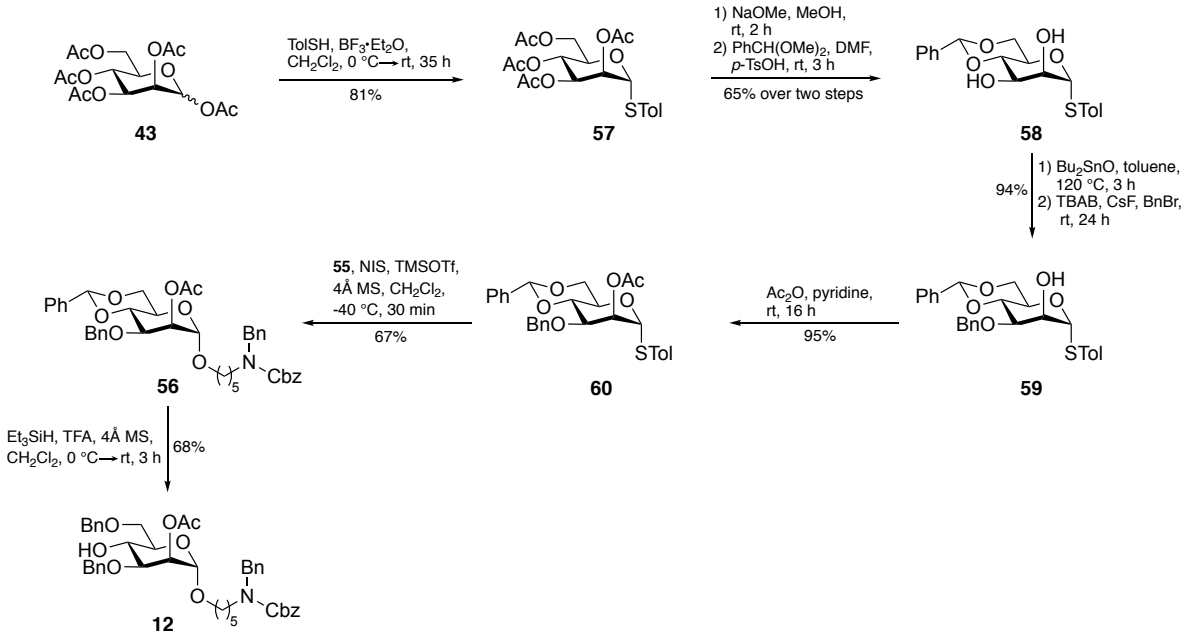
Common promotor acids for glycosyl imidates are for instance trimethylsilyl triflate (TMSOTf), $BF_3 \cdot Et_2O$, triflic acid (TfOH) or silver triflate (AgOTf).[220] For the glycosylation of mannosyl donor **54** with aminopentyl linker **55** in a 1:1 mixture of dichloromethane and diethyl ether, catalytic amounts of TMSOTf were employed at 0 °C. Thereby, TMSOTf initially activates the trichloroacetimidate (**A**) and leads to the formation of an intermediate oxocarbenium ion (**B**) upon release of nonbasic trichloroacetamide (see SCHEME 3.16). The subsequent attack of the amine linker can only take place in the axial position, as the equatorial position is blocked due to the participatory effect of the acetate protecting group at C-2 (**C**). Furthermore, the α-directing solvent effect[221] of diethyl ether (**D**) aids to increase the desired α-diastereoselectivity of the glycosylation reaction and as a consequence compound **56** was obtained in an excellent yield of 94%. The transformation of compound **56** into a suitable glycosyl acceptor for the subsequent galactosylation required the regioselective opening of the 4,6-*O*-benzylidene acetal to liberate the primary benzyl ether. A plethora of regioselective

4,6-*O*-benzylidene acetal opening methods are available,[222] whereby the selection of the acid (Brønsted or Lewis acid) strongly influences the regioselective outcome of the reaction. For instance, a Brønsted acid is usually applied in order to liberate the 4-position, as the acid protonates the more basic oxygen at C-4 and after hydride transfer by a reducing agent furnishes the primary benzyl ether (see SCHEME 3.17).



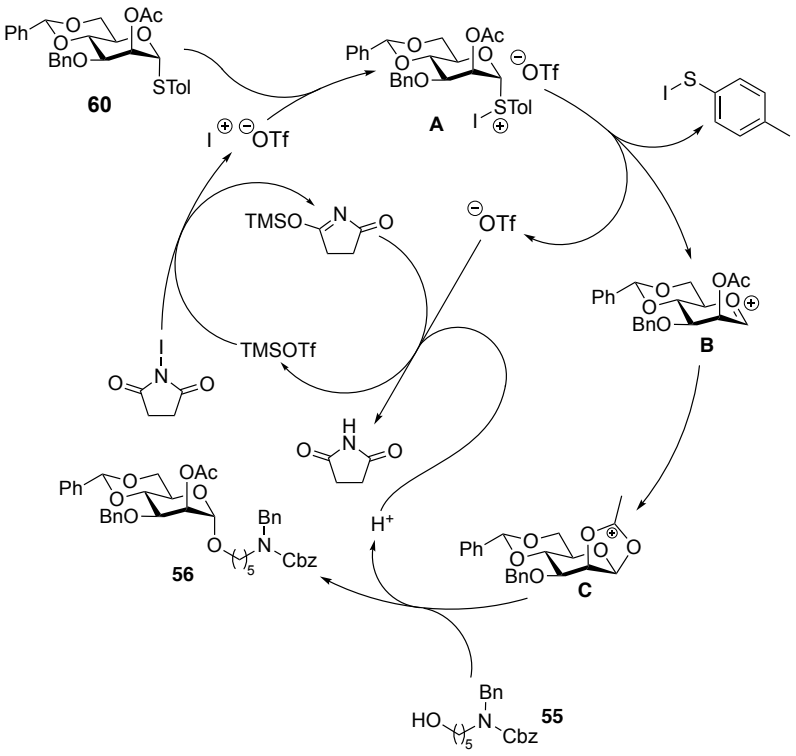
SCHEME 3.17. Mechanism of the regioselective benzylidene opening, either leading to the secondary or primary benzylated hydroxyl group.

In contrast, a sterically demanding Lewis acid prefers coordination of the less hindered oxygen at C-6, which then affords the secondary benzyl ether after hydride transfer by a reducing agent. First mechanistic studies about the reductive regioselective opening of such acetals were carried out by *Garreg et al.*,[223] using hydrogenchloride as the Brønsted acid and sodium cyanoborohydride as the reducing agent. Further intensive studies, shedding light on the influence of the solvent and the nature of the reducing agent on the regioselectivity of Lewis acid-driven acetal openings were carried out by *Ellervik and co-workers*.[222, 224, 225]
In our case, a method described by *DeNinno et al.*[226] was applied, using the Brønsted acid trifluoroacetic acid (TFA) and triethylsilane (Et_3SiH) as reducing agent for the regioselective opening of the 4,6-*O*-benzylidene acetal of **56**[161] to produce glycosyl acceptor **12** in a yield of 68%.



SCHEME 3.18. Synthesis of mannosyl acceptor **12** over a shortened six-step synthetic route.

A second synthetic route using a thioglycoside as anomeric protecting group was also investigated, since it shortened the synthesis of mannosyl acceptor **12** by two steps (see SCHEME 3.18). Thioglycosides can be used as glycosyl donors and thus should allow direct incorporation of aminopentyl linker **55**, which would avoid the excessive use of expensive palladium(II) chloride for the anomeric deallylation. In the first step of this improved route, pentaacetate **43** was reacted with *p*-thiocresol in the presence of $BF_3 \cdot Et_2O$ to afford thioglycoside **57** in 81% yield and exclusively as the α-anomer.[56] Following *Zemplén* transesterification[203] with catalytic amounts of sodium methanolate in methanol led to the cleavage of the acetate protecting groups. The resulting tetrol was stirred in DMF with equimolar amounts of benzaldehyde dimethylacetal and catalytic amounts of *p*-toluenesulfonic acid to give 4,6-*O*-benzylidene derivative **58** in 65% yield over two steps.[196] Selective benzylation of the equatorial hydroxyl group at C-3 was carried out in accordance to the above described procedure with dibutyltin oxide in toluene and subsequent addition of benzyl bromide in the presence of TBAB and cesium fluoride, which afforded compound **59** in excellent 94% yield.[197] Acetylation of the remaining unprotected hydroxyl group was carried out in a mixture of acetic anhydride and pyridine to provide thioglycoside **60** in 95% yield. Subsequent α-selective glycosylation with aminopentyl linker **55** proceeded in the presence of *N*-iodosuccinimide (NIS) and TMSOTf in dichloromethane at −40 °C and furnished the desired glycoside **56** in 67% yield.[227]



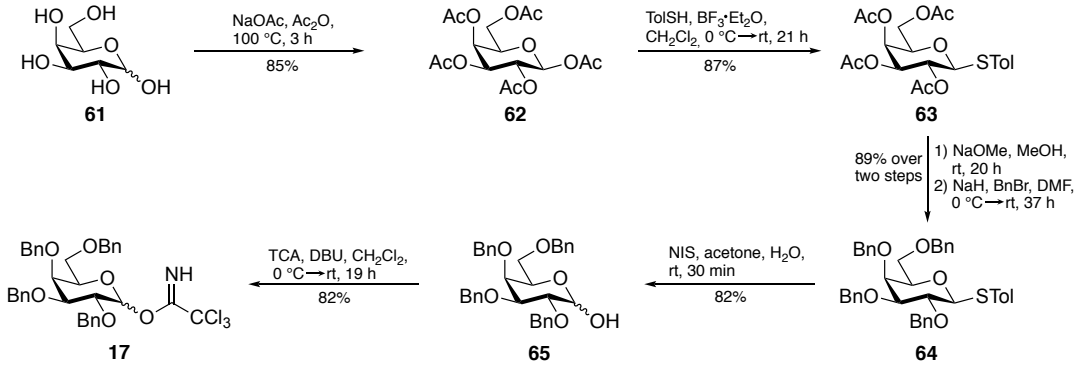
SCHEME 3.19. Mechanism for glycosylation of thiodonor **60** and aminopentyl linker **55** with NIS/TMSOTf.

Thioglycoside activation with NIS in the presence of a suitable acidic promotor was first reported by *van Boom and co-workers*[228] and soon afterwards by *Fraser-Reid and co-workers*.[229] However, a wide range of promotor systems including NIS/$Sn(OTf)_2$,[230] Ph_2O/Tf_2O[231] or Me_2S_2/Tf_2O[232] can be used for the activation of thioglycosides, with all methods requiring at least stoichiometric amounts of the activating reagent (see SCHEME 3.19).[220] For instance, TMSOTf activates NIS and enables the formation of an intermediate

iodonium ion, which can be attacked by the sulfur atom of the thioglycoside. This leads to the formation of an oxocarbenium ion (**B**) and a *p*-toluenesulfenyl iodide. In the presence of an acetate protecting group at C-2, the equatorial position of the oxocarbenium ion can be blocked *via* the formation of an acyloxonium ion (**C**), enabling attack of aminopentyl linker **55** solely in the axial position. This eventually leads to the selective formation of the α-configured glycosidic product. Subsequently, mannosyl acceptor **12** was obtained in 68% yield by regioselective opening of the 4,6-*O*-benzylidene acetal using TFA and Et_3SiH in dichloromethane.[161] Compound **12** represents a key building block for the following preparation of the native and fluorinated oligosaccharides and was afforded either *via* a nine-step synthetic route in 19% overall yield or *via* a shorter time-saving seven-step synthesis in 21% overall yield.

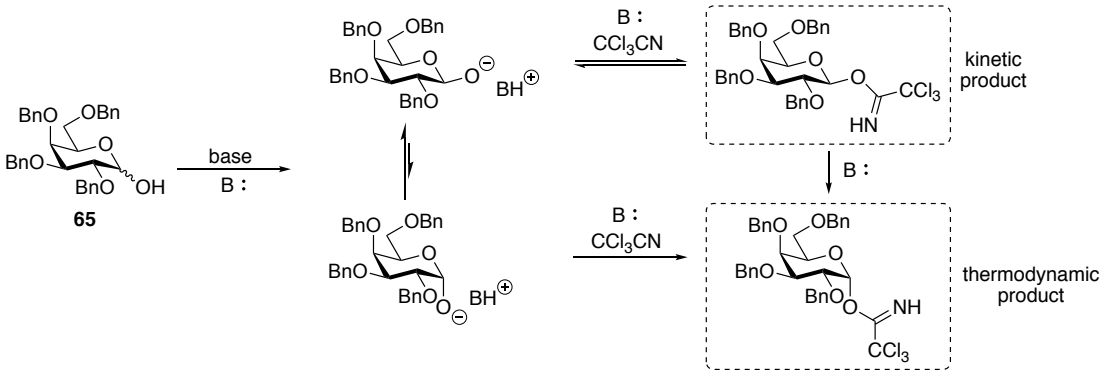
3.2.3 Synthesis of the native Galactosyl donor 17

Galactosyl trichloroacetimidate **17** was prepared over a short six-step synthetic route according to literature known procedures (see SCHEME 3.20).[56, 180, 233-235] In the beginning, peracetylation of commercially available D-galactose **61** with sodium acetate and acetic anhydride furnished penta-*O*-acetyl-β-D-galactopyranoside **62** in 85% yield.[233]



SCHEME 3.20. Synthesis of native galactosyl donors **64** and **17** starting from commercially available D-galactose.

The transformation into a thioglycoside was achieved by reacting pentaacetate **62** with *p*-thiocresol in the presence of $BF_3 \cdot Et_2O$.[234] Thereby, the participatory effect of the acetate protecting group at C-2 led to clean formation of the β-configured product **63**, which was obtained in 87% yield. Afterwards, *Zemplén* transesterification[203] using catalytic amounts of sodium methanolate in methanol and subsequent benzylation of the liberated hydroxyl groups with sodium hydride and benzyl bromide in DMF furnished thiodonor **64** in excellent 89% yield over two steps.[56] Hydrolysis of thioglycoside **64** was accomplished with NIS in aqueous acetone, which gave lactol **65** in 82% yield and as an anomeric mixture.[235]



SCHEME 3.21. Formation of the kinetically controlled β-trichloroacetimidate and the thermodynamically controlled α-trichloroacetimidate.

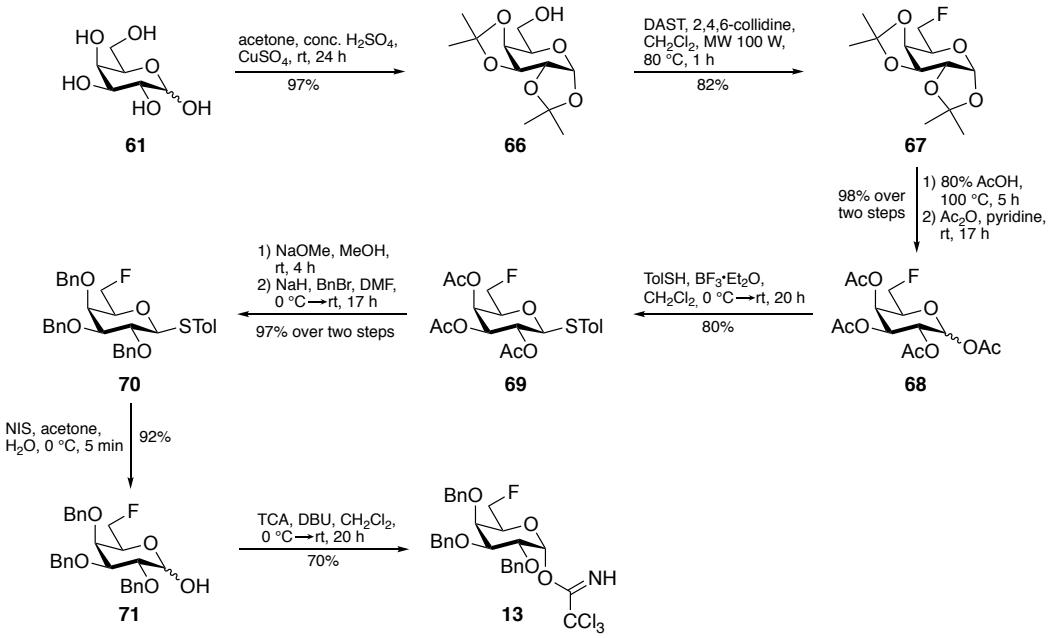
Finally, treatment of hemiacetal **65** with TCA in the presence of DBU afforded galactosyl trichloroacetimidate **17** in 82% yield as a 2:1 anomeric mixture.[180, 193] However, the stereochemical outcome can be usually influenced by reaction conditions, e.g. the choice of base. For instance, *Schmidt et al.*[194] reported that the

base-catalyzed formation of the β-trichloroacetimidate is kinetically preferred due to a fast but reversible addition of the equatorial alkoxide to trichloroacetonitrile (see SCHEME 3.21). However, slow base-catalyzed anomerization of the β-product leads to the thermodynamically favored α-trichloroacetimidate, which is controlled by the anomeric effect. Hence, longer reaction times and stronger bases such as sodium hydride or DBU, which rapidly catalyze the anomerization, lead to the formation of the thermodynamic product. In contrast, weaker bases such as potassium carbonate or cesium carbonate preferentially lead to the formation of the kinetic product, as these bases slowly catalyze the anomerization from β to α.[194]

This short reaction sequence provided galactosyl imidate **17** over six steps in 44% overall yield. Galactosyl trichloroacetimidate **17** and thiodonor **64** represent useful model compounds for subsequent glycosylation studies in order to find the best donor, activator and reaction conditions for the preparation of the native and fluorinated disaccharides.

3.2.4 Synthesis of the 6F-Galactosyl donor 13

The preparation of C-6 fluorinated galactosyl donor **13** was carried out over nine steps according to literature known procedures (see SCHEME 3.22).[178, 180] In order to enable a selective deoxofluorination at the 6-position, the synthesis commenced with protection of the secondary hydroxyl groups *via* isopropylidene acetals.



SCHEME 3.22. Synthesis of 6F-galactosyl donor **13** over eight steps.

This was achieved by stirring commercially available D-galactose **61** in a mixture of anhydrous copper(II) sulfate in acetone with catalytic amounts of sulfuric acid, which gave 1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranoside **66** in an excellent yield of 97%.[205] The remaining free primary hydroxyl group was then deoxofluorinated *via* a nucleophilic substitution reaction. Common nucleophilic fluorination reagents include the organosulfur compounds diethylaminosulfur trifluoride (DAST®),[236] bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor®)[237] and diethylaminodifluorosulfinium tetrafluoroborate (XtalFluor-E®)[238] (see

FIGURE 3.1). The deoxofluorination of compound **66** was carried out with DAST® and 2,4,6-collidine in dichloromethane under microwave irradiation (100 W, 80 °C).[178]

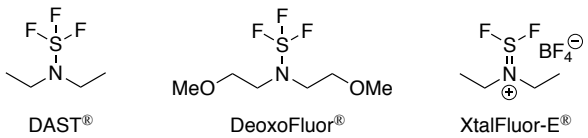
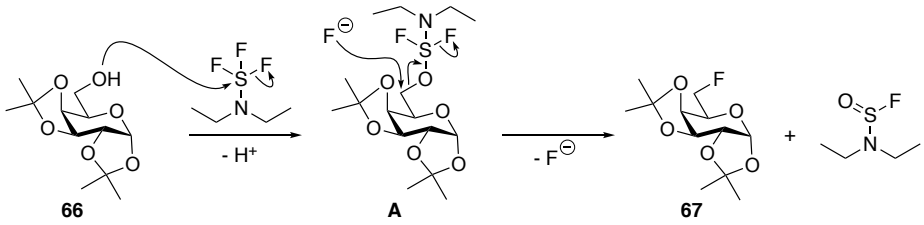


FIGURE 3.1. Nucleophilic fluorination reagents DAST®, Deoxo-Fluor® and XtalFluor-E®.

This reaction proceeded smoothly and gave fluorinated galactose **67** in a very good yield of 82%. Thereby, a nucleophilic attack of the primary alcohol at the sulfur atom leads to the liberation of a fluoride anion (see SCHEME 3.23).[239] The newly formed leaving group in intermediate (**A**) is then replaced by a fluorine atom *via* a S_N2-mechanism under the formation of desired compound **67**.

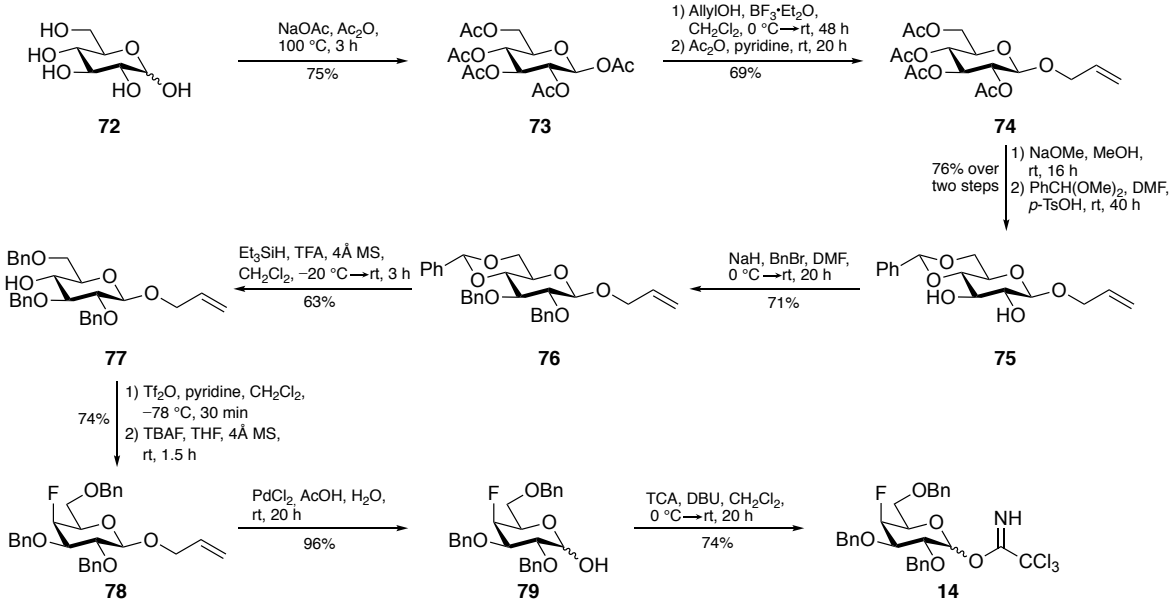


SCHEME 3.23. Mechanism of the deoxofluorination with DAST®.

Subsequently, the isopropylidene acetals of fluorinated compound **67** were removed in aqueous acetic acid (80%) and the unprotected hydroxyl groups were acetylated in a mixture of acetic anhydride and pyridine. Compound **68** was obtained as an anomeric mixture in excellent 98% yield over two steps.[178] Then, transformation into thioglycoside **69** was achieved by stirring fluorinated tetraacetate **68** with *p*-thiocresol and $BF_3 \cdot Et_2O$ in dichloromethane. Thereby, the participatory effect of the acetate protecting group at C-2 led exclusively to the β-anomer, which was obtained in 80% yield. Following deacetylation under *Zemplén* conditions[203] and ensuing benzylation of the quantitatively obtained triol with sodium hydride and benzyl bromide in DMF afforded thioglycoside **70** in an excellent yield of 97% over two steps. Finally, the anomeric center was deproteced with NIS in aqueous acetone (92%)[235] and the resulting lactol **71** was transformed into trichloroacetimidate **13** with TCA in the presence of DBU[180, 193] to provide α-glycosyl donor **13** in 70% yield. In this way, the desired fluorinated galactosyl donor was obtained over nine steps in 39% yield for subsequent preparation of the 6-fluorinated trisaccharide.

3.2.5 Synthesis of the 4F-Galactosyl donor 14

The synthesis of 4-fluorinated galactosyl donor **14** was carried out over nine steps in accordance to literature known procedures (see SCHEME 3.24). The key fluorination proceeded *via* nucleophilic displacement at C-4 of glucose with epimerization to obtain a 4F-galactose derivative. Thus, the synthesis started with the peracetylation of commercially available D-glucose **72** using sodium acetate in acetic anhydride, which afforded pentaacetate **73** in 75% yield.[240]

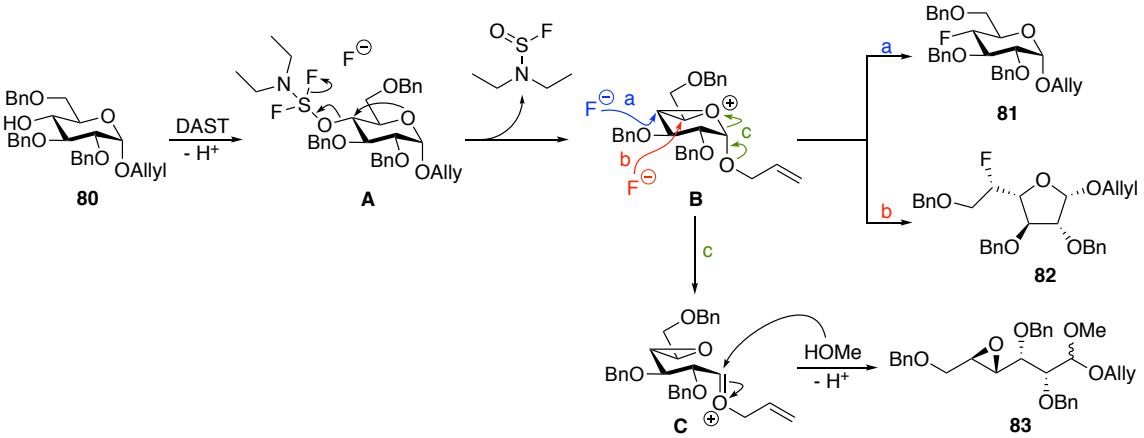


SCHEME 3.24. Synthesis of fluorinated galactosyl donor **14** over nine steps.

Transformation into allyl glycoside **74** was achieved by stirring compound **73** with $BF_3 \cdot Et_2O$ in dichloromethane, followed by reacetylation of transiently incomplete protected by-products in acetic anhydride and pyridine (see SCHEME 3.12).[199, 240] The anchimeric effect again led to β-linked compound **74** in 69% yield. Ensuing *Zemplén* transesterification[203] using catalytic amounts of sodium methanolate in methanol afforded the crude tetrol, which was selectively converted into 4,6-*O*-benzylidene derivative **75** in 76% yield by using a mixture of equimolar amounts of benzaldehyde dimethylacetal in DMF and catalytic amounts of *p*-toluenesulfonic acid.[241] The remaining free hydroxyl groups were benzylated in a mixture of sodium hydride and benzyl bromide in DMF,[242] which led to fully protected compound **76** in 71% yield. The selective opening of the 4,6-*O*-benzylidene acetal of compound **76** was accomplished according to the above described procedure with Et_3SiH and TFA to give primary benzyl ether **77** in 63% yield.[179] This compound provided the possibility to selectively deoxofluorinate the 4-position under epimerization in order to obtain the fluorinated galactose derivative **78**.

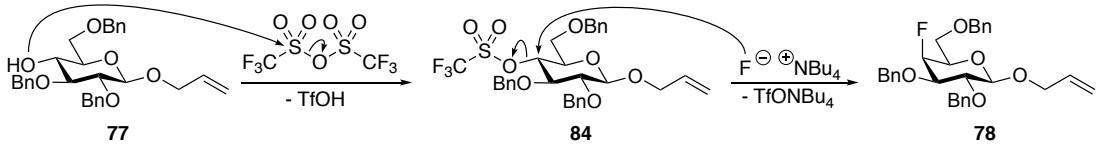
However, the already described fluorination procedure with DAST® had to be avoided, as *Liang and co-workers*[243] described that fluorination of compound **80** with DAST® does not lead to the desired fluoro-galactose (see SCHEME 3.25). In their experiments they only observed the formation of 4-fluorine glucopyranose **81** or 5-fluorine altrofuranose **82** as well as an additional epoxide derivative **83** in polar solvents. Due to the isolated epoxide derivative, they proposed a mechanism that might lead to these by-

products *via* the formation of a bicyclic oxiranium ion intermediate (**B**) (see SCHEME 3.25). A nucleophilic attack of a fluoride anion at C-4 of intermediate (**B**) might then lead to the formation of compounds **81** and **82**. Furthermore, using methanol as scavenger reagent could then lead to epoxide **83** *via* intermediate (**C**).[243]



SCHEME 3.25. Mechanism for the formation of by-products during fluorination of **80** with DAST® proposed by *Liang and co-workers*.[243]

Thus compound **77** was transformed into fluorinated galactose derivative **78** *via* the formation of an intermediate triflate. Therefore, secondary alcohol **77** was stirred at −78 °C with triflic anhydride (Tf_2O) and pyridine in dichloromethane to obtained triflate **84**, which was reacted immediately with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (see SCHEME 3.26). This furnished fluorinated compound **78** in a good yield of 74% yield.[179]



SCHEME 3.26. Introduction of the axial fluorine substituent with TBAF and triflate **84**.

Subsequent deallylation proceeded smoothly in a mixture of sodium acetate and excess amounts of palladium(II) chloride in aqueous acetic acid.[161] Hemiacetal **79** was obtained in excellent 96% yield as an anomeric mixture. Finally, the desired 4-fluorinated galactosyl donor **14** was afforded in 74% yield (α:β = 2.8:1) after treatment of lactol **79** with TCA in the presences of DBU.[180, 193]

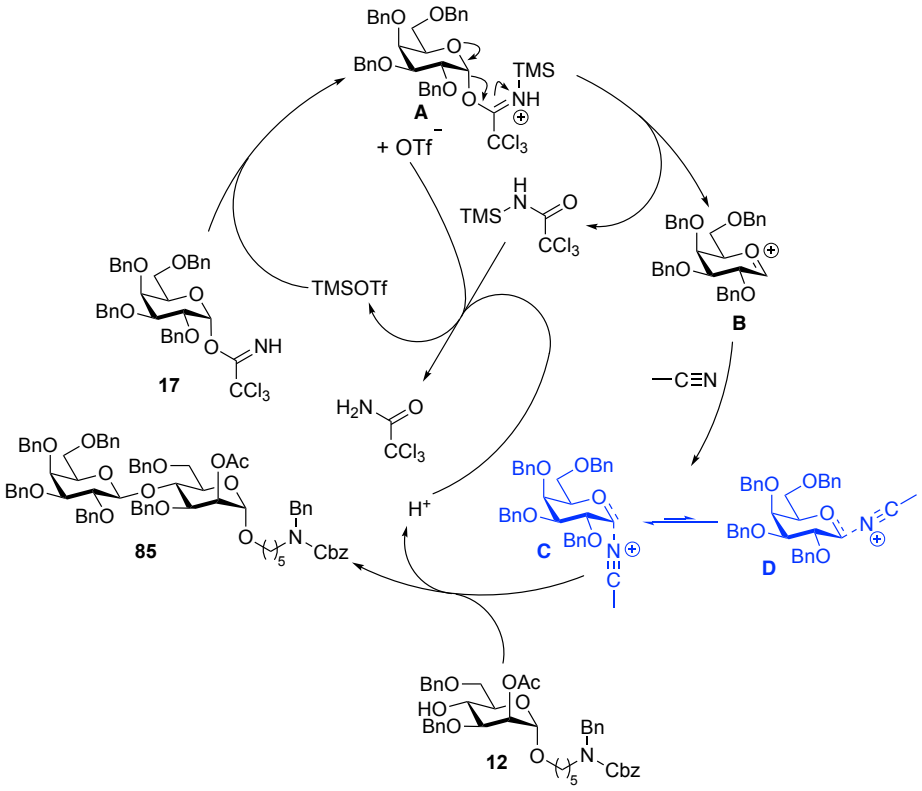
In conclusion, this procedure gave fluorinated donor **14** over a nine-step synthesis in 9% overall yield and was ready to use for the preparation of the corresponding disaccharide.

3.3 SYNTHESIS OF THE DISACCHARIDES

The first step toward the native trisaccharide **1** and its fluorinated derivatives **6–9** required the assembly of the β-D-Gal*p*-(1→4)-α-D-Man*p* subunits *via* glycosylation of galactosyl donors **13–17** with selectively protected mannosyl acceptor **12**. Therefore, an efficient β-selective glycosylation procedure for the preparation of the disaccharides had to be developed, which however turned out to be very challenging due to the donors' lack of participating neighboring groups at C-2. The optimization of the glycosylation conditions was carried out with readily accessable galactosyl trichloroactimidate **17** and thiodonor **64**.

3.3.1 Synthesis of the native Disaccharide

A first attempt to accomplish the β-selective glycosylation employed galactosyl trichloroacetimidate **17** and mannosyl acceptor **12** under *Schmidt* conditions,[193] by dissolving both compounds in a mixture of dichloromethane and acetonitrile at −78 °C, before catalytic amounts of TMSOTf were added as reaction promotor.



SCHEME 3.27. Formation of disaccharide **85** using the nitrile solvent effect to advantage.

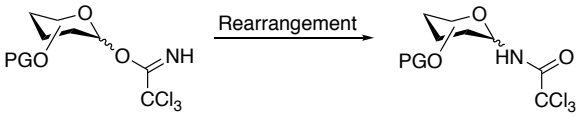
Thereby, the use of acetonitrile should compensate the missing anchimeric assistance of a neighboring group at C-2 due to its β-directing solvent effect.[244] This so-called „nitrile effect" can be attributed to the formation of a stabilizing α-nitrilium ion (**C**) at low temperatures (see SCHEME 3.27). These nitrilium ions, which are formed by coordination of an acetonitrile solvent molecule to an oxocarbenium ion (**B**), are supposed to block

the axial position preferentially at lower temperatures and the equatorial position at higher temperatures. As the reaction temperature was set to −78 °C, the α-nitrilium ion should be formed and thus only allow for an equatorial nucleophilic attack of the acceptor. Although, thin-layer chromatography indicated rapid disappearance of donor **17** under these reaction conditions only formation of undesired by-products was observed. More importantly, large amounts of mannosyl acceptor **12** remained unreacted. Consequently, in a second experiment, extra amounts of donor **17** were added slowly to the reaction mixture at −78 °C. Eventually, this led to the desired consumption of mannosyl acceptor **12** and furnished disaccharide **85** in 67% yield (see TABLE 3.1).

TABLE 3.1. Investigation of suitable reaction conditions for the preparation of disaccharide **85**.

entry	donor	conditions	yield (%)
1	**17**	donor (2.3 equiv.), acceptor **12** (1.0 equiv.), TMSOTf (0.2 equiv.), 4Å MS, −78 °C, 30 min, CH_2Cl_2/MeCN (4:1)	67
2	**17**	inverse conditions: donor (1.3 equiv.), acceptor **12** (1.0 equiv.), TMSOTf (0.2 equiv.), 4Å MS, −78 °C, 30 min, CH_2Cl_2/MeCN (3:1)	73
3	**64**	donor (1.1 equiv.), acceptor **12** (1.0 equiv.), NIS (1.1 equiv.), TMSOTf (0.3 equiv.), 4Å MS, −50 °C, 30 min, CH_2Cl_2/MeCN (3:1)	40[245]
4	**64**	donor (1.1 equiv.), acceptor **12** (1.0 equiv.), NIS (1.1 equiv.), AgOTf (1.1 equiv.), 4Å MS, −55 °C, 30 min, CH_2Cl_2/MeCN (3:1)	57[245]

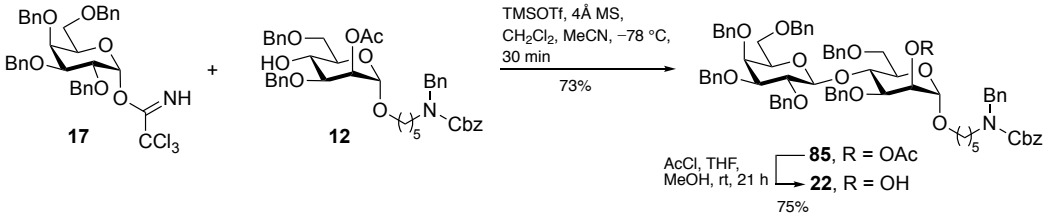
The unambiguous β-connectivity of disaccharide **85** was proven in a ^{1}H-NMR experiment by a large coupling constant between H-1 and H-2 ($J_{H1,H2}$ = 7.6 Hz) and an additional HMBC-NMR spectrum confirmed the correct (1→4)-linkage by crosspeaks between H-1 of galactose and C-4 of mannose as well as between C-1 of galactose and H-4 of mannose. Unfortunately, the amount of the concomittant α-product could not be determined, as it was not possible to separate the α-configured disaccharide from other by-products *via* column chromatography. These by-products probably included the *N*-trichloroacetylglycosyl amine, which generally arises from rearrangement of trichloroacetimidate glycosyl donors. (see SCHEME 3.28).



SCHEME 3.28. *Chapman* rearrangement of trichloroacetimidate glycosyl donor.[62]

This acid-catalyzed rearrangement of an imidate to a stable *N*-acylamide was first reported in 1915 by *Volquartz and co-workers*.[246] Then, 70 years later, *Schmidt and co-workers*[247] reported on first examples of this so-called *Chapman* rearrangement[248] for trichloroacetimidate donors.[62] The formation of stable *N*-trichloroacetylglycosyl amines might also explain the moderate yield obtained in the glycosylation reaction of donor **17** with acceptor **12**. In combination with the observation that slow addition of extra amounts of donor **17** led to complete consumption of acceptor **12** inspired us to use an inverse glycosylation strategy, i.e. slow addition of the glycosyl donor to a mixture of glycosyl acceptor and the acidic promotor. This strategy is

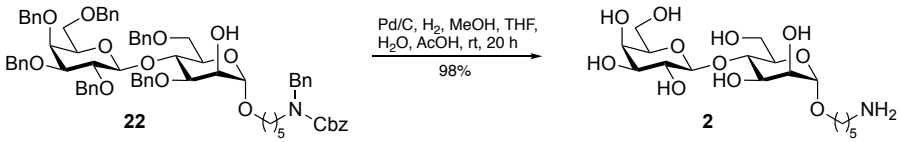
of particular interest for highly reactive (armed) glycosyl donors, which might rather rapidly decompose than react with the acceptor.[249, 250] Under inverse glycosylation conditions the formation of an inital cluster between catalyst and acceptor is anticipated, which could then activate the donor in vicinity to the acceptor. The activated donor could then react quickly with the proximate cluster-acceptor to form a glycosidic bond.[250] Therefore, trichloroacetimidate glycosyl donor **17** was slowly added over 15 minutes to a mixture of mannosyl acceptor **12** and TMSOTf in dichloromethane/acetonitrile (3:1) at −78 °C (see TABLE 3.1). After additional 15 minutes, thin-layer chromatography indicated the complete consumption of the starting materials. The desired β-linked disaccharide was obtained in 73% yield (see SCHEME 3.29). Nevertheless, formation of by-products was also observed, which again hampered isolation of the α-configured disaccharide to determine the anomeric ratio.



SCHEME 3.29. Glycosylation of galactosyl donor **17** with mannosyl acceptor **12** and subsequent selective deprotection.

Further glycosylation studies with thiodonor **64** were carried out by *Stefan Marchner* in the course of his master thesis in the *Hoffmann-Röder* research group.[245] However, neither the use of NIS/TMSOTf nor the use of NIS/AgOTf as promotor system led to a glycosylation outcome that surpassed the yield of the inverse glycosylation procedure with galactosyl trichloroacetimidate **17** as donor (see TABLE 3.1).

Subsequent selective deprotection of the acetate protecting group at C-2 of the mannose residue proceeded according to literature precedent in a mixture of methanol/tetrahydrofuran (8:3) with acetyl chloride (see SCHEME 3.29).[160] This afforded disaccharide **22** as glycosyl acceptor in a good yield of 75% for the subsequent constructions of native trisaccharide **1** and tetrasaccharide **5**. Moreover, compound **22** should also serve for the preparation of β-D-Gal*p*-(1→4)-α-D-Man*p* motif **2**.



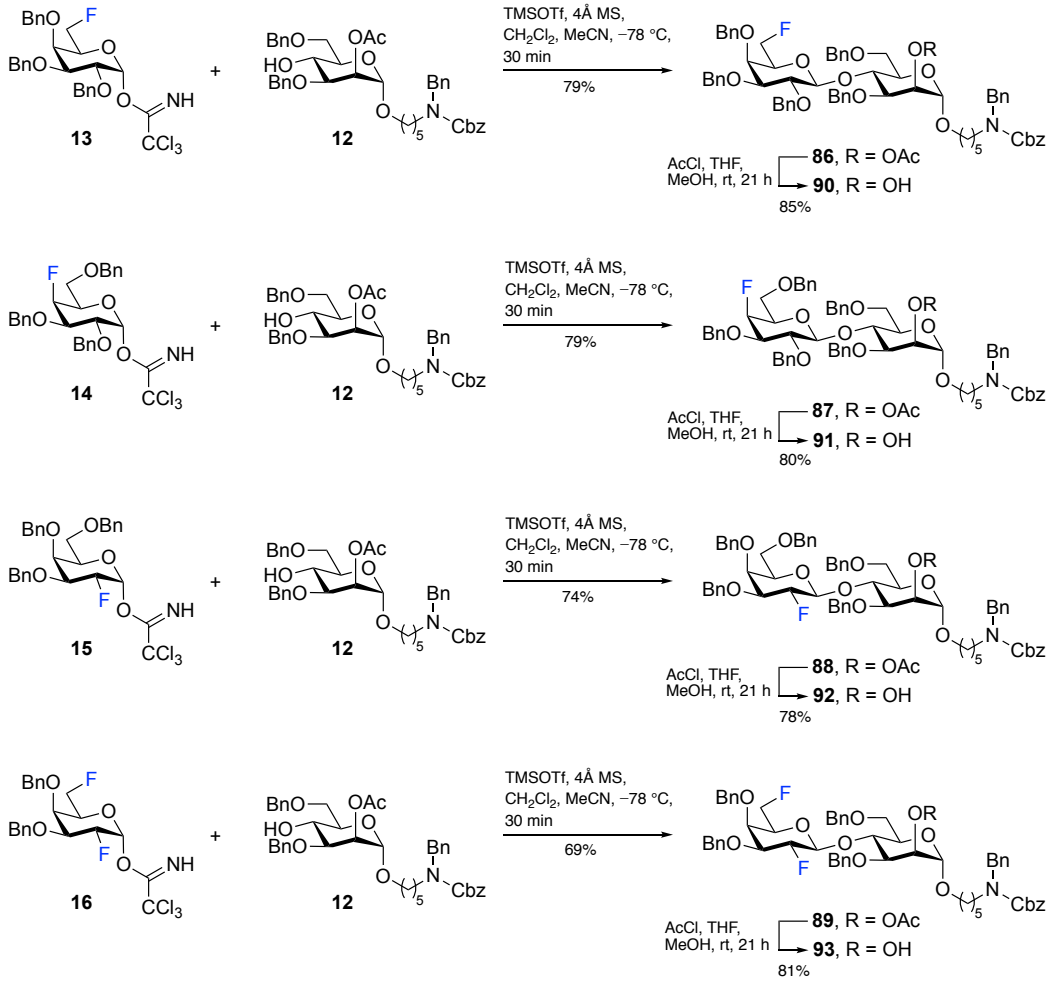
SCHEME 3.30. Global deprotection of disaccharide **22** by hydrogenolysis with palladium on charcoal.

The global deprotection of compound **22** was achieved following a hydrogenolysis protocol developed by *Seeberger and co-workers*,[160] using palladium on charcoal in a mixture of aqueous acetic acid, methanol and tetrahydrofuran (see SCHEME 3.30). This procedure afforded desired compound **2** after reversed-phase flash column chromatography in 98% yield.

Leishmanial capping disaccharide **2** was provided in sufficient amounts (45 mg) for future conjugation reactions and biological evaluations.

3.3.2 Synthesis of the fluorinated Disaccharides

The optimized inverse glycosylation procedure was also used for the preparation of fluorinated disaccharides **86**–**89**, where, in each case the corresponding fluorinated galactosyl donor was slowly added over 15 minutes to a mixture of mannosyl acceptor **12** and TMSOTf in dichloromethane/acetonitrile (3:1) at −78 °C (see SCHEME 3.31).



SCHEME 3.31. Synthesis of fluorinated disaccharide glycosyl acceptors **90**–**93**.

Using this approach, glycosylation with 6F-galactosyl donor **13** afforded the corresponding β-(1→4)-linked disaccharide **86** in 79% yield. Surprisingly, the 6-fluorinated compound **13** led to a slightly better yield in comparison to the native donor **17** (see TABLE 3.2). This might be explained by a decreased reactivity of the fluorinated derivate, as the deactivation with a fluorine substituent also seems to reduce the rapid formation of by-products. The inverse glycosylation procedure with 4F-galactosyl donor **14** afforded the 4-fluorinated disaccharide **87** in 79% yield. Also in this case, the balance between deactivation by the fluorine substituent and activation by electron donating benzyl ether protecting groups seems to render compound **14** a suitable donor for a β-selective glycosylation with mannosyl acceptor **12**. The glycosylation reactions with 2F-galactosyl donor **15** and with 2,6F-galactosyl donor **16**, which were kindly provided by *Markus Daum* from the *Hoffmann-Röder* research group,[251] afforded the corresponding disaccharides **88** and **89** in comparable

good yields of 74% and 69%. A possible explanation for the good yield and β-selectivity with 2F-galactosly donor **15** can be found in the work of *Gilmour and co-workers*,[180, 252, 253] who considered the 2-fluoro substituent as an inert steering group. They were able to show that the diastereocontrol in glycosylation reactions with fluorinated donor **15** is a consequence of the interplay between the electronic nature of the protecting groups and the fluorine substituent as well as the 2*R*,3*S*,4*S* stereotriad of galactose.[180] For instance, they observed that the glycosylation of 2F-galactosyl donor **15** and isopropanol with TMSOTf in dichloromethane at −78 °C afforded the corresponding isopropyl glycoside in high β-selectivity (β/α 150:1), whereas glycosylation with the C-4 epimer at the same temperature led to a lower β-selectivity (β/α 57:1). Furthermore, they reported by far lower levels of diastereocontrol with fully benzylated donor **17**.[253] Thus it can be assumed that the interplay between the fluorine substituent and the protecting group electronics as well as the 2*R*,3*S*,4*S* stereotriad of galactose and the suitable mannosyl acceptor **12** led to these glycosylation results.

TABLE 3.2. Comparison of the glycosylation yields of native donor **17** or fluorinated donors **13**–**16** with mannosyl acceptor **12**.

	native donor **17**	6F-donor **13**	4F-donor **14**	2F-donor **15**	2,6F-donor **16**
Yield (%)	73	79	79	74	69

In all cases, the unambiguous β-(1→4)-connectivity was proven *via* $^{3}J_{H,H}$ coupling constants in ^{1}H-NMR spectra and *via* crosspeaks between H-1 of galactose and C-4 of mannose or between C-1 of galactose and H-4 of mannose in HMBC-NMR spectra. Again, the α/β-ratios were not be determined, as the α-linked disaccharides could not be separated from other by-products *via* flash column chromatography or high performance liquid chromatography (HPLC).

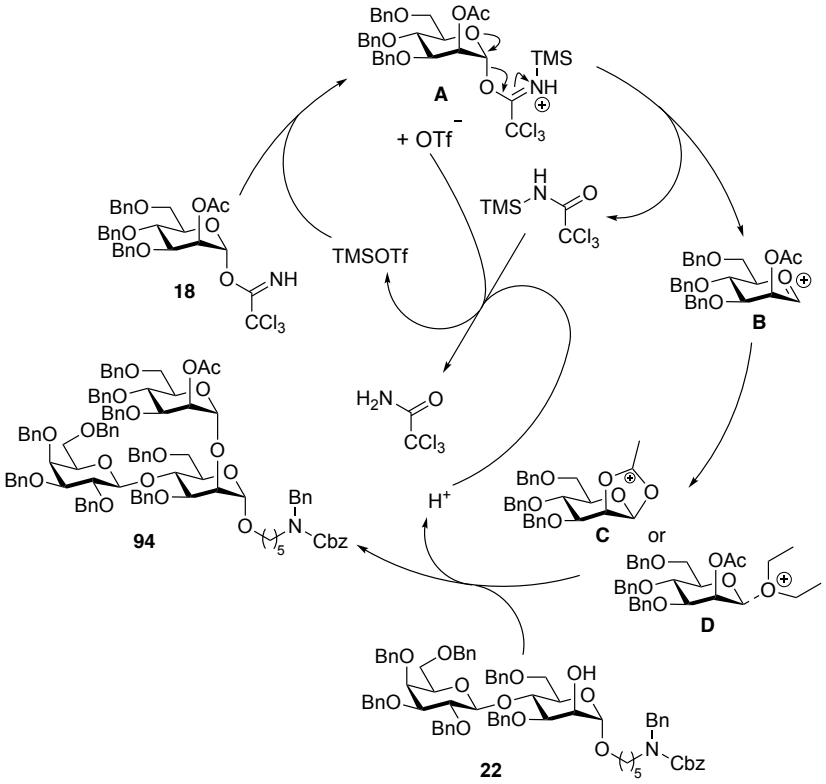
In order to obtain the free hydroxyl group at C-2 of the mannose residue, which was necessary for the following glycosylation reaction with mannosyl donor **18**, a selective deprotection of the acetate protecting group was carried out using acetyl chloride in a mixture of methanol and tetrahydrofuran.[160] This afforded the fluorinated glycosyl acceptor disaccharides **90**–**93** in good yields between 78% and 85% (see SCHEME 3.31) for subsequent preparation of the corresponding trisaccharides.

3.4 SYNTHESIS OF THE TRISACCHARIDES

Finally, it was possible to access the targeted α-D-Man*p*-(1→2)-[β-D-Gal*p*-(1→4)]-α-D-Man*p* subunits *via* α-selective glycosylations of mannosyl donor **18** using the corresponding disaccharide glycosyl acceptors **22** and **90**–**93** followed by global deprotection procedures.

3.4.1 Synthesis of the native Trisaccharide

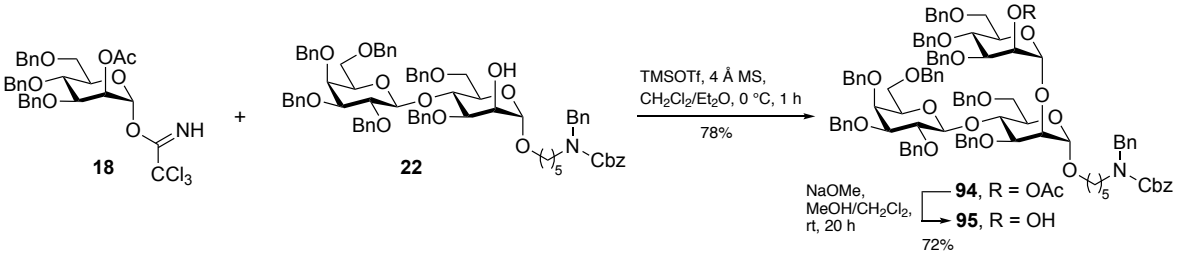
The glycosylation of mannosyl donor **18** with disaccharide glycosyl acceptor **22** was carried out at 0 °C under *Schmidt* conditions[193] using TMSOTf in a mixture of diethyl ether and dichloromethane.[160] Thereby, the addition of diethyl ether should support the anchimeric assistance of the acetate protecting group at C-2 of the mannosyl donor (**C**) due to its α-directing solvent effect (**D**) (see SCHEME 3.32).[221]



SCHEME 3.32. Mechanism for the glycosylation of mannosyl donor **18** and disacchride glycosyl acceptor **22**.

This procedure afforded fully protected trisaccharide **94** in 78% yield (see SCHEME 3.33). Furthermore, in order to validate the influence of the α-directing solvent effect of diethyl ether on the glycosylation outcome, the reaction was additionally carried out in pure dichloromethane. Although one should expect that the participatory effect of the acetate protecting group at C-2 of the mannosyl donor would be strong enough to control the diastereochemical outcome, the glycosylation yield of the desired α-product dropped significantly to 56% and the formation of the undesired β-product was observed. This indicated the necessity of the directing solvent effect for this reaction. However, the correct diastereochemical connectivity of the product could not be verified *via* $^{3}J_{H,H}$ NMR-coupling constants in accordance to the *Karplus* curve,[254, 255] as the

H-C-C-H dihedral angle of α- and β-mannosyl glycosides are very similar (see FIGURE 3.2). Therefore, it was necessary to determine the anomeric configuration *via* ^{1}H-^{13}C coupled HSQC-experiments, based on the fact that $^{1}J_{C,H}$ coupling constants exhibit a structural dependence, which is known as the *Perlin* effect.[256]



SCHEME 3.33. Glycosylation of mannosyl donor **18** with disaccharide glycosyl acceptor **22** and subsequent selective deprotection.

Perlin et al. observed that there is an approximately 10 Hz difference between the $^{1}J_{C1,H1}$ coupling constants of the two different anomeric configurations.[256] Thereby, the $^{1}J_{C1,H1}$ coupling constant of an α-*O*-glycoside ($H1_{eq}$) usally lies in the range of 170 Hz, whereas the $^{1}J_{C1,H1}$ coupling constant of a β-*O*-glycoside ($H1_{ax}$) can be found in the range of 160 Hz.[257]

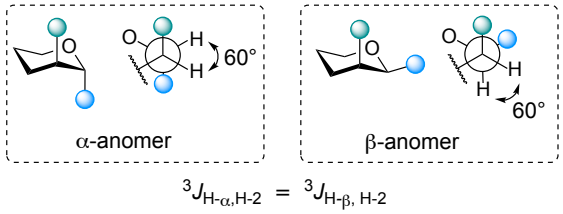


FIGURE 3.2. H-C-C-H dihedral angle of α- and β-mannosylglycosides.

For the synthesized trisaccharide **94**, we observed a $^{1}J_{C1,H1}$ coupling constant of 174.1 Hz for the newly formed D-Man*p*-(1→2)-D-Man*p* linkage, which unambiguously proved the α-connectivity (see FIGURE 3.3). Furthermore, the $^{1}J_{C1,H1}$ coupling constant of 171.4 Hz for the bridging mannose residue and the $^{1}J_{C1,H1}$ coupling constant of 161.4 Hz for the D-Gal*p*-(1→4)-D-Man*p* linkage once again confirmed the correct diastereochemical connectivities for the rest of the trisaccharide.

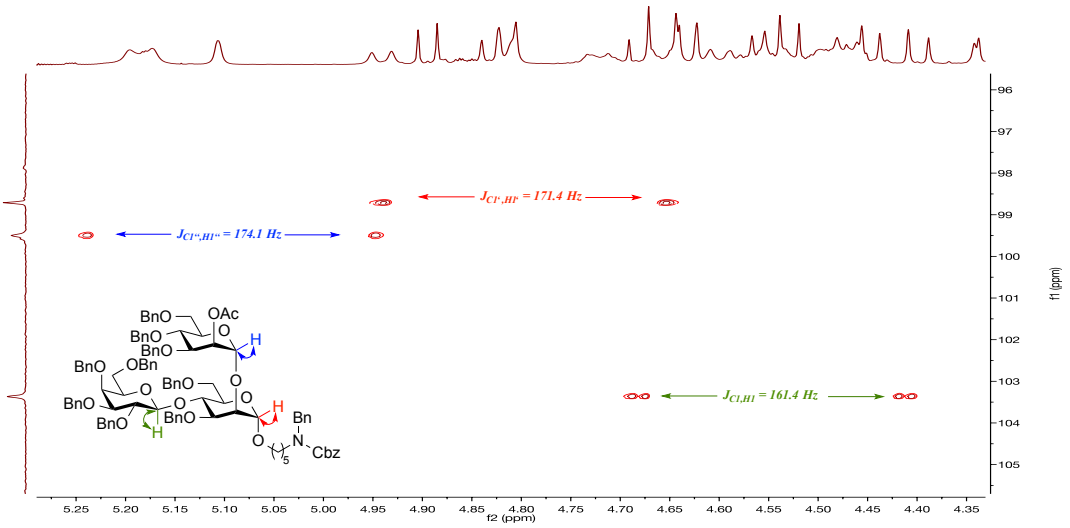
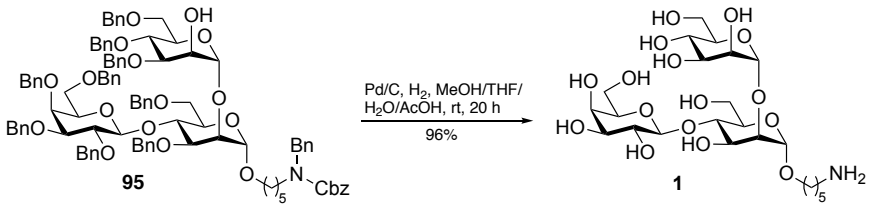


FIGURE 3.3. Extract from a ^{1}H-^{13}C coupled HSQC-NMR spectrum of trisaccharide **94** showing the relevant $^{1}J_{C1,H1}$ coupling constants.

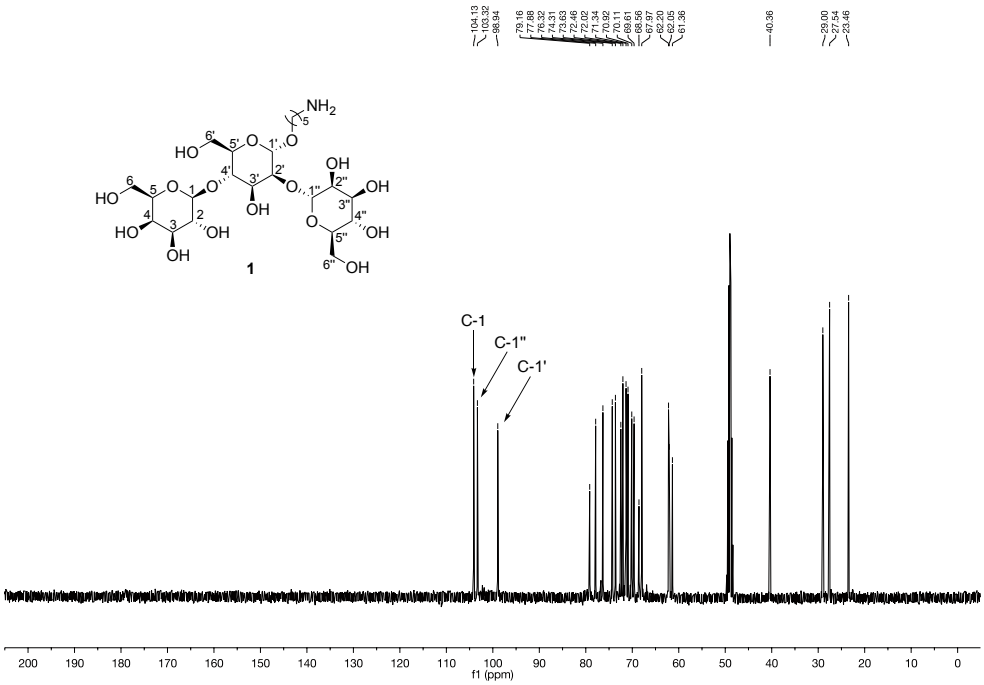
The correct (1→2)-linkage was confirmed by a HMBC-NMR spectrum showing crosspeaks between H-1 of the newly introduced mannose moiety and C-2 of the bridging mannose residue as well as between C-1 of the mannose at the non-reducing and H-2 of the bridging mannosyl residue.

To access the desired native trisaccharidic leishmanial capping structure **1** (see SCHEME 3.34), compound **94** was at first selectively deacetylated under *Zemplén* conditions[203] using catalytic amounts of sodium methanolate in a mixture of methanol and dichloromethane to furnish trisaccharide **95** in 72% yield (see SCHEME 3.33).[160]



SCHEME 3.34. Global deprotection of trisaccharide **95** by hydrogenolysis with palladium on charcoal.

Subsequent cleavage of the benzyl protecting groups as well as of the carboxybenzyl protecting group was achieved by hydrogenolysis with palladium on charcoal in an aqueous mixture of methanol, tetrahydrofuran and acetic acid according to literature precedent,[160] yielding trisaccharide **1** after careful purification by reversed-phase flash column chromatography in 96%.

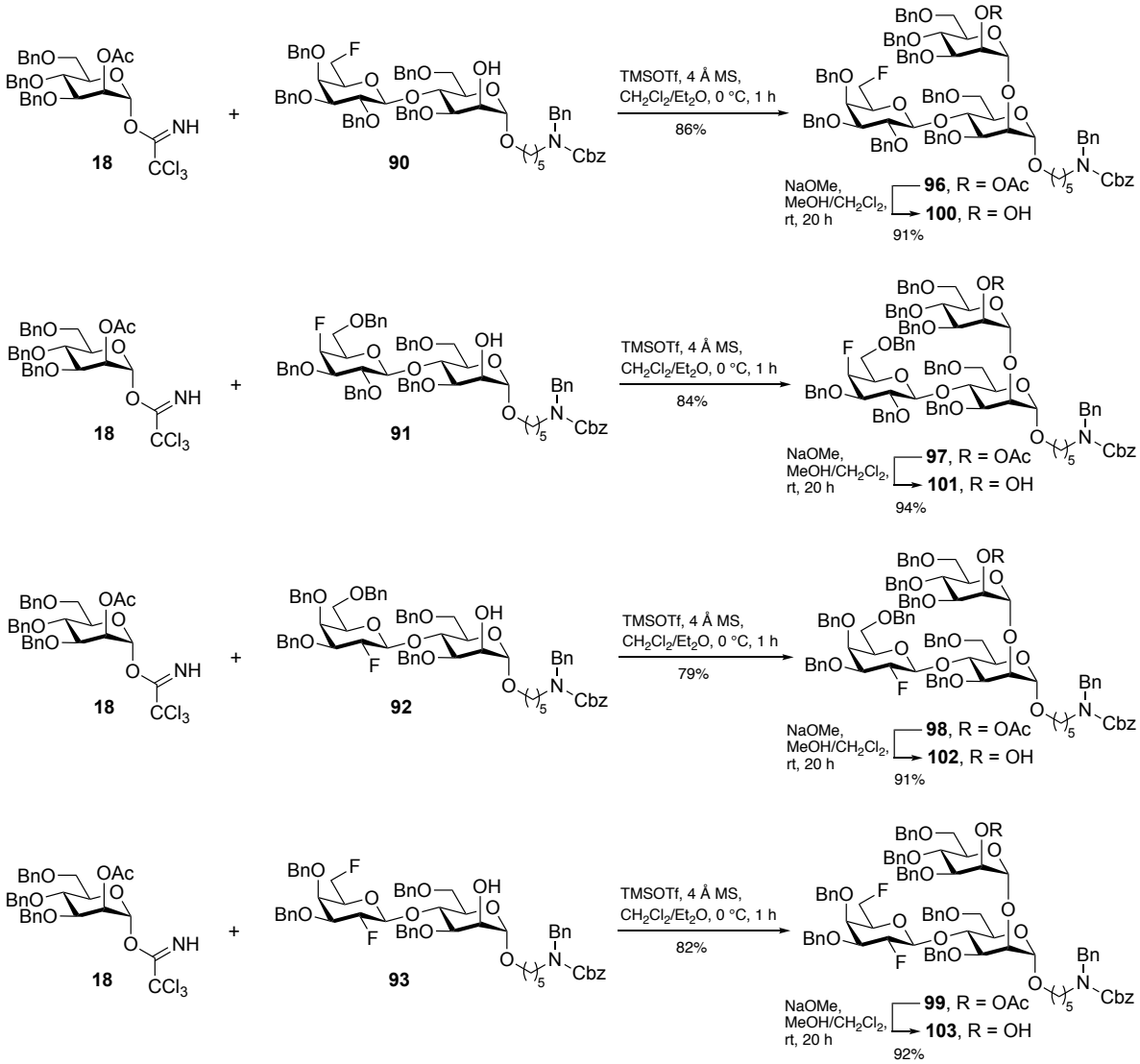


FIRGURE 3.4. ^{13}C-NMR spectrum (101 MHz, D_2O/CD_3OD) of fully deprotected trisaccharide **1**.

In conclusion, 136 mg (30% chemical yield) of the linker-functionalized native trisaccharide **1** were prepared in requisite purity (see FIGURE 3.4) over five steps starting from the synthesized monosaccharide building blocks **12**, **17** and **18** for subsequent conjugation reactions and future biological evaluations.

3.4.2 Synthesis of the fluorinated Trisaccharides

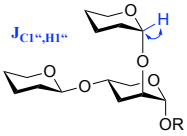
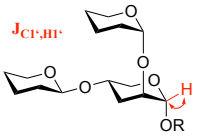
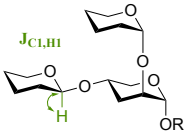
The syntheses of fluorinated trisaccharides **96–99** were carried out in accordance to the glycosylation protocol used for the preparation of nonfluorinated analog **94**. Thus, glycosylation of mannosyl donor **18** with the corresponding fluorinated disaccharide glycosyl acceptors **90–93** in a mixture of diethyl ether and dichloromethane at 0 °C with TMSOTf as promotor gave the desired fully protected trisaccharides in very good yields between 79% and 86% (see SCHEME 3.35).[160]



SCHEME 3.35. Glycosylation of mannosyl donor **18** with fluorinated disaccharide glycosyl acceptors **90–93** and subsequent selective deprotection.

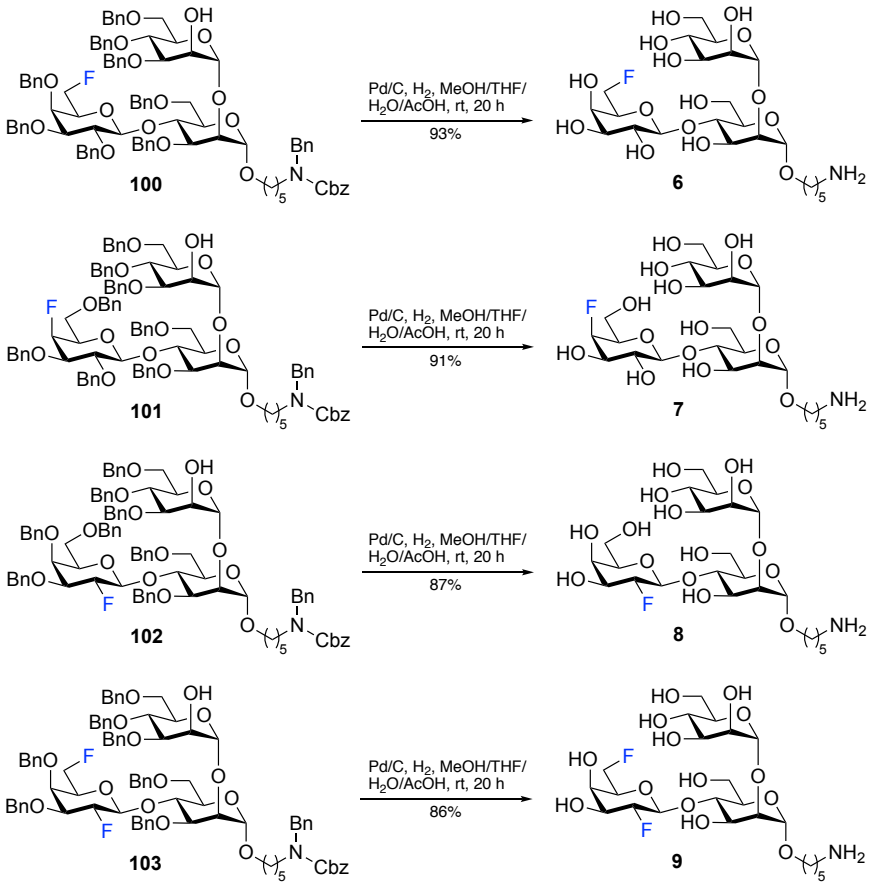
Again, the desired α-connectivity of the newly formed glycosidic bond was confirmed for each compound *via* ^{1}H-^{13}C coupled HSQC-experiments as described before for trisaccharide **94** (see TABLE 3.3). Thereby, also the β-linkage of the Gal*p*-(1→4)-D-Man*p* subunit and of the α-linkage of the bridging mannose moiety were verified. Finally, the (1→2)-connectivity of the two mannose subunits was validated by HMBC-NMR experiments as it was the case for the native structure (*vide supra*).

TABLE 3.3. $^{1}J_{C1,H1}$ coupling constants of compounds **96–99**.[a]

	96	**97**	**98**	**99**
$J_{C1\alpha,H1\alpha}$ 	174.4 Hz	174.6 Hz	173.8 Hz	173.6 Hz
$J_{C1\beta,H1\beta}$ 	171.3 Hz	171.3 Hz	171.5 Hz	171.5 Hz
$J_{C1,H1}$ 	162.4 Hz	161.6 Hz	162.5 Hz	163.1 Hz

[a]Extracts from ^{1}H-^{13}C coupled HSQC-NMR spectra can be found in Appendix.

With the desired trisaccharides **96–99** in hands, deacetylation under *Zemplén* conditions[203] with catalytic amounts of sodium methanolate in a mixture of methanol and dichloromethane[160] was accomplished to furnish fluorinated trisaccharides **100–103** in excellent yields between 91% and 94% (see SCHEME 3.35).



SCHEME 3.36. Global deprotection of fluorinated trisaccharides **100–103** by hydrogenolysis with palladium on charcoal.

The following global deprotection of fluorinated trisaccharides **100**–**103** was achieved according to a literature known procedure.[160] Again, hydrogenolysis was carried out with palladium on charcoal in an aqueous mixture of methanol, tetrahydrofuran and acetic acid (see SCHEME 3.36). The reaction proceeded smoothly in all cases and afforded the target structures **6**–**9** in yields between 86% and 93% after reversed-phase flash column chromatography.

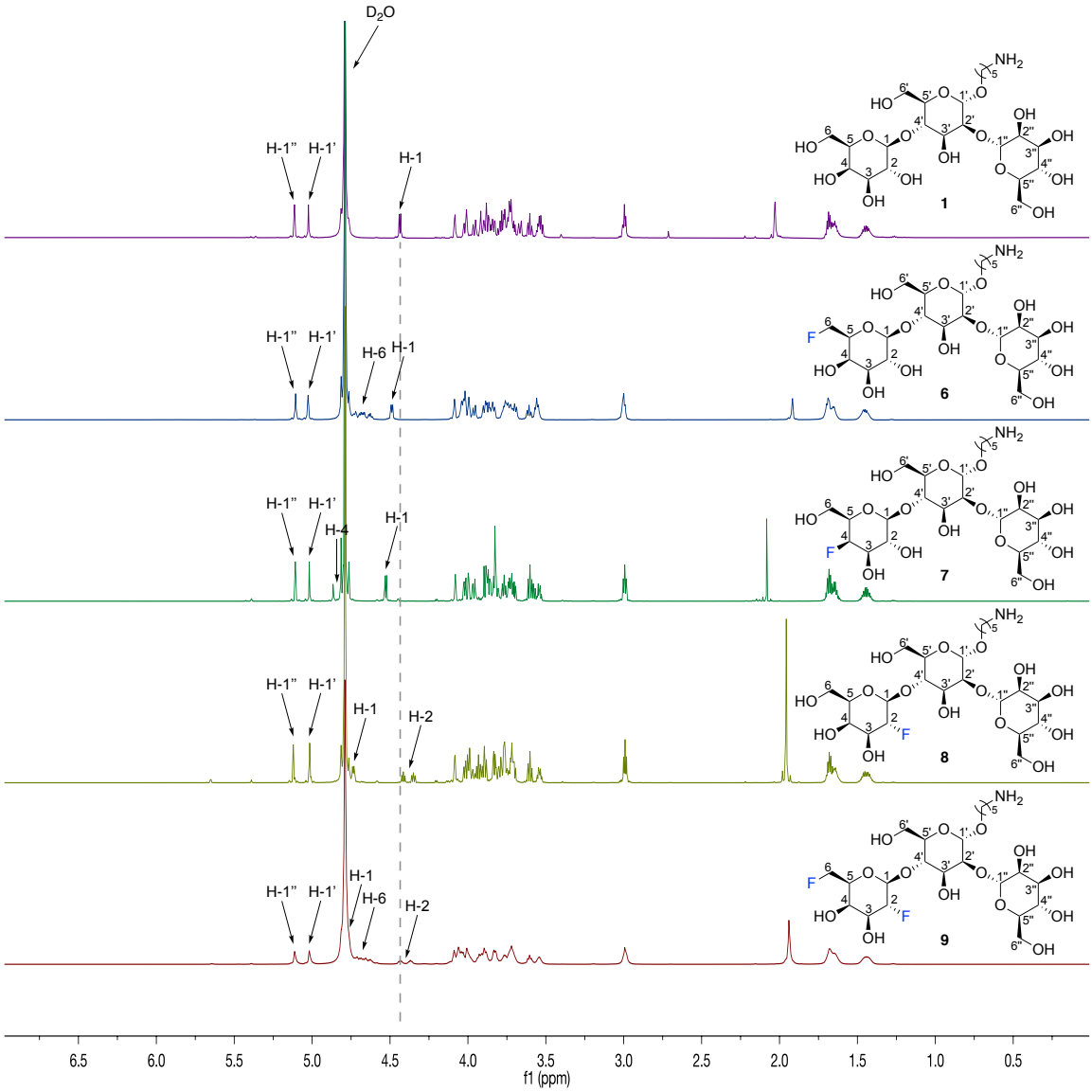


FIGURE 3.5. ^{1}H-NMR spectra (800 MHz, D_2O) of native trisaccharide **1** and of fluorinated trisaccharide **6**–**9**.

Finally, the desired fluorinated leishmanial capping trisaccharides **6**–**9** were obtained in good yields over five steps starting from the monosaccharide building blocks **12**, **18** and **13**–**16**. Thus, sufficient amounts of each trisaccharide are now available for future conjugation reactions and biological evaluations (see TABLE 3.4). Moreover, the requisite purities of all compounds were confirmed in NMR-experiments (see FIGURE 3.5), which also displayed characteristic deviations in the chemical shift values for protons in close proximity to fluorine substituents. For instance, the signals for protons bound to a fluorine-bearing carbon were shifted from the bulk of signals at around 3.5 ppm – 4.0 ppm towards higher ppm-values, as expected. Noteworthy,

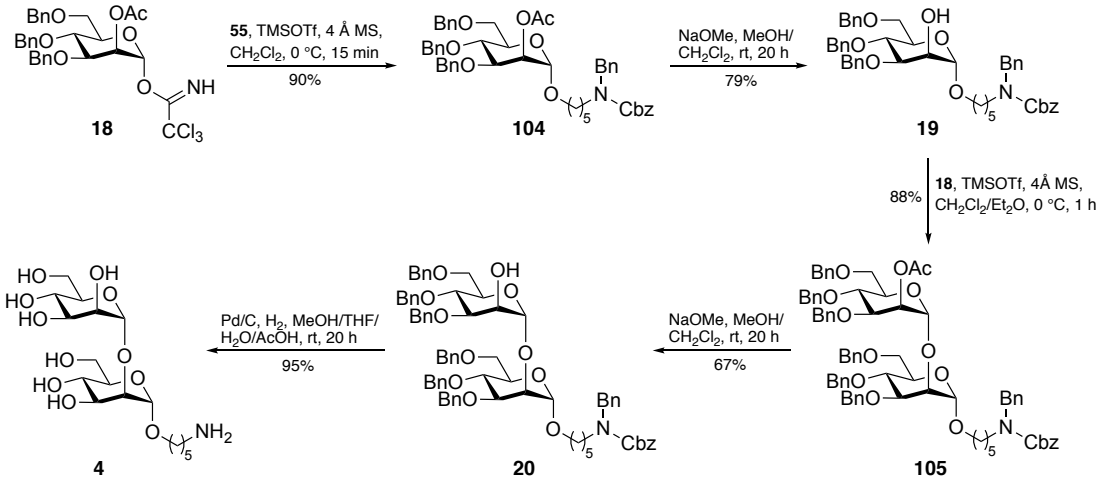
the signals of the anomeric protons of each galactose residue were significantly deep-field shifted due to the electron withdrawing effect of fluorine. The closer the fluorine substituent is placed to the anomeric center, the stronger this effect becomes. Thus, it was even possible to observe a shift for the signal of the anomeric proton of the most distant-substituted 4- and 6- fluorinated derivates. As the shifts are induced by differences in the electronic environment (lower electron density) at the anomeric position, this might already indicate a higher stability of the adjacent glycosidic bond for the fluorinated compounds.

TABLE 3.4. Comparison of the overall yields and isolated amounts for the native trisaccharide and its fluorinated derivatives

	native-Tri **1**	6F-Tri **6**	4F-Tri **7**	2F-Tri **8**	2,6F-Tri **9**
Yield (%) over five steps	30	49	45	36	36
Isolated amount	136 mg	202 mg	173 mg	142 mg	208 mg

3.5 SYNTHESIS OF DIMANNOSE 4 AND TRIMANNOSE 3

The syntheses of dimannose **4** and trimannose **3** were achieved over five steps (see SCHEME 3.37) or over seven steps, respectively (see SCHEME 3.37/3.38) based on literature known procedures.[160] The sythesis of dimannose **4** started with a *Schmidt* glycosylation of mannosyl donor **18** and protected amine linker **55** by stirring the two compounds at 0 °C in dichloromethane with TMSOTf as promotor.[193] The reaction proceeded smoothly and afforded compound **104** in an excellent yield of 90%.



SCHEME 3.37. Synthesis of dimannose **4** over five steps starting from mannosyl donor **18**.

Subsequently, selective deacetylation was carried out under *Zemplén* conditions[203] using catalytic amounts of sodium methanolate in a mixture of dichloromethane and methanol to provide glycosyl acceptor **19** in 79% yield. The dimannose subunit was then built up *via* a *Schmidt* glycosylation of **19** with mannosyl donor **18** in the presence of TMSOTf and in a mixture of dichloromethane and diethyl ether at 0 °C.[193] The use of diethyl ether was necessary due to its α-directing solvent effect, as has been discussed earlier. Thus, the α-linked fully protected dimannose **105** was obtained in a very good yield of 88%. The desired α-connectivity of the newly established glycosidic bond was confirmed in a ^{1}H-^{13}C coupled HSQC-experiment by a $^{1}J_{C1,H1}$ coupling constant of 173.5 Hz. The corresponding (1→2)-linkage between the two mannose residues was further verified in a HMBC-NMR experiment as described earlier. The acetate protecting group at C-2 was then cleaved *via Zemplén* transesterification[203] using catalytic amounts of sodium methanolate in a mixture of dichloromethane and methanol. This furnished compound **20** in 67% yield, which could then either be used for global deprotection to prepare target dimannose **4** or for the construction of the corresponding trimannose moiety. The removal of the benzyl protecting groups and the carboxybenzyl protecting group was achieved by hydrogenolysis with palladium on charcoal in an aqueous mixture of methanol, tetrahydrofuran and acetic acid.[160] Again, this reaction proceeded smoothly and target compound **4** was obtained in an excellent yield of 95% after purification by reversed-phase flash column chromatography.

Linker-functionalized dimannose **4** was obtained in 40% yield over five steps. Sufficient amounts (118 mg) of this compound were provided in requisite purity (see FIGURE 3.6) for future conjugation reactions or biological evaluations.

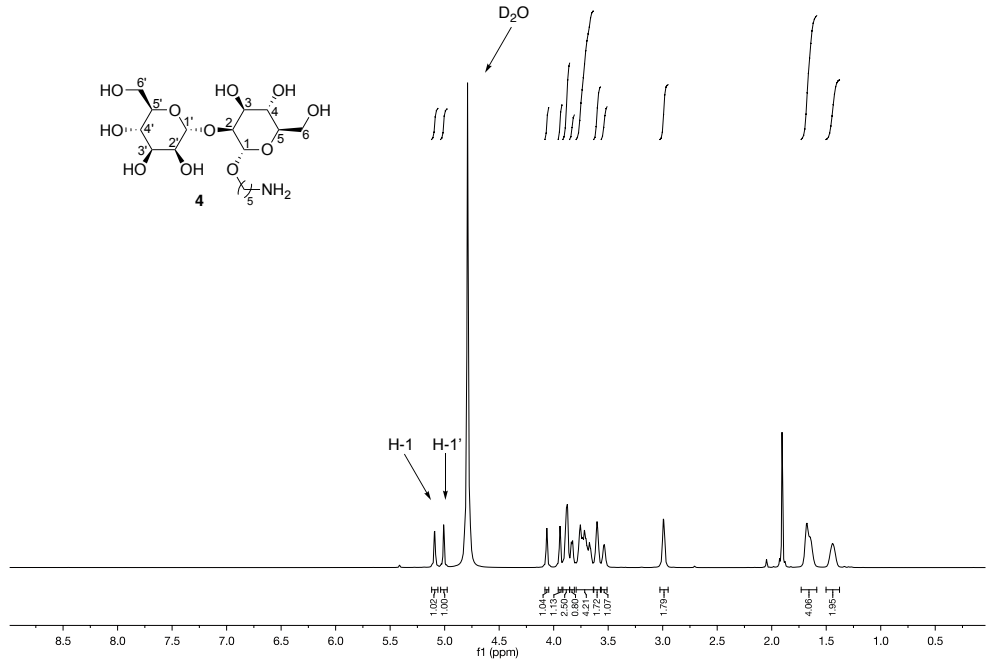
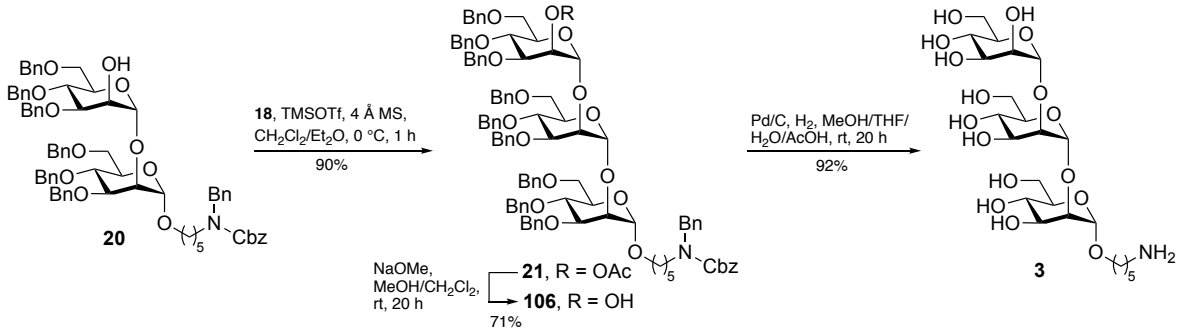


FIGURE 3.6. ^{1}H-NMR spectrum (800 MHz, D_2O) of dimannose **4**.

Selectively deprotected mannose building block **20** was then used for the preparation of trimannose **3**. Therefore, it was reacted with mannosyl donor **18** at 0 °C under *Schmidt* conditions with TMSOTf as promotor and dichloromethane and diethyl ether as solvents. This furnished fully protected trimannose **21** in an excellent yield of 90% (see SCHEME 3.38). The α-connectivities of all glycosidic bonds were verified as before using ^{1}H-^{13}C coupled HSQC-experiments showing large $^{1}J_{C1,H1}$ coupling constants between 172.1 Hz and 173.4 Hz (see FIGURE 3.7). Moreover, the (1→2)-linkage of the newly formed glycosidic bond was confirmend on the basis of a HMBC-NMR experiment as described above.



SCHEME 3.38. Synthesis of fully deprotected trimannose **3**.

Global deprotection commenced with cleavage of the acetate protecting group at C-2 of the terminal mannose residue by stirring compound **21** with catalytic amounts of sodium methanolate in a mixture of dichloromethane and methanol.[203] Subsequent hydrogenolysis of deacetylated trimannose **106** proceeded in 71% yield by using palladium on charcoal in an aqueous mixture of methanol, tetrahydrofuran and acetic acid.[160] This afforded fully deprotected trimannose **3** in an excellent yield of 92% after reversed-phase flash column chromatography.

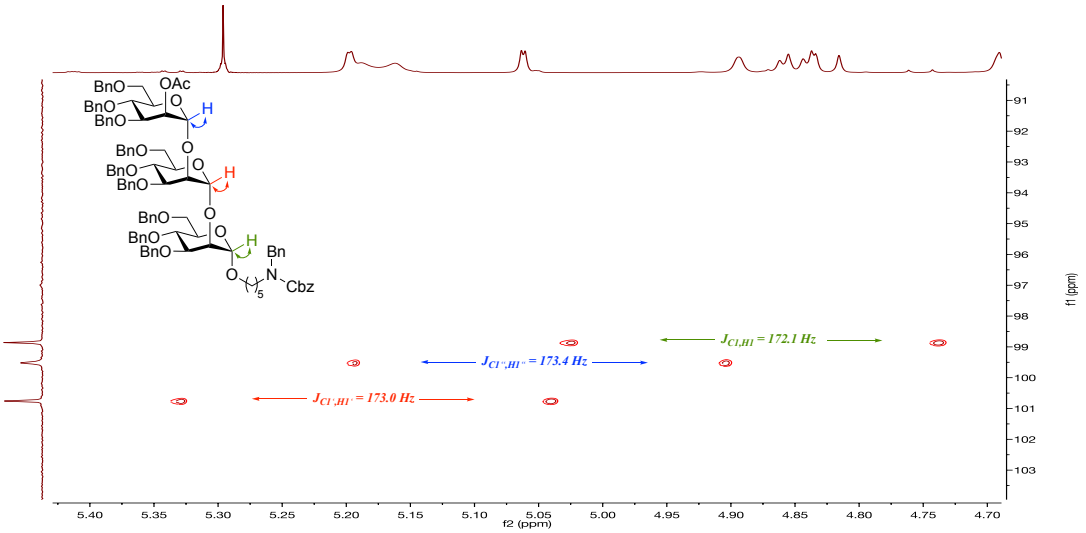


FIGURE 3.7. Extract from a ^{1}H-^{13}C coupled HSQC-NMR spectrum of trimannose **21** showing the relevant $^{1}J_{C1,H1}$ coupling constants.

Thus, the targeted leishmanial capping trimannose **3** was obtained in 25% yield over seven steps starting from monosaccharide building block **18**. This compound was also provided in sufficient amounts (124 mg) and with the requisite purity (see FIGURE 3.8) for future conjugation reactions or biological evaluations.

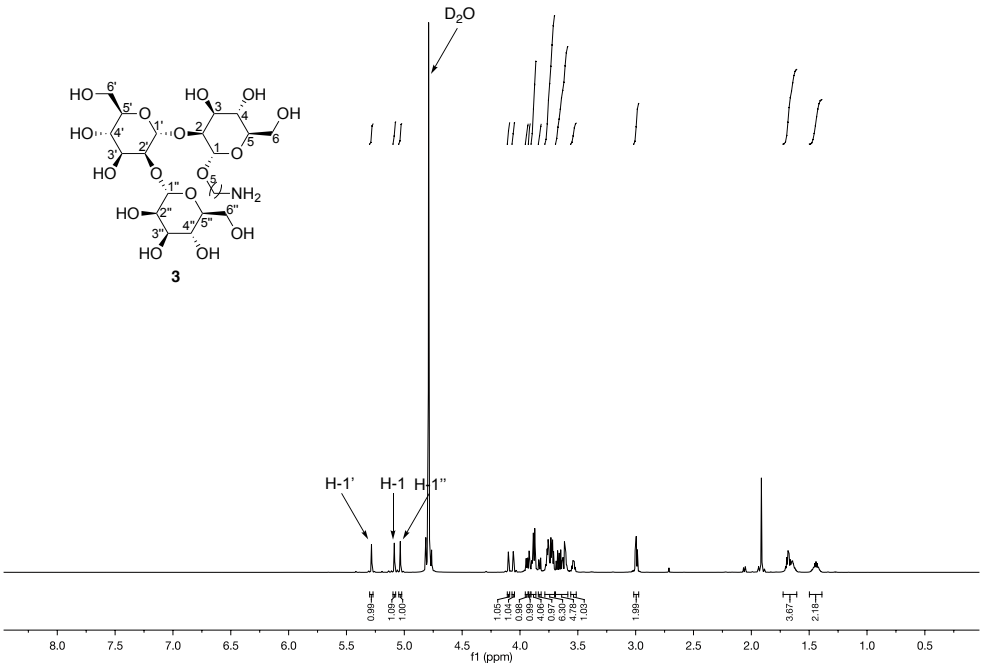
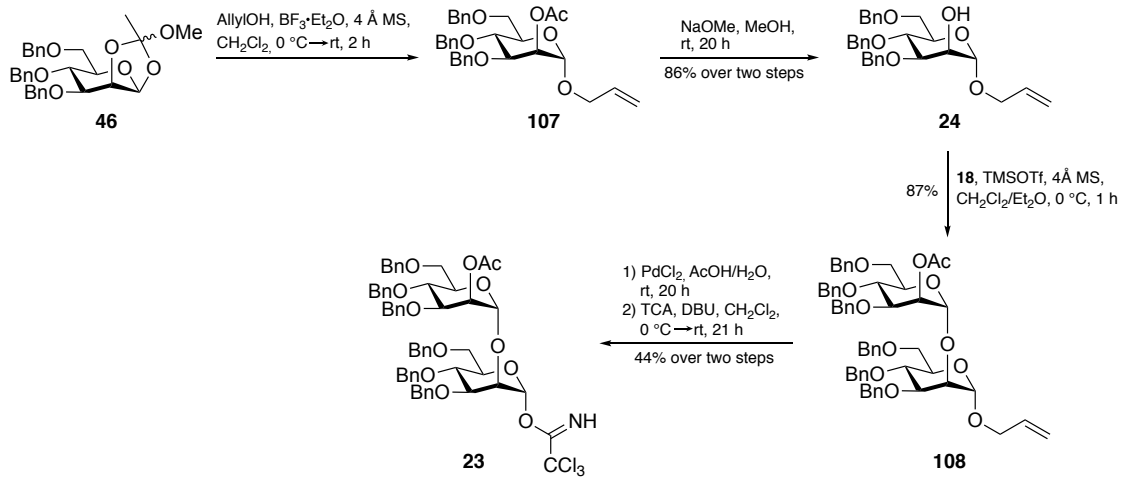


FIGURE 3.8. ^{1}H-NMR spectrum (800 MHz, D_2O) of trimannose **3**.

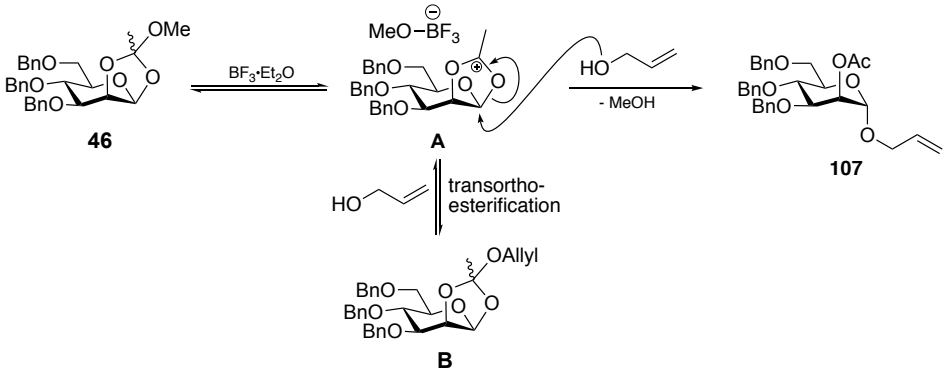
3.6 SYNTHESIS OF TETRASACCHARIDE 5

The synthesis of leishmanial tetrasaccharide **5** was carried out on the basis of a [2+2]-glycosylation strategy, using a suitable dimannosyl donor **23** (see SCHEME 3.39) and the already described disaccharide glycosyl acceptor **22** (see SCHEME 3.41). The preparation of dimannosyl donor **23** proceeded *via* five steps, starting from already synthesized monosaccharide building blocks **18** and **46**.



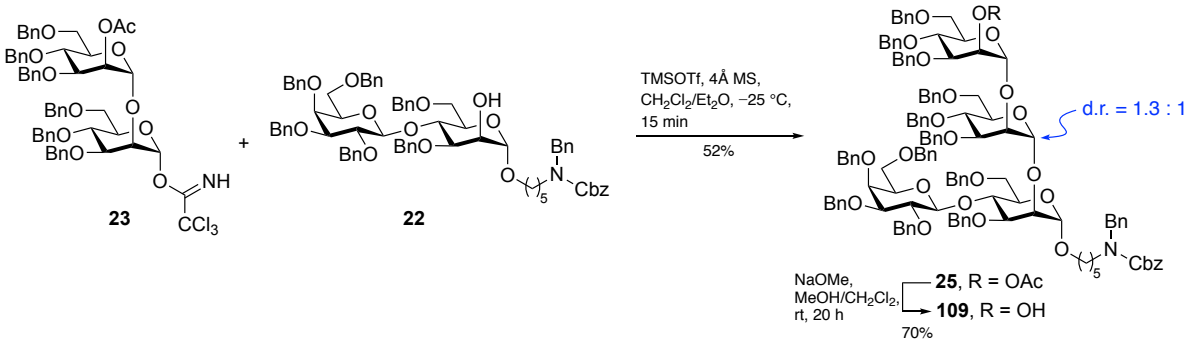
SCHEME 3.39. Synthesis of mannosyl donor **23** over five steps.

In the first step of the synthesis, a temporary allyl protecting group was introduced at the anomeric center of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranoside **46** *via* an orthoester glycosylation method. This method was developed in the 1960s by *Kochetkov and co-workers*[258-260] for the synthesis of 1,2-*trans* glycosides and has been often applied over the years.[202, 261, 262] In this case here, $BF_3 \cdot Et_2O$ was used for the activation of the orthoester.[263] Coordination of the Lewis acid leads to a reversible formation of an acyloxonium ion (**A**), which can then either transform into intermediate (**B**) *via* reversible transorthoesterification[260] or undergo a nucleophilic attack in axial position by allylic alcohol, furnishing α-allylglycoside **107** (see SCHEME 3.40).[202] The formation of a concomittant methylglycoside by-product can be suppressed by using a large excess of the nucleophile, here allylic alcohol.



SCHEME 3.40. Possible reaction pathway for the orthoester glycosylation with compound **46**.[202]

Subsequently, the newly formed acetate protecting group at C-2 was cleaved under *Zemplén* conditions[203] using catalytic amounts of sodium methanolate in methanol to furnish glycosyl acceptor **24** in 86% yield over two steps. Glycosylation of mannosyl donor **18** with mannosyl acceptor **24** was carried out at 0 °C in a mixture of dichloromethane and diethyl ether by employing TMSOTf as promotor. The desired α-linked disaccharide **108** was obtained in a good yield of 87%. Afterwards, selective deallylation with excess amounts of palladium(II) chloride and sodium acetate in aqueous acetic acid furnished an intermediate lactol,[161] which was directly transformed into the corresponding dimannosyl trichloroacetimidate **23** by stirring it in a mixture of TCA and DBU in dichloromethane.[193] The resulting α-trichloroacetimidate donor **23** was obtained in 44% yield over two steps and was coupled to the β-D-Gal*p*-(1→4)-α-D-Man*p* subunit to give the fully protected tetrasaccharide **25**. This [2+2]-glycosylation reaction of dimannosyl donor **23** and glycosyl acceptor **22** was carried out at −25 °C in a mixture of dichloromethane and diethyl ether with TMSOTf as promotor (see SCHEME 3.41).[264] Although it was supposed, that the α-directing solvent effect of diethyl ether as well as the steric hindrance of the axial mannosyl residue at C-2 of the donor would strongly favor axial attack of the disaccharide glycosyl acceptor, an α/β-mixture (1.3:1) of the tetrasaccharide was obtained.



SCHEME 3.41. Synthesis of tetrasaccharide **109** via a [2+2]-glycosylation and subsequent selective deacetylation.

A similar effect was observed by *Teumelsan et al.*,[265] who applied a related dimannosyl thiodonor for the preparation of HIV-derived oligomannose compounds and obtained predominantly the β-glycoside instead of the desired α-linked product. The authors proposed that the predominant formation of the β-product might be the result of an intermediate α-mannosyl triflate,[266] which is stabilized by the electron-withdrawing mannosyl residue at C-2. As the large carbohydrate moiety can also point away from the anomeric center and thus reduce the steric hindrance, the triflate can be replaced easily in a S_N2 mode leading to the observed equatorial glycosidic linkage.[265] In this approach, trichloroacetimidate activator *tert*-butyldimethylsilyl triflate (TBSOTf)[264, 267, 268] was also used but did not result in the expected α-selectivity.[245] Interestingly and despite all these observations, *Mukherjee et al.*[174] were able to synthesize the properly connected leishmanial capping tetrasaccharide in 82% yield by applying a one-pot glycosylation strategy with a related dimannosyl thiodonor and NIS/TMSOTf as promotor system. However, since it was possible to separate both diastereomeric tetrasaccharides by flash column chromatography, the desired α-linked compound **25** was finally obtained in a fair yield of 52%. The α-connectivity of the newly formed glycosidic bond was proven by a ^{1}H-^{13}C coupled HSQC-experiment revealing a $^{1}J_{C1,H1}$ coupling constants of 170.0 Hz (see FIGURE 3.9) for compound **25**. The spectrum also confirmed the anomeric configuration of the galactose residue by a $^{1}J_{C1,H1}$ coupling constant of

161.3 Hz as well as the α-connectivies of the remaining mannose residues by $^{1}J_{C1,H1}$ coupling constants of 170.0 Hz and 173.2 Hz. Furthermore, a HMBC-NMR experiment confirmed the newly formed (1→2)-connectivity as described before.

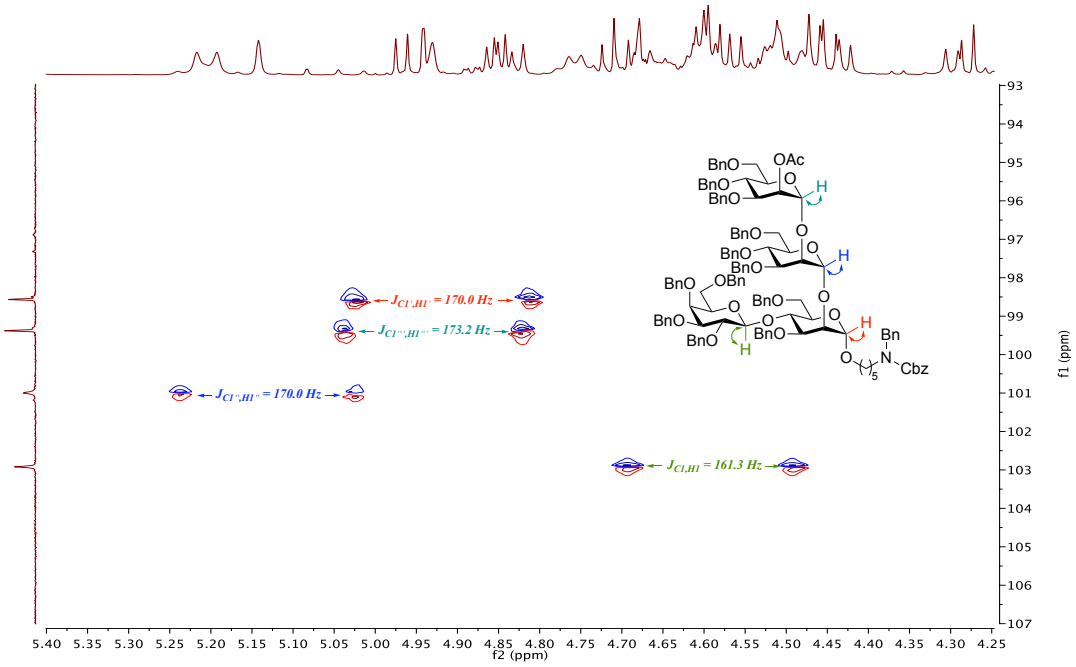
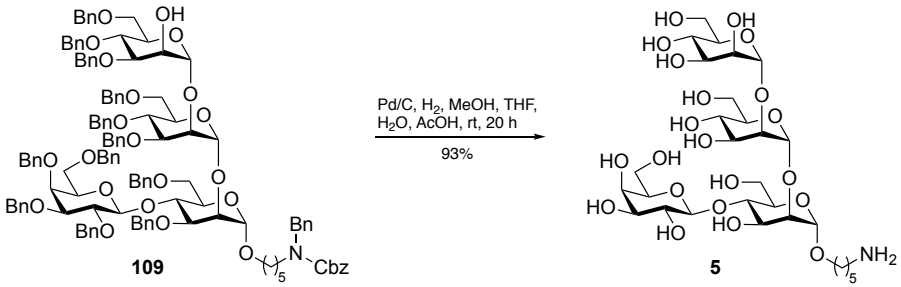


FIGURE 3.9. Extract from a ^{1}H-^{13}C coupled HSQC-NMR spectrum of tetrasaccharide **25** showing the relevant $^{1}J_{C1,H1}$ coupling constants.

The following deacetylation step proceeded *via Zemplén* transesterification[203] with catalytic amounts of sodium methanolate in a mixture of methanol and dichloromethane to afford tetrasaccharide **109** in 70% yield. The subsequent global deprotection was carried out under reductive conditions (see SCHEME 3.42) using a hydrogenolysis protocol developed by *Seeberger and co-workers*.[160] Therefore, deacetylated compound **109** was stirred with palladium on charcoal in a mixture of aqueous acetic acid, methanol and tetrahydrofuran to provide fully deprotected tetrasaccharide **5** in 93% yield after purification by reversed-phase column chromatography.



SCHEME 3.42. Global deprotection of tetrasaccharide **109** by hydrogenolysis with palladium on charcoal.

In summary, the leishmanial capping tetrasaccharide **5** was obtained in 11% yield and in sufficient amounts (63mg) over eight steps starting from orthoester **46**. The requisite purity of compound **5** (see FIGURE 3.10) for future conjugation reactions and biological evaluations was confirmed by NMR-experiments.

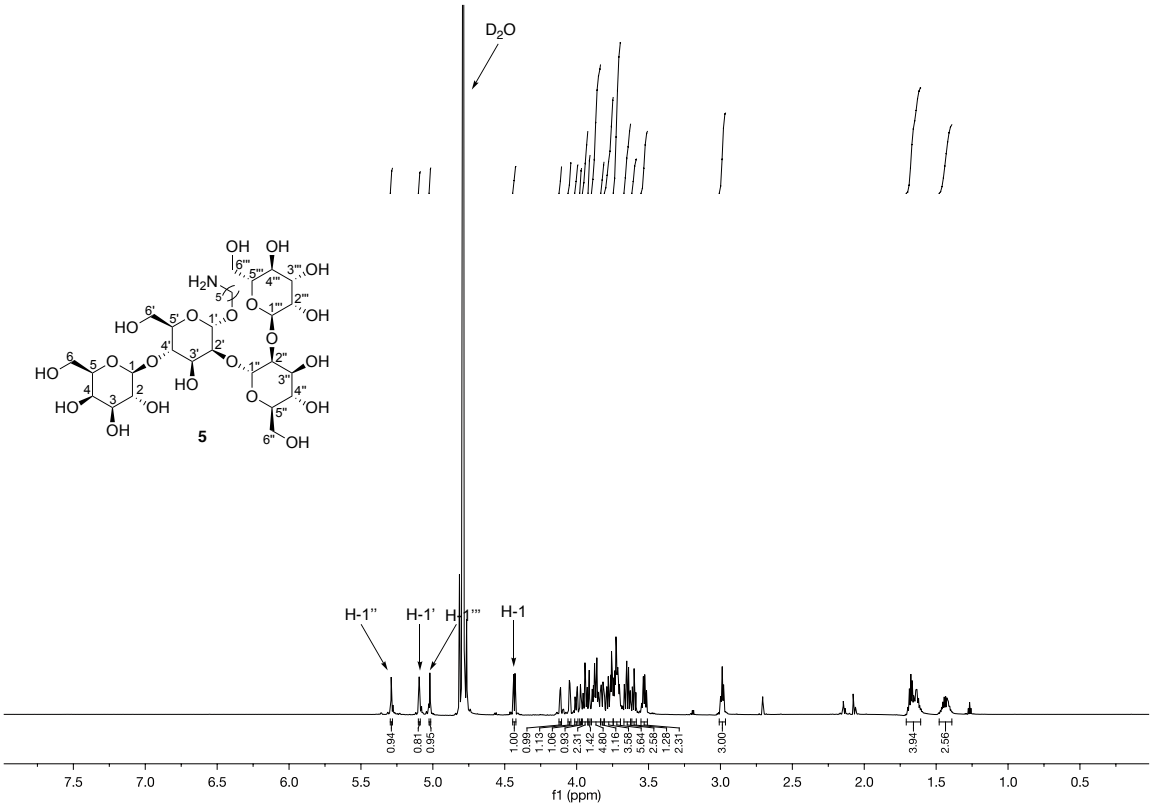
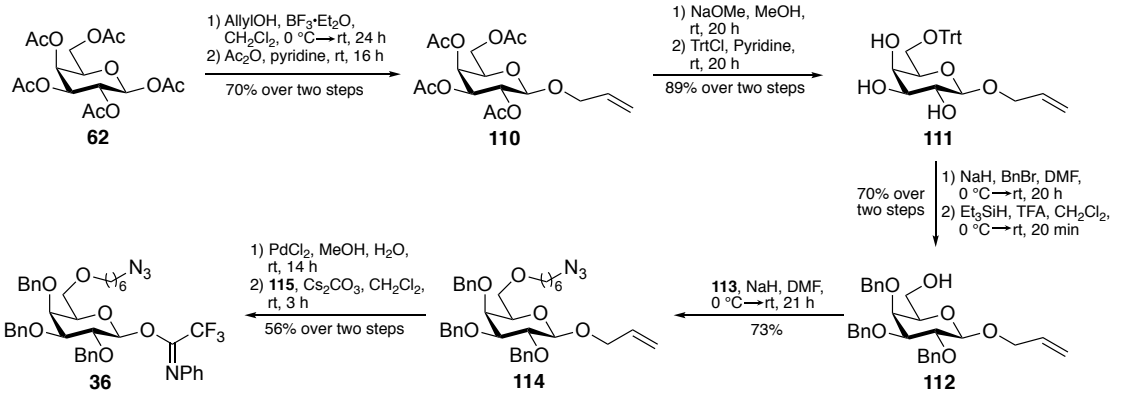


FIGURE 3.10. ^{1}H-NMR spectrum (800 MHz, D_2O) of tetrasaccharide **5**.

3.7 SYNTHESIS OF α-GALCER DERIVATIVE 11

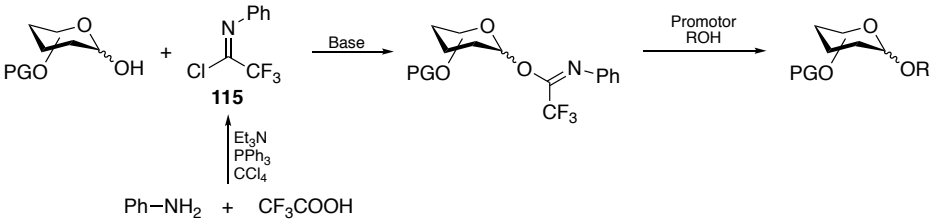
The α-GalCer derivative **11**, which should be used as T helper-like epitope, was assembled from three different parts: the galactosyl donor **36**, the phytosphingosine derivative **35** and the self-immolative Val-Cit linker **28**. The galactosyl donor **36**, which represents the core synthon, was synthesized over eight steps according to a literature know procedure (see SCHEME 3.43).[89]



SCHEME 3.43. Synthesis of galactosyl donor **36** over eigth steps according to literature precedent.[89]

The synthesis commenced with the formation of an allyl glycoside starting from readily available pentaacetate **62**. Therefore, this peracetylated compound was reacted with allylic alcohol in the presence of $BF_3 \cdot Et_2O$ in dichloromethane.[240] As described earlier, thin-layer chromatography indicated at first the presence of several by-products, which however eventually diminished upon reacetylation in acetic anhydride and pyridine[199] to provide allyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **110** in a good yield of 70%. As expected, the anchimeric assistance of the acetate protecting group at C-2 exclusively led to formation of the β-anomer. Subsequent deacetylation proceeded *via Zemplén* transesterification[203] using catalytic amounts of sodium methanolate in methanol furnishing an intermediate tetrol, which was used in the next step without further purification. In order to transiently protect the 6-position of the tetrol, a trityl ether group was chosen. Therefore, the crude product was stirred in pyridine with a slight excess of trityl chloride to afford triol **111** in 89% yield over two steps. Subsequently, the remaining unprotected hydroxyl groups of **111** were benzylated with sodium hydride and benzyl bromide in DMF. Removal of the trityl protecting group was next achieved in a mixture of TFA and Et_3SiH in dichloromethane to furnish compound **112** in 70% yield over two steps. The selectively deprotected hydroxyl group at C-6 then allowed for the introduction of an azide linker at this position by stirring primary alcohol **112** with sodium hydride in DMF prior to the addition of 6-azidohexyl 4-methylbenzenesulfonate **113**.[89] Thereby, the resulting alkoxide reacted with the azide linker in a S_N2 mode to give compound **114**, which was obtained in 73% yield and should later serve as a masked amine for conjugation to the Val-Cit linker. Ensuing deprotection of the anomeric center was necessary in order to enable the formation of a glycosyl *N*-phenyltrifluoroacetimidate (PTFAI) donor. The deallylation reaction proceeded smoothly according to a literature known protocol with catalytic amounts of palladium(II) chloride in aqueous methanol,[89] furnishing the desired lactol in 63% yield. Subsequent synthesis of PTFAI donor **36** was carried out in dichloromethane with the obtained hemiacetal, 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride

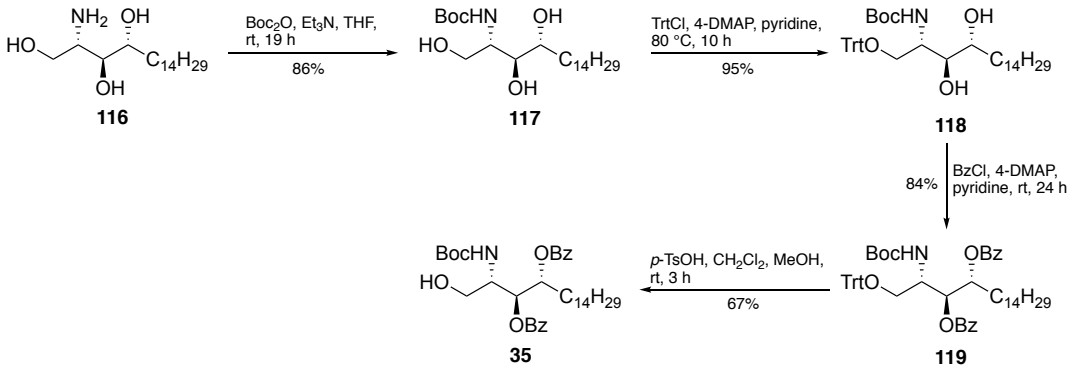
115[269] (see SCHEME 3.43) and cesium carbonate to afford the target compound in a very good yield of 89%. The use of PTFAI donors was first reported by *Yu et al.*[270] in 2001 and has been widely exploited since then for the preparation of oligosaccharides and glycoconjugates.[271] They are usually synthesized by the treatment of a lactol with *N*-arylacetimidoyl chlorides in the presence of a base, such as potassium carbonate,[272] cesium carbonate,[89, 273] sodium hydride[274] or DBU[275] in dichloromethane or tetrahydrofuran. The necessary *N*-arylacetimidoyl chlorides are easily accessible by reacting the corresponding aniline derivatives with trifluoroacetic acid in tetrachloromethane in the presence of triethylamine and triphenylphosphine (see SCHEME 3.44).[269, 271]



SCHEME 3.44. Preparation of a glycosyl *N*-phenyltrifluoroacetimidate (PTFAI) and subsequent glycosylation. (Adapted from ref.[271])

In contrast to trichloroacetimidate donors, whose anomeric configuration can be influenced during preparation by the choice of base and reaction conditions,[194] PTFAI donors are usually obtained as α/β-mixtures due to the irreversibility of the condensation reaction of the hemiacetals with *N*-arylacetimidoyl chlorides. These donors are also activated by catalytic amounts of TMSOTf or other Lewis acid promotor.[271] Furthermore, PTFAI donors do not undergo a *Chapman* rearrangement[248] and thus simplify the separation from by-products during purification. Here, PTFAI donor **36** was obtained over eight steps in 18% overall yield and was ready to use for the preparation of KRN7000 derivative **11**.

Acceptor **35** was accessed in four steps starting from commercially available phytosphingosine **116** in accordance to literature known procedures (see SCHEME 3.45).[276, 277]

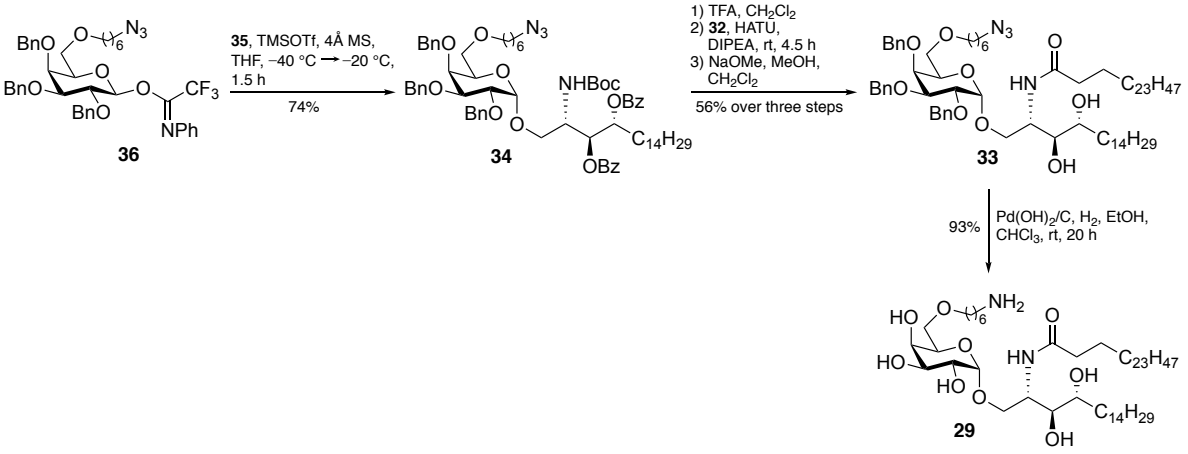


SCHEME 3.45. Synthesis of phytosphingosine glycosyl acceptor **35** over four steps.

A suitable phytosphingosine acceptor required a free primary alcohol in the presence of protected secondary hydroxyl groups and an orthogonally blocked amine. Therefore, the amine was at first selectively protected by stirring phytosphingosine **116** in tetrahydrofuran with triethylamine and di-*tert*-butyl dicarbonate (Boc_2O)[276] to provid carbamate **117** after recrystallization in 86% yield. The primary alcohol was then transformed into a

trityl ether by stirring triol **117** with trityl chloride and catalytic amounts of 4-dimethylaminopyridine (4-DMAP) in pyridine,[277] furnishing diol **118** in an excellent yield of 95%. The remaining free hydroxyl groups were blocked orthogonally by benzoyl esters, which was achieved by reacting compound **118** in pyridine with benzoyl chloride and catalytic amounts of 4-dimethylaminopyridine (4-DMAP).[277] The fully protected phytosphingosine derivative **119** was thus obtained in a very good yield of 84%. Subsequent selective deprotection of the primary hydroxyl group was accomplished in a mixture of dichloromethane and methanol in the presence of equimolar amounts of *para*-toluenesulfonic acid (*p*-TsOH).[277] However, the mixture had to be carefully neutralized in order to reduce cleavage of the Boc protecting group during work-up. Targeted glycosyl acceptor **35** was obtained in 67% yield. The moderate yield most presumably results from the aforementioned loss of the amine protecting group. However, phytosphingosine derivate **35** was obtained over four steps in 46% yield for subsequent glycosylation with PTFAI donor **36**.

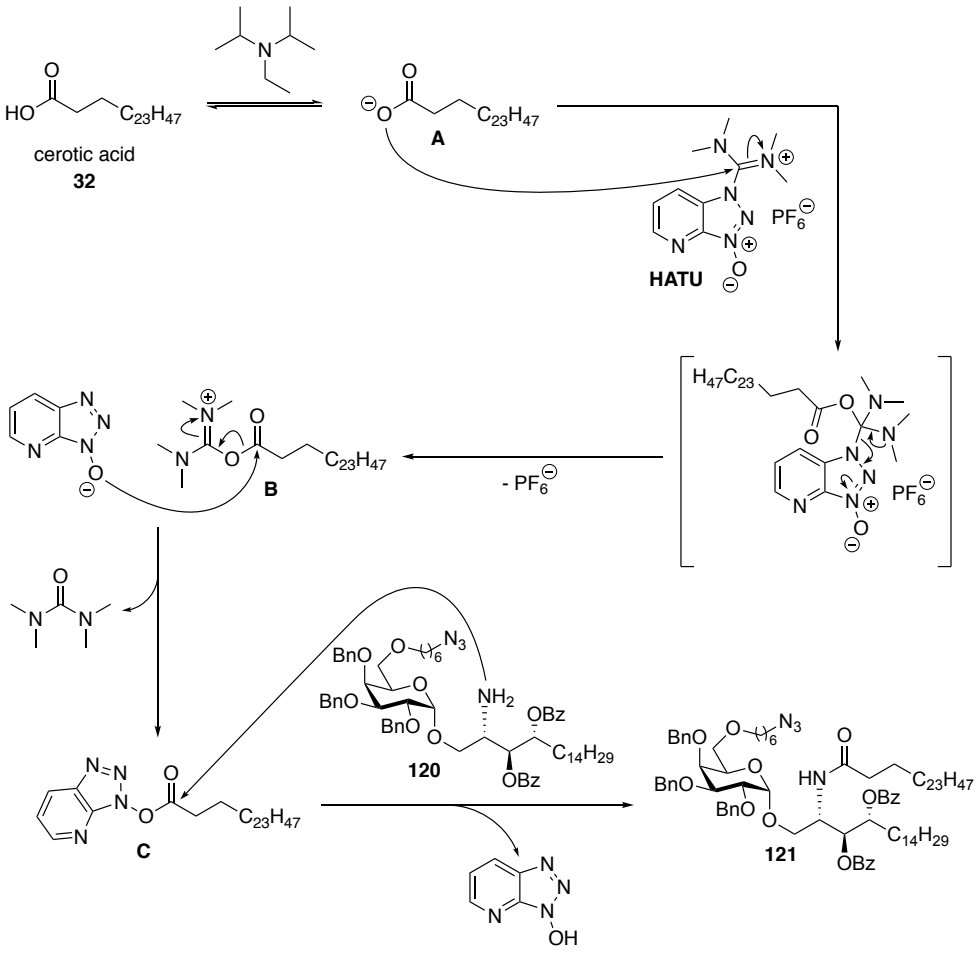
The KRN7000 derivative **29** was prepared over four steps followed by a global deprotection in accordance to literature known procedures (see SCHEME 3.46).[89, 276] The glycosylation of galactosyl donor **36** with phytosphingosine derivative **35** was carried out in tetrahydrofuran with TMSOTf as promotor. The reaction temperature was allowed to raise from initial −40 °C to −20 °C over 1.5 hours. This procedure and the α-directing solvent effect of tetrahydrofuran led to the formation of the α-glycoside in 74% yield. The observed small coupling constant between H-1 and H-2 ($J_{H1,H2}$ = 3.8 Hz) unambiguously proved the desired α-connectivity of compound **34**.



SCHEME 3.46. Synthesis of KRN7000 derivative **29**.

Afterwards, the Boc-protecting group was selectively cleaved in order to enable amide bond formation between the phytosphingosine residue and the carboxyl group of cerotic acid **32**. Boc deprotection was accomplished by stirring carbamate **34** in dichloromethane with TFA.[276] The reaction proceeded smoothly and completed within two hours. The crude amine was used without any further purification in the following amide bond coupling reaction, in which the carboxylic acid usually has to be acitvated at first. A plethora of activating reagents is available for this purpose, but most approaches use coupling reagents which transform the carboxyl group into an acid chloride, mixed anhydride or activated ester.[278-280] In this case, we turned the carboxyl group of cerotic acid into an activated ester using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexafluorphosphat (HATU) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) (see

SCHEME 3.47). This amide coupling reagent allows for mild reaction conditions, a fast amide bond formation and is supposed to be particular efficient for sterically hindered couplings.[281, 282] Furthermore, HATU is of interest as activator for carboxylic acids possessing α-stereocenters, as it leads to less epimerization during the course of the coupling.[278]

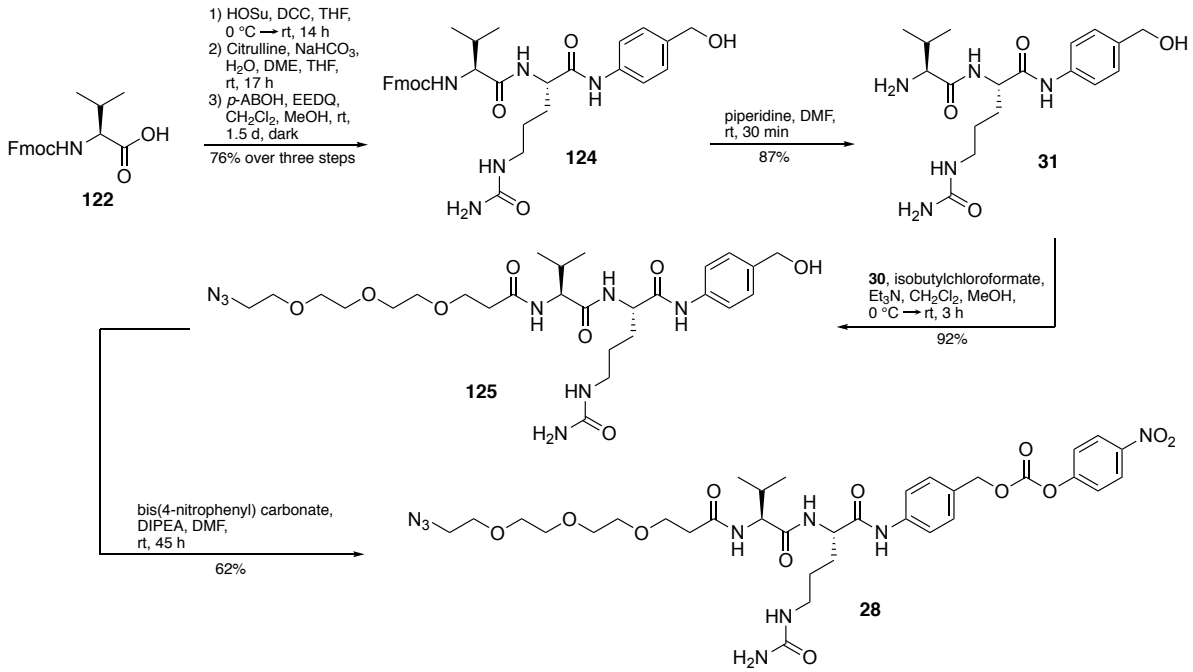


SCHEME 3.47. Formation of the amide bond in compound **33** *via* an activated ester.

In the first step of the activation mechanism, cerotic acid is deprotonated by DIPEA in order to give the corresponding caboxylate anion (**A**), which then reacts with HATU under formation of an unstable *O*-acyl(tetramethyl)isouronium ion (**B**). This species rapidly reacts with the concomittantly formed azabenzotriazolyloxy anion to give the activated ester (**C**) and tetramethylurea as the by-product. Subsequent reaction with the secondary amine group of compound **120** leads to the targeted fully protected KRN7000 derivative **121** and 1-Hydroxy-7-azabenzotriazole (HOAt). However, since purification by flash column chromatography could not deliver the desired product in high purity, crude compound **121** was directly used in the next reaction. In this step, cleavage of the benzoyl esters was carried out under *Zemplén* conditions[203] using catalytic amounts of sodium methanolate in a mixture of dichloromethane and methanol. This afforded diol **33** in 56% yield over three steps. The following global deprotection proceeded once more *via* hydrogenolysis according to literature precedent.[89] Therefore, compound **33** was stirred under a hydrogen atmosphere with palladium(II) hydroxide on charcoal in a mixture of ethanol and chloroform to furnish KRN7000 derivative **29** in an excellent yield of 93%. The aforementioned acyl shift[94] of cerotic acid was not

observed and a HMBC-NMR spectrum demonstrated the desired amide bond connectivity. In summary, compound **29** was obtained over five steps starting from galactosyl donor **36** and phytosphingosine derivative **35** in satisfying 39% yield to be employed for the following linker conjugation.

The valine-citrulline (Val-Cit) linker **28**, which should connect KRN7000 derivative **29** and the B cell epitope or the multivalent scaffold, respectively, was prepared over six steps in accordance to literature known procedures (see SCHEME 3.48).[94, 182] The synthesis started with the formation of a *N*-hydroxysuccinimide ester (NHS-ester) from commercially available Fmoc-protected L-Valine **122**.



SCHEME 3.48. Synthesis of self-immolative valine-citrulline linker **28** over six steps.

NHS-esters have been widely used as active esters for amide bond formation since their advent in 1963.[283, 284] Their preparation requires *N*-hydroxysuccinimide (HOSu) and the presence of an amide coupling reagent, but many other efficient methods are available in the literature.[284] In this case, Fmoc-protected L-Valine **122** was stirred together with *N*,*N*′-dicylcohexylcarbodiimide (DCC) and HOSu in tetrahydrofuran to give the corresponding NHS-ester,[182] which was immediately used without further purification for amide coupling with L-Citrulline. For this coupling, both compounds were stirred with sodium bicarbonate in a mixture of water, tetrahydrofuran and 1,2-dimethoxyethane.[182] When thin-layer chromatography indicated completeness of the reaction, work-up and recrystallization afforded the desired dipeptide **123** in a very good yield of 91% over two steps. The carboxyl group of the citrulline moiety was then transformed into a mixed anhydride for the subsequent amide bond formation between dipeptide **123** and *p*-aminobenzylalcohol (*p*-ABOH). Mixed anhydrides, which are used for amide coupling can be divided into two different subclasses: mixed carboxylic acid anhydrides and mixed carbonic acid anhydrides.[278]

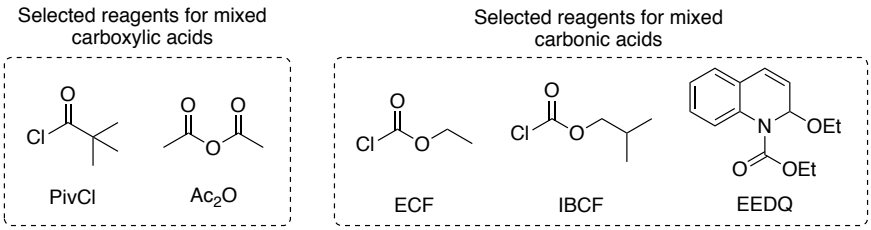
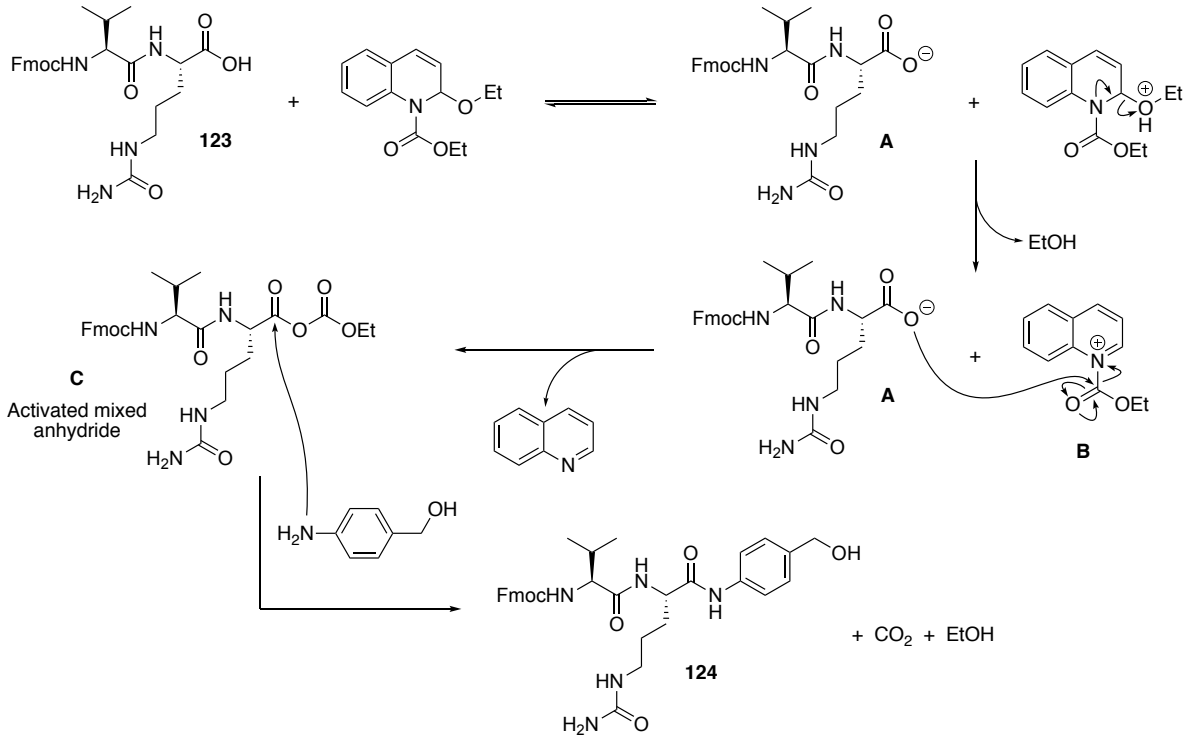


FIGURE 3.11. Reagents for the preparation of mixed caboxylic and mixed carbonic acid anhydrides.[278]

The carboxylic acid anhydrides are usually prepared with reagents such as acetic anhydride (Ac_2O)[285] or pivaloyl chloride (PivCl),[286] whereas the preparation of carbonic acid anhydrides requires chloroformates, such as ethyl chloroformate (ECF)[287] and isobutyl chloroformate (IBCF)[288, 289] or *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[290] (see FIGURE 3.11).[278]

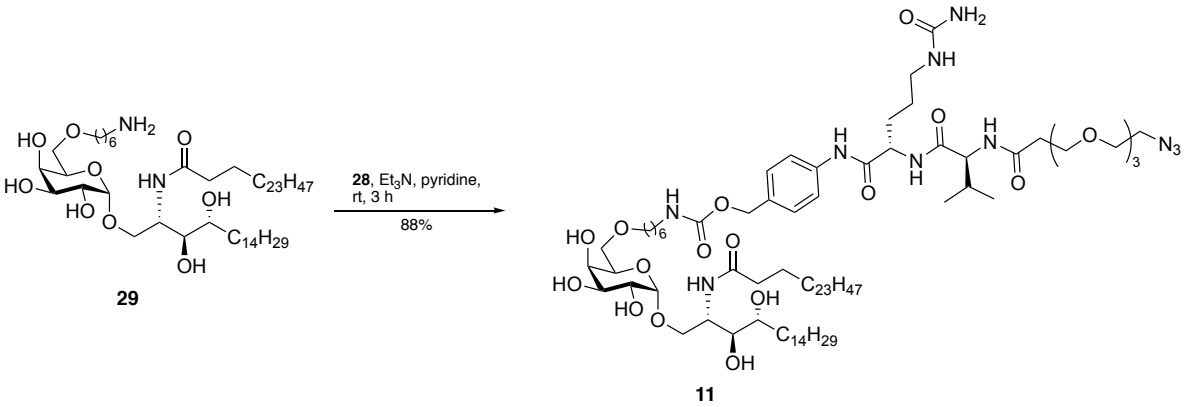


SCHEME 3.49. Mechanism for the amide bond formation with EEDQ.

EEDQ was used for the preparation of the amide bond in compound **124** (see SCHEME 3.49) following a literature known protocol.[182] This inexpensive coupling reagent does not require additional base and transforms the carboxyl group slowly into the mixed anhydride. Rapid consumption of the latter avoids side-reactions, e.g. epimerizations.[279] During the course of the reaction, ethanol is expelled from EEDQ under the formation of an ethyl formate quinolinium ion (**B**). The carboxylate is then transformed by (**B**) into the activated ethoxycarbonyl anhydride (**C**), which can be attacked by *p*-ABOH in order to build up compound **124**.[280] Thus, Fmoc-Val-Cit-4-aminobenzyl alcohol **124** was obtained in a very good yield of 84%. In order to enable the subsequent installation of an azide polyethyleneglycol (PEG) spacer for future conjugation reactions, it was necessary to cleave the Fmoc-protecting group. Therefore, compound **124** was stirred in DMF in the presence of piperidine, which afforded free amine **31** *via* an E_1cb-mechanism in 87% yield.[182] Prior to amide coupling between azide PEG spacer **30**[291] and free amine **31**, the carboxyl group was activated by

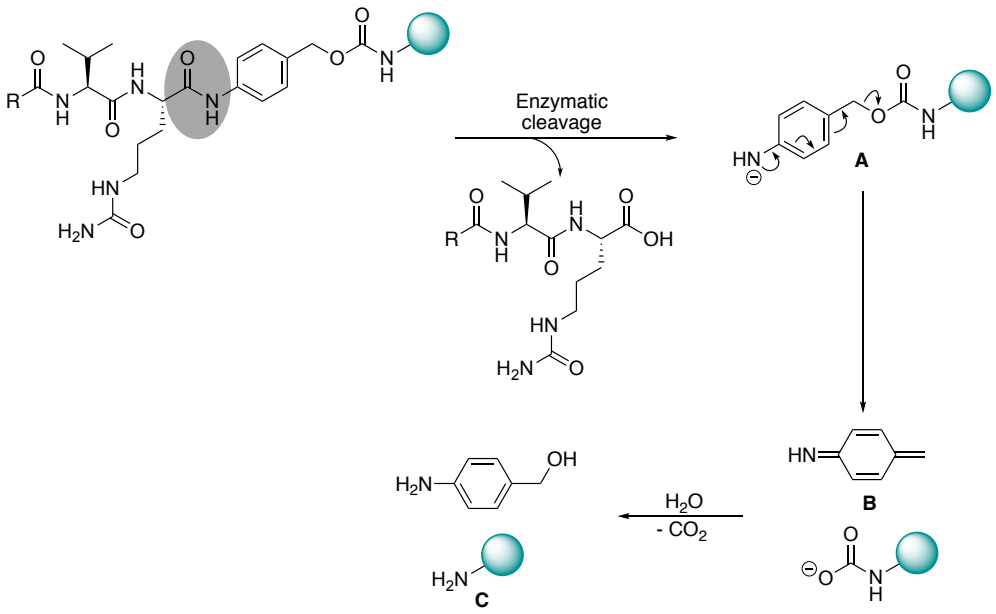
transformation into a mixed carbonic acid anhydride with IBCF in the presence of triethylamine in dichloromethane. Addition of the activated mixed anhydride to free amine **31** in a mixture of methanol and dichloromethane then afforded desired compound **125** in 92% yield.[94] Finally, the unprotected benzylic alcohol was transformed into an activated mixed carbonate with a *p*-nitrophenyl moiety[292] for subsequent coupling to the free amine of KRN7000 derivative **29**. Therefore, compound **125** was reacted with bis(4-nitrophenyl) carbonate in the presence of DIPEA in DMF to give mixed carbonate **28** in 62% yield.[94] This way, the desired self-immolative Val-Cit linker **28** was obtained in 38% yield over six steps and was ready to use for the assembly of T helper-like epitope **11**.

Activated mixed carbonate **28** and KRN7000 derivative **29** were coupled in a mixture of triethylamine and pyridine according to literature precedent,[94] which afforded the desired T helper epitope in a good yield of 88% (see SCHEME 3.50). The required purity and correct connectivity of this compound was confirmed usind 2D-NMR-experiments.



SCHEME 3.50. Assembly of T helper-like epitope **11** from self-immolative linker **28** and KRN7000 derivative **29**.

Self-immolative Val-Cit-4-aminobenzyl carbamate linkers are known substrates for lysosomal proteases such as cathepsin B (Cat B).[182, 293] These proteases are usually not active in the plasma due to adverse pH conditions and the presence of protease inhibitors, which leads to a high plasma stability of the dipeptide linkers.[294] Therefore, this class of linkers has particulary raised attention for the preparation of antibody-drug conjugates (ADCs), as these compounds require an intracellular release of the drug payload.[295] However, a Val-Cit linker has also been used for the preparation of an α-GalCer conjugate vaccine by *Anderson et al.*[94] They observed a high stability of the Val-Cit linker at physiological pH and only a small impact on vaccine efficacy, which makes this linker a promising candidate for the preparation of a fully synthetic vaccine. *Katzenellenbogen and co-workers*[296] supposed that the enzymatic cleavage of the Val-Cit linker leads to the release of the intermediate *p*-aminobenzyl residue (**A**) (see SCHEME 3.51), which then undergoes a 1,6-elimination with liberation of carbon dioxide and the free amine (**C**). Concomittantly formed intermediate iminoquinone methide (**B**) is hydrolysed to give *p*-aminobenzylalcohol.



SCHEME 3.51. Possible mechanism for the cleavage of a self-immolative Val-Cit-4-aminobenzyl carbamate linker.[296]

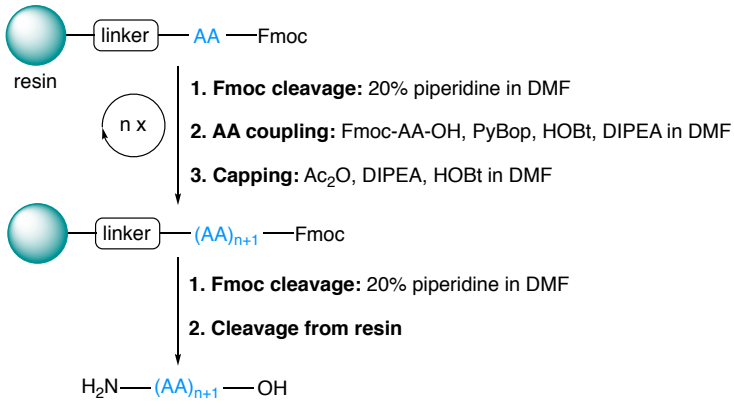
However, the applicability of this self-immolative linker has to be re-evaluated for the planned vaccine candidate in order to determine compatibility of each individual component. Therefore, an iNKT cell activation assay should be carried out with the vaccine construct beyond the scope of this thesis.

3.8 SYNTHESIS OF THE MULTIVALENT SCAFFOLD

The synthesized T helper-like epitope as well as the B cell epitopes were used for the final assembly on a multivalent scaffold by employing a regio-selectively addressable cyclic decapeptide as linkage unit to allow for tetravalent display of the B cell epitope. The first step toward the RAFT scaffold required preparation of a linear ten amino acid (AA) sequence *via* solid-phase peptide synthesis (SPPS).

3.8.1 Basic principles of solid-phase peptide synthesis

In 1963, *Bruce Merrifield*[297, 298] laid the foundation for SPPS through the first assembly of a peptide on an insoluble resin.[299] This pioneering work as well as subsequent developmental progresses have made it possible to generate longer and more complex peptides with less effort. Before the advent of this technique, peptide synthesis was carried out in homogenous solution and required extensive protecting group manipulations, as well as time-consuming purification and isolation procedures for each peptidic intermediate. In contrast, SPPS enables the assembly of peptides in a single vessel. Excess amounts of reagents and reactants as well as by-products can be easily removed by washing and filtration, which overcomes the repetitive purification steps for each intermediate peptide. Furthermore, the use of excess amounts of coupling partners and reagents guarantees high coupling yields and also allows for efficient *on-resin* modifications of the peptide backbone.[299]



SCHEME 3.52. General protocol for SPPS applying the Fmoc-protocol.

Nowadays, SPPS most commonly utilizes commercially available polystyrene resins pre-loaded with the starting amino acid (AA). Thereby, the *C*-terminus is typically anchored to the solid support *via* a cleavable linker (see SCHEME 3.52) and the *N*-terminus as well as all reactive side chains of trifunctional AAs are blocked with temporary or semi-permanent protecting groups, respectively. However, it is important that the protecting group pattern and the cleavable handle allow for an orthogonal and selective deprotection of the *N*-terminal end after each successful coupling step. Usually, the α-amino group of the resin-bound AA as well as of each incoming AA is blocked with a base labile 9*H*-fluorenylmethyloxycarbonyl (Fmoc) protecting group.[300] In contrast, side chain functionalities are blocked with a diverse repertoire of mostly acid labile protecting groups (see FIGURE 3.12). In order to enable the first amide coupling in the C→N direction, it is

necessary to remove the Fmoc protecting group with a secondary base, such as piperidine or morpholine. Afterwards, the first amide bond can be formed between the free *N*-terminus of the resin-bound AA and the carboxyl group of the incoming AA using suitable activating reagents (see above).

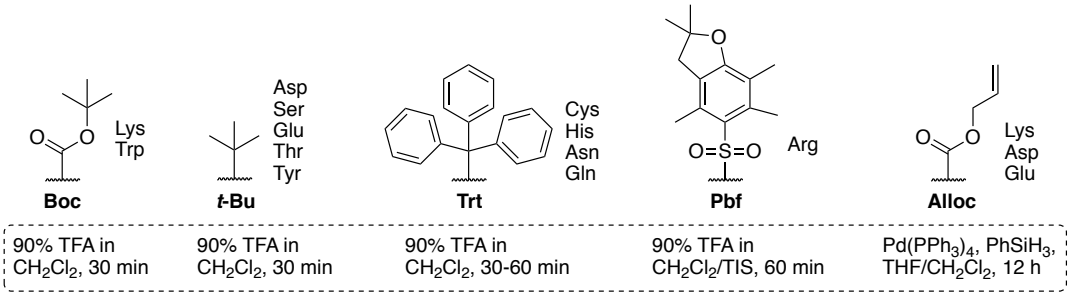


FIGURE 3.12. Selection of side chain protecting groups commonly used for the SPPS Fmoc protocol and description of appropriate deprotection conditions. Boc: *tert*-butyloxycarbonyl; *t*-Bu: *tert*-butyl; Trt: trityl; Pbf: pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl; Alloc: allyloxycarbonyl.[299, 301]

Subsequent washing and filtration steps remove possible by-products, as well as excess amounts of coupling reactants and reagents. The sequence of α-amino deprotection, activation, coupling and washing steps is then repeated until the desired peptide chain is assembled. Furthermore, an additional capping step can be applied in the SPPS protocol in order to make unreacted α-amino groups inaccessible for subsequent couplings, which avoids the formation of undesired (incomplete) peptide sequences. Capping is usually achieved by reacting the free *N*-terminus with acetic anhydride in the presence of DIPEA. Finally, the peptide is usually cleaved from the resin under acidic conditions with trifluoroacetic acid and the released peptide is collected after filtration. As the linker determines the cleavage conditions (see FIGURE 3.13), side chain functional groups can be either simultaneously deprotected or stay blocked during this step.

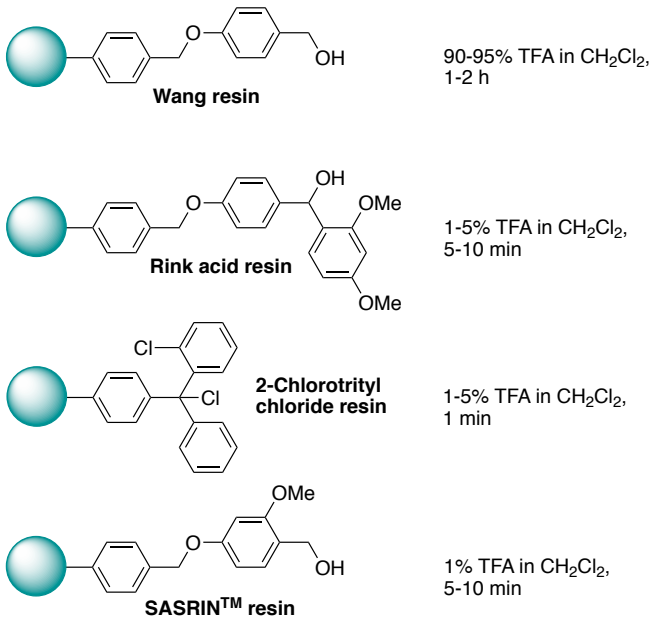
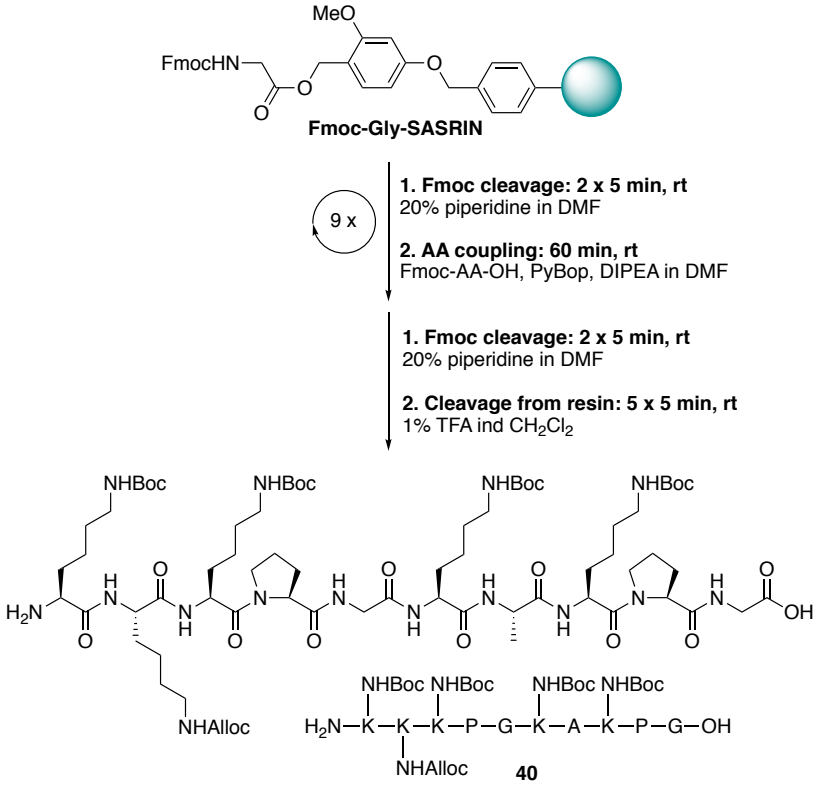


FIGURE 3.13. Selection of different commonly used cleavable linkers for SPPS and description of appropriate cleavage conditions.[301]

For instance, the use of a Wang resin, which is cleaved with a mixture of 90-95% TFA in dichloromethane, will also lead to the cleavage of all acid labile side chain protecting groups, whereas the use of a super acid-sensitive resin (SASRIN™),[302] which is cleaved with a mixture of 1% TFA in dichloromethane, will keep the side chain protecting group pattern intact.[301] This is of particular interest, if the synthesized peptide has to undergo further modifications after cleavage. Furthermore, the automation of this process,[299] the application of microwave synthesizers[303] as well as diverse modern chromatographic methods, such as high performance liquid chromatography (HPLC), have also strongly contributed to the success of SPPS.

3.8.2 Synthesis of the cyclic decapeptide (RAFT)

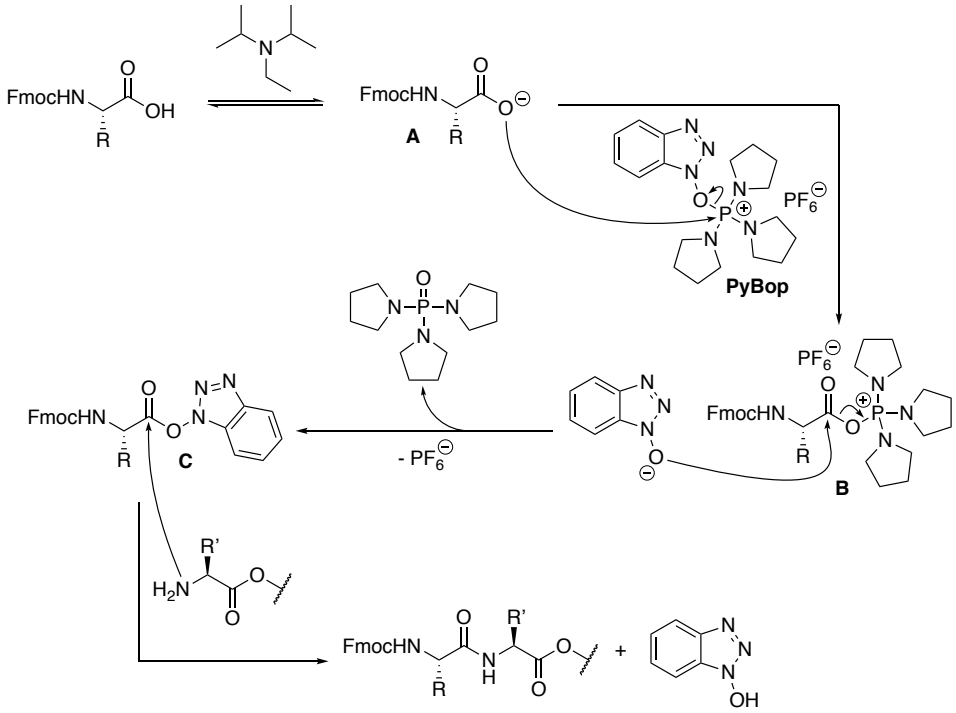
The automated SPPS of the linear amino acid sequence **40** (see SCHEME 3.53) was carried out on a *CEM Liberty Blue* peptide synthesizer using a Fmoc protocol. A commercially available SASRIN™ resin pre-loaded with the *C*-terminal starting AA Fmoc-Gly (*BACHEM*, loading 0.79 mmol/g) was employed.[183]



SCHEME 3.53. Solid-phase peptide synthesis of linear decapeptide **40**.

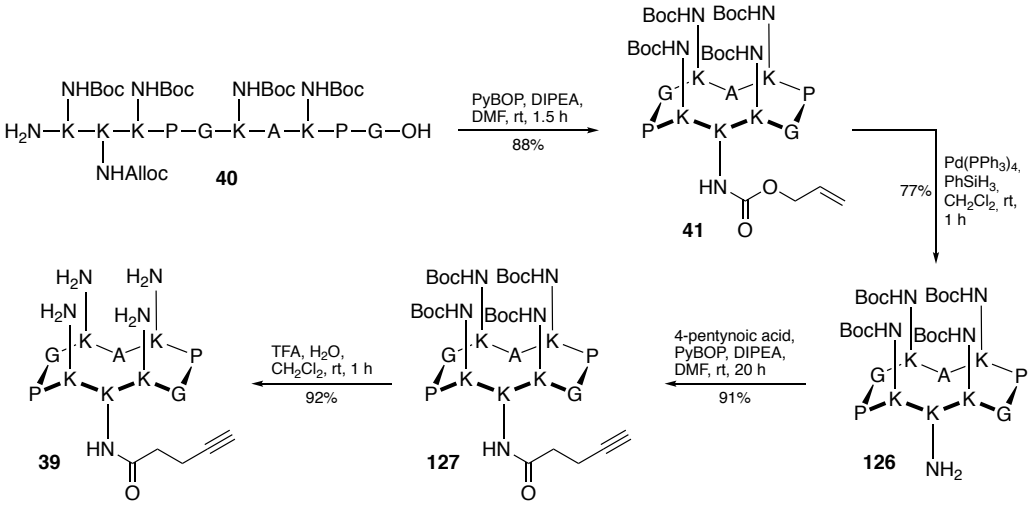
Thereby, the super acid-sensitive resin should enable mild cleavage conditions, without affecting the protecting groups of the lysine side chains. The latter should remain intact in order to allow the subsequent intramolecular cyclization reaction to proceed smoothly an without formation of unwanted by-products. The automated synthesis started with deprotection of the *N*-terminus in a mixture of 20% piperidine in DMF. This basic treatment was repeated twice in each cycle in order to assure complete deprotection. The following amide coupling was carried out according to a literature known protocol with benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP)[304] and DIPEA.[183] In contrast to carbodiimide

reagents, such as *N*,*N*′-dicylcohexylcarbodiimide (DCC),[305] *N*,*N*′-diisopropylcarbodiimide (DIC) or *N*-ethyl-*N*-(3-dimethyl-aminopropyl) carbodiimide (EDC),[306] the phosphonium salt PyBOP avoids side reactions and epimerization.[278] In the first step of the mechanism, the carboxylate anion (**A**) reacts with PyBOP under formation of an unstable acyloxyphosphonium intermediate (**B**) (see SCHEME 3.54). This species immediately reacts with the simultaneously formed benzotriazolyloxy anion to furnish the activated benzotriazole ester (**C**) and tris(pyrrolidin-1-yl)phosphine oxide.



SCHEME 3.54. Schematic depiction of the amide bond formation with PyBOP during solid-phase peptide synthesis.

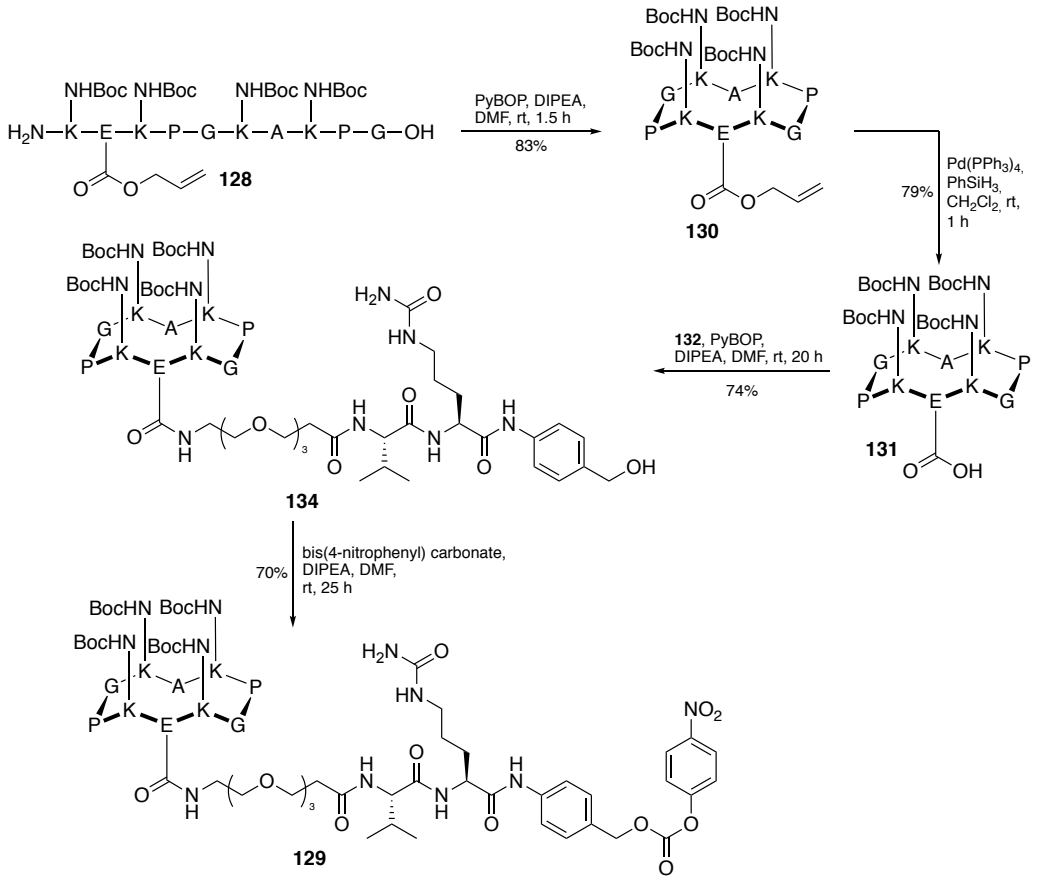
In the final step, the free α-amino group of the resin-bound AA reacts with the activated ester to give the desired amide bond. Afterwards, the Fmoc protecting group was removed and the resin-bound peptide was prone to enter the next coupling cycle. After completion of the peptide sequence and final Fmoc deprotection, the decapeptide was cleaved from the resin using a mixture of 1% TFA in dichloromethane. Therefore, the resin was transferred into a *Merrifield* reactor and treated five times with the cleavage cocktail for five minutes. The resulting solution was immediately neutralized with a mixture of pyridine in methanol and the peptide was precipitated from cold diethyl ether. Decapeptide **40** was obtained in 80% yield and in sufficient purity, as indicated by high performance liquid chromatography (HPLC) and high resolution mass spectrometry (HRMS). Subsequently, the linear peptide was cyclized with a mixture of PyBOP and DIPEA in DMF (see SCHEME 3.55).[183] Thereby, it is necessary to employ low peptide concentrations throughout the reaction course, as this will favor the desired intramolecular amide bond formation over intermolecular side reactions. Cyclic decapeptide **41** was obtained in 88% yield after precipitation from cold diethyl ether. In order to enable the introduction of an alkyne moiety for envisaged copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) conjugation, the allyloxycarbonyl (Alloc) protecting group at the „lower“ side of the RAFT had to be selectively removed. Therefore, fully protected cyclic decapeptide **41** was stirred with tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) and phenylsilane in dichloromethane.[183] Compound **126** was obtained after precipitation and excessive washing in 77% yield.



SCHEME 3.55. Synthesis of cyclic decapeptide **39** over four steps starting from linear peptide **40**.[183]

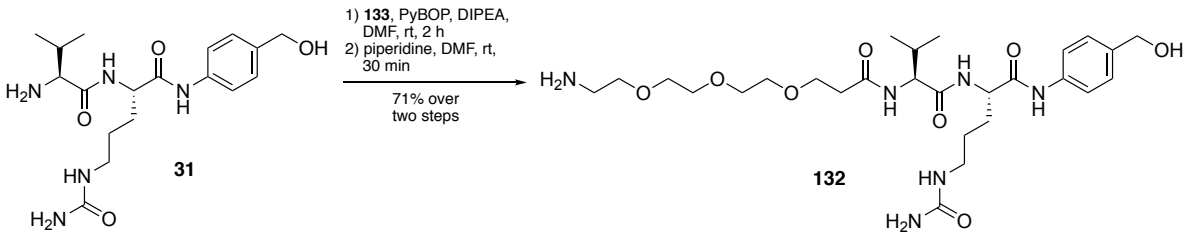
In the following step, the free amine was reacted with 4-pentynoic acid in the presence of PyBOP and DIPEA in DMF. The crude peptide was obtained by precipitation from cold diethyl ether and was subsequently purified by semipreparative HPLC. Thus, alkyne-bearing cyclic decapeptide **127** was afforded in 91% yield and in high purity. Finally, the Boc protecting groups were removed with TFA in a mixture of water and dichloromethane. The desired RAFT **39** was obtained after precipitation from cold diethyl ether in 92% yield and in high purity. The overall yield was determined as 45% after four steps.

The automated SPPS of a second linear ten amino acid sequence **128** (see SCHEME 3.56) was also carried out on the commercially available SASRIN™ resin pre-loaded with the *C*-terminal starting AA Fmoc-Gly (*BACHEM*, loading 0.79 mmol/g) according to the above described synthesis of **40**.[183] This peptide should lead to alternative cyclic decapeptide **129** (see SCHEME 3.56) over four steps and should furnish a comparable multivalent construct as back-up strategy. After cleavage from the resin with a mixture of 1% TFA in dichloromethane, decapeptide **128** was obtained by precipitation from cold diethyl ether in 72% yield and in high purity. The subsequent cyclization was carried out in a mixture of PyBOP and DIPEA in DMF,[183] to give cyclic decapeptide **130** in 83% yield after precipitation from cold diethyl ether. The Alloc protecting group at the glutamic acid side chain was selectively removed by using $Pd(PPh_3)_4$ and phenylsilane in dichloromethane.[183] Compound **131** was obtained after precipitation and excessive washing in 79% yield. The next step required an amide bond formation between the carboxylic acid of **131** and the primary amine of dipeptide linker **132** (see SCHEME 3.57). The latter was prepared from already available compound **31** and literature known Fmoc-protected PEG spacer **133**.[307] Amide bond formation with PyBOP and DIPEA in DMF followed by removal of the Fmoc protecting group gave the desired linker **132** in 71% yield over two steps. Both compounds **131** and **132** were coupled in the presence of PyBOP and DIPEA in DMF. The resulting crude peptide was precipitated from cold diethyl ether and was purified by semipreparative HPLC to afford **134** in 74% yield.



SCHEME 3.56. Synthesis of cyclic decapeptide **129** over four steps starting from linear peptide **128**.

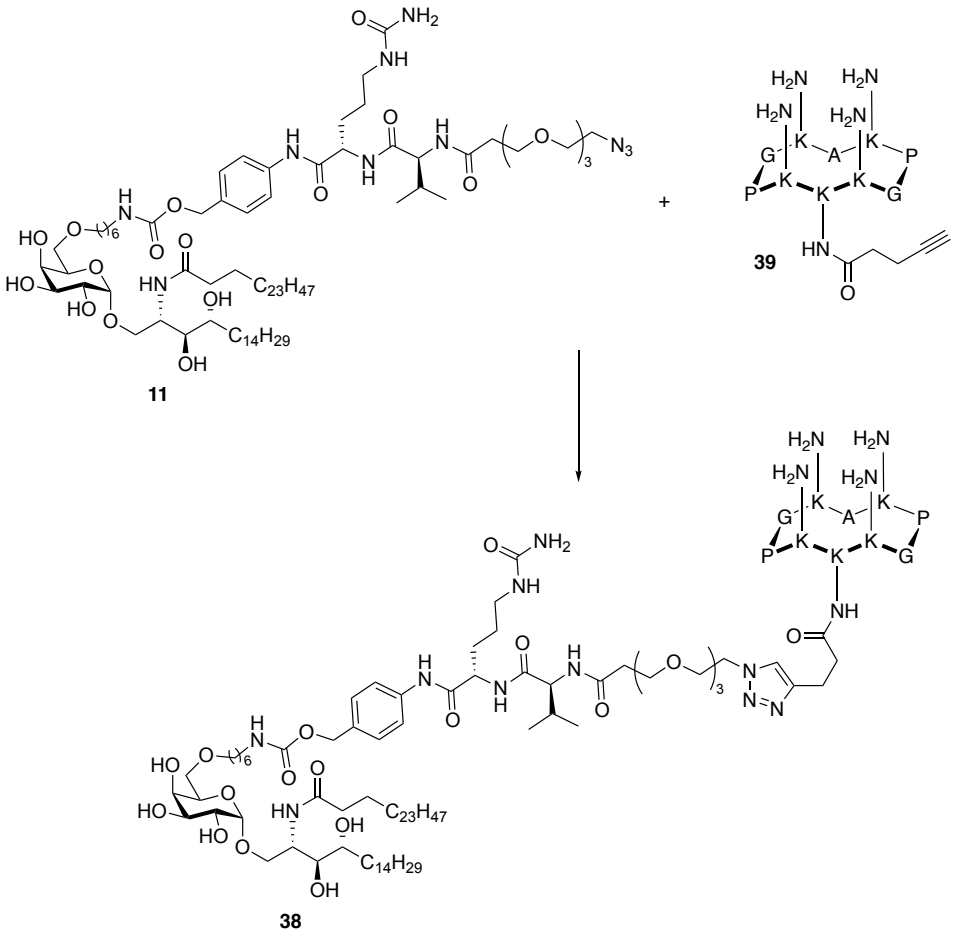
In the following, the unprotected benzylic alcohol of the linker residue was transformed into an activated mixed *p*-nitrophenyl carbonate. Therefore, peptide **134** was reacted with bis(4-nitrophenyl) carbonate in the presence of DIPEA in DMF to give compound **129** in 70% yield after purification by semipreparative HPLC.[94] This compound could then be used for the conjugation to the KRN7000 derivative **29**.



SCHEME 3.57. Synthesis of dipeptide linker **132**.

3.9 ASSEMBLY OF A FULLY SYNTHETIC VACCINE CANDIDATE

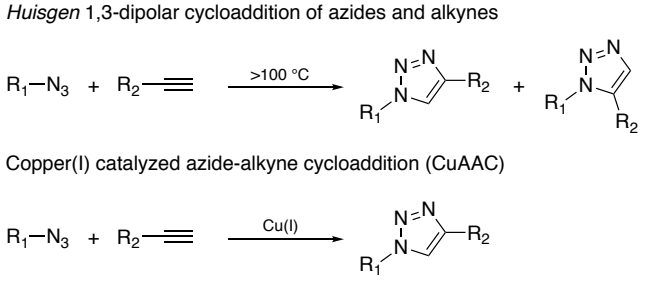
Finally, with all building blocks for the fully synthetic vaccine candidates in hand, the assembly of the multivalent construct *via* copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) between the azide moiety of T helper-like epitope **11** and the alkyne moiety of cyclic decapeptide **39** was tackled. Upon successful conjugation a novel versatile T helper epitope scaffold **38** would result, which could be used as starting point for the preparation of a variety of multivalent carbohydrate-based vaccines (see SCHEME 3.58).



SCHEME 3.58. Synthesis of T helper epitope scaffold **38** *via* a copper(I)-catalyzed azide-alkyne cycloaddtion (CuAAC).

The CuAAC reaction was independently discovered by the research groups of *Meldal*[308] and *Sharpless*[309] in 2002. This reaction is based on the *Huisgen* 1,3-dipolar cycloaddition[310] between azide and alkyne moieties (see SCHEME 3.59), which is usually a slow and unselective reaction and requires high temperatures. However, the addition of a copper(I) species significantly accelerates the reaction rate and increases the regioselectivity, so that the 1,4-disubstituted 1,2,3-triazole isomer becomes the only product.[311, 312] Click chemistry has found wide-spread application in drug discovery, biochemistry, dendrimer chemistry and many more disciplines, because the reaction is compatible with aqueous systems and several unprotected functionalities. Moreover, the obtained 1,2,3-triazole exhibits excellent chemical stability, biomimetic characteristics and biological properties.[311] The required Cu(I) species can be directly applied by the use of Cu(I) salts or they can be generated from diverse pre-catalysts during the course of reaction. Different Cu(I) sources and suitable

additives have been largely summarized by *Meldal and Tornøe*.[313] However, the most common method employs the *in situ* reduction of copper(II) sulfate with excess amounts of sodium ascorbate in aqueous medium. Thereby, the ascorbate not only allows for a mild reduction of the copper(II) sulfate, but also enables a viable alternative to oxygen-free conditions and suppresses any copper-mediated oxidative side reactions.[312]



SCHEME 3.59. Depiction of thermal and copper(I)-catalized azide-alkyne cycloaddition.

Another possibility to prevent Cu(I)-mediated oxidative damage lies within the use of coordinating polydentate *N*-donor ligands, such as tris(benzyltriazolylmethyl)amine (TBTA) or tris(hydroxypropyl-triazolylmethyl)amine (THPTA) (see FIGURE 3.14/3.15). These compounds stabilize Cu(I) species by coordination and reduce the concentration of toxic Cu(I) in solution .[311, 312] Besides, it has been shown by *Sharpless and co-workers*, that such ligands also have an accelerating effect on 1,3-dipolar cycloadditions.[314]

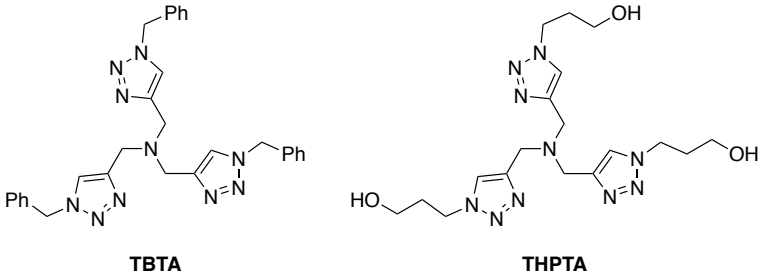


FIGURE 3.14 Selection of polydentate *N*-donor ligands used for CuAAC reactions.

Further common CuAAC methods directly apply Cu(I) salts (CuBr, CuI, CuCl), which however requires an inert oxygen-free atmosphere to prevent oxidation of the unstable +1 oxidation state. In this case, excess amounts of a nitrogen base, such as DIPEA, are usually added to form the necessary Cu(I)-acetylide intermediate and prevent formation of unreactive polymeric copper acetylides.[311, 315]

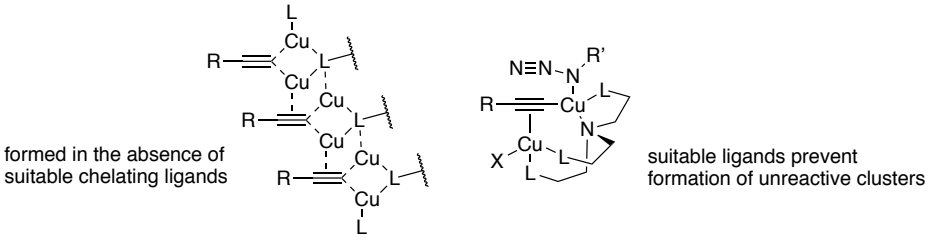
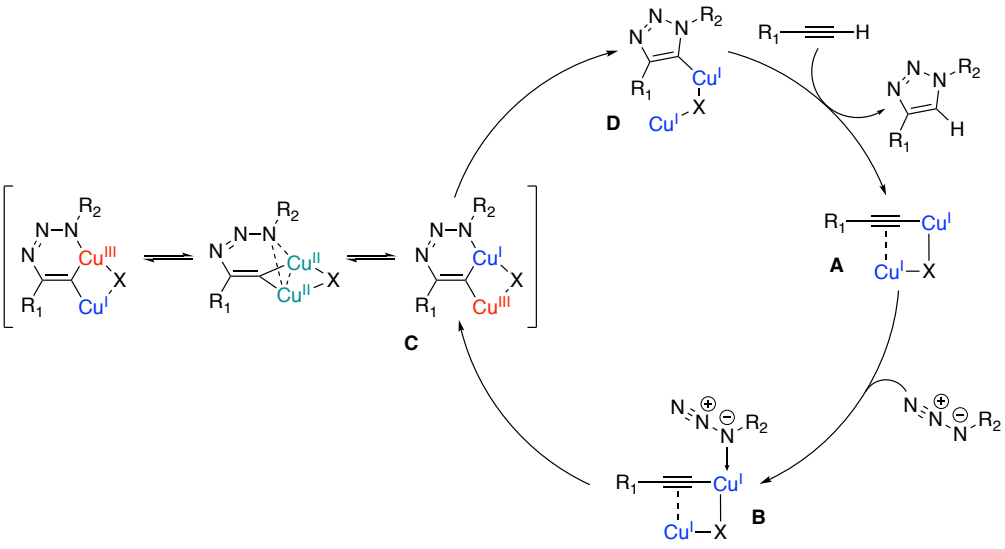


FIGURE 3.15. Depiction of polymeric copper(I) acetylide species (left) and composition of a reactive copper(I) acetylide ligand cluster breaking complex (right).[312, 315]

The application of a copper wire or copper turning also catalyzes 1,3-dipolar cycloaddition reactions by comproportionation to Cu(I). This approach is accompanied by longer reaction times but also leads to lower copper contamination. Fortunately, in some cases the reaction can be accelerated by the use of ultrasound or microwave irradiation.[311]



SCHEME 3.60. Dinuclear mechanistic proposal for CuAAC; (X = bridging ligand). (Adapted from ref.[316])

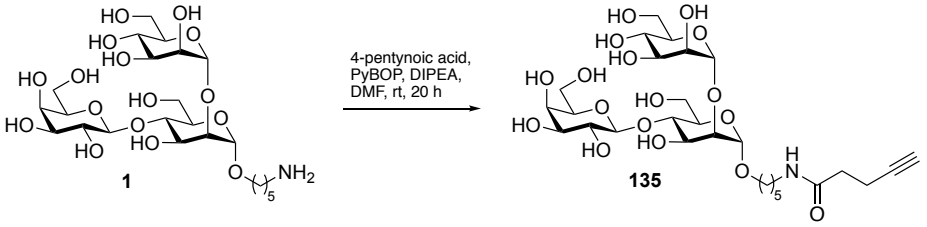
Regarding its mechanism, it is assumed that the CuAAC reaction is initiated by the formation of a σ,π-di(copper) acetylide (**A**), which engages in a complex (**B**) with the azide residue (see SCHEME 3.60).[317] Presumably, this intermediate arranges in a metallacycle (**C**), in which one copper(I) atom is oxidized to copper(III).[316] *Fokin and co-workers*[318] used copper isotope labeling to show, that the dicopper metallacycle rapidly rearranges under exchange of the two copper centers. Subsequent reductive ring contraction furnishes a copper(I) triazolide (**D**), which is able to deprotonate a terminal alkyne. However, this deprotonation step can also be catalyzed by additional base, a conjugate base of a protic solvent or by additives, such as sodium ascorbate.[315, 316]

In this work, first attempts towards a CuAAC click reaction between alkyne **39** and azide **11** were carried out in accordance to a literature known protocol.[94] Therefore, both reactants were dissolved in a mixture of DMSO/$CHCl_3$/MeOH/H_2O, to which a copper wire, copper(II) sulfate and TBTA were added. In a second approach the use of copper(II) sulfate was avoided in order to lower putative copper contaminations of the product. After 20 h at room temperature, high resolution mass spectrometry (HRMS) indicated the presence of the desired product **38** in both reaction mixtures (see TABLE 3.4, entry 1/2). However, the pure compound could not be obtained *via* HPLC, flash column chromatography, precipitation, size exclusion chromatography or molecular weight cut off (MWCO) spin filters. Most presumably the poor solubility of the product and the presence of remaining azide **11** and TBTA hamper the purification. In order to solve this problem, complete conversion of azide **11** to the desired product would be required. Moreover, protocols employing more soluble ligands or procedures omitting these ligands were tested. Thus, several different Cu(I) sources, reaction temperatures, stoichometric ratios and solvents as well as different ligands were screened and the results are depicted in TABLE 3.5.

TABLE **3.5**. Investigation of suitable reaction conditions for the preparation of compound **38**.

entry	azide **11**	alkyne **39**	ligand	Cu(I) source	conditions	found in HRMS
1	1.0 equiv.	2.0 equiv.	TBTA	Cu wire $CuSO_4$	DMSO/$CHCl_3$/MeOH/H_2O (3:3:3:1), rt, 20 h	✓
2	1.0 equiv.	2.0 equiv.	TBTA	Cu wire	DMSO/$CHCl_3$/MeOH (1:1:1), rt, 20 h	✓
3	1.0 equiv.	2.0 equiv.	THPTA	Cu wire	DMSO/$CHCl_3$/MeOH (1:1:1), rt, 20 h	✗
4	1.0 equiv.	2.0 equiv.	THPTA	Cu wire	DMSO/$CHCl_3$/MeOH (1:1:1), 35 °C, 21 h	✗
5	1.0 equiv.	2.0 equiv.	THPTA	Cu wire $CuSO_4$	DMSO/$CHCl_3$/MeOH/H_2O (3:3:3:1), rt, 21 h	✗
6	1.0 equiv.	2.0 equiv.	THPTA	Cu wire $CuSO_4$	DMSO/$CHCl_3$/MeOH/H_2O (3:3:3:1), 60 °C, 24 h	✗
7	1.2 equiv.	1.0 equiv.	—	$CuSO_4$ NaAscorbat	DMSO/$CHCl_3$/MeOH/H_2O (3:3:3:1), rt, 21 h	✗
8	1.0 equiv.	1.0 equiv.	—	CuBr	DMSO/$CHCl_3$/MeOH/H_2O (3:3:3:1), rt, 21 h	✗
9	1.0 equiv.	2.0 equiv.	—	Cu wire	Pyridine, rt, 21 h	✗
10	1.0 equiv.	2.0 equiv.	resin-bound TBTA	Cu wire	DMSO/$CHCl_3$/MeOH (1:1:1), rt, 20 h	✗
11	1.0 equiv.	2.0 equiv.	resin-bound TBTA	Cu wire	Pyridine, rt, 21 h	✗

Unfortunately, neither the use of easier removable ligands (THPTA or resin-bound TBTA) nor the absence of such ligands or changes in the reaction conditions led to the formation of compound **38**. Similarly, longer reaction times proved unsuccessful so far (not listed in TABLE 3.5) and consequently further reaction and purification conditions have to be evaluated for this challenging step.



SCHEME 3.61. Synthesis of alkyne-linker bearing B cell epitope **135**.

Despite these hurdles, synthesis of a monovalent fully synthetic vaccine candidate was also tackled *via* CuAAC click conditions, for which the amine linker of trisaccharide **1** was equipped with an alkyne moiety (see SCHEME 3.61).

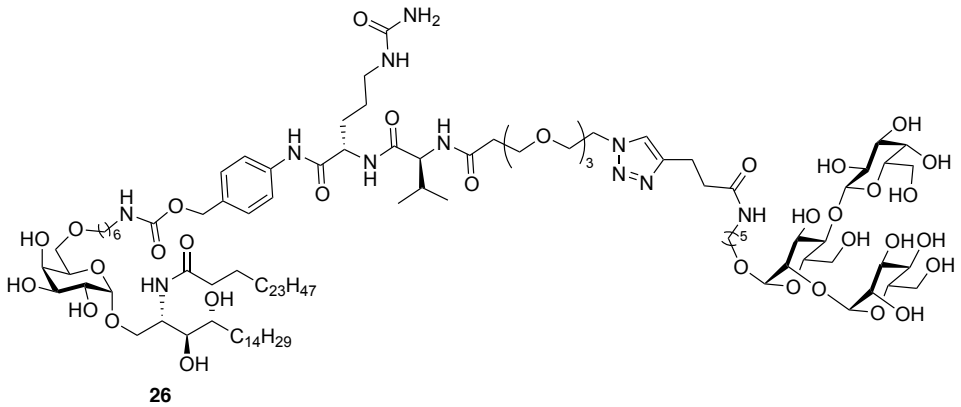


FIGURE 3.16. Targeted monovalent full synthetic vaccine candidate **26**.

The resulting alkyne **135** was precipitated from cold diethyl ether, dried and immediately dissolved with azide **11** in a mixture of DMSO/$CHCl_3$/MeOH. After stirring in the presence of a copper wire and TBTA for 20 h at room temperature, HRMS indicated the presence of desired product **26** (see FIGURE 3.16). However, purification *via* HPLC was again impossible due to the poor solubility and a lack of UV-absorption. Click conditions similar to those employed in the approaches toward multivalent construct **38** were again applied to the assembly of compound **26** but proved unsuccessful.

Therefore, application of a copper free strain-promoted alkyne-azide cycloaddition (SPAAC) was envisaged, as recently described by *Anderson et al*. for a related construct.[95]

This chemistry uses highly strained and medium-sized cyclic alkynes, e.g. cyclooctyne, as functional handles.[319] The cycloaddition reaction between cyclooctyne and an organic azide has found wide application among chemists and biologists over the last decades, because it offers a clean bioconjugation tool, which is easy to handle, does not require any additives, possesses a broad solvent compatibility and delivers stable products.[319]

Already in 1953, *Blomquist et al.*[320] assumed cyclooctyne to be highly strained as they observed an „explosive“ reaction between this compound and phenylazide. The acetylene bond angle of 163° in cyclooctyne significantly differs from the favorable linear arrangement and therefore delivers a suitable release of ring-strain when being transformed to a system with sp^2-hybridized carbon atoms.[319] The fact that the driving force of this reaction lies in the release of ring-strain and does not require toxic Cu(I)-species as catalyst makes the SPAAC reaction particularly interesting for metabolic labeling studies. In 2004, *Bertozzi and co-workers*[321] firstly reported on the bioorthogonality of this [3+2]-cycloaddition under physiological conditions by selective chemical modification of living cells. In the following, many research groups have focused on the synthesis of novel cyclooctyne-derivatives to improve reactivity without affecting stability. Nowadays, in particular aliphatic and dibenzoannulated cyclooctynes are in use (see FIGURE 3.17).

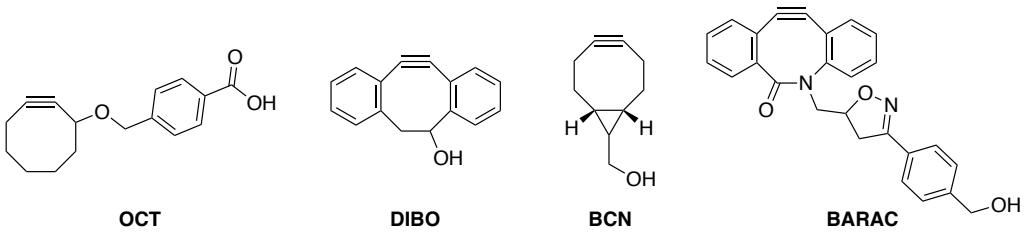
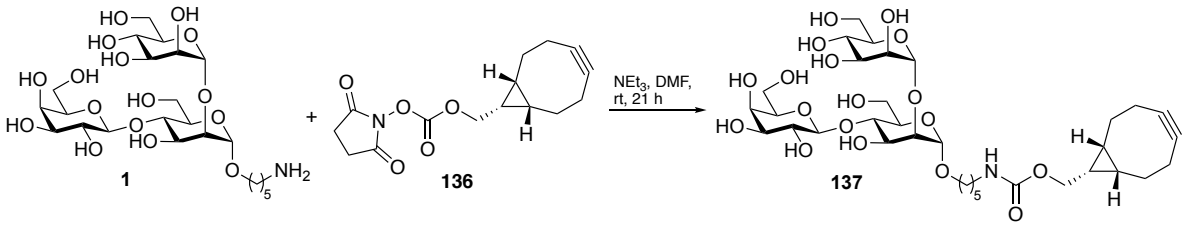


FIGURE 3.17. Selection of functionalized cyclooctyne derivatives suitable for conjugations reactions: cyclooctyne OCT,[321] dibenzocyclooctyne DIBO,[322] bicyclononyne BCN,[323] biarylazacyclooctyne BARAC.[324]

A broader overview of such compounds and a detailed discussion on the characteristics of these is given in a recent review by *Dommerholt et al*.[319] It was also this group that developed bicyclo[6.1.0]nonyne (BCN) as a readily accessible functionalized handle with low lipophilicity and high reactivity in [3+2]-cycloadditions.[323] *Anderson et al.* used BCN to assemble an α-GalCer derivative with antigenic peptides derived from influenza A virus (IAV) proteins.[95] We therefore decided to apply their approach to our target vaccine candidate, despite the potential risk of generating antibodies directed to the strained ring moiety.



SCHEME 3.62. Synthesis of cycloalkyne-functionalized B cell epitope **137**.

Furthermore, we assumed that in the case of the monovalent vaccine candidate, liposomal formation could also lead to the desired multivalent surface presentation of the B cell epitopes and thus might reduce the immune response against the triazole moiety as a result of poorer accessibility for the immune system. Therefore, and to equip compound **1** with a BCN handle, the former was reacted with commercially available NHS-ester **136** (see SCHEME 3.62). The desired compound **137** was then precipitated from cold diethyl ether, dried and immediately used for the subsequent click reaction, in which a four-molar excess of **137** should guarantee full conversion of azide **11** to the desired vaccine product **138** (see FIGURE 3.18). After stirring both compounds in DMSO for four days, the monovalent fully synthetic vaccine construct **138** was obtained after precipitation from water and excessive washings with an excellent yield of 94% and sufficient purity (see FIGURE 3.18).

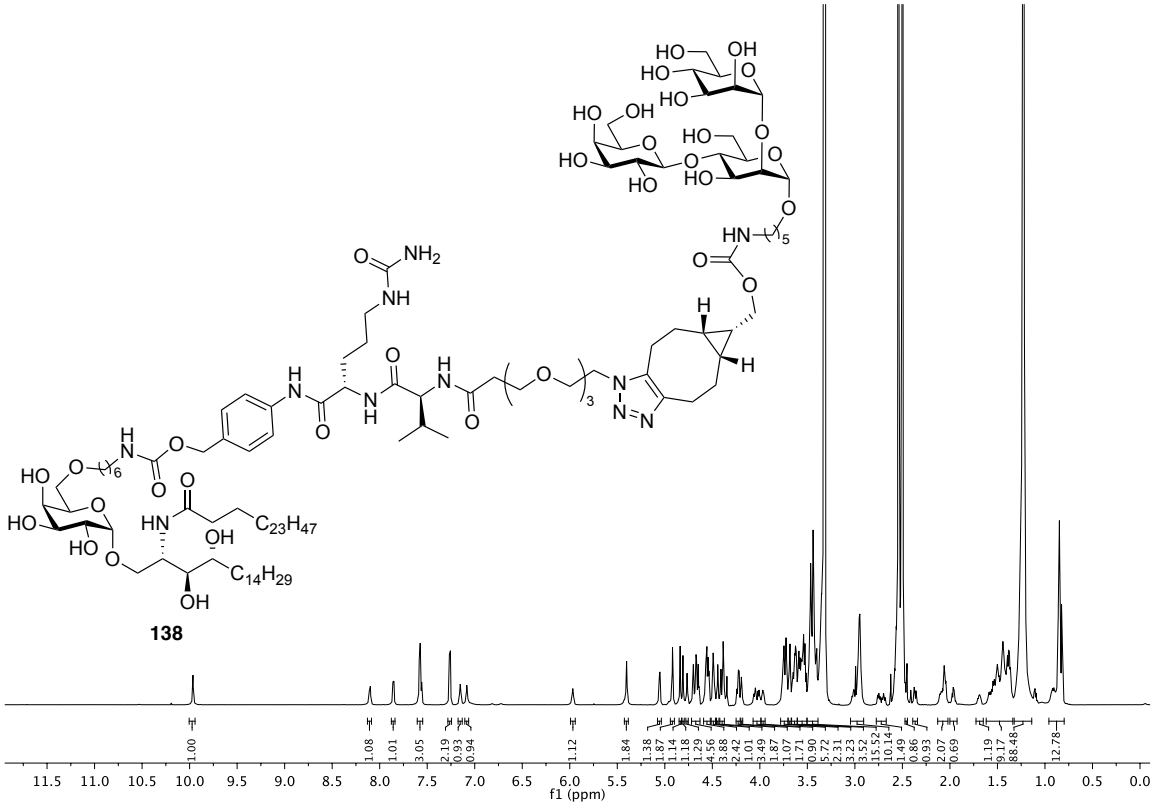
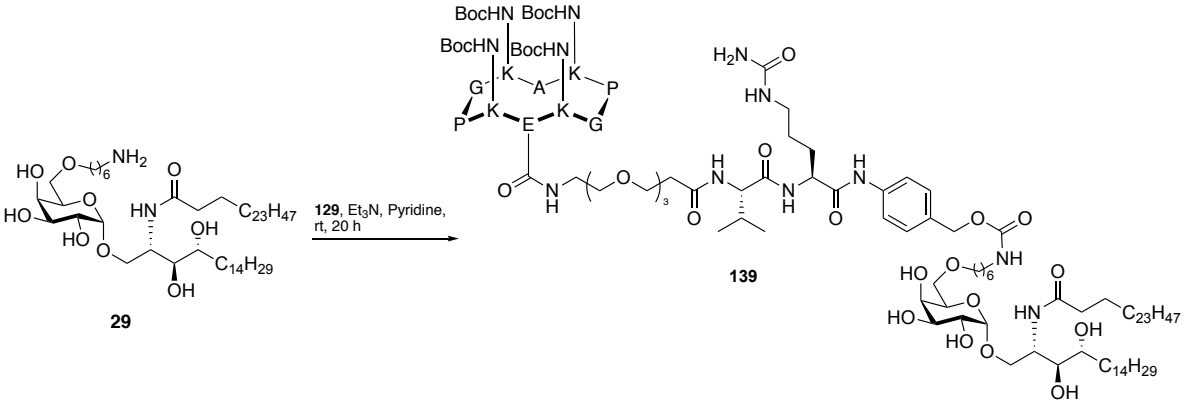


FIGURE 3.18. ^{1}H-NMR spectrum (800 MHz, $(CD_3)_2SO$) of fully synthetic vaccine candidate **138**.

Sufficient amounts of this compound are now at hands for future vaccination studies, whereby the KRN7000 moiety can be used as liposomal carrier vehicle as described by *Yin et al*.[96]

A further approach toward a multivalent fully synthetic vaccine candidate was carried out using KRN7000 derivative **29** and linker-bearing cyclic decapeptide **129** (see SCHEME 3.63). The use of an activated carbonate should avoid problems encountered during CuAAC click chemistry. Unfortunately, it was not possible to observe the formation of desired compound **139** under the conditions applied, presumably due to steric hindrance. Thus, reaction conditions will have to be evaluated for the preparation of **139** in the near future.



SCHEME 3.63. Tested conditions for the preparation of **139**.

4 SUMMARY AND OUTLOOK

The dense distribution of unique glycans on the surface of pathogens and malignant cells has made carbohydrate epitopes attractive target structures for vaccine development. In particular, fully synthetic carbohydrate-based vaccines have gained major attention over the last decades, since they provide excellent structural homogeneity, a high immunological reproducibility and the advantage of a tailor-made epitope design. Fully synthetic glycoconjugate constructs are usually comprised of defined and characterized chemical units, whereby each part plays a well-founded and specific role during the immunization event. Ideally, they should contain a smallest carbohydrate epitope that is able to induce protective immunity, a non-immunogenic multivalent scaffold, which enables the formation of a glycocluster imitating the dense surface distribution of pathogenic surface glycans, as well as an additional T helper epitope, such as a defined immunogenic peptide, oligosaccharide or glycolipid, which helps to overcome the often insufficient immunogenicity of carbohydrate epitopes and induces immunological memory. Besides insufficient immunogenicity, carbohydrate antigens may suffer from rapid *in vivo* degradation, leading inevitably to a limited bioavailability and a reduced immunological potency. However, this drawback might be overcome by strategic fluorine incorporation, as selectively fluorinated glycotopes have already been proven to exhibit an enhanced metabolic stability, an increased “non-self”-character and comparable or even increased immunogenicities.

4.1 SYNTHESIS OF NATIVE AND FLUORINATED LPG CAPPING STRUCTURES OF *LEISHMANIA DONOVANI*

Within this work, synthetic routes toward native and selectively fluorinated lipophosphoglycan capping structures of *Leishmania donovani* were carried out to set the stage for their future use in immunological studies. The severe pathophysiology of the protozoan parasite *Leishmania*, which is endemic in more than 88 countries and leads to approximately 70.000 deaths annually among the permanent 12 million infected people, has already turned attention of several research groups to the synthesis of leishmanial carbohydrate epitopes for vaccine and diagnostic tool development. In particular substructures of the promastigote lipophosphoglycans (LPG), which belong to the most abundant leishmanial surface glycoconjugates, have been synthesized so far and have been applied in preliminary immunological evaluations. Inspired by these efforts, we decided to focus on the LPG terminating neutral cap structures of *Leishmania donovani*, which is the main species causing fatal anthroponotic visceral leishmaniasis. In that regard, we developed and executed synthetic strategies for the preparation of novel fluorinated leishmanial carbohydrate epitopes comprising a β-Gal-(1→4)-[α-Man-(1→2)]-α-Man trisaccharide (see FIGURE 4.1). This glycan epitope not only contains the most crucial LPG elements but also represents the most abundant terminating neutral cap structure of *Leishmania donovani*. Moreover, fluorine incorporation was envisaged to take place at the galactose moiety in order to stabilize and maintain the important β-Gal-(1→4)-α-Man glycosidic linkage *in vivo*, which is also unique among eukaryotic organisms. The targeted structures **6**–**10** included distinct fluorine incorporation at the 2-, 3-, 4-, 6- and 2,6-position of the galactose moiety (see FIGURE 4.1) to address the question of how fluorine incorporation affects binding affinity and antibody selectivity in comparison to native trisaccharide **1**.

However, as the work-flow of rational vaccine design requires comparison of all terminating neutral cap structures in biological evaluations, syntheses of the remaining leishmanial neutral cap structures **2**–**5** were also accomplished (see FIGURE 4.1).

The synthetic concept toward the fluorinated and non-flourinated neutral cap structures was based on a literature known convergent strategy and started from suitably protected monosaccharide building blocks. These were assembled in glycosylation reactions to provide the desired di-, tri- or tetrasaccharide derivatives **1**–**10** additionally equipped with amine linkers for future conjugation reactions (see FIGURE 4.1). Therefore, the different galactosyl and mannosyl building blocks had to be accessed *via* linear synthetic routes. In the design process of the individual syntheses, the choice of an orthogonal protecting group pattern, as well as the use of common precursor molecules for a modular approach was of particular interest.

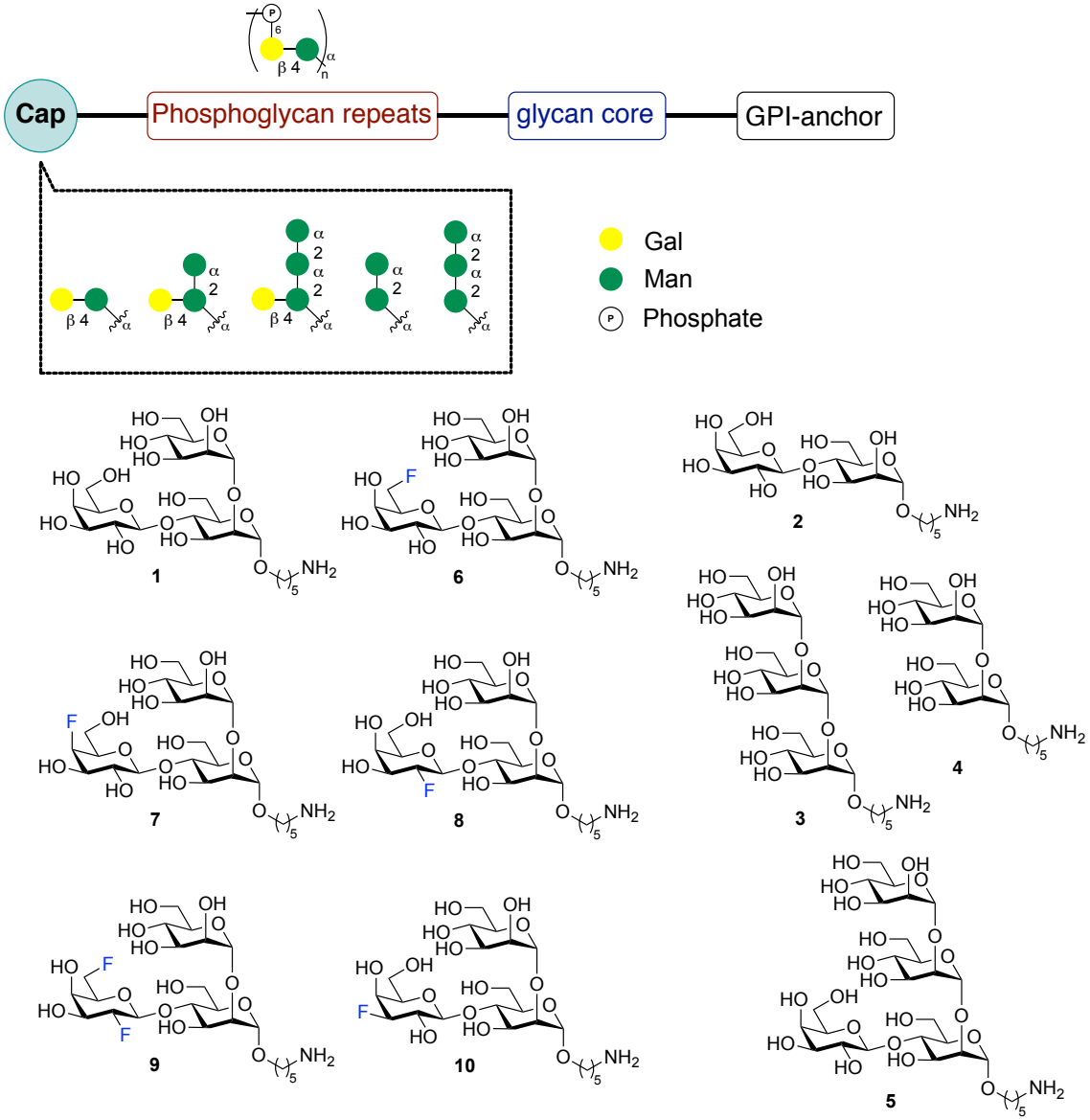
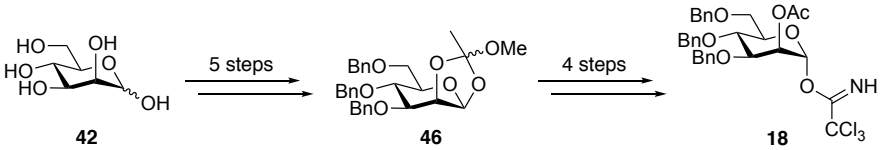


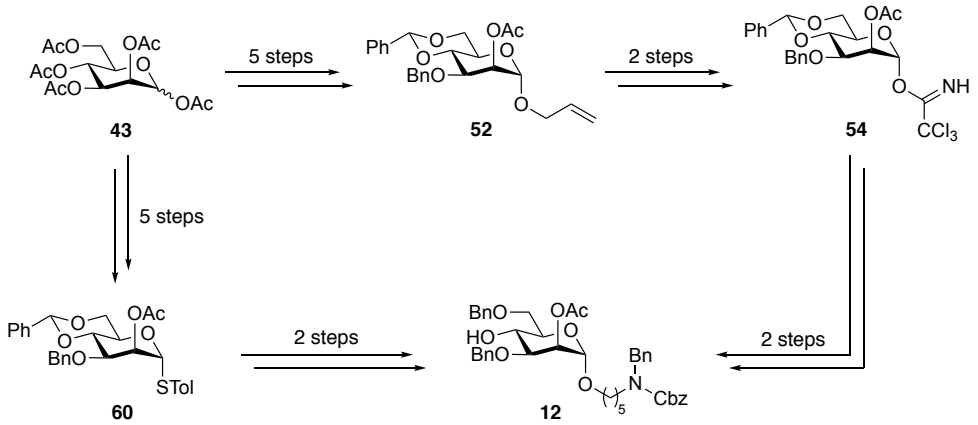
FIGURE 4.1. Synthesized leishmanial native and fluorinated LPG cap structures.

Mannosyl donor **18**, which was used for the preparation of all neutral cap structures, was synthesized over nine steps starting from commercially available D-mannose **42** and was obtained in an overall yield of 32% (see SCHEME 4.1). The intermediate compound **46** was additionally used as a precursor molecule for the synthesis of nonfluorinated tetrasaccharide **5**.



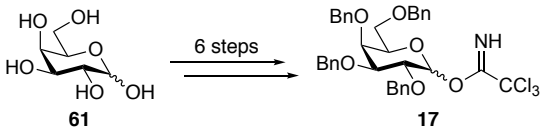
SCHEME 4.1. Synthesis of mannosyl donor **18** over nine steps.

The preparation of the bridging mannosyl acceptor **12**, which was equipped with a protected aminopentyl linker, started from readily available pentaacetate **43** and was accomplished using two different synthetic strategies (see SCHEME 4.2). The first synthetic route followed literature precedent by using an allylic ether as orthogonal temporary protecting group at the anomeric center. This synthesis provided the desired mannosyl acceptor **12** in 19% yield over nine steps. Alternatively, by using a corresponding thioglycoside donor, the desired compound was prepared *via* a shorter seven-step synthetic route in 21% overall yield.



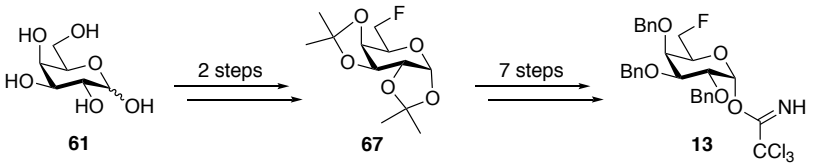
SCHEME 4.2. Synthesis of mannosyl acceptor **12** over nine or seven steps, respectively.

The syntheses of the nonfluorinated and the corresponding fluorinated galactosyl donors **17**, **13** and **14** were carried out based on literature known protocols (see SCHEME 4.3/4.4/4.5). Thus, preparation of the native trichloroacetimidate donor **17** started from commercially available D-galactose **61** to furnish this compound over six steps and in 44% overall yield (see SCHEME 4.3).



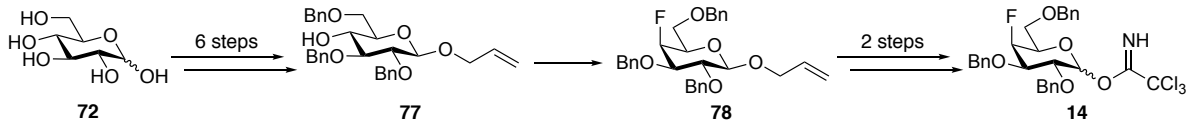
SCHEME 4.3. Synthesis of native galactosyl donor **17** over six steps.

The synthesis toward 6F-galactosyl trichloroacetimidate **13** commenced with D-galactose **61** and used the nucleophilic fluorination reagent diethylaminosulfur trifluoride (DAST®) to introduce a fluorine at C-6 (see SCHEME 4.4). The desired compound was obtained over nine steps in 39% overall yield.



SCHEME 4.4. Synthesis of 6F-galactosyl donor **13** over nine steps.

In contrast, the fluorination strategy for preparation of 4F-galactosyl donor **14** required a classical nucleophilic displacement at C-4 of glucose under epimerization. Therefore, glucose-derived secondary alcohol **77** was first transformed into a triflate, which was immediately reacted with tetrabutylammonium fluoride (TBAF) to give 4-fluorinated galactose derivative **78** (see SCHEME 4.5). Fluorinated donor **14** was obtained over nine steps in an overall yield of 9%.

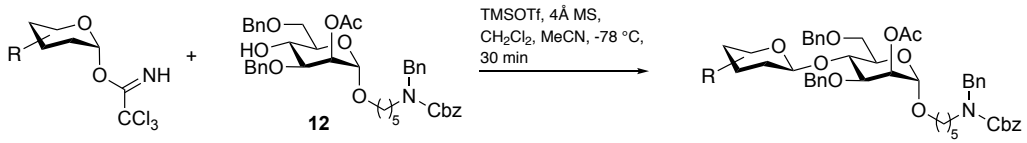


SCHEME 4.5. Synthesis of 4F-galactosyl donor **14** over nine steps.

The 2F-galactosyl donor **15** and the 2,6F-galactosyl donor **16** were kindly provided by *Markus Daum* from the *Hoffmann-Röder* research group.

To install the first mannosyl units, galactosyl donors **13**–**17** were subjected to coupling reactions with mannosyl acceptor **12** under optimized inverse glycosylation conditions. The desired β-linked disaccharide building blocks **85**–**89** were obtained in yields between 69% and 79% (see TABLE 4.1).

TABLE **4.1**. Comparison of the glycosylation yields of native donor **17** and fluorinated donors **13**–**16** with mannosyl acceptor **12**.

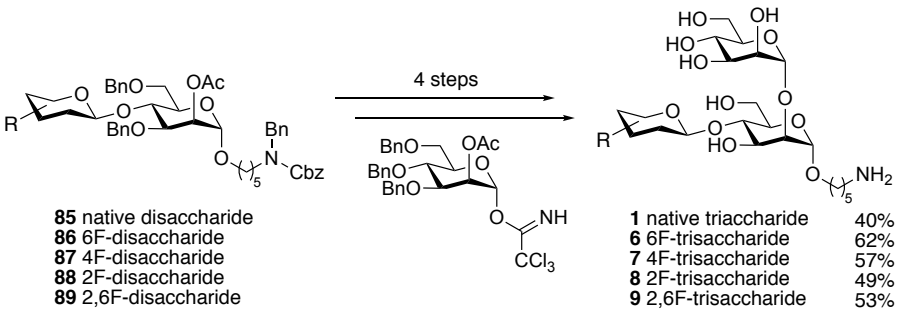


17 native donor
13 6F-donor
14 4F-donor
15 2F-donor
16 2,6F-donor

85 native disaccharide
86 6F-disaccharide
87 4F-disaccharide
88 2F-disaccharide
89 2,6F-disaccharide

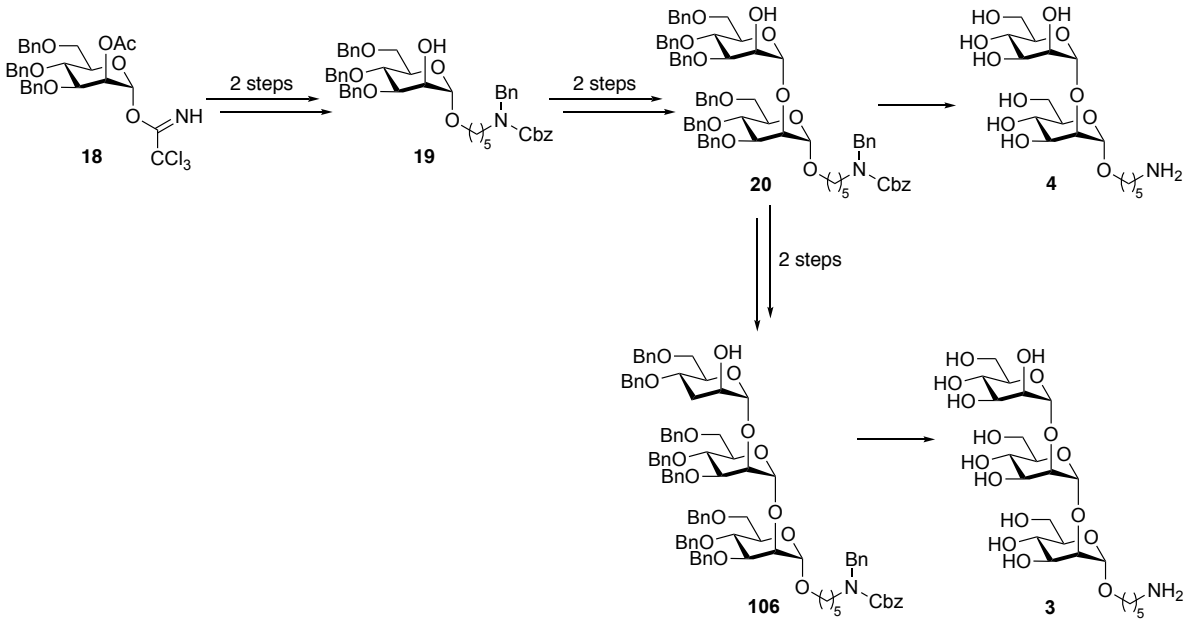
	85	**86**	**87**	**88**	**89**
Yield (%)	73	79	79	74	69

These disaccharides were then transformed into the fully deprotected trisaccharides *via* a four step reaction sequence including an α-selective [2+1]-glycosylation procedure with mannosyl donor **18** and a final global deprotection (see SCHEME 4.6). The corresponding trisaccharides **1** and **6**–**9** were obtained over four steps in overall yields between 40% and 62%. The remaining 3F-trisaccharide **10** was prepared by *Stefan Marchner* in the course of his Master thesis in the *Hoffmann-Röder* research group to complete the envisaged library of flourinated compounds.



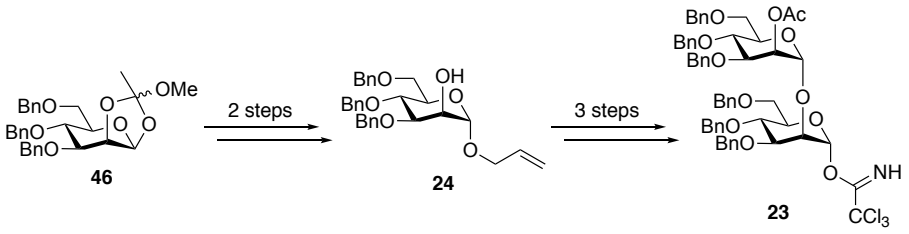
SCHEME 4.6. Synthesis of the targeted native trisaccharide **1** and its fluorinated derivatives **6**–**9** starting from the fully protected disaccharides **85**–**89**.

Additionally, dimannose and trimannose neutral cap structures **4** and **3** were prepared starting from already available mannosyl donor **18** (see SCHEME 4.7). After protecting group manipulations and a [1+1]-glycosylation reaction followed by global deprotection, the targeted dimannose **4**, equipped with an amine linker, was obtained in 40% yield over five steps. Intermediate **20** was further elongated using mannosyl trichloroacetimidate **18** to provide trimannose **3** after global deprotection in 25% yield over seven steps.



SCHEME 4.7.Synthesis of dimannose **4** and trimannose **3** over five or seven steps, respectively, starting from mannosyl donor **18**.

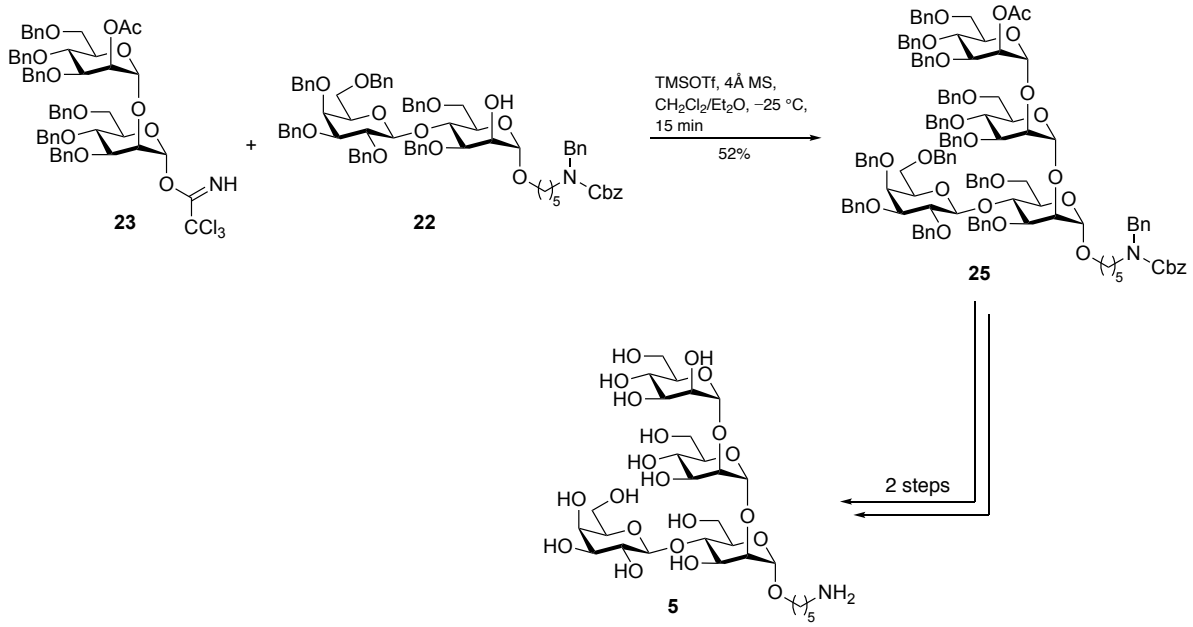
Finally, a tetrasaccharidic neutral cap structure was assembled based on a [2+2]-glycosylation strategy and starting from already available disaccharide glycosyl acceptor **22**, as well as dimannosyl donor **23** (see SCHEME 4.8). The latter was prepared using orthoester **46** and already known mannosyl donor **18** over five steps and in an overall yield of 33%.



SCHEME 4.8. Synthesis of dimannosyl donor **23** over five steps starting from orthoester **46**.

The glycosylation of both compounds afforded the fully protected tetrasaccharide **25** in 52%, which was transformed by two following deprotection steps into the desired target compound **5** in 65% yield (see SCHEME 4.9).

All of the prepared compounds, i.e. the native β-Gal-(1→4)-[α-Man-(1→2)]-α-Man trisaccharide **1**, fluorinated derivatives **6**–**10** and the other terminating neutral cap structures **2**–**5** are now ready to use in future biological screening assays (microarry/SPR) to evaluate their potential for diagnostic tool development and for preparation of fully synthetic vaccine candidates.



SCHEME 4.9. Synthesis of tetrasaccharide **5** *via* a [2+2]-glycosylation.

4.2 SYNTHESIS OF A MONOVALENT FULLY SYNTHETIC VACCINE CANDIDATE AND STUDIES TOWARD A MULTIVALENT FULLY SYNTHETIC VACCINE CONSTRUCT AGAINST *LEISHMANIA DONOVANI*

In a second project of this thesis, synthetic access to a monovalent vaccine candidate against *Leishmania donovani* comprising native B cell epitope **1** conjugated to an α-GalCer derivative as T helper-like epitope was developed (see FIGURE 4.2). Furthermore, initial syntetic studies toward a multivalent fully synthetic vaccine construct based on a Regioselectively Addressable Functionalized Template (RAFT) (see FIGURE 4.2) were conducted.

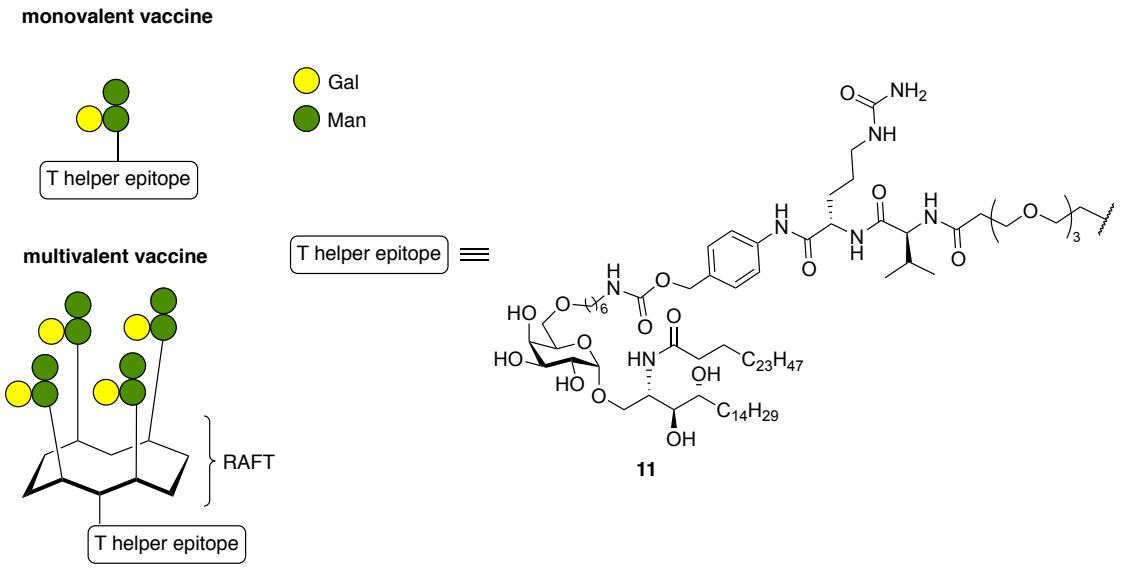
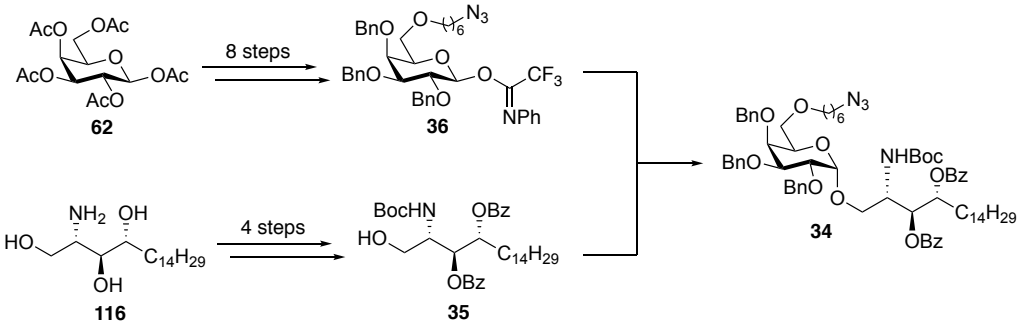


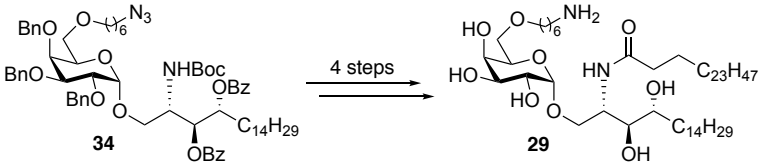
FIGURE 4.2. Schematic depiction of the targeted monovalent and multivalent fully synthetic vaccine candidates.

The synthetic strategy toward these constructs involved an α-GalCer derivative, which has been proven by several research groups to exhibit excellent T helper-like characteristics with a rapid IgM to IgG class switch. Furthermore, a multivalent cyclcopetide scaffold developed by *Dumy and co-workers* should enable the formation of a glycocluster imitating the dense surface distribution of pathogenic surface glycans. The T helper-like epitope was designed to be linked either to the B cell epitope or the RAFT, respectively, *via* a self-immolative Val-Cit linker in accordance to literature precedent. This approach should lay the foundation for future vaccination studies to evaluate the efficacy of covalently-attached NKT cell glycolipid agonists in synthetic vaccines and further confirm the excellent T helper characteristics of this class of compound.



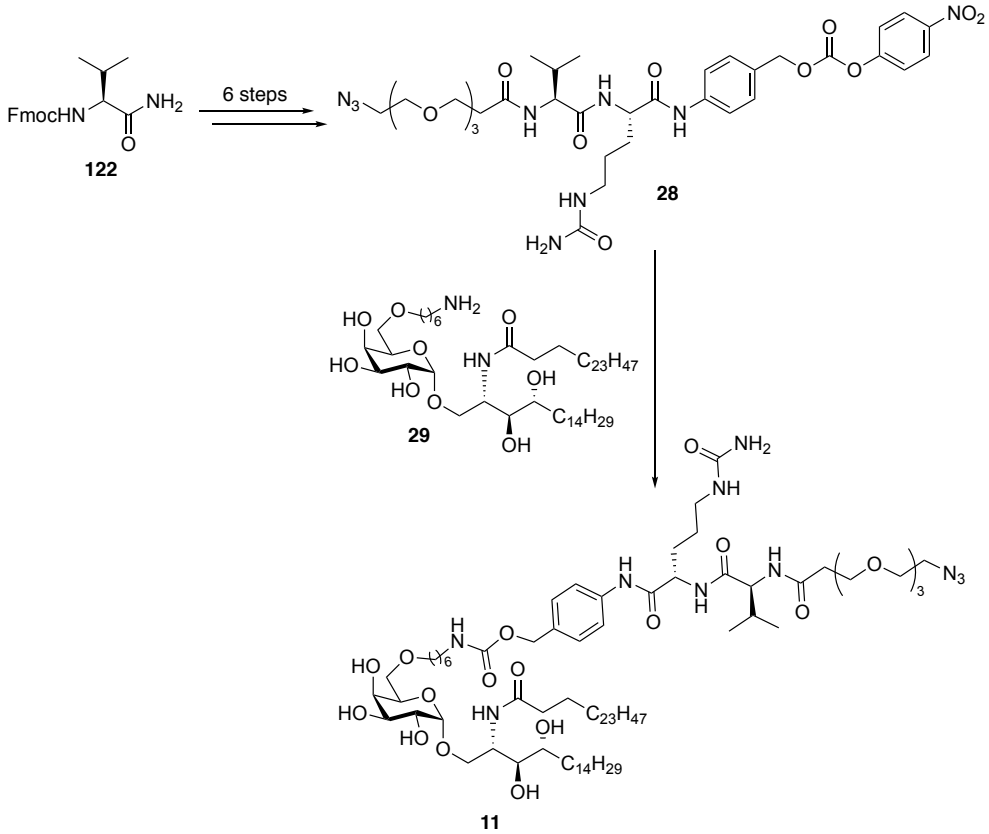
SCHEME 4.10. Convergent synthesis of α-GalCer precursor **34**.

The synthetic route toward the α-GalCer derivative **29** closely followed a literature known convergent strategy starting from pentaacetate **62** and commercially available phytosphingosine **116** (see SCHEME 4.10/4.11). The required *N*-phenyltrifluoroacetimidate (PTFAI) donor **36** was synthesized over eight steps with an overall yield of 18% in accordance to literature precedent. A suitably protected phytosphingosine acceptor **35** was readily available over a short four-step synthesis and was obtained in 46% overall yield.



SCHEME 4.11. Synthesis of compound **29** over four steps starting from **34**.

Both compounds were subsequently merged together *via* an α-selective glycosylation reaction to furnish the α-GalCer precursor **34** in 74% yield (see SCHEME 4.11). A following four step reaction sequence, including the introduction of a cerotic acid moiety, allowed for transformation of this compound into literature known α-GalCer derivative **29** in 52% overall yield (see SCHEME 4.11).

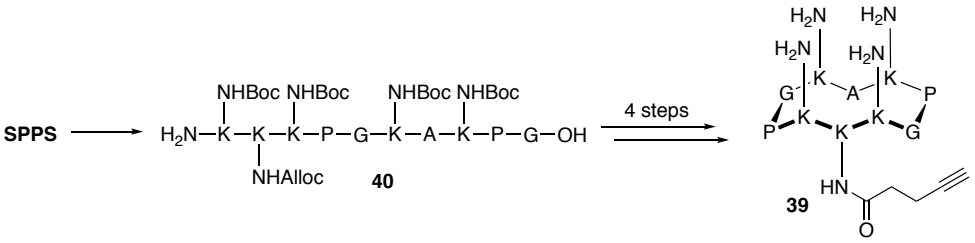


SCHEME 4.12. Synthesis of T helper epitope **11** starting from **28** and **29**.

Self-immolative linker **28** was prepared over six steps starting from commercially available Fmoc-protected L-valine **122** (see SCHEME 4.12). Thereby, an azide polyethyleneglycol (PEG) spacer was introduced, which should not only enable the conjugation by a *Huisgen* 1,3-dipolar cycloaddition with the B cell epitope or the cyclic decapeptide, respectively, but also to improve the solubility of the construct in polar solvents. Coupling

of the functionalized linker to α-GalCer derivative **29** was accomplished with intermediate *para*-nitrophenyl (*p*NP) carbonate **28**, which was obtained in 38% overall yield and allowed for the assembly of T helper-like epitope **11** in 88% (see SCHEME 4.12).

To attach cyclic decapeptide **39** (see SCHEME 4.13) to the construct, the former was prepared over four steps starting from a linear ten amino acid sequence **40** *via* solid-phase peptide synthesis (SPPS). This linear peptide precursor **40** was cyclized and equipped with an alkyne moiety for subsequent click reaction with T helper-like epitope **11**. This synthetic approach afforded RAFT **39** in 45% overall yield over five steps.



SCHEME 4.13. Synthesis of RAFT **39** *via* SPPS and four following steps.

Subsequently, many conditions were screened for the CuAAC click reaction between azide **11** and RAFT **39** or an alkyne-functionalized derivative of native trisaccharide **1**, respectively, to access multivalent construct **38** and monovalent vaccine candidate **26** (see FIGURE 4.3). However, although some conditions led to formation of the desired click products, the isolation of the compounds was hampered by purification problems. Therefore, the preparation of **26** and multivalent vaccine candidate **37** (see FIGURE 4.3) in requisite purity and sufficient amounts requires further optimization, which is part of ongoing studies.

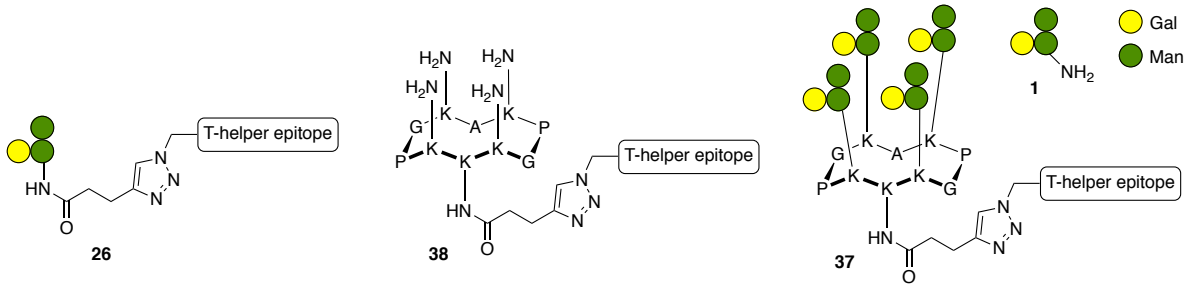
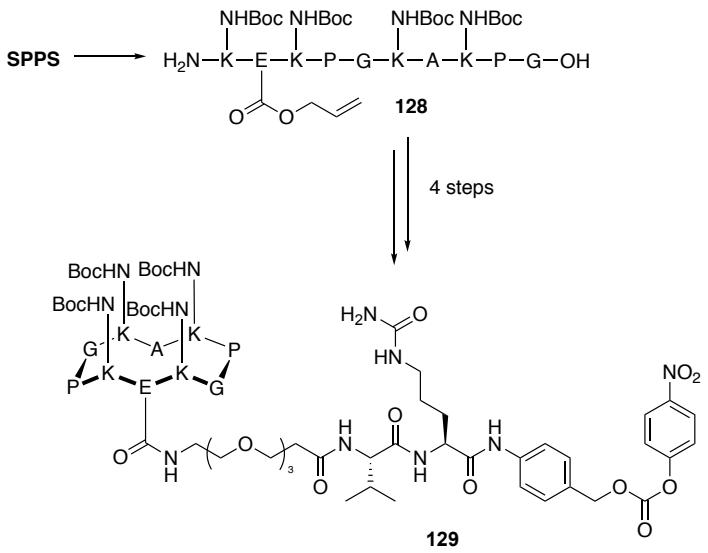


FIGURE 4.3. Targeted monovalent and multivalent vaccines candidates **26** and **37**.

A related RAFT **129** (see SCHEME 4.14) was prepared in order to avoid the problems hampering successful CuAAC conjugation of RAFT **39** to functionalized α-GalCer derivative **11**. In that regard, linear peptide **128** was assembled *via* SPPS, cyclized and directly eqipped with the enzymatically cleavable dipeptide linker, so that the conjugation could directly take place at the free amine of compound **29** *via* an activated carbonate (see SCHEME 4.14). Yet, this coupling approach did not afford the corresponding multivalent construct and thus requires redesign of the synthetic route.



SCHEME 4.14. Synthesis of RAFT **129** *via* SPPS and four following steps.

A possible solution to the problem was presented by the work of *Anderson et al.*, who applied a copper-free strain-promoted azide alkyne cycloaddition (SPAAC) for conjugation instead of CuAAC click conditions. Inspired by their efforts, preparation of an intermediate cyclooctyne derivative of native trisaccharide **1** was accomplished and subsequent optimized click reaction with azide **11** finally furnished the desired monovalent vaccine candidate **138** in requisite purity and sufficient amounts for vaccination studies (see FIGURE 4.4). This established protocol can now be used for the preparation of diverse anti-pathogenic monovalent vaccine candidates bearing a cyclooctyne-functionalized antigenic determinant. Moreover, this conjugation method is also currently evaluated for the preparation of a multivalent construct.

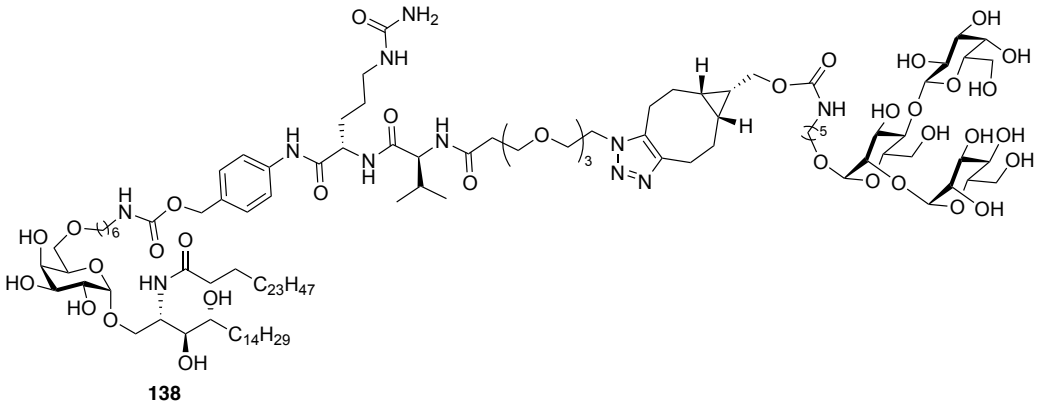


FIGURE 4.4. Monovalent fully synthetic vaccine candidate **138**.

Vaccine candidate **138** should be used in future vaccination studies, whereby the KRN7000 moiety could be used as liposomal carrier vehicle. The liposomal formulation might be carried out with 1,2-distearoyl-*sn*-glycero-3-phospho-choline (DSPC) and cholesterol as described by *Yin et al*. The immune response of such a liposomal formulation should then be compared with the immune response elicited by a KLH conjugate of trisaccharide **1** and thus give further insights into the vaccination efficacy of fully synthetic vaccine candidates using the αGalCer-moiety as T helper-like epitope (see FIGURE 4.5). The synthesized native and fluorinated LPG capping structures could then be used for epitope screening with patient sera, enzymatic degradation

studies as well as for epitope mapping (SPR, STD NMR) with antibodies obtained from vaccination studies in order to find the best glycotope for redesigning a more efficient fluorinated fully synthetic vaccine candidate. Moreover, further synthetic studies toward a multivalent construct exploiting the SPAAC reaction are underway in order to provide access to a tetravalent vaccine candidate with the most promising carbohydrate B cell epitope in near future (see FIGURE 4.5).

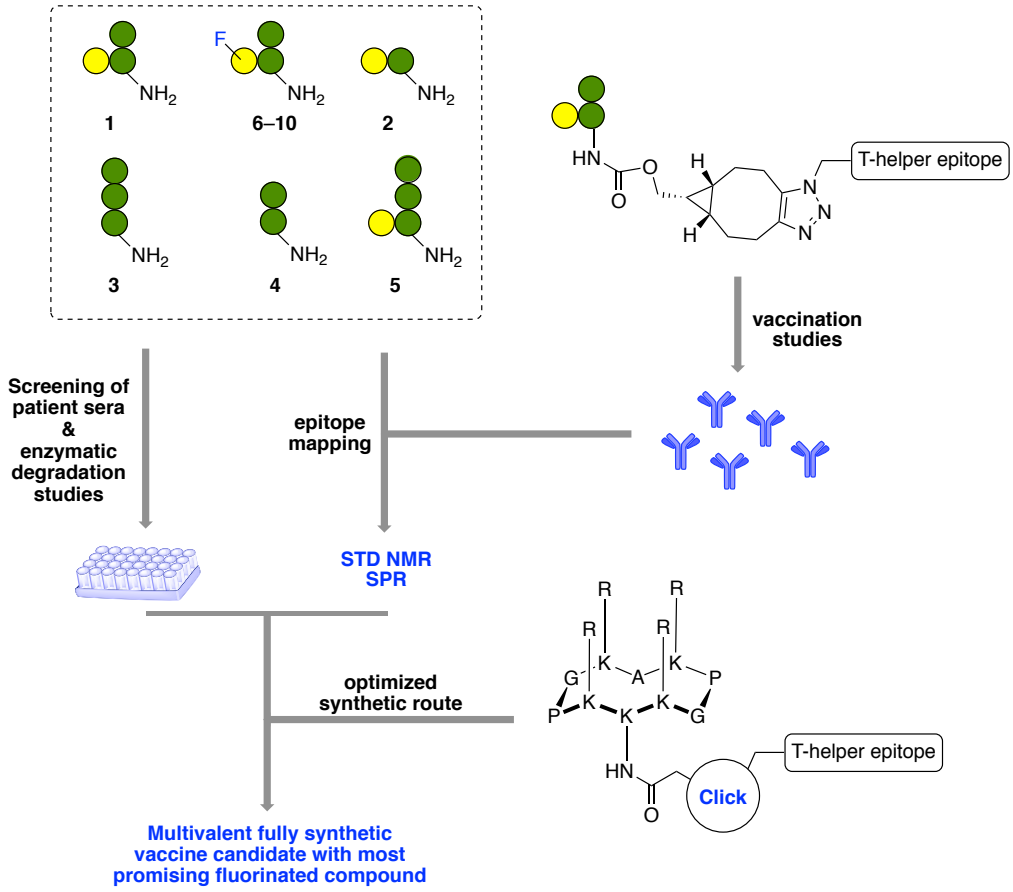


FIGURE 4.5. Project outline for future vaccination studies and redesign of a multivalent fully synthetic vaccine candidate with the most promising glycotope from epitope screening and enzymatic degradation studies.

5 EXPERIMENTAL PROCEDURES

5.1 REAGENTS AND GENERAL PROCEDURES

When indicated, reactions were carried out with standard Schlenk techniques under argon atmosphere in flame-dried glassware (100°C oven temperature) that was further dried using a heat gun (set to 650°C). Solvents for moisture sensitive reactions were distilled and dried according to standard procedures. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled prior to use from sodium and benzophenone, toluene, dichloromethane (CH_2Cl_2) and acetonitrile (MeCN) were distilled from calcium hydride. Commercially available reagents and solvents were used without further purification. Reaction progress was monitored by analytical thin-layer chromatography (TLC) with pre-coated silica gel 60 F_{254} aluminium plates (*Merck KGaA*) using ultraviolet light (254 nm) for visualization where applicable and by staining the plate with a 1:1 mixture of 1 M H_2SO_4 in EtOH and 3% 4-methoxyphenol solution in EtOH. The crude products were purified by flash column chromatography using silica gel (35 – 70 μm) from *Acros Organics* or by reversed phase column chromatography using silica gel C_8-reversed phase from *Sigma-Aldrich*. Microwave assisted reactions were performed in a *CEM Discover* microwave system. Solid-phase peptide synthesis (SPPS) was carried out on *CEM Liberty Blue* peptide synthesizer. Analytical RP-HPLC was measured on a *JASCO* system with a *Phenomenex Luna* C18 column (5 μm, 250 × 4.6 mm) or a *Phenomenex Aeris* C18 column (5 μm, 250 × 4.6 mm). In all cases mixtures of water and acetonitrile were used as solvents. High resolution mass spectra (HRMS) were recorded with a Thermo Finnigan LTQ FT (electronspray ionization, ESI) instrument. ^{1}H, ^{13}C, ^{19}F and 2D NMR spectra were recorded on a *Varian* 400 MHz and 600 MHz spectrometer or on a *Bruker* Avance III 800 MHz spectrometer. The chemical shifts (δ scale) are expressed in parts per million (ppm) and reported relative to the signal of the deuterated solvent: $CDCl_3$ (δ_H = 7.26 ppm, δ_C = 77.16 ppm), D_2O (δ_H = 4.79 ppm), CD_3OD (δ_H = 3.31 ppm, δ_C = 49.00 ppm) and $(CD_3)_2SO$ (δ_H = 2.50 ppm, δ_C = 39.52 ppm). The following abbreviations are used to indicate the multiplicities: s (singlet), br s (broad singlet), d (doublet), t (triplett), q (quartett) and m (multiplet). Assignments of proton and carbon signals were achieved by addtional COSY, HSQC and HMBC experiments. Anomeric configurations were proved with proton-coupled HSQC experiments when necessary. Optical rotations were measured at 598 nm with a *Perkin-Elmer* polarimeter 241 and a concentration c given in g/100 mL.

5.2 ANALYTICAL DATA

5.2.1 Synthesis of native and fluorinated LPG capping structures of *Leishmania donovani*

General Procedure A (Glycosylation with galactosyl trichloroacetimidate)

Galactosy trichloroacetimidate (1.3–1.4 equiv.) and mannosyl acceptor (1.0 equiv.) were co-evaporated seperately with toluene three times and once with CH_2Cl_2, dried under high vacuum and then dissolved seperately in a mixture of CH_2Cl_2/MeCN (3:1) under argon. The mannosyl acceptor solution (90 mM) was stirred with freshly activated 4Å MS at room temperature for 30 min, before the reaction vessel was cooled to −78 °C. After TMSOTf (0.2 equiv.) was added, the galactosyl donor solution (400–500 mM) was added dropwise and the mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with CH_2Cl_2, quenched by the addition of NEt_3 and filtered through a pad of celite. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc).

General Procedure B (Acetyl deprotection with AcCl)[160]

The starting material (1.0 eqiv.) was dissolved in a mixture of THF/MeOH (3:8, 30–40 mM) and cooled to 0 °C. Then AcCl (10 equiv.) was added and the reaction mixture was stirred over night at room temperature. After the addition of NEt_3 the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc).

General Procedure C (Glycosylation with mannosyl trichloroacetimidate)[160]

Mannosyl trichloroacetimidate (1.5–3.0 equiv.) and the acceptor (1.0 equiv.) were combined and co-evaporated with toluene three times and once with CH_2Cl_2, dried under high vacuum and then dissolved in a mixture of CH_2Cl_2/Et_2O (1:2, 60 mM) under argon. The mixture was stirred with freshly activated 4Å MS at room temperature for 30 min, before the reaction vessel was cooled to 0 °C. TMSOTf (0.2 equiv.) was added and the reaction mixture was stirred for 1 h at the same temperature. The reaction mixture was diluted with CH_2Cl_2, quenched by the addition of NEt_3 and filtered through a pad of celite. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc).

General Procedure D (Acetyl deprotection with NaOMe)[160]

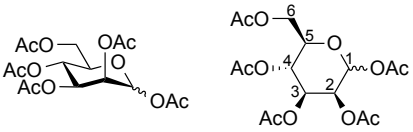
To a stirred solution of the starting material in a mixture of MeOH/CH_2Cl_2 (4:1, 30–50 mM), a catalytic amount of NaOMe was added at room temperature. After complete conversion was monitored by TLC, the reaction mixture was neutralized by adding *Amberlite IR120*. The ion-exchange resin was then filtered off and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc).

General Procedure E (Hydrogenolysis/Global Deprotection)[160]

The starting material was dissolved (2–4 mM) in a mixture of MeOH/THF/H_2O/AcOH (10:5:4:1) and purged with argon. Then 10% Pd/C (same weight as starting material) was added and the reaction mixture was purged with H_2 four times and then stirred under an H_2 atmosphere for 20 h. The reaction mixture was filtered and the solvents were removed under reduced pressure. The residue was dissolved in H_2O and reversed phase column chromatography (C8) and lyophilization afforded the pure product.

5.2.1.1 Synthesis of Mannosyl donor 18

Penta-*O*-acetyl-α/β-D-mannopyranoside (43)[184, 185]



A suspension of NaOAc (25.0 g, 305 mmol, 1.0 equiv.) in Ac_2O (300 mL) was heated to 100 °C and D-Mannose **42** (55.0 g, 305 mmol, 1.0 equiv.) was added portionwise. The reaction mixture was stirred at 100 °C for another 3 h and was then poured onto ice (800 g). After stirring for 1 h, the obtained emulsion was extracted with CH_2Cl_2 (4 × 200 mL) and the combined organic layers were washed with sat. aq. $NaHCO_3$ (4 × 200 mL). The organic phase was dried with $MgSO_4$ and solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to give **43** (104 g of anomeric mixture α/β 70:30, 266 mmol, 87%) as a colorless oil.

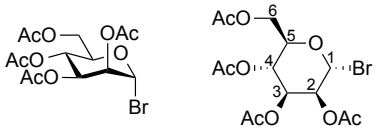
R_f = 0.24 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ (β-Anomer) = 6.03 (d, $J_{H1,H2}$ = 1.7 Hz, 1H, H-1), 5.31–5.28 (m, 2H, H-3/H-4), 5.22–5.20 (m, 1H, H-2), 4.23 (dd, $J_{H6a,H6b}$ = 12.3 Hz, $J_{H6a,H5}$ = 4.8 Hz, 1H, H-6a), 4.05 (dd, $J_{H6b,H5}$ = 12.3 Hz, $J_{H6b,H5}$ = 2.5 Hz, 1H, H-6b), 4.02–3.98 (m, 1H, H-5), 2.13, 2.12, 2.04, 2.00, 1.96 (5s, 15H, 5 × CH_3-OAc) ppm; δ (α-Anomer) = 5.83 (d, $J_{H1,H2}$ = 1.2 Hz, 1H, H-1), 5.43 (dd, $J_{H2,H3}$ = 3.3 Hz, $J_{H2,H1}$ = 1.1 Hz, 1H, H-2), 5.25 (d, $J_{H4,H3/5}$ = 9.7 Hz, 1H, H-4), 5.10 (dd, $J_{H3,H4}$ = 10.0 Hz, $J_{H3,H2}$ = 3.3 Hz, 1H, H-3), 4.26 (dd, $J_{H6a,H6b}$ = 12.4 Hz, $J_{H6a,H5}$ = 5.3 Hz, 1H, H-6a), 4.09 (dd, $J_{H6b,H5}$ = 13.5 Hz, $J_{H6b,H5}$ = 2.2 Hz, 1H, H-6b), 3.77 (ddd, $J_{H5,H4}$ = 9.8 Hz, $J_{H5,H6a}$ = 5.3 Hz, $J_{H5,H6b}$ = 2.4 Hz, 1H, H-5), 2.16, 2.05 , 2.01, 1.99, 1.95 (5s, 15H, 5 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ (β-Anomer) =170.6, 167.0, 169.7, 169.5, 168.1 (5 × C=O-OAc), 90.6 (C-1), 70.6 (C-5), 68.8 (C-3), 68.3 (C-2), 65.5 (C-4), 62.1 (C-6), 20.9, 20.8, 20.7, 20.6 (5 × CH_3-OAc) ppm; δ (α-Anomer) = 170.6, 170.2, 169.8, 169.6, 168.4 (5 × C=O-OAc), 90.4 (C-1), 73.3 (C-5), 70.7 (C-3), 68.2 (C-2), 65.4 (C-4), 62.1 (C-6), 20.8, 20.7, 20.6, 20.5 (5 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{16}H_{26}O_{11}N^+$ $[M+NH_4]^+$: 408.1500, found 408.1499.

1-Bromo-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (44)[184]

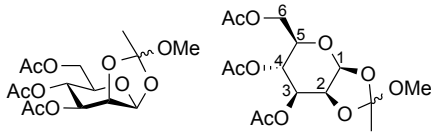


A stirred solution of pentaacetate **43** (13.7 g, 35.1 mmol) in AcOH (75 mL) was cooled to 0 °C and HBr in AcOH (30 mL of a 30% solution, 156 mmol) was added slowly. The reaction mixture was stirred for 4 h at room temperature and was then poured onto ice-water (150 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL) and the organic layer was washed with sat. aq. $NaHCO_3$ (2 × 150 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was quickly purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to furnish **44** (10.7 g, 25.9 mmol, 74%) as a colorless oil.

$\boldsymbol{R_f}$ = 0.67 (^{c}Hex/EtOAc, 4:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.24 (d, $J_{H1,H2}$ = 1.1 Hz, 1H, H-1), 5.65 (dd, $J_{H3,H4}$ = 10.2 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 5.38 (dd, $J_{H2,H3}$ = 3.4 Hz, $J_{H2,H1}$ = 1.6 Hz, 1H, H-2), 5.31 (t, $J_{H4,H3/H5}$ = 10.2 Hz, 1H, H-4), 4.27 (dd, $J_{H6a,H6b}$ = 12.5 Hz, $J_{H6a,H5}$ = 4.9 Hz, 1H, H-6a), 4.16 (ddd, $J_{H5,H4}$ = 10.2 Hz, $J_{H5,H6a}$ = 4.9 Hz, $J_{H1,H6b}$ = 2.2 Hz, 1H, H-5), 4.08 (dd, $J_{H6b,H6a}$ = 12.5 Hz, $J_{H6b,H5}$ = 2.2 Hz, 1H, H-6b), 2.11, 2.04, 2.01, 1.95 (4s, 12H, 4 × CH_3-OAc) ppm.

3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranoside (45)[184, 187]



To a stirred solution of bromide **44** (10.4 g, 25.3 mmol, 1.0 equiv.) in $CHCl_3$ (100 mL) and 2,6-lutidine (5.87 mL, 50.6 mmol, 2.0 equiv.) was added MeOH (100 mL) and the reaction mixture was stirred for 19 h at room temperature. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with sat. aq. $NaHCO_3$ (2 × 100 mL) and brine (100 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The oily residue was co-evaporated with toluene (2 × 80 mL) and dried under high vaccum for one day. A few drops of MeOH were added and colorless crystals started to form. The solid was filtered off and washed with ice-cold Et_2O (3 × 30 mL). The mother liquor was concentrated and the procedure was repeated two more times. The batches of colorless crystalline needles were combined to give **45** (7.10 g, 19.6 mmol, 77%).

$\boldsymbol{R_f}$ = 0.45 (^{c}Hex/EtOAc, 1:1).

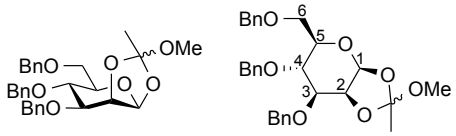
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.49 (d, $J_{H1,H2}$ = 2.6 Hz, 1H, H-1), 5.30 (t, $J_{H4,H3/H5}$ = 9.7 Hz, 1H, H-4), 5.14 (dd, $J_{H3,H4}$ = 9.9 Hz, $J_{H3,H2}$ = 4.0 Hz, 1H, H-3), 4.61 (dd, $J_{H2,H3}$ = 4.0 Hz, $J_{H2,H1}$ = 2.6 Hz, 1H, H-4), 4.23 (dd, $J_{H6a,H6b}$ = 12.1 Hz, $J_{H6a,H5}$ = 4.9 Hz, 1H, H-6a), 4.14 (dd, $J_{H6b,H6a}$ = 12.1 Hz, $J_{H6b,H5}$ = 2.7 Hz, 1H, H-6b), 3.68

(ddd, $J_{H5,H4}$ = 9.6 Hz, $J_{H5,H6a}$ = 4.9 Hz, $J_{H5,H6b}$ = 2.7 Hz, 1H, H-5), 3.27 (s, 3H, -OCH_3), 2.12, 2.07, 2.05 (3s, 9H, 3 × CH_3-OAc), 1.74 (s, 3H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.7, 170.4, 169.4 (3 × C=O-OAc), 124.5 (C_q), 97.4 (C-1), 76.6 (C-2), 71.3 (C-5), 70.6 (C-3), 65.5 (C-4), 62.3 (C-6), 49.9 (-OCH_3), 24.4 (-CH_3), 20.8, 20.7, 20.7 (3 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{15}H_{22}O_{10}Na^+$ $[M+Na]^+$: 385.1105, found 385.1109.

3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranoside (46)[184]



To a solution of orthoester **45** (17.0 g, 46.9 mmol, 1.0 equiv.) in MeOH (100 mL) was added anhydrous K_2CO_3 (0.52 g, 3.8 mmol, 0.08 equiv.) and the reaction mixture was stirred for 3 h at room temperature. The solvents were removed under reduced pressure to give an amorphous off-white solid (quant.). The crude product was used without further purification in the next step. It was dissolved in DMF (HPLC grade, 250 mL) under an argon atmosphere and cooled to 0 °C. NaH (60% dispersion in oil, 6.96 g, 174 mmol, 3.7 equiv.) was added portionwise and the reaction mixture was stirred for 1 h at 0 °C. Benzylbromide (21.1 mL, 174 mmol, 3.7 equiv.) was added slowly and the mixture was allowed to warm to room temperature and it was stirred for another 3 h. The mixture was poured onto ice (200 g) and Et_2O (250 mL) was added. The aqueous phase was extracted with Et_2O (3 × 200 mL) and the combined organic phases were washed with water (3 × 100 mL) and brine (100 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to give **46** (23.2 g, 45.8 mmol, 97% over two steps) as a colorless solid.

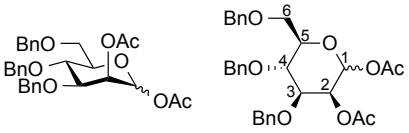
R_f = 0.45 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.43–7.14 (m, 15H, Ar-H), 5.35 (d, $J_{H1,H2}$ = 2.6 Hz, 1H, H-1), 4.90 (d, J = 10.8 Hz, 1H, CH_{Bn}), 4.79 (d, J = 2.4 Hz, 2H, CH_{Bn}), 4.63–4.59 (m, 2H, CH_{Bn}), 4.55 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.40 (dd, $J_{H2,H3}$ = 3.9 Hz, $J_{H2,H1}$ = 2.6 Hz, 1H, H-2), 3.93 (t, $J_{H4,H3/H5}$ = 9.3 Hz, 1H, H-4), 3.77–3.70 (m, 3H, H-3/H-6a/ H-6b), 3.42 (ddd, $J_{H5,H4}$ = 9.4 Hz, $J_{H5,H6a}$ = 4.5 Hz, $J_{H5,H6b}$ = 2.3 Hz, 1H, H-5), 3.29 (s, 3H, -OCH_3), 1.74 (s, 3H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 138.2, 137.8 (3 × C_q), 128.5, 128.39, 128.3, 128.0, 127.8, 127.5, 127.3 (15 × C_{Ar}), 124.0 (C_q), 97.6 (C-1), 79.0 (C-3), 77.1 (C-2), 75.2 (CH_{Bn}), 74.2 (C-4/C-5), 73.4, 72.4 (2 × CH_{Bn}), 69.0 (C-6), 49.8 (-OCH_3), 24.4 (-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{30}H_{34}O_7Na^+$ $[M+Na]^+$: 529.2197, found 529.2201.

1,2-Di-*O*-acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranoside (47)[181]



Compound **46** (13.2 g, 25.9 mmol, 1.0 equiv.) was dissolved in a mixture of AcOH (140 mL) and water (90 mL) and it was stirred for 20 h at room temperature. The solvents were removed under reduced pressure and the oily residue was dissolved in CH_2Cl_2 (100 mL), washed with sat. aq. $NaHCO_3$ (2 × 100 mL) and water (2 × 100 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The resultant syrup was used without further purification in the next step. A solution of the crude product in Ac_2O (19.6 mL, 207 mmol, 8.0 equiv.) and pyridine (20.9 mL, 259 mmol, 10.0 equiv.) was stirred for 2 h at room temperature. Then water (35 mL) was added and the mixture was extracted with $CHCl_3$ (3 × 150 mL). The combined organic phases were washed with sat. aq. $NaHCO_3$ (2 × 150 mL) and dried with $MgSO_4$. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 4:1) to give **47** (11.9 g of anomeric mixture, 22.3 mmol, 86% over two steps) as a colorless oil.

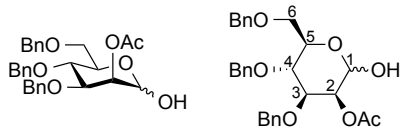
$\boldsymbol{R_f}$ = 0.60 (α-anomer), 0.50 (β-anomer) (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ (α-anomer) = 7.40–7.14 (m, 15H, Ar-H), 6.15 (d, $J_{H1,H2}$ = 2.3 Hz, 1H, H-1), 5.39 (d, $J_{H2,H1}$ = 2.3 Hz, 1H, H-2), 4.89 (d, J = 10.6 Hz, 1H, CH_{Bn}), 4.79–4.66 (m, 2H, CH_{Bn}), 4.61–4.49 (m, 3H, CH_{Bn}), 4.02–3.97 (m, 2H, H-3/H-5), 3.94–3.69 (m, 3H, H-4/H-6a/H-6b), 2.19, 2.09 (2s, 6H, 2 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ (α-anomer) = 170.2, 168.4 (2 × C=O-OAc), 138.2, 137.7 (3 × C_q), 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7 (15 × C_{Ar}), 91.4 (C-1), 77.8 (C-3), 75.5 (CH_{Bn}), 73.9 (C-4), 73.8 (C-5), 73.7, 72.1 (2 × CH_{Bn}), 68.6 (C-6), 67.6 (C-2), 21.1, 21.00 (2 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{31}H_{38}NO_8^+$ $[M+NH_4]^+$: 552.2592, found 552.2592.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranose (48)[181]



To a stirred solution of compound **47** (11.9 g, 22.3 mmol, 1.0 equiv.) in DMF (HPLC grade, 150 mL) was added hydrazinium acetate (2.46 g, 26.7 mmol, 1.2 equiv.) and the mixture was stirred for 4 h at 50 °C. Then it was allowed to cool to room temperature, water (600 mL) was added and the mixture was extracted with EtOAc (4 × 200 mL). The combined organic phases were washed with water (2 × 150 mL) and dried with $MgSO_4$. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to give lactol **48** (9.78 g of anomeric mixture, 19.9 mmol, 89%) as a colorless oil.

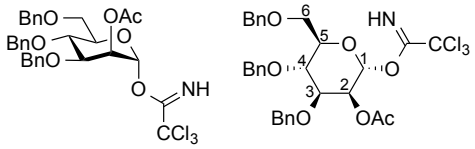
R_f = 0.31 (α-anomer), 0.13 (β-anomer) (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ (α-anomer) = 7.36–7.16 (m, 15H, Ar-H), 5.38 (dd, $J_{H2,H1}$ = 3.3 Hz, $J_{H2,H3}$ = 1.9 Hz, 1H, H-2), 5.21 (br s, 1H, H-1), 4.88 (d, J = 10.9 Hz, 1H, CH_{Bn}), 4.72 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.61 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.54 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.52 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.48 (d, J = 10.9 Hz, 1H, CH_{Bn}), 4.12–4.07 (m, 1H, H-5), 4.05 (dd, $J_{H3,H4}$ = 9.4 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 4.01 (d, J = 2.6 Hz, 1H, -OH), 3.75 (t, $J_{H4,H3/H5}$ = 9.7 Hz, 1H, H-4), 3.72–3.66 (m, 2H, H-6a/H-6b), 2.16 (s, 3H, CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ (α-anomer) = 170.6 (C=O-OAc), 138.4, 138.0, 137.9 (3 × C_q), 128.5, 128.4, 128.2, 128.0, 127.8, 127.7 (15 × C_{Ar}), 92.5 (C-1), 77.8 (C-3), 75.2 (CH_{Bn}), 74.7 (C-5), 73.5, 71.9 (2 × CH_{Bn}), 71.1 (C-4), 69.4 (C-2), 69.3 (C-6), 21.3 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{29}H_{36}NO_7^+$ $[M+NH_4]^+$: 510.2486, found 510.2484.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (18)[181]



To a solution of lactol **48** (9.78 g, 19.9 mmol, 1.0 equiv.) in CH_2Cl_2 (150 mL) at 0 °C was added trichloroacetonitrile (5.00 mL, 49.9 mmol, 2.5 equiv.) and DBU (0.74 mL, 4.98 mmol, 0.25 equiv.) in sequential order. The mixture was allowed to warm to room temperature and it was stirred for 17 h. Then sat. aq. NH_4Cl (50 mL) and water (50 mL) were added. The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 4:1) to give **18** (11.1 g, 17.4 mmol, 87%) as a colorless oil.

R_f = 0.31 (^{c}Hex/EtOAc, 4:1).

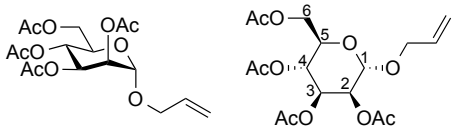
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.70 (s, 1H, C=NH), 7.41–7.16 (m, 15H, Ar-H), 6.33 (d, $J_{H1,H2}$ = 1.9 Hz, 1H, H-1), 5.52 (t, $J_{H2,H1/H3}$ = 2.4 Hz, 1H, H-2), 4.90 (d, J = 10.6 Hz, 1H, CH_{Bn}), 4.76 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.71 (d, J = 12.0 Hz, 1H, CH_{Bn}), 4.60 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.56 (d, J = 10.6 Hz, 1H, CH_{Bn}), 4.53 (d, J = 12.0 Hz, 1H, CH_{Bn}), 4.08–4.05 (m, 2H, H-3/H-4), 4.05–3.98 (m, 1H, H-5), 3.87 (dd, $J_{H6a,H6b}$ = 11.2 Hz, $J_{H6a,H5}$ = 3.6 Hz, 1H, H-6a), 3.74 (dd, $J_{H6b,H6a}$ = 11.2 Hz, $J_{H6b,H5}$ = 1.7 Hz, 1H, H-6b), 2.21 (s, 3H, CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.2 (C=O-OAc), 160.1 (C=NH), 138.3, 138.22, 137.6 (3 × C_q), 128.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.7 (15 × C_{Ar}), 95.5 (C-1), 90.9 (CCl_3), 77.5 (C-3), 75.6 (CH_{Bn}), 74.5 (C-5), 73.8 (C-4), 73.5, 72.2 (2 × CH_{Bn}), 68.5 (C-6), 67.4 (C-2), 21.1 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{31}H_{32}Cl_3NO_7Na^+$ $[M+Na]^+$: 660.1107, found 660.1121.

5.2.1.2 Synthesis of the bridging Mannosyl acceptor 12

Allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (49)[195, 199]



A stirred solution of penta-*O*-acetyl-α/β-D-mannopyranoside **43** (35.5 g, 90.9 mmol, 1.0 equiv.) and allylic alcohol (12.4 mL, 182 mmol, 3.0 equiv.) in CH_2Cl_2 (300 mL) under an argon atmosphere was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (14.6 mL, 118 mmol, 1.3 equiv.) was added. The solution was allowed to warm to room temperature and was stirred for 21 h. The reaction mixture was poured onto ice-water (300 mL) and the organic phase was washed with sat. aq. $NaHCO_3$ (3 × 150 ml). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was redissolved in pyridine (100 mL) and cooled to 0 °C. Ac_2O (60 mL) was added dropwise and the reaction mixture was stirred for 17 h. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to furnish **49** (26.8 g, 69.1 mmol, 76%) as a colorless oil.

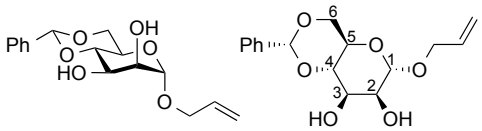
$\boldsymbol{R_f}$ = 0.35 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 5.87 (dddd, $J_{CH,CH2trans}$ = 16.8 Hz, $J_{CH,CH2cis}$ = 10.4 Hz, $J_{CH,CH2b}$ = 6.3 Hz, $J_{CH,CH2a}$ = 5.3 Hz, 1H, CH_2CH=CH_2), 5.34 (dd, $J_{H3,H4}$ = 10.0 Hz, $J_{H3,H2}$ = 3.5 Hz, 1H, H-3), 5.28 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.5 Hz, 1H, CH_2CH=CH_{2trans}), 5.26 (dd, $J_{H4,H3/H5}$ = 10.1 Hz, 1H, H-4), 5.24–5.20 (m, 2H, H-2/CH_2CH=CH_{2cis}), 4.84 (d, $J_{H1,H2}$ = 1.6 Hz, 1H, H-1), 4.26 (dd, $J_{H6a,H6b}$ = 12.2 Hz, $J_{H6a,H5}$ = 5.3 Hz, 1H, H-6a), 4.16 (ddt, $J_{CH2a,CH2b}$ = 12.7 Hz, $J_{CH2a,CH}$ = 5.2 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.3 Hz, 1H, $CH_{2a}CH$=CH_2), 4.08 (dd, $J_{H6b,H6a}$ = 12.3 Hz, $J_{H6b,H5}$ = 2.4 Hz, 1H, H-6b), 4.03–3.97 (m, 2H, H-5/$CH_{2b}CH$=CH_2), 2.12, 2.08, 2.01, 1.96 (4s, 12H, 4 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.7, 170.1, 169.9, 169.8 (4 × C_q), 133.0 (CH_2CH=CH_2), 118.5 (CH_2CH=CH_2), 96.7 (C-1), 69.7 (C-2), 69.2 (C-3), 68.7 (CH_2CH=CH_2), 68.6 (C-5), 66.3 (C-4), 62.6 (C-6), 21.0, 20.8, 20.7 (4 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{17}H_{24}O_{10}Na^+$ $[M+Na]^+$: 411.1262, found 411.1259.

Allyl 4,6-*O*-benzylidene-α-D-mannopyranoside (50)[195]



To a stirred solution of compound **49** (19.4 g, 50.0 mmol, 1.0 equiv.) in methanol (250 mL) were added catalytic amounts of NaOMe. The reaction mixture was stirred for 2 h at room temperature and was then neutralized by the addition of *Amberlite IR120*. The ion-exchange resin was filtered off and the solvents were

removed under reduced pressure to give an amorphous off-white solid (quant.). The crude product was dissolved in DMF (HPLC grade, 150 mL) and benzaldehyde dimethylacetal (8.21 mL, 55.0 mmol, 1.1 equiv.) and a catalytic amounts of *p*-toluenesulfonic acid were added. The reaction mixture was stirred for 3 h at room temperature. The solvents were removed under reduced pressure and the oily residue was dissolved in CH_2Cl_2 (100 mL) and washed with sat. aq. $NaHCO_3$ (2 × 100 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 1:1) to give **50** (12.7 g, 41.3 mmol, 83% over two steps) as a colorless solid.

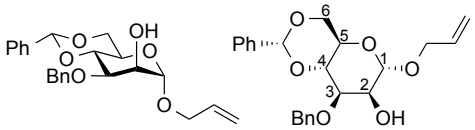
$\boldsymbol{R_f}$ = 0.14 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.52–7.33 (m, 5H, Ar-H), 5.93–5.86 (m, 1H, CH_2CH=CH_2), 5.55 (s, 1H, Ar-CH), 5.30 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.6 Hz, 1H, CH_2CH=CH_{2trans}), 5.22 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.2 Hz, 1H, CH_2CH=CH_{2cis}), 4.86 (d, $J_{H1,H2}$ = 1.2 Hz, 1H, H-1), 4.26–4.24 (m, 1H, H-6a), 4.18 (ddt, $J_{CH2a,CH2b}$ = 12.9 Hz, $J_{CH2a,CH}$ = 5.2 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.4 Hz, 1H, CH_{2a}CH=CH_2), 4.06 (d, $J_{H3,H4}$ = 9.6 Hz, 1H, H-3), 4.00–3.97 (m, 2H, H-2/CH_{2b}CH=CH_2), 3.91 (t, $J_{H4,H3/H5}$ = 9.3 Hz, 1H, H-4), 3.84 (dd, $J_{H5,H4/H6a/H6b}$ = 10.2 Hz, $J_{H5,H6a/H6b}$ = 4.0 Hz, 1H, H-5), 3.83–3.78 (m, 1H, H-6b) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 137.3 (C_q), 133.6 (CH_2CH=CH_2), 129.4, 128.5, 126.4 (5 × C_{Ar}), 117.9 (CH_2CH=CH_2), 102.4 (Ar-CH), 99.6 (C-1), 79.0 (C-4), 71.1 (C-2), 68.9 (C-6), 68.7 (C-3), 68.4 (CH_2CH=CH_2), 63.3 (C-5) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{16}H_{20}O_6Na^+$ $[M+Na]^+$: 331.1152, found 331.1152.

Allyl 3-*O*-benzyl-4,6-benzylidene-α-D-mannopyranoside (51)[195]



To a solution of compound **50** (12.7 g, 41.3 mmol, 1.0 equiv.) in dry toluene (150 mL) was added Bu_2SnO (10.5 g, 42.1 mmol, 1.02 equiv.) and the mixture was stirred under an argon atmosphere for 3 h at 120 °C. The reaction mixture was allowed to cool down to room temperature and Bu_4NBr (14.1 g, 43.8 mmol, 1.06 equiv.), CsF (6.39 g, 42.1 mmol, 1.02 equiv.) and BnBr (5.15 mL, 43.4 mmol, 1.05 equiv.) were added. Then it was stirred for 24 h at room temperature, diluted with EtOAc (100 mL) and sat. aq. $NaHCO_3$ (100 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL) and the combined organic layers were washed with water (100 mL) and brine (100 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **51** (13.3 g, 33.4 mmol, 81%) as a colorless oil.

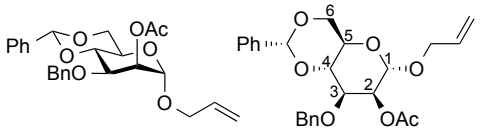
$\boldsymbol{R_f}$ = 0.66 (^{c}Hex/EtOAc, 1:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.52–7.25 (m, 10H, Ar-H), 5.89 (dddd, $J_{CH,CH2trans}$ = 17.4 Hz, $J_{CH,CH2cis}$ = 10.3 Hz, $J_{CH,CH2b}$ = 6.2 Hz, $J_{CH,CH2a}$ = 5.2 Hz, 1H, CH_2CH=CH_2), 5.61 (s, 1H, Ar-CH), 5.28 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.6 Hz, 1H, CH_2CH=CH_{2trans}), 5.21 (dq, $J_{CH2cis,CH}$= 10.5 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.4 Hz, 1H, CH_2CH=CH_{2cis}), 4.90 (d, $J_{H1,H2}$ = 1.5 Hz, 1H, H-1), 4.86 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.71 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.29–4.24 (m, 1H, H-6a), 4.17 (ddt, $J_{CH2a,CH2b}$ = 12.9 Hz, $J_{CH2a,CH}$ = 5.2 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.5 Hz, 1H, $CH_{2a}CH$=CH_2), 4.11 (t, $J_{H4,H3/H5}$ = 9.2 Hz, 1H, H-4), 4.06 (dd, $J_{H2,H3}$ = 3.5 Hz, $J_{H2,H1}$ = 1.5 Hz, 1H, H-2), 4.02–3.96 (m, 1H, $CH_{2b}CH$=CH_2), 3.94 (dd, $J_{H3,H4}$ = 9.6 Hz, $J_{H3,H2}$ = 3.5 Hz, 1H), 3.88–3.83 (m, 2H, H-5/H-6b), 2.74 (s, 1H, -OH) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 138.2, 137.67 (2 × C_q), 133.6 (CH_2CH=CH_2), 129.0, 128.6, 128.3, 128.0, 127.9, 126.1 (10 × C_{Ar}), 117.9 (CH_2CH=CH_2), 101.7 (Ar-CH), 99.3 (C-1), 79.0 (C-4), 75.8 (C-3), 73.2 (CH_{Bn}), 70.1 (C-2), 69.0 (C-5), 68.3 (CH_2CH=CH_2), 63.5 (C-5) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{23}H_{30}O_6N^+$ $[M+NH_4]^+$: 416.2068, found: 416.2071.

Allyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (52)[161]



To a solution of compound **51** (9.40 g, 23.6 mmol, 1.0 equiv.) in pyridine (70 mL) was added Ac_2O (2.34 mL, 24.8 mmol, 1.05 equiv.) and the reaction mixture was stirred for 16 h at room temperature. The solvents were removed under reduced pressure and the resultant syrup was dissolved in CH_2Cl_2 (100 mL), washed with water (2 × 100 mL) and brine (100 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **52** (10.2 g, 23.1 mmol, 98%) as a colorless oil.

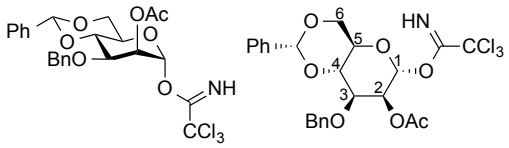
R_f = 0.41 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.57–7.23 (m, 10H, Ar-H), 5.90 (dddd, $J_{CH,CH2trans}$ = 17.2 Hz, $J_{CH,CH2cis}$ = 10.4 Hz, $J_{CH,CH2b}$ = 6.2 Hz, $J_{CH,CH2a}$ = 5.2 Hz, 1H, CH_2CH=CH_2), 5.65 (s, 1H, Ar-CH), 5.44 (dd, $J_{H2,H3}$ = 3.0 Hz, $J_{H2,H1}$ = 1.7 Hz, 1H, H-2), 5.30 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.6 Hz, 1H, CH_2CH=CH_{2trans}), 5.23 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.3 Hz, 1H, CH_2CH=CH_{2cis}), 4.85 (d, $J_{H1,H2}$ = 1.6 Hz, 1H, H-1), 4.73 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.68 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.31–4.26 (m, 1H, H-6a), 4.18 (ddt, $J_{CH2a,CH2b}$ = 12.8 Hz, $J_{CH2a,CH}$ = 5.2 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.5 Hz, 1H, $CH_{2a}CH$=CH_2), 4.10–4.05 (m, 2H, H-3/H-4), 4.00 (ddt, $J_{CH2b,CH2a}$ = 12.9 Hz, $J_{CH2b,CH}$ = 6.2 Hz, $J_{CH2b,CH2cis/CH2trans}$ = 1.4 Hz, 1H, $CH_{2b}CH$=CH_2), 3.94–3.82 (m, 2H, H-5/H-6b), 2.17 (s, 3H, CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.3 (C=O-OAc), 138.1, 137.6 (2 × C_q), 133.3 (CH_2CH=CH_2), 129.0, 128.4, 128.3, 127.8, 126.2 (10 × C_{Ar}), 118.2 (CH_2CH=CH_2), 101.7 (Ar-CH), 98.0 (C-1), 78.5 (C-4), 74.0 (C-3), 72.3 (CH_{Bn}), 69.9 (C-2), 68.8 (C-6), 68.5 (CH_2CH=CH_2), 64.1 (C-5), 21.1 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{25}H_{32}NO_7^+$ $[M+NH_4]^+$: 458.2173, found 458.2176.

2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl trichloroacetimidate (54)[161]



Before being dissolved in AcOH/H_2O (19:1, 12 mL), compound **52** (1.76 g, 4.00 mmol, 1.0 equiv.) was mixed with NaOAc (1.20 g, 14.6 mmol, 3.66 equiv.) and $PdCl_2$ (1.21 g, 6.80 mmol, 1.7 equiv.). The reaction mixture was stirred for 17 h at room temperature. Excess of AcOH was carefully neutralized by the addition of sat. aq. $NaHCO_3$ and solid $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic phases were washed with sat. aq. $NaHCO_3$ (3 × 100 mL) and brine (100 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **53** (1.34 g of anomeric mixture, 3.35 mmol, 84%) as a colorless solid. To a solution of this compound (1.34 g, 3.35 mmol, 1.0 equiv.) in CH_2Cl_2 (20 mL) at 0 °C was added trichloroacetonitrile (1.68 mL, 16.7 mmol, 5.0 equiv.) and DBU (0.125 mL, 0.84 mmol, 0.25 equiv.) in sequential order. The mixture was allowed to warm to room temperature and it was stirred for 19 h. Then sat. aq. NH_4Cl (15 mL) was added and the aqeous phase was extracted with CH_2Cl_2 (30 mL). The combined organic phases were washed with brine (2 × 30 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **54** (1.28 g, 2.35 mmol, 70%) as a colorless oil.

R_f = 0.50 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.71 (s, 1H, C=NH), 7.54–7.26 (m, 10H, Ar-H), 6.23 (d, $J_{H1,H2}$ = 1.8 Hz, 1H, H-1), 5.66 (s, 1H, Ar-CH), 5.51 (dd, $J_{H2,H3}$ = 3.4 Hz, $J_{H2,H1}$ = 1.8 Hz, 1H; H-2), 4.75 (d, J = 12.0 Hz, 1H, CH_{Bn}), 4.71 (d, J = 12.0 Hz, 1H, CH_{Bn}), 4.32 (dd, $J_{H6a,H6b}$ = 10.3 Hz, $J_{H6a,H5}$ = 4.7 Hz, 1H, H-6a), 4.21–4.14 (m, 1H, H-4), 4.09 (dd, $J_{H3,H4}$ = 10.0 Hz, $J_{H3,H2}$ = 3.5 Hz, 1H, H-3), 4.02 (td, $J_{H5,H4/H6b}$ = 9.7 Hz, $J_{H5,H6a}$ = 4.7 Hz, 1H, H-5), 3.86 (t, $J_{H6b,H5/H6a}$ = 10.2 Hz, 1H, H-6b), 2.21 (CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.0 (C=O-OAc), 160.1 (C=NH), 137.7, 137.3 (2 × C_q), 129.2, 128.6, 128.4, 128.1, 128.0, 126.2 (10 × C_{Ar}), 101.7 (Ar-CH), 95.8 (C-1), 90.7 (CCl_3), 78.0 (C-4), 73.3 (C-3), 72.8 (CH_{Bn}), 68.5 (C-2/C-6), 66.7 (C-5), 21.1 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{24}H_{24}Cl_3NO_7Na^+$ $[M+Na]^+$: 566.0511, found 566.0518.

4-Methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (57)[56]

A solution of penta-*O*-acetyl-α/β-D-mannopyranoside **43** (22.6 g, 57.9 mmol, 1.0 equiv.) and *p*-thiocresol (10.8 g, 86.8 mmol, 1.5 equiv.) in CH_2Cl_2 (80 mL) was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (11.3 mL, 91.3 mmol, 1.58 equiv.) was added slowly. After the addition, the solution was allowed to warm to room temperature and was stirred for 35 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and carefully neutralized with sat. aq. $NaHCO_3$ (100 ml) and solid $NaHCO_3$. The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to furnish **57** (21.4 g, 47.0 mmol, 81%) as a colorless oil.

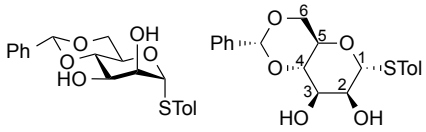
$\boldsymbol{R_f}$ = 0.62 (^{c}Hex/EtOAc, 1:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.37 (d, J = 8.1 Hz, 2H, Ar-H), 7.11 (d, J = 7.9 Hz, 2H, Ar-H), 5.48 (dd, $J_{H2,H3}$ = 2.8 Hz, $J_{H2,H1}$ = 1.7 Hz, 1H), 5.40 (d, $J_{H1,H2}$ = 1.6 Hz, 1H), 5.33–5.29 (m, 2H, H-3/H-4), 4.57–4.51 (m, 1H, H-5), 4.29 (dd, $J_{H6a,H6b}$ = 12.2 Hz, $J_{H6a,H5}$ = 5.9 Hz, 1H, H-6a), 4.09 (dd, $J_{H6b,H6a}$ = 12.2 Hz, $J_{H6b,H5}$ = 2.4 Hz, 1H, H-6b), 2.32 (s, 3H, Ar-CH_3), 2.13, 2.06, 2.05, 2.00 (4s, 12H, 4 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.6, 170.0, 169.9, 169.8 (4 × C=O-OAc), 138.5 (C_q), 132.7, 130.1 (4 × C_{Ar}), 128.9 (C_q), 86.1 (C-1), 71.0 (C-2), 69.5 (C-3/C-5), 66.5 (C-4), 62.6 (C-6), 21.2 (Ar-CH_3), 21.0, 20.8, 20.8, 20.7 (4 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{21}H_{30}NO_9S^+$ $[M+NH_4]^+$: 472.1636, found 472.1637.

4-Methylphenyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (58)[196]



To a solution of compound **57** (14.3 g, 31.5 mmol, 1.0 equiv.) in MeOH (200 mL) were added catalytic amounts of NaOMe and the reaction mixture was stirred for 2 h at room temperature. The mixture was neutralized by the addition of *Amberlite IR120* and the ion-exchange resin was filtered off. The solvents were removed under reduced pressure to give an amorphous off-white solid (quant.). The crude product was dissolved in DMF (HPLC grade, 150 mL) and benzaldehyde dimethylacetal (5.17 mL, 55.0 mmol, 1.1 equiv.) and catalytic amounts of *p*-toluenesulfonic acid were added. The reaction mixture was stirred for 37 h at room temperature. The solvents were removed under reduced pressure and the oily residue was dissolved in CH_2Cl_2 (100 mL) and washed with sat. aq. $NaHCO_3$ (2 × 100 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 1:1) to give **58** (7.67 g, 20.5 mmol, 65% over two steps) as a colorless solid.

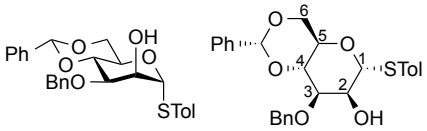
R_f = 0.67 (EtOAc).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ = 7.50–7.34 (m, 7H, Ar-H), 7.21–7.16 (m, 2H, Ar-H), 5.62 (s, 1H, Ar-CH), 5.51 (d, $J_{OH,H2}$ = 4.2 Hz, 1H, -OH), 5.37 (d, $J_{H1,H2}$ = 1.3 Hz, 1H; H-1), 5.19 (d, $J_{OH,H3}$ = 6.2 Hz, 1H, -OH), 4.11–4.03 (m, 2H, H-5/H-6a), 4.01–3.98 (m, 1H, H-2), 3.94 (t, $J_{H4,H3/H5}$ = 9.4 Hz, 1H, H-4), 3.81–3.71 (m, 2H, H-3/H-6b), 2.29 (s, 3H, Ar-CH_3) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $(CD_3)_2SO$): δ = 137.8, 137.3 (2 × C_q), 132.0 (2 × C_{Ar}), 129.9 (C_q/2 × C_{Ar}), 128.0, 126.4 (5 × C_{Ar}), 101.2 (Ar-CH), 89.7 (C-1), 78.5 (C-4), 72.4 (C-2), 68.1 (C-3), 67.6 (C-6), 65.2 (C-5), 20.6 (Ar-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{20}H_{26}NO_5S^+$ $[M+NH_4]^+$: 392.1526, found 392.1526.

4-Methylphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (59)[197]



A solution of compound **58** (5.90 g, 15.8 mmol, 1.0 equiv.) and Bu_2SnO (4.01 g, 16.1 mmol, 1.02 equiv.) in dry toluene (100 mL) was stirred under an argon atmosphere for 3 h at 120 °C. The reaction mixture was allowed to cool to room temperature and Bu_4NBr (5.38 g, 16.7 mmol, 1.06 equiv.), CsF (2.45 g, 16.1 mmol, 1.02 equiv.) and BnBr (1.97 mL, 16.6 mmol, 1.05 equiv.) were added. Afterwards it was stirred for another 3 h at 120 °C, then allowed to cool to room temperature and diluted with EtOAc (100 mL) and sat. aq. $NaHCO_3$ (100 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL) and the combined organic layers were washed with water (100 mL) and brine (100 mL). The organic phase was filtered over a plug of celite and dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to give **59** (6.91 g, 14.9 mmol, 94%) as a colorless oil.

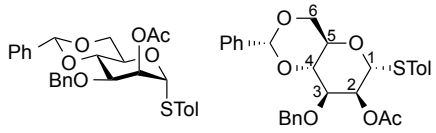
R_f = 0.82 (^{c}Hex/EtOAc, 1:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.56–7.52 (m, 2H, Ar-H), 7.45–7.31 (m, 10H, Ar-H), 7.16–7.12 (m, 2H, Ar-H), 5.64 (s, 1H, Ar-CH), 5.54 (d, $J_{H1,H2}$ = 1.1 Hz, 1H; H-1), 4.91 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.76 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.38 (td, $J_{H5,H4/H6b}$ = 9.9 Hz, $J_{H5,H6a}$ = 4.9Hz, 1H, H-5), 4.29–4.26 (m, 1H, H-2), 4.26–4.17 (m, 2H, H-4/H-6a), 3.99 (dd, $J_{H3,H4}$ = 9.5 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 3.87 (t, $J_{H6b,H5/H6a}$ = 10.2 Hz, 1H, H-6b), 2.95 (d, $J_{OH,H2}$ = 1.5 Hz, 1H, -OH), 2.35 (s, 3H, Ar-CH_3) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 138.1, 137.9, 137.6 (3 × C_q), 132.5, 130.1 (4 × C_{Ar}), 129.5 (C_q), 129.1, 128.7, 128.4, 128.2, 128.0, 126.2 (10 × C_{Ar}), 101.7 (Ar-CH), 88.3 (C-1), 79.2 (C-4), 75.9 (C-3), 73.3 (CH_{Bn}), 71.4 (C-2), 68.7 (C-6), 64.6 (C-5), 21.3 (Ar-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{27}H_{32}NO_5S^+$ $[M+NH_4]^+$: 482.1996, found 482.1998.

4-Methylphenyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (60)[197]



To a solution of compound **59** (6.69 g, 14.7 mmol, 1.0 equiv.) in pyridine (60 mL) was added Ac_2O (1.46 mL, 15.5 mmol, 1.08 equiv.). The reaction mixture was stirred for 1 h at room temperature. The solvents were removed under reduced pressure and the oily residue was dissolved in CH_2Cl_2 (100 mL), washed with water (2 × 100 mL) and brine (100 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **60** (6.96 g, 13.7 mmol, 95%) as a colorless oil.

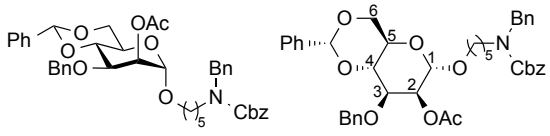
R_f = 0.83 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.56–7.51 (m, 2H, Ar-H), 7.44–7.27 (m, 10H, Ar-H), 7.16–7.11 (m, 2H, Ar-H), 5.65 (s, 1H, Ar-CH), 5.63 (dd, $J_{H2,H3}$ = 3.4 Hz, $J_{H2,H1}$ = 1.4 Hz, 1H, H-2), 5.40 (d, $J_{H1,H2}$ = 1.4 Hz, 1H, H-1), 4.74 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.70 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.38 (td, $J_{H5,H4/H6b}$ = 9.8 Hz, $J_{H5,H6a}$ = 4.8 Hz, 1H, H-5), 4.25 (dd, $J_{H6a,H6b}$ = 10.3 Hz, $J_{H6a,H5}$ = 4.9 Hz, 1H, H-6a), 4.15 (t, $J_{H4,H3/H5}$ = 9.6 Hz, 1H, H-4), 4.03 (dd, $J_{H3,H4}$ = 9.9 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 3.87 (t, $J_{H6b,H5/H6a}$ = 10.3 Hz, 1H, H-6b), 2.34 (s, 3H, Ar-CH_3), 2.16 (s, 3H, CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.2 (C=O-OAc), 138.5, 137.8, 137.5 (3 × C_q), 132.9, 130.1 (4 × C_{Ar}), 129.3 (C_q), 129.1, 128.5, 128.3, 127.9, 126.2 (10 × C_{Ar}), 101.7 (Ar-CH), 87.6 (C-1), 78.7 (C-4), 74.2 (C-3), 72.5 (CH_{Bn}), 71.5 (C-2), 68.6 (C-6), 65.2 (C-5), 21.3 (Ar-CH_3), 21.2 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{29}H_{34}NO_6S^+$ $[M+NH_4]^+$: 524.2101, found 524.2105.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (56)**



Method A[160]

Mannosyl trichloroacetimidate **54** (1.14 g, 2.09 mmol, 1.0 equiv.) and *N*-benzyl-*N*-benzyloxycarbonyl-5-aminopentanol **55** (1.03 g, 3.14 mmol, 1.5 equiv.) were combined and co-evaporated with toluene (2 × 7.5 mL) and with CH_2Cl_2, (7.5 mL), dried under high vacuum and then dissolved in a mixture of CH_2Cl_2/Et_2O (1:1, 15 mL) under an argon atmosphere. The mixture was stirred with freshly activated 4Å MS at room temperature for 30 min, before the reaction vessel was cooled to 0 °C. TMSOTf (38 µL, 0.21 mmol, 0.1 equiv.) was added and the reaction mixture was stirred for 20 min at the same temperature. The reaction mixture was diluted with CH_2Cl_2 (15 mL), quenched by the addition of NEt_3 and filtered through a pad of

celite. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **56** (1.39 g, 1.97 mmol, 94%) as a colorless oil.

Method B[227]

4-Methylphenyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **60** (2.03 g, 4.01 mmol, 1.0 equiv.) and *N*-benzyl-*N*-benzyloxycarbonyl-5-aminopentanol **55** (1.58 g, 4.82 mmol, 1.2 equiv.) were combined and co-evaporated with toluene (3 × 10 mL) and CH_2Cl_2 (10 mL), dried under high vacuum and then dissolved in CH_2Cl_2 (30 mL) under an argon atmosphere. The solution was stirred with freshly activated 4Å MS (2 g) at room temperature for 30 min, before NIS (1.35 g, 6.02 mmol, 1.5 equiv.) was added. The reaction vessel was cooled to −40 °C, TMSOTf (0.94 mL, 5.21 mmol, 1.3 equiv.) was added dropwise and the mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with CH_2Cl_2 (30 mL), quenched by the addition of NEt_3 and filtered through a pad of celite. The filtrate was washed with sat. aq. $NaHCO_3$ (50 mL), sat. aq. $Na_2S_2O_3$ (50 mL) and brine (50 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **56** (3.93 g, 5.54 mmol, 67%) as a colorless oil.

$\boldsymbol{R_f}$ = 0.52 (^{c}Hex/EtOAc, 3:1).

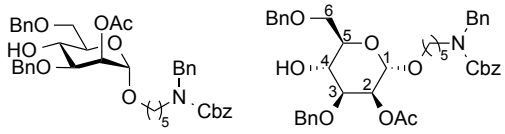
$\boldsymbol{[\alpha]_D^{22}}$ = + 2.0 (c = 0.5, $CHCl_3$).

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.56–7.13 (m, 20H, Ar-H), 5.64 (s, 1H, Ar-CH), 5.38 (s, 1H, H-2), 5.18 (d, J = 20.0 Hz, 2H, CH_{Cbz}), 4.74 (d, $J_{H1,H2}$ = 11.5 Hz, 1H, H-1), 4.71 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.67 (d, J = 12.0 Hz, 1H, CH_{Bn}), 4.51 (d, J = 12.0 Hz, NCH_{Bn}), 4.31–4.22 (m, 1H, H-6a), 4.10–4.03 (m, 1H, H-4), 4.03–3.97 (m, 1H, H-3), 3.88–3.78 (m, 2H, H-5/H-6b), 3.69–3.54 (m, 1H, CH_{Linker}), 3.42–3.15 (m, 3H, CH_{Linker}), 2.17 (s, 3H, CH_3-OAc), 1.65–1.44 (m, 4H, CH_{Linker}), 1.36–1.19 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.2 (C=O-OAc), 156.7/156.2 (C=O-Cbz), 138.0, 137.9, 137.4, 136.9/136.7 (4 × C_q), 128.9, 128.5, 128.4, 128.3, 128.1, 127.8, 127.6, 127.3, 127.2, 127.2, 126.0 (11 × C_{Ar}), 101.5 (Ar-CH), 98.7 (C-1), 78.4 (C-4), 74.0 (C-3), 72.2 (CH_{Bn}), 69.8 (C-2), 68.7 (C-6), 67.9 (CH_{Linker}), 67.1 (CH_{Cbz}), 63.8 (C-5), 50.6/50.2 (NCH_{Bn}), 47.1/46.1 (CH_{Linker}), 29.0 (CH_{Linker}), 27.9/27.5 (CH_{Linker}), 23.3 (CH_{Linker}), 21.0 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{42}H_{51}N_2O_9^+$ $[M+NH_4]^+$: 727.3589, found 727.3593.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (12)**[160]



Benzylidene mannoside **56** (1.03 g, 1.45 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (20 mL) under an argon atmosphere and freshly activated 4Å MS (1 g) was added. The mixture was stirred at room temperature for 30 min before it was cooled to 0 °C. Then triethylsilane (1.16 mL, 7.25 mmol, 5.0 equiv.) and trifluoroacetic acid (0.56 mL, 7.25 mmol, 5.0 equiv.) were added in sequential order. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was diluted with CH_2Cl_2 (20 mL), quenched by the addition of NEt_3 and filtered through a pad of celite. The filtrate was washed with sat. aq. $NaHCO_3$ (50 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **12** (705 mg, 0.990 mmol, 68%) as a colorless oil.

R_f = 0.40 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 2.4 (c = 1.0, $CHCl_3$).

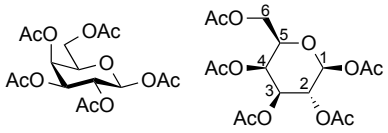
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.43–7.10 (m, 20H, Ar-H), 5.34–5.31 (m, 1H, H-2), 5.18 (d, J = 15.1 Hz, 2H, CH_{Cbz}), 4.80 (s, 1H, H-1), 4.72 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.64 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.57 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.54–4.44 (m, 3H, CH_{Bn}), 3.99–3.88 (m, 1H, H-4), 3.82–3.71 (m, 4H, H-3/H-5/H-6a/H-6b), 3.71–3.56 (m, 1H, CH_{Linker}), 3.44–3.29 (m, 1H, CH_{Linker}), 3.30–3.13 (m, 1H, CH_{Linker}), 2.12 (s, 3H, CH_3-OAc), 1.64–1.44 (m, 4H, CH_{Linker}), 1.37–1.19 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (100 MHz, $CDCl_3$): δ = 170.5 (C=O-OAc), 156.9/156.3 (C=O-Cbz), 138.3, 138.0, 137.8, 137.0/136.9 (4 × C_q), 128.7, 128.6, 128.6, 128.4, 128.2, 128.1, 128.0, 127.7, 127.7, 127.4, 127.3 (20 × C_{Ar}), 98.0 (C-1), 77.8 (C-3), 73.7, 71.8 (2 × CH_{Bn}), 71.4 (C-5), 70.0 (C-6), 68.3 (C-2), 67.8 (CH_{Linker}), 67.5 (C-4), 67.3 (CH_{Cbz}), 50.6/50.4 (NCH_{Bn}), 47.3 (CH_{Linker}), 46.2 (CH_{Linker}), 29.2 (CH_{Linker}), 28.0/27.6 (CH_{Linker}), 23.6/23.5 (CH_{Linker}), 21.1 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{42}H_{53}N_2O_9^+$ $[M+NH_4]^+$: 729.3746, found 729.3751.

5.2.1.3 Synthesis of the native Galactosyl donor 17

Penta-*O*-acetyl-β-D-galactopyranoside (62)[233]



A suspension of NaOAc (50 g, 555 mmol, 1.0 equiv.) in Ac_2O (600 mL) was heated to 100 °C and D-Galactose **61** (100 g, 555 mmol, 1.0 equiv.) was added portionwise. The reaction mixture was stirred at 100 °C for another 3 h and was then poured on ice (800 g). After stirring for 1 h, the colorless precipitate was filtered off, washed with water (4 × 200 mL) and dried *in vacuo* to give **62** (184 g, 471 mmol, 85%) as a colorless solid.

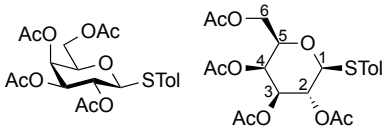
R_f = 0.43 (^{c}Hex/EtOAc = 1:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.36 (d, 1H, $J_{H1,H2}$ = 1.7 Hz, H-1), 5.50-5.47 (m, 1H, H-4), 5.33-5.31 (m, 2H, H-2/H-3), 4.37-4.29 (m, 1H, H-5), 4.13-4.03 (m, 2H, H-6a/H-6b), 2.15, 2.14, 2.03, 2.01, 1.99 (5s, 15H, 5 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (100 MHz, $CDCl_3$): δ = 170.3, 170.1, 169.9, 168.9 (5 × C=O-OAc), 89.7 (C-1), 68.8 (C-5), 67.4 (C-4), 67.3(C-2/C-3), 66.4 (C-2/C-3), 61.2 (C-6), 20.9, 20.7, 20.6, 20.5 (5 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{16}H_{26}NO_{11}^+$ $[M+NH_4]^+$: 408.1500, found: 408.1504.

4-Methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (63)[234]



A solution of penta-*O*-acetyl-β-D-galactopyranoside **62** (6.00 g, 15.4 mmol, 1.0 equiv.) and *p*-thiocresol (2.87 g, 23.1 mmol, 1.5 equiv.) in CH_2Cl_2 (80 mL) under argon was cooled to 0 °C and $BF_3 \cdot Et_2O$ (2.8 mL, 22.1 mmol, 1.4 equiv.) was added slowly. After the addition, the solution was allowed to warm to room temperature and it was stirred for 21 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), carefully neutralized with sat. aq. $NaHCO_3$ (100 ml) and solid $NaHCO_3$. The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to furnish **63** (6.09 g, 13.4 mmol, 87%) as an amorphous off-white solid.

R_f = 0.35 (^{c}Hex/EtOAc, 2:1).

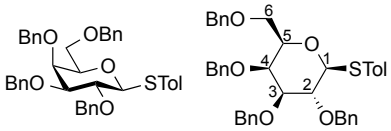
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.41 (d, J = 8.1 Hz, 2H, Ar-H), 7.12 (d, J = 7.9 Hz, 2H, Ar-H), 5.40 (dd, $J_{H4,H3}$ = 3.3 Hz, $J_{H4,H5}$ = 1.1 Hz, 1H, H-4), 5.21 (t, $J_{H2,H1/H3}$ = 10.0 Hz, 1H, H-2), 5.03 (dd, $J_{H3,H2}$ = 9.9 Hz,

$J_{H3,H4}$ = 3.3 Hz, 1H, H-3), 4.64 (d, $J_{H1,H2}$ = 10.0 Hz, 1H, H-1), 4.18 (dd, $J_{H6a,H6b}$ = 11.3 Hz, $J_{H6a,H5}$ = 6.9 Hz, 1H, H-6a), 4.10 (dd, $J_{H6b,H6a}$ = 11.3 Hz, $J_{H6b,H5}$ = 6.3 Hz, 1H, H-6b), 3.90 (dt, $J_{H5,H6a/b}$ = 6.7 Hz, $J_{H5,H4}$ = 1.1 Hz, 1H, H-5), 2.34 (s, 3H, Ar-CH_3), 2.11, 2.09, 2.04, 1.97 (4s, 12H, 4 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.5, 170.3, 170.2, 169.6 (4 × C=O-OAc), 138.6 (C_q), 133.3, 129.8 (4 × C_{Ar}), 128.8 (C_q), 87.1 (C-1), 74.5 (C-5), 72.2 (C-3), 67.5 (C-2/4), 67.4 (C-2/C-4), 61.7 (C-6), 21.3 (Ar-CH_3), 21.0, 20.8, 20.7 (4 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{21}H_{26}O_9SNa^+$ $[M+Na]^+$: 477.1190, found: 477.1198.

4-Methylphenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (64)[56]



To a solution of compound **63** (9.56 g, 21.0 mmol, 1.0 equiv.) in MeOH (180 mL) were added catalytic amounts of NaOMe. The reaction mixture was stirred at room temperature for 20 h and neutralized by adding *Amberlite IR120*. The ion-exchange resin was filtered off and the solvents were removed under reduced pressure to give an amorphous off-white solid (quant.). The deprotected compound was then dissolved in DMF (HPLC grade, 200 mL) under an argon atmosphere and cooled to 0 °C. NaH (60% dispersion in oil, 3.95 g, 98.7 mmol, 4.7 equiv.) was added portionwise and the reaction mixture was stirred for 1 h at 0 °C. BnBr (7.92 mL, 65.3 mmol, 3.5 equiv.) was slowly added and the mixture was allowed to warm to room temperature and was stirred for 37 h. The solvents were removed under reduced pressure and the sticky yellow residue was dissolved in EtOAc (200 mL) and poured onto ice-cold water (200 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL) and the combined organic layers were washed with brine (2 × 100 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **64** (12.1 g, 18.6 mmol, 89% over two steps) as an amorphous yellow solid.

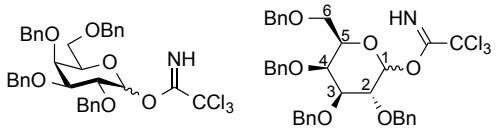
R_f = 0.86 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.51 (d, J = 8.1 Hz, 2H, Ar-H), 7.46–7.29 (m, 20H, Ar-H), 7.03 (d, J = 7.9 Hz, 2H, Ar-H), 5.00 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.84 (d, J = 10.2 Hz, 1H, CH_{Bn}), 4.79–4.73 (m, 3H, CH_{Bn}), 4.66–4.61 (m, 2H, H-1/CH_{Bn}), 4.53–4.44 (m, 2H, CH_{Bn}), 4.01 (d, $J_{H4,H3}$ = 2.8 Hz, $J_{H4,H5}$ = 1.0 Hz, 1H, H-4), 3.94 (t, $J_{H2,H1/H3}$ = 9.4 Hz, 1H, H-2), 3.71–3.68 (m, 2H, H-6a/H-6b), 3.65–3.61 (m, 2H, H-3/H-5), 2.32 (s, 3H, Ar-CH_3) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 138.9, 138.5, 138.4, 138.0, 137.3 (5 × C_q), 132.3 (2 × C_{Ar}), 130.3 (C_q), 129.7, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.7, 127.5 (22 × C_{Ar}), 88.2 (C-1), 84.3 (C-3), 77.4 (C-2/C-5), 75.7, 74.5 (2 × CH_{Bn}), 73.7 (C-4/CH_{Bn}), 72.8 (CH_{Bn}), 68.9 (C-6), 21.2 (Ar-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{41}H_{46}NO_6S^+$ $[M+NH_4]^+$: 664.3091, found 664.3093.

2,3,4,6-Tetra-*O*-benzyl-α/β-D-galactopyranosyl trichloroacetimidate (17)[180, 235]



To a stirred solution of thioglycoside **64** (6.26 g, 9.68 mmol, 1.0 equiv.) in acetone (1% aq., 100 mL) at 0 °C was added NIS (3.27 g, 14.8 mmol, 1.5 equiv.). The reaction mixture was stirred for 30 min and was then quenched by the addition of sat. aq. $Na_2S_2O_3$ (50 mL). The mixture was diluted with EtOAc (150 mL) and was washed with sat. aq. $NaHCO_3$ (3 × 80 mL) and brine (80 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give lactol **65** (4.35 g of anomeric mixture, 8.05 mmol, 82%) as a colorless oil. To a solution of lactol **65** (3.82 g, 7.06 mmol, 1.0 equiv.) in CH_2Cl_2 (30 mL) at 0 °C was added trichloroacetonitrile (1.77 mL, 17.7 mmol, 2.5 equiv.) and DBU (0.26 mL, 1.77 mmol, 0.25 equiv.) in sequential order. The mixture was allowed to warm to room temperature and stirred for 17 h. Then sat. aq. NH_4Cl (50 mL) was added and the aqeous phase was extracted with CH_2Cl_2 (100 mL). The combined organic layers were washed with brine (2 × 50 mL) and dried with $MgSO_4$. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 5:1) to give **17** (3.98 g of anomeric mixture α/β 2:1, 5.81 mmol, 82%) as a colorless oil.

R_f = 0.69 (α-anomer), 0.50 (β-anomer) (^{c}Hex/EtOAc, 3:1).

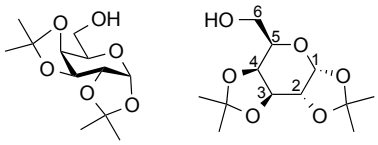
^{1}H-NMR (400 MHz, $CDCl_3$): δ (α-Anomer) = 8.55 (s, 1H, C=NH), 7.43–7.24 (m, 20H, Ar-H), 6.56 (d, $J_{H1,H2}$ = 3.4 Hz, 1H, H-1), 5.01 (d, J = 11.3 Hz, 1H, CH_{Bn}), 4.86 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.81–4.76 (m, 3H, CH_{Bn}), 4.63 (d, J = 11.3 Hz, 1H, CH_{Bn}), 4.50 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.43 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.28 (dd, $J_{H2,H3}$ = 10.0 Hz, $J_{H2,H1}$ = 3.5 Hz, 1H, H-2), 4.20 (ddd, $J_{H5,H6a}$ = 7.1 Hz, $J_{H5,H6b}$ = 5.2 Hz, $J_{H5,H4}$ = 1.1 Hz, 1H, H-5), 4.09 (dd, $J_{H4,H3}$ = 2.9 Hz, $J_{H4,H5}$ = 1.3 Hz, 1H, H-4), 4.06 (dd, $J_{H3,H2}$ = 10.0 Hz, $J_{H3,H4}$ = 2.8 Hz, 1H, H-3), 3.68–3.56 (m, 2H, H-6a/H-6b) ppm; δ (β-Anomer) = 8.65 (s, 1H, C=NH), 7.44–7.19 (m, 20H, Ar-H), 5.78 (d, $J_{H1,H2}$ = 8.0 Hz, 1H, H-1), 4.98 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.94 (d, J = 10.7 Hz, 1H, CH_{Bn}), 4.84 (d, J = 10.7 Hz, 1H, CH_{Bn}), 4.76 (s, 2H, CH_{Bn}), 4.66 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.50 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.45 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.12 (dd, $J_{H2,H3}$ = 9.7 Hz, $J_{H2,H1}$ = 8.0 Hz, 1H, H-2), 4.02 (dd, $J_{H4,H3}$ = 3.0 Hz, $J_{H4,H5}$ = 1.1 Hz, 1H, H-4), 3.78 (ddd, $J_{H5,H6a}$ = 7.0 Hz, $J_{H5,H6b}$ = 5.7 Hz, $J_{H5,H4}$ = 1.2 Hz, 1H, H-5), 3.73–3.62 (m, 3H, H-3/H-6a/H-6b) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ (α-Anomer) = 161.4 (C=NH), 138.7, 138.6, 138.5, 138.0 (4 × C_q), 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6 (20 × C_{Ar}), 95.3 (C-1), 91.6 (CCl_3), 78.0 (C-3), 76.0 (C-2), 75.1 (CH_{Bn}), 74.6 (C-4), 73.6, 73.1, 73.0 (3 × CH_{Bn}), 72.3 (C-5), 68.4 (C-6) ppm; δ (β-Anomer) = 161.6 (C=NH), 138.6, 138.4, 138.0 (4 × C_q), 128.5, 128.4, 128.1, 127.9, 127.8, 127.7 (20 × C_{Ar}), 98.8 (C-1), 91.1 (CCl_3), 82.3 (C-3), 78.2 (C-2), 75.4, 74.9 (2 × CH_{Bn}), 74.6 (C-5), 73.6 (CH_{Bn}), 73.5 (C-4), 73.2 (CH_{Bn}), 68.2 (C-6) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{36}H_{36}Cl_3NO_6Na^+$ $[M+Na]^+$: 706.1500, found 706.1524.

5.2.1.4 Synthesis of the 6F-Galactosyl donor 13

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranoside (66)[205]



To a stirred suspension of D-galactose **61** (15.0 g, 83.3 mmol, 1.0 equiv.) and anhydrous $CuSO_4$ (35.5 g, 0.21 mol, 2.5 equiv.) in acetone (500 mL) was added conc. H_2SO_4 (2.0 mL). The mixture was stirred at room temperature for 24 h. Then all solids were filtered off and the filtrate was neutralized by the addition of $Ca(OH)_2$. After filtration, all solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to furnish **66** (21.0 g, 80.6 mmol, 97%) as a colorless oil.

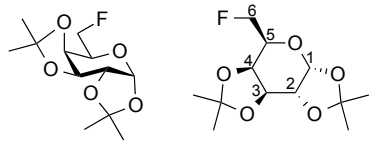
R_f = 0.41 (^{c}Hex/EtOAc, 1:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.55 (d, $J_{H1,H2}$ = 5.0 Hz, 1H, H-1), 4.60 (dd, $J_{H3,H4}$ = 8.0 Hz, $J_{H3,H2}$ = 2.3 Hz, 1H, H-3), 4.32 (dd, $J_{H2,H1}$ = 5.2 Hz, $J_{H2,H3}$ = 2.6 Hz, 1H, H-2), 4.26 (d, $J_{H4,H3}$ = 7.9 Hz, 1H, H-4), 3.90–3.68 (m, 3H, H-5/H-6a/H-6b), 1.52, 1.44, 1.33, 1.32 (4s, 12H, 4 × -CH_3) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 109.6, 108.8 (2 × C_q), 96.4 (C-1), 71.7 (C-4), 70.9 (C-3), 70.7 (C-2), 68.2 (C-5), 62.5 (C-6), 26.2, 26.1, 25.1, 24.4 (4 × -CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{12}H_{20}O_6Na^+$ $[M+Na]^+$: 283.1152, found: 283.1157.

1,2:3,4-Di-*O*-isopropylidene-α-D-6-deoxy-6-fluorogalactopyranoside (67)[178]



A solution of compound **66** (1.50 g, 5.76 mmol, 1.0 equiv.), DAST (0.91 mL, 6.92 mmol, 1.2 equiv.) and 2,4,6-Collidine (1.53 mL, 11.5 mmol, 2.0 equiv.) in CH_2Cl_2 (2.5 mL) was exposed to microwave irradiation (100 W, 80 °C) for 1 h. The reaction mixture was quenched by the addition of MeOH (5 mL) and was washed with HCl (1 M, 10 mL) and water (10 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 5:1) to furnish **67** (1.24 g, 4.73 mmol, 82%) as a colorless oil.

R_f = 0.70 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{21}$ = − 45.4 (c = 1.00, $CHCl_3$).

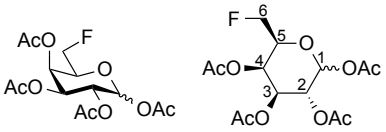
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.54 (d, $J_{H1,H2}$ = 5.0 Hz, 1H, H-1), 4.69–4.56 (m, 2H, H-3/H-6a), 4.53–4.40 (m, 1H, H-6b), 4.33 (dd, $J_{H2,H1}$ = 5.0 Hz, $J_{H2,H3}$ = 2.5 Hz, 1H, H-2), 4.26 (dd, $J_{H4,H3}$ = 7.9 Hz, $J_{H4,H5}$ = 2.0 Hz, 1H, H-4), 4.13–4.01 (m, 1H, H-5), 1.54, 1.44, 1.33 (3s, 12H, 4 × -CH_3) ppm.

^{13}C-{^{1}H}-NMR (75 MHz, $CDCl_3$): δ = 109.9, 108.9 (2 × $C(CH_3)_2$), 96.3 (C-1), 82.2 (d, $J_{C6,F}$ = 168.1 Hz, C-6), 70.7, 70.6 (C-2/C-3/C-4), 66.8 (d, $J_{C5,F}$ = 22.6 Hz, C-5), 26.2, 26.1, 25.1, 24.6 (4 × -CH_3) ppm.

^{19}F-NMR (282 MHz, $CDCl_3$): δ = − 231.2 (dt, $J_{F,H6}$ = 47.3 Hz, $J_{F,H5}$ = 13.6 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{12}H_{24}FNO_5^+$ $[M+NH_4]^+$: 280.1555, found 280.1555.

1,2,3,4-Tetra-*O*-acetyl-α/β-D-6-deoxy-6-fluorogalactopyranoside (68)[178]



A solution of fluorinated compound **67** (1.24 g, 4.73 mmol, 1.0 equiv.) in 80% AcOH (40 mL) was stirred for 5 h at 100 °C. The solvents were removed under reduced pressure and the oily residue was co-evaporated with toluene (2 × 10 mL) and CH_2Cl_2 (2 × 10 mL). The crude product was dissolved in pyridine (20 mL) and cooled to 0 °C. Ac_2O (4.47 mL, 47.3 mmol, 10 equiv.) was slowly added and the mixture was stirred for 17 h at room temperature. The solvents were removed under reduced pressure and the residue was co-evaporated with toluene (2 × 10 mL) and CH_2Cl_2 (2 × 10 mL). The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 1:1) to furnish **68** (1.62 g of anomeric mixture, 4.62 mmol, 98% over two steps) as a colorless oil.

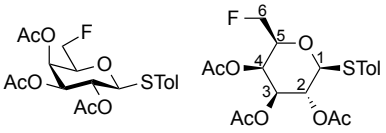
R_f = 0.33 (^{c}Hex/EtOAc, 1:1);

^{1}H-NMR (400 MHz, $CDCl_3$): characteristic signals δ = 5.72 (d, $J_{H1,H2}$ = 8.3 Hz, 1H, H-1β), 5.48 (d, $J_{H1,H2}$ = 3.5 Hz, 1H, H-1α) ppm.

^{19}F-NMR (282 MHz, $CDCl_3$): δ = − 232.2 (dt, $J_{F,H6}$ = 45.1 Hz, $J_{F,H5}$ = 15.4 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{14}H_{23}FNO_9^+$ $[M+NH_4]^+$: 368.1351, found 368.1355.

4-Methylphenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-6-deoxy-6-fluorogalactopyranoside (69)



A solution of tetra-*O*-acetyl-α/β-D-6-deoxy-6-fluorogalactopyranoside **68** (15.5 g, 44.2 mmol, 1.0 equiv.) and *p*-thiocresol (8.24 g, 66.4 mmol, 1.5 equiv.) in CH_2Cl_2 (200 mL) was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (11.2 mL, 88.4 mmol, 2.0 equiv.) was added slowly. After the addition, the solution was allowed to warm to room

temperature and stirred for 20 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), carefully neutralized with sat. aq. $NaHCO_3$ (100 ml) and solid $NaHCO_3$. The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to furnish **69** (14.6 g, 35.2 mmol, 80%) as an amorphous off-white solid.

R_f = 0.53 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 84.2 (c = 1.0, $CHCl_3$).

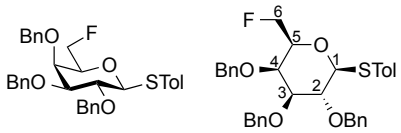
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.41 (d, J = 8.3 Hz, 2H, Ar-H), 7.13 (d, J = 8.4 Hz, 2H, Ar-H), 5.45 (dd, $J_{H4,H3}$ = 3.3 Hz, $J_{H4,H5}$ = 1.0 Hz, 1H, H-4), 5.23 (t, $J_{H2,H1/H3}$ = 9.9 Hz, 1H, H-2), 5.05 (dd, $J_{H3,H2}$ = 9.9 Hz, $J_{H3,H4}$ = 3.3 Hz, 1H, H-3), 4.67 (d, $J_{H1,H2}$ = 9.9 Hz, 1H, H-1), 4.61–4.28 (m, 1H, H-6a/H-6b), 4.00–3.91 (m, 1H, H-5), 2.34 (Ar-CH_3), 2.10, 1.97, 1.42 (3s, 9H, 3 × CH_3 OAc) ppm.

^{13}C-{^{1}H}-NMR (75 MHz, $CDCl_3$): δ = 170.2, 170.1, 169.5 (3 × C=O-OAc), 138.6 (2 × C_q), 133.2, 129.8, 128.8 (4 × C_{Ar}), 87.3 (C-1), 80.9 (d, $J_{C6,F}$ = 172.3 Hz, C-6), 75.3 (d, $J_{C5,F}$ = 23.2 Hz, C-5), 72.1 (C-3), 67.4 (C-2), 67.3 (C-4), 27.1 (CH_3-OAc), 21.3 (Ar-CH_3), 21.0, 20.7 (2 × CH_3-OAc) ppm.

^{19}F-NMR (282 MHz, $CDCl_3$): δ = − 230.8 (dt, $J_{F,H6}$ = 46.5 Hz, $J_{F,H5}$ = 12.1 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{19}H_{27}FNO_7S^+$ $[M+NH_4]^+$: 432.1492, found 432.1492.

4-Methylphenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-6-deoxy-6-fluorogalactopyranoside (70)



To a stirred solution of fluorinated compound **69** (7.73 g, 18.7 mmol, 1.0 equiv.) in MeOH (150 mL) were added catalytic amounts of NaOMe at room temperature. After complete conversion within 4 h, the reaction mixture was neutralized by adding *Amberlite IR120*. The ion-exchange resin was filtered off and the solvents were removed under reduced pressure to give an amorphous off-white solid (quant.). The crude product was used without further purification in the next step. The deprotected compound was then dissolved in DMF (HPLC grade, 150 mL) under an argon atmosphere and the solution was cooled to 0 °C. At this temperature, sodium hydride (60% dispersion in oil, 2.61 g, 65.3 mmol, 3.5 equiv.) was added portionwise and the reaction mixture was stirred for 1 h at 0 °C. After the addition of benzylbromide (7.92 mL, 65.3 mmol, 3.5 equiv.) the mixture was allowed to warm to room temperature and it was stirred for 17 h. The solvents were then removed under reduced pressure, the sticky yellow residue was dissolved in EtOAc (200 mL) and poured onto ice-cold water (200 mL). The aqueous phase was extracted with EtOAc (3 × 80 mL) and the combined organic layers were washed with brine (2 × 50 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **69** (10.1 g, 18.1 mmol, 97% over two steps) as an amorphous yellow solid.

R_f = 0.77 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = − 2.6 (c = 1.0, $CHCl_3$).

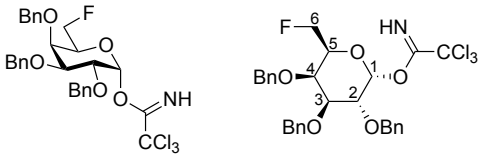
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.49 (d, J = 8.1 Hz, 2H, Ar-H), 7.45–7.28 (m, 15H, Ar-H), 7.05 (d, J = 7.9 Hz, 2H, Ar-H), 5.02 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.85 (d, J = 10.2 Hz, 1H, CH_{Bn}), 4.80–4.75 (m, 3H, CH_{Bn}), 4.67–4.36 (m, 4H, H-1/H-6a/H-6b/CH_{Bn}), 3.97–3.90 (m, 2H, H-2/H-4), 3.73–3.66 (m, 1H, H-5), 3.63 (dd, $J_{H3,H2}$ = 9.2 Hz, $J_{H3,H4}$ = 2.8 Hz, 1H, H-3), 2.33 (Ar-CH_3) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 138.5, 138.4, 138.2, 137.6 (4 × C_q), 132.4 (2 × C_{Ar}), 130.1 (C_q), 129.7, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7 (17 × C_{Ar}), 88.3 (C-1), 84.0 (C-3), 81.7 (d, $J_{C6,F}$ = 167.8 Hz, C-6), 77.4 (C-2), 76.4 (d, $J_{C5,F}$ = 24.0 Hz, C-5), 75.8, 74.5 (2 × CH_{Bn}), 73.2 (d, $J_{C4,F}$ = 4.0 Hz, C-4), 73.1 (CH_{Bn}), 21.3 (Ar-CH_3) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 229.68 (dt, $J_{F,H6}$ = 46.6 Hz, $J_{F,H5}$ = 10.0 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{34}H_{39}FNO_4S^+$ $[M+NH_4]^+$: 576.2584, found 576.2585.

2,3,4-Tri-*O*-benzyl-α-D-6-deoxy-6-fluorogalactopyranosyl trichloroacetimidate (13)[180, 235]



NIS (604 mg, 2.68 mmol, 1.5 equiv.) was added to a stirred solution of thioglycoside **69** (1.00 g, 1.79 mmol, 1.0 equiv.) in acetone (1% aq., 20 mL) at 0 °C. The reaction mixture was stirred for 5 min and then quenched by the addition of sat. aq. $Na_2S_2O_3$ (20 mL). The solution was diluted with EtOAc (100 mL) and washed with sat. aq. $NaHCO_3$ (3 × 50 mL) and brine (50 mL). The organic phase was dried with $MgSO_4$ and solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **71** (740 mg of anomeric mixture, 1.64 mmol, 92%) as a colorless oil. To a solution of this compound (4.00 g, 8.84 mmol, 1.0 equiv.) in CH_2Cl_2 (50 mL) at 0 °C was added trichloroacetonitrile (4.43 mL, 44.2 mmol, 5.0 equiv.) and DBU (0.33 mL, 2.21 mmol, 0.25 equiv.) in sequential order. The mixture was allowed to warm to room temperature and stirred for 20 h. Then sat. aq. NH_4Cl (30 mL) was added and the aqeous phase was extracted with CH_2Cl_2 (50 mL). The combined organic layers were washed with brine (2 × 30 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 5:1) to give **13** (3.70 g, 6.20 mmol, 70%) as a colorless oil.

R_f = 0.73 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 62.2 (c = 1.0, $CHCl_3$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.58 (s, 1H, C=NH), 7.43–7.26 (m, 15H, Ar-H), 6.57 (d, $J_{H1,H2}$ = 3.4 Hz, 1H, H-1), 5.03 (d, J = 11.3 Hz, 1H, CH_{Bn}), 4.89 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.83–4.77 (m, 3H, CH_{Bn}), 4.66 (d, J = 11.3 Hz, 1H, CH_{Bn}), 4.50 (ddd, $J_{H6a,F}$ = 47.1 Hz, $J_{H6a,H6b}$ = 9.3 Hz, $J_{H6a,H5}$ = 6.3 Hz, 1H, H-6a), 4.39 (ddd, $J_{H6b,F}$ = 46.1 Hz, $J_{H6b,H6a}$ = 9.3 Hz, $J_{H6b,H5}$ = 6.1 Hz, 1H, H-6b), 4.28 (dd, $J_{H2,H3}$ = 10.0 Hz, $J_{H2,H1}$ = 3.5 Hz, 1H, H-2), 4.26–4.19 (m, 1H, H-5), 4.07 (dd, $J_{H3,H2}$ = 10.0 Hz, $J_{H3,H4}$ = 2.8 Hz, 1H, H-3), 4.02–3.99 (m, 1H, H-4) ppm.

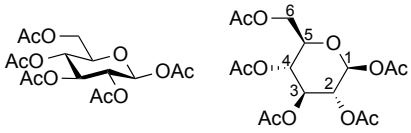
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 161.2 (C=NH), 138.5, 138.4, 138.2 (3 × C_q), 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6 (15 × C_{Ar}), 95.0 (C-1), 91.5 (CCl_3) 81.7 (d, $J_{C6,F}$ = 167.7 Hz, C-6), 77.7 (C-3), 75.9 (C-2), 75.0 (CH_{Bn}), 74.3 (d, $J_{C4,F}$ = 4.5 Hz, C-4), 73.5, 73.1 (2 × CH_{Bn}), 71.7 (d, $J_{C5,F}$ = 24.5 Hz, C-5) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 230.30 (dt, $J_{F,H6}$ = 46.6 Hz, $J_{F,H5}$ = 11.4 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{29}H_{33}Cl_3FN_2O_5^+$ $[M+NH_4]^+$: 613.1439, found 613.1458.

5.2.1.5 Synthesis of the 4F-Galactosyl donor 14

Penta-*O*-acetyl-α/β-D-glucopyranoside (73)[240]



A suspension of NaOAc (50 g, 555 mmol, 1.0 equiv.) in Ac_2O (600 mL) was heated to 100 °C and D-Glucose **72** (100 g, 555 mmol, 1.0 equiv.) was added portionwise. The reaction mixture was stirred at 100 °C for another 3 h and was then poured on ice (800 g). After stirring for 1 h, the colorless precipitate was filtered off, washed with water (4 × 200 mL) and dried under high vacuum to give **73** (162 g, 416 mmol, 75%).

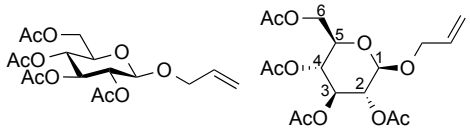
R_f = 0.35 (^{c}Hex/EtOAc, 1:1).

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 5.70 (d, $J_{H1,H2}$ = 8.3 Hz, 1H, H-1), 5.24 (t, $J_{H3,H2/H4}$ = 9.5 Hz, 1H, H-3), 5.17–5.04 (m, 1H, H-2/H-4), 4.27 (dd, $J_{H6a,H6b}$ = 12.5 Hz, $J_{H6a,H5}$ = 4.6 Hz, 1H, H-6a), 4.12–4.08 (m, 1H, H-6b), 3.85–3.80 (m, 1H, H-5), 2.17, 2.10, 2.07, 2.02, 2.00 (5s, 15H, 5 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.5, 170.0, 169.3, 169.2, 168.9 (5 × C=O-OAc), 91.7 (C-1), 72.8 (C-5), 72.7 (C-3), 70.2 (C-2/C-4), 67.7 (C-2/C-4), 61.4 (C-6), 20.8, 20.7, 20.5, 20.4 (5 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{16}H_{22}O_{11}Na^+$ $[M+Na]^+$: 413.1054, found: 413.1059.

Allyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (74)[199, 240]



A solution of penta-*O*-acetyl-α/β-D-glucopyranoside **73** (20.0 g, 51.2 mmol, 1.0 equiv.) and allylic alcohol (7.00 mL, 102.4 mmol, 2.0 equiv.) in CH_2Cl_2 (300 mL) under an argon atmosphere was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (12.6 mL, 102.4 mmol, 1.3 equiv.) was added slowly. The solution was allowed to warm to room temperature and it was stirred for 48 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and carefully neutralized with sat. aq. $NaHCO_3$ (100 ml) and solid $NaHCO_3$. The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was redissolved in pyridine (75 mL) and cooled to 0 °C. Ac_2O (45 mL) was added dropwise and the reaction mixture was stirred for 20 h. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 2:1) to furnish **74** (13.8 g, 35.5 mmol, 69%) as a colorless oil.

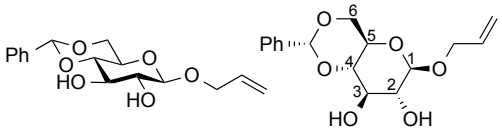
R_f = 0.35 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.85 (dddd, $J_{CH,CH2trans}$ = 17.1 Hz, $J_{CH,CH2cis}$ = 10.8 Hz, $J_{CH,CH2b}$ = 6.1 Hz, $J_{CH,CH2a}$ = 4.9 Hz, 1H, CH_2CH=CH_2), 5.30–5.18 (m, 3H, H-3/CH_2CH=CH_{2trans}/CH_2CH=CH_{2cis}), 5.09 (t, $J_{H4,H3/H5}$ = 9.6 Hz, 1H, H-4), 5.02 (dd, $J_{H2,H3}$ = 9.6 Hz, $J_{H2,H1}$ = 8.0 Hz, 1H, H-2), 4.55 (d, $J_{H1,H2}$ = 8.0 Hz, 1H), 4.34 (ddt, $J_{CH2a,CH2b}$ = 13.1 Hz, $J_{CH2a,CH}$ = 4.9 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.6 Hz, 1H, CH_{2a}CH=CH_2), 4.26 (dd, $J_{H6a,H6b}$ = 12.3 Hz, $J_{H6a,H5}$ = 4.8 Hz, 1H, H-6a), 4.16–4.07 (m, 2H, H-6b/CH_{2b}CH=CH_2), 3.71–3.65 (m, 1H, H-5), 2.09, 2.04, 2.02, 2.00 (4s, 12H, 4 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.8, 170.4, 169.5 (4 × C=O-OAc), 133.5 (CH_2*C*H=CH_2), 117.8 (CH_2CH=*C*H_2), 99.7 (C-1), 73.0 (C-3), 72.0 (C-5), 71.5 (C-2), 70.2 (*C*H_2CH=CH_2), 68.6 (C-4), 62.1 (C-6), 20.9, 20.8, 20.7 (4 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{17}H_{28}NO_{10}^+$ $[M+NH_4]^+$: 406.1708, found 406.1705.

Allyl 4,6-*O*-benzylidene-β-D-glucopyranoside (75)[241]



To a stirred solution of compound **74** (11.6 g, 29.9 mmol, 1.0 equiv.) in MeOH (200 mL) were added catalytic amounts of NaOMe and the reaction mixture was stirred for 16 h at room temperature. The mixture was neutralized by the addition of *Amberlite IR120* and the ion-exchange resin was filtered off. The solvents were removed under reduced pressure to give an amorphous off-white solid (quant.). The crude product was dissolved in DMF (HPLC grade, 150 mL) and benzaldehyde dimethylacetal (8.92 mL, 59.8 mmol, 2.0 equiv.) and catalytic amounts of *p*-toluenesulfonic acid were added. The reaction mixture was stirred for 40 h at room temperature. The solvents were removed under reduced pressure and the oily residue was dissolved in CH_2Cl_2 (100 mL) and washed with sat. aq. $NaHCO_3$ (2 × 100 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (EtOAc) to give **75** (6.96 g, 22.6 mmol, 76% over two steps) as a colorless solid.

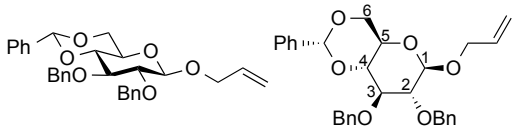
R_f = 0.70 (EtOAc).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.50–7.47 (m, 2H, Ar-H), 7.37–7.33 (m, 3H, Ar-H), 5.98–5.88 (m, 1H, CH_2C*H*=CH_2), 5.49 (s, 1H, Ar-CH), 5.32 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.6 Hz, 1H, CH_2CH=CH_{2trans}), 5.22 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.2 Hz, 1H, CH_2CH=CH_{2cis}), 4.41 (d, $J_{H1,H2}$ = 7.8 Hz, 1H, H-1), 4.38–4.33 (m, 1H, CH_{2a}CH=CH_2), 4.31 (dd, $J_{H6a,H6b}$ = 10.5 Hz, $J_{H6a,H5}$ = 5.0 Hz, 1H, H-6a), 4.18–4.07 (m, 1H, CH_{2b}CH=CH_2), 3.80–3.71 (m, 2H, H-2/6b), 3.55–3.47 (m, 2H, H-3/4), 3.43–3.36 (m, 1H, H-5) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 137.1 (C_{Ar}), 133.6 (CH_2*C*H=CH_2), 129.4, 128.4, 126.4 (3 × C_{Ar}), 118.4 (CH_2CH=*C*H_2), 102.2 (C-1), 102.0 (Ar-CH), 80.6 (C-4), 74.5 (C-3), 73.2 (C-2), 70.7 (*C*H_2CH=CH_2), 68.7 (C-6), 66.4 (C-5) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{16}H_{20}O_6Na$ $[M+Na]^+$: 331.1152, found: 331.1151.

Allyl 2,3-di-*O*-benzyl-4,6-benzylidene-β-D-glucopyranoside (76)[242]



A solution of compound **75** (1.70 g, 5.51 mmol, 1.0 equiv.) in DMF (HPLC grade, 100 mL) under an argon atmosphere was cooled to 0 °C. NaH (60% dispersion in oil, 551 mg, 13.8 mmol, 2.5 equiv.) was added portionwise and the reaction mixture was stirred for 1 h at 0 °C. BnBr (1.67 mL, 13.8 mmol, 2.5 equiv.) was slowly added and the mixture was allowed to warm to room temperature and was stirred for 20 h. The solvents were removed under reduced pressure and the sticky yellow residue was dissolved in EtOAc (100 mL) and poured onto ice-cold water (100 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL) and the combined organic phase was washed with brine (2 × 50 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 5:1) to give **76** (1.90 g, 3.90 mmol, 71%) as an amorphous colorless solid.

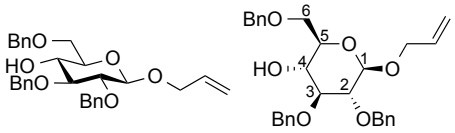
R_f = 0.76 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.53–7.49 (m, 2H, Ar-H), 7.44–7.26 (m, 13H, Ar-H), 5.98 (ddt, $J_{CH,CH2trans}$ = 17.2 Hz, $J_{CH,CH2cis}$ = 10.4 Hz, $J_{CH,CH2a/b}$ = 5.2 Hz, 1H, CH_2CH=CH_2), 5.59 (s, 1H, Ar-CH), 5.38 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.6 Hz, 1H, CH_2CH=CH_{2trans}), 5.25 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.4 Hz, 1H, CH_2CH=CH_{2cis}), 4.94 (d, J = 11.1 Hz, 2H, CH_{Bn}), 4.85–4.77 (m, 2H, CH_{Bn}), 4.59 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.43 (ddt, $J_{CH2a,CH2b}$ = 12.9 Hz, $J_{CH2a,CH}$ = 5.3 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.5 Hz, 1H, CH_{2a}CH=CH_2), 4.38 (dd, $J_{H6a,H6b}$ = 10.5 Hz, $J_{H6a,H5}$ = 5.0 Hz, 1H, H-6a), 4.19 (ddt, $J_{CH2b,CH2a}$ = 12.8 Hz, $J_{CH2b,CH}$ = 6.0 Hz, $J_{CH2b,CH2cis/CH2trans}$ = 1.4 Hz, 1H, CH_{2b}CH=CH_2), 3.85–3.68 (m, 3H), H-3/H-4/H-6b), 3.52 (t, $J_{H2,H1}$ = 8.1 Hz, 1H, H-2), 3.47–3.39 (m, 1H, H-5) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 138.7, 138.5, 137.5 (3 × C_q), 133.9 (CH_2CH=CH_2), 129.1, 128.5, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.8, 127.7, 126.1 (15 × C_{Ar}), 117.8 (CH_2CH=CH_2), 103.3 (C-1), 101.3 (Ar-CH), 82.3 (C-2), 81.6 (C-4), 81.0 (C-3), 75.5, 75.3 (2 × CH_{Bn}), 70.9 (CH_2CH=CH_2), 68.9 (C-6), 66.2 (C-5) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{30}H_{36}NO_6^+$ $[M+NH_4]^+$: 506.2537, found 506.2542.

Allyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (77)[179]



Compound **76** (2.50 g, 5.12 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (40 mL) under an argon atmosphere and freshly activated 4Å MS were added. The mixture was stirred at room temperature for 30 min before it was cooled to −20 °C. Then triethylsilane (4.09 mL, 25.6 mmol, 5.0 equiv.) and trifluoroacetic acid (1.96 mL, 25.6 mmol, 5.0 equiv.) were added in sequential order. The reaction mixture was allowed to warm to room temperature and was stirred for 3 h. The mixture was diluted with CH_2Cl_2 (50 mL), quenched by the addition of NEt_3 and filtered through a pad of celite. The filtrate was washed with sat. aq. $NaHCO_3$ (80 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 × 80 mL). The combined organic phases were dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 4:1) to give **77** (1.58 g, 3.22 mmol, 63%) as a colorless solid.

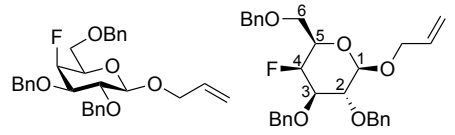
R_f = 0.42 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.44–7.24 (m, 15H, Ar-H), 5.99 (dddd, $J_{CH,CH2trans}$ = 17.2 Hz, $J_{CH,CH2cis}$ = 10.4 Hz, $J_{CH,CH2b}$ = 6.0 Hz, $J_{CH,CH2a}$ = 5.2 Hz, 1H, CH_2CH=CH_2), 5.37 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.7 Hz, 1H, CH_2CH=CH_{2trans}), 5.24 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.4 Hz, 1H, CH_2CH=CH_{2cis}), 5.02–4.95 (m, 2H, CH_{Bn}), 4.80–4.73 (m, 2H, CH_{Bn}), 4.64 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.60 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.52–4.49 (m, 1H, H-1), 4.45 (ddt, $J_{CH2a,CH2b}$ = 12.9 Hz, $J_{CH2a,CH}$ = 5.2 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.6 Hz, 1H, CH_{2a}CH=CH_2), 4.17 (ddt, $J_{CH2b,CH2a}$ = 12.9 Hz, $J_{CH2b,CH}$ = 6.0 Hz, $J_{CH2b,CH2cis/CH2trans}$ = 1.4 Hz, 1H, CH_{2b}CH=CH_2), 3.80 (dd, $J_{H6a,H6b}$ = 10.5 Hz, $J_{H6a,H5}$ = 3.8 Hz, 1H, H-6a), 3.73 (dd, $J_{H6b,H6a}$ 10.4 Hz, $J_{H6b,H5}$ = 5.3 Hz, 1H, H-6b), 3.66–3.59 (m, 1H, H-4), 3.53–3.43 (m, 3H, H-2/H-3/H-5), 2.59 (d, J = 2.2 Hz, 1H, -OH) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 138.7, 138.5, 138.1 (3 × C_q), 134.1 (CH_2CH=CH_2), 128.6, 128.5, 128.3, 128.1, 127.9, 127.8, 127.8 (15 × C_{Ar}), 117.4 (CH_2CH=CH_2), 102.9 (C-1), 84.2 (C-3), 81.8 (C-2), 75.4, 74.9 (2 × CH_{Bn}), 74.2 (C-5), 73.8 (CH_{Bn}), 71.7 (C-4), 70.4 (C-6/CH_2CH=CH_2) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{30}H_{38}NO_6^+$ $[M+NH_4]^+$: 508.2694, found 508.2699.

Allyl 2,3,6-tri-*O*-benzyl-β-D-4-deoxy-4-fluorogalactopyranoside (78)[179]



To a solution of allyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **77** (2.66 g, 5.42 mmol, 1.0 equiv.) in CH_2Cl_2 (30 mL) under argon was added pyridine (3.20 mL) and the mixture was cooled to −78 °C. Triflic anhydride (1.10 mL, 6.51 mmol, 1.2 equiv.) was added and the reaction mixture was allowed to warm to room temperature and it was stirred for 30 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed

with sat. aq. $NaHCO_3$ (2 × 10 mL) and brine (10 mL). The organic phase was dried with $MgSO_4$ and solvents were removed under reduced pressure. The sticky yellow residue was dried under high vaccum for 1 h. Meanwhile, TBAF·$3H_2O$ (2.22 g, 7.05 mmol, 1.3 equiv.) was co-evaporated with toluene (3 × 10 mL) and THF (10 mL), dried under high vacuum and taken up in THF (20 mL). Freshly activated 4Å MS (2 g) were added and the mixture was stirred for 1.5 h. Subsequently, the triflate was dissolved in THF (20 mL) and added to the TBAF-solution dropwise at room temperature. The mixture was stirred for 1.5 h and was then filtered over a pad of celite, before all solvents were removed under reduced pressure. The resulting brown residue was dissolved in CH_2Cl_2 (30 mL) and washed with brine (2 × 10 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 6:1) to furnish **78** (1.98 g, 4.02 mmol, 74%) as an amorphous colorless solid.

R_f = 0.59 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = − 19.2 (c = 0.5, $CHCl_3$).

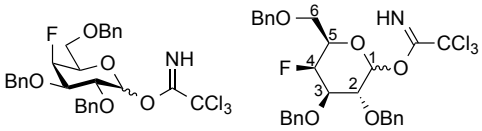
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.44–7.25 (m, 15H, Ar-H), 5.97 (dddd, $J_{CH,CH2trans}$ = 17.3 Hz, $J_{CH,CH2cis}$ = 10.8 Hz, $J_{CH,CH2b}$ = 6.0 Hz, $J_{CH,CH2a}$ = 5.2 Hz, 1H, CH_2CH=CH_2), 5.36 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.7 Hz, 1H, CH_2CH=CH_{2trans}), 5.22 (dq, $J_{CH2cis,CH}$= 10.5 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.4 Hz, 1H, CH_2CH=CH_{2cis}), 4.95 (d, J = 10.8 Hz, 1H, CH_{Bn}), 4.91–4.73 (m, 4H, H-4/3 × CH_{Bn}), 4.59 (s, 2H, CH_{Bn}), 4.48–4.39 (m, 2H, H-1/CH_{2a}CH=CH_2), 4.16 (ddt, $J_{CH2b,CH2a}$ = 12.9 Hz, $J_{CH2b,CH}$ = 6.0 Hz, $J_{CH2b,CH2cis/CH2trans}$ = 1.4 Hz, 1H, CH_{2b}CH=CH_2), 3.81–3.74 (m, 2H, H-2/H-6a), 3.70 (ddd, $J_{H6b,H6a}$ = 9.4 Hz, $J_{H6b,H5}$ = 5.7 Hz, $J_{H6b,F}$ = 1.5 Hz, 1H, H-6b), 3.65–3.46 (m, 2H, H-3/H-5) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 138.6, 138.0, 137.9 (3 × C_q), 134.0 (CH_2CH=CH_2), 128.6, 128.4, 128.3, 128.0, 127.9, 127.8 (15 × C_{Ar}), 117.5 (CH_2CH=CH_2), 102.7 (C-1), 86.1 (d, $J_{C4,F}$ = 183.6 Hz, C-4), 79.1 (d, $J_{C3,F}$ = 18.0 Hz, C-3), 79.1 (C-2), 75.6, 73.9, 72.6, 72.3 (d, $J_{C5,F}$ = 18.3 Hz, C-5), 70.5 (CH_2CH=CH_2), 67.9 (d, $J_{C6,F}$ = 5.3 Hz, C-6) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 216.8 (dt, $J_{F,H4}$ = 50.1 Hz, $J_{F,H3/H5}$ = 27.8 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{30}H_{37}FNO_5^+$ $[M+NH_4]^+$: 510.2656, found 510.2658.

2,3,6-Tri-O-benzyl-α-D-4-deoxy-4-fluorogalactopyranosyl trichloroacetimidate (14)[161, 180]



Fluorinated compound **78** (1.86 g, 3.78 mmol, 1.0 equiv.) was mixed with NaOAc (1.13 g, 13.8 mmol, 3.7 equiv.) and $PdCl_2$ (1.14 g, 6.43 mmol, 1.7 equiv.). The mixture was then dissolved in AcOH (19 mL) and H_2O dest. (1 mL) and it was stirred at room temperature for 20 h. The reaction mixture was diluted with

CH_2Cl_2 (50 mL) and carefully neutralized with sat. aq. $NaHCO_3$ (150 ml) and solid $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic layers were washed with brine (2 × 80 mL). The organic phase was dried with $MgSO_4$ and solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give the lactol (1.64 g mixture of anomers, 3.63 mmol, 96%) as a colorless oil. To a solution of this lactol (1.47 g, 3.25 mmol, 1.0 equiv.) in CH_2Cl_2 (30 mL) at 0 °C was added trichloroacetonitrile (1.63 mL, 16.3 mmol, 5.0 equiv.) and DBU (0.12 mL, 2.21 mmol, 0.25 equiv.) in sequential order. The mixture was allowed to warm to room temperature and stirred for 20 h. Then sat. aq. NH_4Cl (20 mL) was added and the aqeous phase was extracted with CH_2Cl_2 (50 mL). The combined organic layers were washed with brine (2 × 30 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 5:1) to give **14** (1.44 g (α:β = 2.8:1), 2.40 mmol, 74%) as a colorless oil.

R_f (α-anomer)= 0.86 (^{c}Hex/EtOAc, 3:1).
R_f (β-anomer)= 0.69 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{21}$ = + 43.6 (c = 0.5, $CHCl_3$) (α-anomer).

^{1}H-NMR (400 MHz, $CDCl_3$): δ (β-anomer) = 8.70 (s, 1H, C=NH), 7.42–7.28 (m, 15H, Ar-H), 5.80 (d, $J_{H1,H2}$ = 8.1 Hz, 1H), 5.01–4.86 (m, 2H, H-4/CH_{Bn}), 4.86–4.72 (m, 3H, CH_{Bn}), 4.58 (s, 2H, CH_{Bn}), 4.03 (t, $J_{H2,H1/H3}$ = 8.7 Hz, 1H, H-2), 3.86–3.60 (m, 5H, H-3/H-4/H-5/H-6a/H-6b) ppm; δ (α-anomer) = 8.62 (s, 1H, C=NH), 7.44–7.27 (m, 15H, Ar-H), 6.53 (d, $J_{H1,H2}$ = 3.4 Hz, 1H, H-1), 5.01 (dd, $J_{H4,F}$ = 49.9 Hz, $J_{H4,H3/H5}$ = 2.3 Hz, 1H, H-4), 4.82 (s, 2H, CH_{Bn}), 4.80–4.78 (m, 2H, CH_{Bn}), 4.57 (s, 2H, CH_{Bn}), 4.24–4.11 (m, 2H, H-2/H-5), 4.02 (ddd, $J_{H3,F}$ = 28.0 Hz, $J_{H3,H2}$ = 10.1 Hz, $J_{H3,H4}$ = 2.4 Hz, 1H, H-3), 3.73 (dd, $J_{H6a,H6b}$ = 9.5 Hz, $J_{H6a,H5}$ = 7.8 Hz, 1H, H-6a), 3.66 (ddd, $J_{H6b,H6a}$ = 9.5 Hz, $J_{H6b,H5}$ = 5.8 Hz, $J_{H6b,F}$ = 1.5 Hz, 1H, H-6b) ppm.

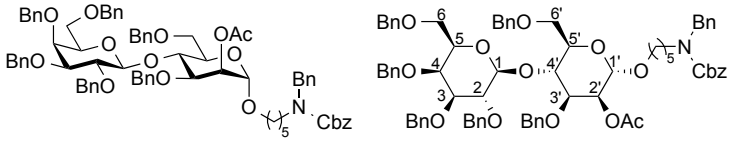
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ (β-anomer) = 161.4 (C=NH), 138.1, 137.8, 137.7 (3 × C_q), 128.6, 128.5, 128.1, 128.0, 127.9 (15 × C_{Ar}), 98.2 (C-1), 91.0 (CCl_3), 85.6 (d, $J_{C4,F}$ = 184.3 Hz, C-4), 79.3 (d, $J_{C3,F}$ = 18.1 Hz, C-3), 77.8 (C-2), 75.6, 73.8 (2 × CH_{Bn}), 73.2 (d, $J_{C5,F}$ = 18.3 Hz, C-5), 72.5 (CH_{Bn}), 67.2 (d, $J_{C6,F}$ = 5.5 Hz, C-6) ppm; δ (α-anomer) = 161.2 (C=NH), 138.2, 137.9, 137.8 (3 × C_q), 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.7, 127.6 (15 × C_{Ar}), 94.8 (C-1), 91.3 (CCl_3), 87.0 (d, $J_{C4,F}$ = 183.4 Hz, C-4), 75.9 (d, $J_{C2,F}$ = 2.6 Hz, C-2), 74.9 (d, $J_{C3,F}$ = 17.7 Hz, C-3), 73.7, 73.4, 72.7 (3 × CH_{Bn}), 70.8 (d, $J_{C5,F}$ = 18.5 Hz, C-5), 67.4 (d, $J_{C6,F}$ = 5.9 Hz, C-6) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ (β-anomer) = − 217.2 (dt, $J_{F,H4}$ = 49.1 Hz, $J_{F,H3/H5}$ = 27.7 Hz) ppm; δ (α-anomer) = − 218.9 (dt, $J_{F,H4}$ = 49.8 Hz, $J_{F,H3/H5}$ = 28.6 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{30}H_{30}Cl_3FNO_7^-$ [M+FA-H]$^-$: 640.1072, found 640.1085 (β-anomer), 640.1083 (α-anomer).

5.2.1.6 Synthesis of 5-Aminopentyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranoside 2

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (85)**



According to general procedure (**A**), galactosyl imidate **17** (625 mg, 0.913 mmol, 1.3 equiv.) and mannosyl acceptor **12** (500 mg, 0.702 mmol, 1.0 equiv.) were reacted under inverse glycosylation conditions to give the corresponding disaccharide **85** (634 mg, 0.514 mmol, 73%) as a colorless oil.

R_f = 0.58 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{21}$ = + 6.8 (c = 0.5, $CHCl_3$).

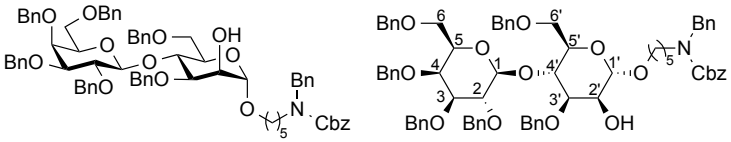
^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.42–7.13 (m, 40H, Ar-H), 5.32–5.27 (m, 1H, H-2‘), 5.20 (d, J = 16.7 Hz, 2H, CH_{Cbz}), 4.97 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.82 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.78 (d, $J_{H1‘,H2‘}$ = 4.6 Hz, 1H, H-1‘), 4.75–4.65 (m, 5H, CH_{Bn}), 4.58 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.56–4.51 (m, 3H, CH_{Bn}/2 × NCH_{Bn}), 4.50 (d, $J_{H1,H2}$ = 7.6 Hz, 1H, H-1), 4.41 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.40 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.25 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.13 (t, $J_{H4‘,H3‘/H5‘}$ = 9.9 Hz, 1H, H-4‘), 3.91–3.88 (m, 2H, H-3‘/H-4), 3.85 (dd, $J_{H6a‘,H6b‘}$ = 10.5 Hz, $J_{H6a‘,H5‘}$ = 4.2 Hz, 1H, H-6a‘), 3.79–3.77 (m, 1H, H-5‘), 3.75 (dd, $J_{H2,H3}$ = 9.7 Hz, $J_{H2,H1}$ = 7.6 Hz, 1H, H-2), 3.69 (dd, $J_{H6b‘,H6a‘}$ = 10.8 Hz, $J_{H6b‘,H5‘}$ = 1.3 Hz, 1H, H-6b‘), 3.63 (m, 1H, CH_{Linker}), 3.58 (dd, $J_{H6a,H6b}$ = 9.4 Hz, $J_{H6a,H5}$ = 7.3 Hz, 1H, H-6a), 3.44 (dd, $J_{H6b,H6a}$ = 9.5 Hz, $J_{H6b,H5}$ = 5.6 Hz, 1H, H-6b), 3.42 (dd, $J_{H3,H2}$ = 9.8 Hz, $J_{H3,H4}$ = 2.8 Hz, 1H, H-3), 3.40–3.35 (m, 1H, H-5), 3.34–3.18 (m, 3H, CH_{Linker}), 1.98 (s, 3H, CH_3-OAc), 1.64–1.48 (m, 4H, CH_{Linker}), 1.37–1.22 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.6 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 139.0, 138.9, 138.6, 138.5, 138.3, 138.0, 137.0, 136.9 (8 × C_q), 128.6, 128.5, 128.3, 128.1, 128.0, 127.7, 127.6, 127.5, 127.4, 127.2, 126.9 (40 × C_{Ar}), 103.0 (C-1), 97.6 (C-1‘), 82.9 (C-3), 80.1 (C-2), 76.3 (C-3‘), 75.2, 74.7 (2 × CH_{Bn}), 74.6 (C-4‘), 73.8 (C-4), 73.6 (C-5/CH_{Bn}), 73.2, 72.7, 72.2 (3 ×CH_{Bn}), 71.4 (C-5‘), 69.7 (C-2‘), 68.88 (C-6‘), 68.85 (C-6), 67.8 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.7/50.4 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.5 (CH_{Linker}), 21.1 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{76}H_{87}N_2O_{14}^+$ $[M+NH_4]^+$: 1251.6152, found 1251.6149.

RP-HPLC: t_R = 25.3 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (22)**



The acetyl protecting group of disaccharide **85** (940 mg, 0.761 mmol) was removed according to general procedure (**B**) to give the corresponding disaccharide **22** (680 mg, 0.570 mmol, 75%) as a colorless oil.

R_f = 0.27 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{21}$ = + 12.6 (c = 1.0, $CHCl_3$).

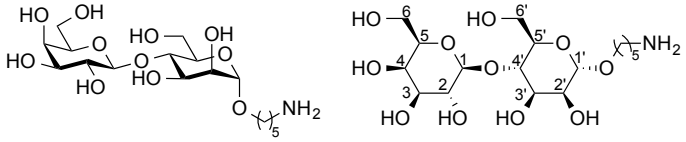
^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.36–7.16 (m, 40H, Ar-H), 5.17 (d, J = 14.8 Hz, 2H, CH_{Cbz}), 4.96 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.90 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.84–4.80 (m, 1H, H-1‘), 4.78 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.73 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.70 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.67 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.62 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.57 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.54 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.48 (d, J = 15.0 Hz, 2H, NCH_{Bn}), 4.43 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.40–4.36 (m, 2H, CH_{Bn}), 4.27 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.12 (t, $J_{H4‘,H3‘/H5‘}$ = 9.3 Hz, 1H, H-4‘), 3.97 (br s, 1H, H-2‘), 3.89 (d, $J_{H4,H3/H5}$ = 2.4 Hz, 1H, H-4), 3.80 (dd, $J_{H6a,H6b}$ = 10.7 Hz, $J_{H6a,H5}$ = 4.6 Hz, 1H, H-6a), 3.77–3.68 (m, 3H, H-2/H-3‘/H-5‘), 3.68–3.56 (m, 3H, H-6b/H-6a‘/CH_{Linker}), 3.44 (dd, $J_{H6b‘,H6a‘}$ = 9.1 Hz, $J_{H6b‘,H5‘}$ = 5.4 Hz, 1H, H-6b‘) 3.41–3.29 (m, 3H, H-3/H-5/CH_{Linker}), 3.28–3.15 (m, 2H, CH_{Linker}), 2.49 (d, $J_{OH,H2‘}$ = 1.2 Hz, 1H, -OH), 1.61–1.46 (m, 4H, CH_{Linker}), 1.34–1.20 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 139.1, 139.0, 138.9, 138.6, 138.2, 138.0, 137.0 (8 × C_q), 128.7, 128.5, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4 (40 × C_{Ar}), 103.3 (C-1), 99.1 (C-1‘), 82.7 (C-3), 80.1 (C-2), 78.3 (C-3‘), 75.3 (CH_{Bn}), 74.8 (CH_{Bn}), 74.4 (C-4‘), 73.8 (C-4), 73.6 (CH_{Bn}), 73.3 (CH_{Bn}), 72.9 (CH_{Bn}), 72.8 (CH_{Bn}), 71.3 (C-5‘), 69.6 (C-2‘), 68.7 (C-6), 68.6 (C-6‘), 67.5 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{74}H_{85}N_2O_{13}^+$ $[M+NH_4]^+$: 1209.6046, found 1209.6051.

RP-HPLC: t_R = 20.2 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 30 min.

5-Aminopentyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranoside (2)



According to general procedure (**E**), disaccharide **22** (128 mg, 0.107 mmol) was globally deprotected by hydrogenolysis to give the corresponding disaccharide **2** (45 mg, 0.105 mmol, 98%) as a colorless solid.

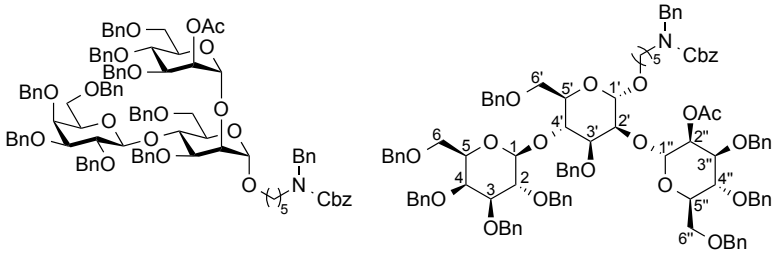
^{1}H-NMR (599 MHz, D_2O): δ = 4.88 (s, 1H, H-1‘), 4.46 (d, $J_{H1,H2}$ = 7.6 Hz, 1H, H-1), 4.01 (s, 1H, H-2‘), 3.99–3.90 (m, 3H, H-4/H-3‘/H-6a‘), 3.89–3.82 (m, 2H, H-4‘/H-6b‘), 3.82–3.72 (m, 5H, H-3/H-6a/H-6b/H-5‘/CH_{Linker}), 3.71–3.63 (m, 1H, H-5), 3.60–3.52 (m, 2H, H-2/CH_{Linker}), 3.04–2.97 (m, 2H, CH_{Linker}), 1.75–1.61 (m, 4H, CH_{Linker}), 1.52–1.39 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, D_2O/CD_3OD): δ = 104.4 (C-1), 100.7 (C-1‘), 77.9 (C-4‘), 76.6 (C-3), 74.0 (C-5), 72.5 (C-5‘), 72.2 (C-2), 70.9 (C-2‘), 70.7 (C-3‘), 69.8 (C-4), 68.4 (CH_{Linker}), 62.2 (C-6), 61.5 (C-6‘), 40.5 (CH_{Linker}), 29.3 (CH_{Linker}), 27.8 (CH_{Linker}), 23.7 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{17}H_{34}NO_{11}^+$ $[M+H]^+$: 428.2126, found 428.2132.

5.2.1.7 Synthesis of 5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside 1

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (94)**



According to general procedure (**C**), disaccharide **22** (191 mg, 0.160 mmol, 1.0 equiv.) was reacted with mannosyl imidate **18** (306 mg, 0.480 mmol, 3.0 equiv.) to give the corresponding trisaccharide **94** (208 mg, 0.125 mmol, 78%) as a colorless oil.

R_f = 0.45 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 14.4 (c = 0.5, $CHCl_3$).

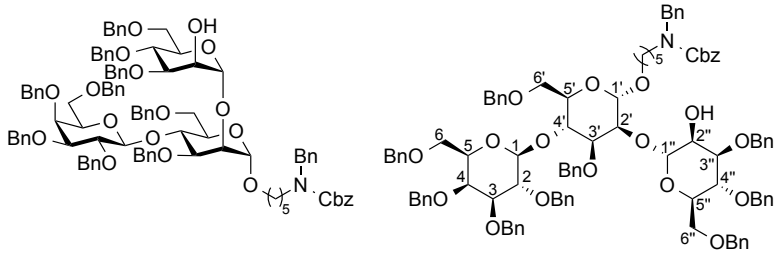
^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.37–7.11 (m, 55H, Ar-H), 5.55 (dd, $J_{H2'',H3''}$ = 3.2 Hz, $J_{H2'',H1''}$ = 1.9 Hz, 1H, H-2''), 5.18 (d, J = 13.7 Hz, 2H, CH_{Cbz}), 5.11 (s, 1H, H-1''), 4.94 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.89 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.85–4.79 (m, 3H, H-1'/2 × CH_{Bn}), 4.74–4.62 (m, 5H, CH_{Bn}), 4.60 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.56 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.55–4.51 (m, 2H, CH_{Bn}), 4.50–4.43 (m, 4H, 2 × CH_{Bn}/2 × NCH_{Bn}), 4.40 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.35–4.31 (m, 2H, CH_{Bn}), 4.24 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.13 (t, $J_{H4',H3'/H5'}$ = 7.6 Hz, 1H, H-4'), 4.02 (s, 1H, H-2'), 3.98 (dd, $J_{H3'',H4''}$ = 9.4 Hz, $J_{H3'',H2''}$ = 3.3 Hz, 1H, H-3''), 3.94 (ddd, $J_{H5'',H4''}$ = 10.0 Hz, $J_{H5'',H6a''}$ = 4.4 Hz, $J_{H5'',H6b''}$ = 1.6 Hz, 1H, H-5''), 3.90 (d, $J_{H4,H3/H5}$ = 2.8 Hz, 1H, H-4), 3.88–3.85 (m, 1H, H-3'), 3.82 (t, $J_{H4'',H3''/H5''}$ = 9.7 Hz, 1H, H-4''), 3.79–3.67 (m, 6H, H-2/H-5'/H-6a'/H-6b'/H-6a''/H-6b''), 3.64–3.52 (m, 2H, H-6a/CH_{Linker}), 3.46 (dd, $J_{H3,H2}$ = 9.8 Hz, $J_{H3,H4}$ = 2.8 Hz, 1H, H-3), 3.45–3.42 (m, 1H, H-6b), 3.39 (dd, $J_{H5,H6a}$ = 8.6 Hz, $J_{H5,H6b}$ = 5.3 Hz, 1H, H-5), 3.30–3.15 (m, 3H, CH_{Linker}), 2.06 (s, 3H, CH_3-OAc), 1.59–1.41 (m, 4H, CH_{Linker}), 1.33–1.16 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 169.9 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 139.3, 139.1, 139.0, 138.9, 138.65, 138.6, 138.4, 138.2, 138.0, 136.9 (11 × C_q), 128.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 126.9 (55 × C_{Ar}), 103.4 (C-1), 99.5 (C-1''), 98.7 (C-1'), 82.9 (C-3), 80.1 (C-2), 78.5 (C-3''), 78.1 (C-3'), 75.4 (C-4'), 75.2 (C-2'/2 × CH_{Bn}), 74.7 (CH_{Bn}), 74.4 (C-4''), 73.5 (CH_{Bn}), 73.4 (C-4/CH_{Bn}), 73.1 (CH_{Bn}), 72.8 (C-5), 72.6, 72.5, 72.0 (3 × CH_{Bn}), 71.9 (C-5''), 71.7 (C-5'), 69.2 (C-6'/C-6''), 69.1 (C-6'/C-6''), 68.5 (C-2''), 68.2 (C-6), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.2 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{103}H_{115}N_2O_{19}^+$ $[M+NH_4]^+$: 1684.8122, found 1684.8146.

RP-HPLC: t_R = 32.0 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (95)**



The acetyl protecting group of trisaccharide **94** (489 mg, 0.293 mmol) was removed according to general procedure (**D**) to give the corresponding trisaccharide **95** (343 mg, 0.211 mmol, 72%) as a colorless oil.

R_f = 0.35 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 16.4 (c = 0.5, $CHCl_3$).

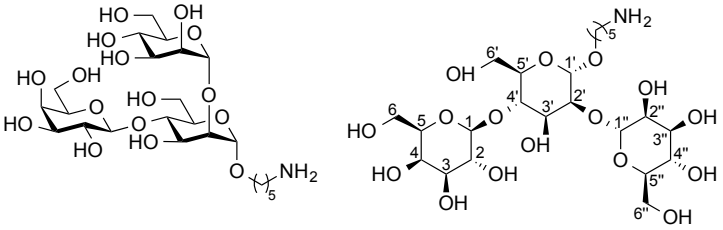
^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.41–7.10 (m, 55H, Ar-H), 5.18 (d, J = 18.7 Hz, 2H, CH_{Cbz}), 5.14 (s, 1H, H-1‘‘), 4.95 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.89 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.84 (s, 1H, H-1‘), 4.80 (d, J = 11.1 Hz, 1H, CH_{Bn}), 4.78 (d, J = 11.0 Hz, 1H, CH_{Bn}), 4.76–4.72 (m, 1H, CH_{Bn}), 4.71 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.67 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.65–4.57 (m, 3H, 3 × CH_{Bn}), 4.57–4.53 (m, 2H, H-1/CH_{Bn}), 4.52–4.42 (m, 6H, 4 × CH_{Bn}/2 × NCH_{Bn}), 4.40 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.34 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.23 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.13 (t, $J_{H4‘,H3‘/H5‘}$ = 9.0 Hz, 1H, H-4‘), 4.04 (s, 1H, H-2‘), 4.00 (q, $J_{H2‘‘,H1‘‘/H3‘‘/OH}$ = 2.5 Hz, 1H, H-2‘‘), 3.92 (ddd, $J_{H5‘‘,H4‘‘}$ = 9.7 Hz, $J_{H5‘‘,H6a‘‘}$ = 4.8 Hz, $J_{H5‘‘,H6b‘‘}$ = 2.2 Hz, 1H, H-5‘‘), 3.88 (d, $J_{H4,H3/H5}$ = 2.9 Hz, 1H, H-4), 3.87–3.84 (m, 1H, H-3‘), 3.84–3.80 (m, 2H, H-6a‘/H-3‘‘), 3.80–3.74 (m, 2H, H-2/H-4‘‘), 3.74–3.67 (m, 4H, H-5’/H-6b‘/H-6a‘‘/H-6b‘‘), 3.59–3.51 (m, 2H, H-6a/CH_{Linker}), 3.46 (dd, $J_{H3,H2}$ = 9.8, $J_{H3,H4}$ = 2.9 Hz, 1H, H-3), 3.42–3.37 (m, 2H, H-5/H-6b), 3.28–3.13 (m, 3H, CH_{Linker}), 2.19 (d, $J_{OH,H2‘}$ = 2.6 Hz, 1H, -OH), 1.57–1.42 (m, 4H, CH_{Linker}), 1.30–1.15 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 139.3, 139.1, 138.9, 138.6, 138.4, 138.2, 138.1, 137.0, 136.9 (11 × C_q), 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2 (55 × C_{Ar}), 103.2 (C-1), 101.0 (C-1‘‘), 98.9 (C-1‘), 82.9 (C-3), 80.1 (C-2/C-3‘‘), 78.1 (C-3‘), 75.6 (C-2‘), 75.3 (CH_{Bn}), 75.2 (C-4‘), 75.0, 74.7 (2 × CH_{Bn}), 74.6 (C-4‘‘), 73.7 (C-4), 73.5 (2 × CH_{Bn}), 73.3 (C-5), 73.1, 72.9, 72.7, 72.0 (4 × CH_{Bn}), 71.9 (C-5‘), 71.7 (C-5‘‘), 69.3 (C-6‘‘), 69.1 (C-6‘), 68.8 (C-6), 68.4 (C-2‘‘), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.4 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{101}H_{113}N_2O_{18}^+$ $[M+NH_4]^+$: 1682.8016, found 1642.8038.

RP-HPLC: t_R = 34.2 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside (1)



According to general procedure (**E**), trisaccharide **95** (390 mg, 0,240 mmol) was globally deprotected by hydrogenolysis to give the corresponding trisaccharide **1** (136 mg, 0.231 mmol, 96%) as a colorless solid.

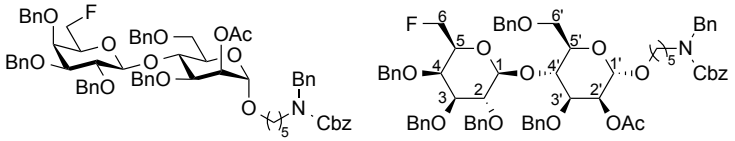
^{1}H-NMR (800 MHz, D_2O): δ = 5.11 (s, 1H, H-1‘), 5.02 (s, 1H, H-1‘‘), 4.44 (d, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1), 4.09–4.07 (m, 1H, H-2‘‘), 4.04–4.00 (m, 2H, H-2‘/H-3‘), 3.96 (dd, $J_{H6a‘,H6b‘}$ = 12.3 Hz, $J_{H6a‘,H5‘}$ =2.2 Hz, 1H, H-6a‘), 3.92 (d, $J_{H4,H3/H5}$ = 3.5 Hz, 1H, H-4), 3.90–3.84 (m, 3H, H-6a/H-4‘/H-6b‘), 3.83 (dd, $J_{H3‘‘,H4‘‘}$ = 9.8 Hz, $J_{H3‘‘,H2‘‘}$ = 3.4 Hz, 1H, H-3‘‘), 3.80 (dd, $J_{H6b,H6a}$ = 11.8 Hz, $J_{H6b,H5}$ = 8.3 Hz, 1H, H-6b), 3.78–3.69 (m, 5H, H-5/H-5‘/H-5‘‘/H-6a‘‘/ H-6b‘‘), 3.67 (dd, $J_{H3,H2}$ = 10.0 Hz, $J_{H3,H4}$ = 3.4 Hz, 1H, H-3), 3.60 (t, $J_{H4‘‘,H3‘‘/H5‘‘}$ = 9.8 Hz, 1H, H-4‘‘), 3.56–3.51 (m, 2H, H-2/CH_{Linker}), 3.00 (t, J = 7.7 Hz, 2H, CH_{Linker}), 1.71–1.62 (m, 4H, CH_{Linker}), 1.49–1.39 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, D_2O/CD_3OD): δ = 104.1 (C-1), 103.3 (C-1‘‘), 98.9 (C-1‘), 79.2 (C-2‘), 77.9 (C-4‘), 76.3 (C-5), 74.3 (C-5‘‘), 73.6 (C-3), 72.5 (C-5‘), 72.0 (C-2), 71.3 (C-3‘‘), 70.9 (C-2‘‘), 70.1 (C-3‘), 69.6 (C-4), 68.6 (CH_{Linker}), 68.0 (C-4‘‘), 62.2 (C-6‘‘), 62.1 (C-6), 61.4 (C-6‘), 40.4 (CH_{Linker}), 29.0 (CH_{Linker}), 27.5 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{23}H_{44}NO_{16}^+$ $[M+H]^+$: 590.2655, found 590.2647.

5.2.1.8 Synthesis of 5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-6-deoxy-6-fluorogalactopyranosyl)-α-D-mannopyranoside 6

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,4-tri-*O*-benzyl-β-D-6-deoxy-6-fluorogalactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (86)**



According to general procedure (**A**), fluorinated galactosyl imidate **13** (545 mg, 0.913 mmol, 1.3 equiv.) and mannosyl acceptor **12** (500 mg, 0.702 mmol, 1.0 equiv.) were reacted under inverse glycosylation conditions to give the corresponding disaccharide **86** (633 mg, 0.552 mmol, 79%) as a colorless oil.

R_f = 0.47 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 27.2 (c = 0.5, $CHCl_3$).

^{1}H-NMR (800 MHz, $CDCl_3$): δ = 7.41–7.14 (m, 35H, Ar-H), 5.29 (br s, 1H, H-2‘), 5.18 (d, J = 24.9 Hz, 2H, CH_{Cbz}), 4.97 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.80 (d, J = 11.1 Hz, 1H, CH_{Bn}), 4.77–4.76 (m, 1H, H-1‘), 4.73–4.67 (m, 3H, CH_{Bn}), 4.65 (br s, 2H, CH_{Bn}), 4.60 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.57 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.53–4.46 (m, 3H, H-1/2 × NCH_{Bn}), 4.39 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.29 (dd, $J_{H6,F}$ = 46.6 Hz, $J_{H6a/b,H5}$ = 6.4 Hz, 2H, H-6a/H-6b), 4.11 (t, $J_{H4‘,H3‘/H5‘}$ = 9.3 Hz, 1H, H-4‘), 3.89 (dd, $J_{H3‘H4‘}$ = 9.2 Hz, $J_{H3‘,H2‘}$ = 3.2 Hz, 1H, H-3‘), 3.85–3.81 (m, 1H, H-6a‘), 3.80 (d, $J_{H4,H3/H5}$ = 2.7 Hz, 1H, H-4), 3.78–3.74 (m, 1H, H-5‘), 3.73 (dd, $J_{H2,H3}$ = 9.7 Hz, $J_{H2,H1}$ = 7.8 Hz, 1H, H-2), 3.67 (dd, $J_{H6b‘,H6a‘}$ = 10.9 Hz, $J_{H6b‘,H5‘}$ = 1.8 Hz, 1H, H-6b‘), 3.65–3.57 (m, 1H, CH_{Linker}), 3.39 (dd, $J_{H3,H2}$ = 9.7 Hz, $J_{H3,H4}$ = 2.4 Hz, 1H, H-3), 3.37–3.33 (m, 1H, H-5), 3.32–3.17 (m, 3H, CH_{Linker}), 2.09 (s, 3H, CH_3-OAc), 1.61–1.47 (m, 4H, CH_{Linker}), 1.36–1.20 (m, 2H, CH_{Linker}) ppm.

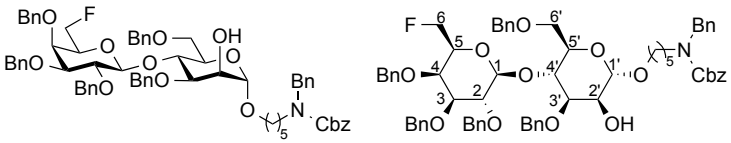
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.6 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 138.9, 138.8, 138.6, 138.4, 138.0, 137.9 (7 × C_q), 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.3, 127.2 (35 × C_{Ar}), 102.9 (C-1), 97.6 (C-1‘), 82.5 (C-3), 81.4 (d, $J_{C6,F}$ = 166.8 Hz, C-6), 79.9 (C-2), 76.1 (C-3‘), 75.3, 74.7 (C-4‘/2 × CH_{Bn}), 73.3 (CH_{Bn}), 73.1 (d, $J_{C4,F}$ = 2.9 Hz, C-4), 73.0 (CH_{Bn}), 72.3 (d, $J_{C5,F}$ = 24.9 Hz, C-5), 71.9 (CH_{Bn}), 71.4 (C-5‘), 69.6 (C-2‘), 68.9 (C-6‘), 67.8 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.7/50.4 (NCH_{Bn}), 47.2/46.4 (CH_{Linker}), 29.2 (CH_{Linker}), 28.0/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.2 (CH_3-OAc) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 229.9 (t, $J_{F,H6}$ = 48.6 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{69}H_{80}FN_2O_{13}^+$ $[M+NH_4]^+$: 1163.5639, found 1163.5648.

RP-HPLC: t_R = 19.9 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,4-tri-*O*-benzyl-β-D-6-deoxy-6-fluorogalactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (90)**



The acetyl protecting group of fluorinated disaccharide **86** (1.00 g, 0.872 mmol) was removed according to general procedure (**B**) to give the corresponding disaccharide **90** (820 mg, 0.743 mmol, 85%) as a colorless oil.

R_f = 0.31 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{21}$ = + 17.4 (c = 1.0, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.39–7.20 (m, 35H, Ar-H), 5.19 (d, J = 15.2 Hz, 2H, CH_{Cbz}), 5.00 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.89 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.86–4.83 (m, 1H, H-1‘), 4.80 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.76 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.73 (s, 2H, CH_{Bn}), 4.65 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.61 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.59 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.51 (d, J = 15.2 Hz, 2H, NCH_{Bn}), 4.45 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.41–4.35 (m, 2H, H-6a/CH_{Bn}), 4.33–4.27 (m, 1H, H-6b), 4.15 (t, $J_{H4',H3'/H5'}$ = 9.3 Hz, 1H, H-4‘), 4.00 (br s, 1H, H-2‘), 3.86–3.81 (m, 2H, H-4/H-6a‘), 3.80–3.70 (m, 3H, H-2/H-3‘/H-5‘), 3.69–3.60 (m, 2H, H-6b‘/CH_{Linker}), 3.43–3.31 (m, 3H, H-3/H-5/CH_{Linker}), 3.30–3.18 (m, 2H, CH_{Linker}), 2.57 (d, $J_{OH,H2'}$ = 2.2 Hz, 1H, -OH), 1.62–1.48 (m, 4H, CH_{Linker}), 1.38–1.22 (m, 2H, CH_{Linker}) ppm.

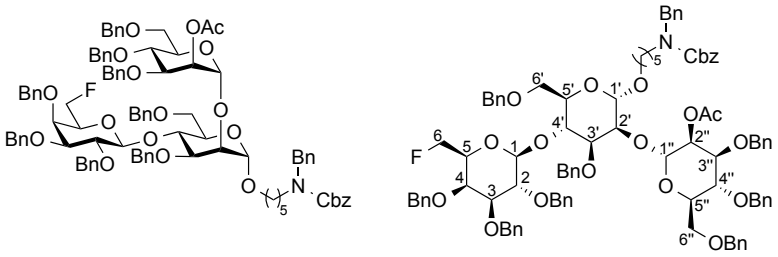
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 156.9/156.28 (C=O-Cbz), 138.8, 138.6, 138.4, 138.0, 136.9 (7 × C_q), 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5 (35 × C_{Ar}), 103.1 (C-1), 99.1 (C-1‘), 82.3 (C-3), 81.3 (d, $J_{C6,F}$ = 167.0 Hz, C-6), 79.9 (C-2), 78.2 (C-3‘), 75.3 (CH_{Bn}), 74.8 (CH_{Bn}), 74.5 (C-4‘), 73.3 (C-4/CH_{Bn}), 73.0 (CH_{Bn}), 72.8 (CH_{Bn}), 72.3 (d, $J_{C5,F}$ = 24.8 Hz, C-5), 71.2 (C-5‘), 69.5 (C-2‘), 68.7 (C-6‘), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 229.8 (t, $J_{F,H6}$ = 46.1 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{67}H_{78}FN_2O_{12}^+$ $[M+NH_4]^+$: 1121.5533, found 1121.5536.

RP-HPLC: t_R = 14.8 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 30 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-D-6-deoxy-6-fluorogalactopyranosyl)-α-D-mannopyranoside (96)**



According to general procedure (**C**), fluorinated disaccharide **90** (715 mg, 0.647 mmol, 1.0 equiv.) was reacted with mannosyl imidate **18** (825 mg, 1.29 mmol, 2.0 equiv.) to give the corresponding trisaccharide **96** (876 mg, 0.555 mmol, 86%) as a colorless oil.

R_f = 0.47 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 16.4 (c = 0.5, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.39–7.09 (m, 50H, Ar-H), 5.53 (dd, $J_{H2'',H3''}$ = 2.9 Hz, $J_{H2'',H1''}$ = 2.0 Hz, 1H, H-2''), 5.18 (d, J = 14.0 Hz, 2H CH_{Cbz}), 5.11 (s, 1H, H-1''), 4.95–4.88 (m, 2H, CH_{Bn}), 4.84–4.78 (m, 3H, H-1'/ 2 × CH_{Bn}), 4.77–4.72 (m, 1H, CH_{Bn}), 4.69 (s, 2H, CH_{Bn}), 4.67–4.63 (m, 1H, CH_{Bn}), 4.62–4.55 (m, 4H, CH_{Bn}), 4.54 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.51–4.42 (m, 4H, 2 × CH_{Bn}/2 × NCH_{Bn}), 4.41–4.20 (m, 4H, H-6a/H-6b/ 2 × CH_{Bn}), 4.17 (t, $J_{H4',H3'/H5}$ = 9.2 Hz, 1H, H-4'), 4.01 (s, 1H, H-2'), 3.97 (dd, $J_{H3'',H4''}$ = 9.4 Hz, $J_{H3'',H2''}$ = 3.3 Hz, 1H, H-3''), 3.95–3.92 (m, 1H, H-5''), 3.86–3.83 (m, 1H, H-3'), 3.83–3.74 (m, 4H, H-4/H-4''/ H-6a'/H-6a''), 3.74–3.67 (m, 4H, H-2/H-5'/H-6b'/H-6b''), 3.62–3.52 (m, 1H, CH_{Linker}), 3.44 (dd, $J_{H3,H2}$ = 9.7 Hz, $J_{H3,H4}$ = 2.7 Hz, 1H, H-3), 3.42–3.37 (m, 1H, H-5), 3.30–3.13 (m, 3H, CH_{Linker}), 2.07 (s, 3H, CH_3-OAc), 1.57–1.43 (m, 4H, CH_{Linker}), 1.30–1.16 (m, 2H, CH_{Linker}) ppm.

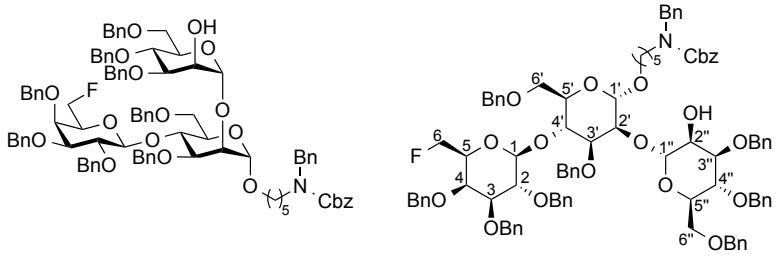
^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 169.9 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 139.2, 138.9, 138.7, 138.6, 138.4, 138.2, 138.0, 137.0, 136.9 (10 × C_q), 128.7, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 127.3, 127.0 (50 × C_{Ar}), 103.2 (C-1), 99.4 (C-1''), 98.8 (C-1'), 82.5 (C-3), 81.2 (d, $J_{C6,F}$ = 166.8 Hz, C-6), 80.0 (C-2), 78.6 (C-3''), 77.9 (C-3'), 75.3 (C-2'/CH_{Bn}), 75.2 (C-4'/CH_{Bn}), 74.6 (CH_{Bn}), 74.4 (C-4''), 73.5 (CH_{Bn}), 73.1 (C-4/CH_{Bn}), 73.0, 72.6 (2 × CH_{Bn}), 72.3 (d, $J_{C5,F}$ = 25.4 Hz, C-5), 72.0 (CH_{Bn}), 71.9 (C-5''), 71.8 (C-5'), 69.1 (C-6'/C-6''), 68.5 (C-2''), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.2 (CH_3-OAc) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 229.9 (t, $J_{F,H6}$ = 47.2 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{96}H_{108}FN_2O_{18}^+$ $[M+NH_4]^+$: 1596.7609, found 1596.7628.

RP-HPLC: t_R = 27.0 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-D-6-deoxy-6-fluorogalactopyranosyl)-α-D-mannopyranoside (100)**



The acetyl protecting group of trisaccharide **96** (822 mg, 0.521 mmol) was removed according to general procedure (**D**) to give the corresponding trisaccharide **100** (727 mg, 0.473 mmol, 91%) as a colorless oil.

R_f = 0.47 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 24.4 (c = 0.5, $CHCl_3$).

^{1}H-NMR (800 MHz, $CDCl_3$): δ = 7.39–7.10 (m, 50H, Ar-H), 5.21–5.14 (m, 3H, H-1‘‘/2 × CH_{Cbz}), 4.96 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.87 (d, J = 11.0 Hz, 1H, CH_{Bn}), 4.83 (s, 1H, H-1‘), 4.81–4.75 (m, 3H, CH_{Bn}), 4.72 (s, 2H, CH_{Bn}), 4.65–4.59 (m, 3H, CH_{Bn}), 4.58 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.54–4.51 (m, 2H, H-1/CH_{Bn}), 4.51–4.44 (m, 5H, 3 × CH_{Bn}/2 × NCH_{Bn}), 4.40 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.29 (ddd, $J_{H6a,F6}$ = 47.2 Hz, $J_{H6a,H6b}$ = 9.1 Hz, $J_{H6a,H5}$ = 6.3 Hz, 1H, H-6a), 4.24 (ddd, $J_{H6b,F6}$ = 46.3 Hz, $J_{H6b,H6a}$ = 9.1 Hz, $J_{H6b,H5}$ = 6.1 Hz, 1H, H-6b), 4.20–4.15 (m, 1H, H-4‘), 4.10 (q, $J_{H2‘‘,H1‘‘/H3‘‘/OH}$ = 2.5 Hz, 1H, H-2‘‘), 4.03 (s, 1H, H-2‘), 3.94 (ddd, $J_{H5‘‘,H4‘‘}$ = 9.9 Hz, $J_{H5‘‘,H6a‘‘}$ = 5.0 Hz, $J_{H5‘‘,H6b‘‘}$ = 2.1 Hz, 1H, H-5‘‘), 3.86–3.81 (m, 3H, H-3‘/H-6a‘/H-3‘‘), 3.81–3.77 (m, 2H, H-4/H-4‘‘), 3.76 (dd, $J_{H2,H3}$ = 9.7, $J_{H2,H1}$ = 7.7 Hz, 1H, H-2), 3.73–3.67 (m, 4H, H-5‘/H-6b‘/H-6a‘‘/H-6b‘‘), 3.59–3.51 (m, 1H, CH_{Linker}), 3.44 (dd, $J_{H3,H2}$ = 9.7 Hz, $J_{H3,H4}$ = 2.9 Hz, 1H, H-3), 3.41–3.37 (m, 1H, H-5), 3.29–3.13 (m, 3H, CH_{Linker}), 2.32 (d, $J_{OH,H2‘}$ = 2.5 Hz, 1H, -OH), 1.57–1.43 (m, 4H, CH_{Linker}), 1.30–1.15 (m, 2H, CH_{Linker}) ppm.

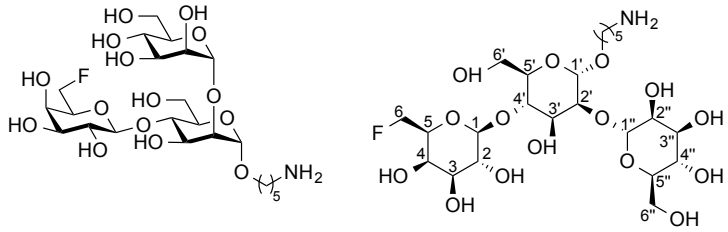
^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 139.3, 138.9, 138.8, 138.6, 138.5, 138.4, 138.2, 138.1, 137.0, 136.9 (10 × C_q), 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2 (50 × C_{Ar}), 103.0 (C-1), 101.0 (C-1‘‘), 98.9 (C-1‘), 82.5 (C-3), 81.8 (d, $J_{C6,F}$ = 167.5 Hz, C-6), 80.2 (C-3‘‘), 80.0 (C-2), 77.8 (C-3‘), 75.9 (C-2‘), 75.3, 75.0 (2 × CH_{Bn}), 74.9 (C-4‘), 74.7 (CH_{Bn}), 74.5 (C-4‘‘), 73.5 (CH_{Bn}), 73.3 (C-4), 73.2, 73.1, 72.8 (3 × CH_{Bn}), 72.6 (d, $J_{C5,F}$ = 24.0 Hz, C-5), 72.0 (CH_{Bn}), 71.9 (C-5‘), 71.6 (C-5‘‘), 69.3 (C-6‘‘), 69.1 (C-6‘), 68.4 (C-2‘‘), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.4 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 229.3 (t, $J_{F,H6}$ = 47.4 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{94}H_{106}FN_2O_{17}^+$ $[M+NH_4]^+$: 1554.7504, found 1554.7510.

RP-HPLC: t_R = 27.9 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-6-deoxy-6-fluorogalactopyranosyl)-α-D-mannopyranoside (6)



According to general procedure (**E**), fluorinated trisaccharide **100** (565 mg, 0.368 mmol) was globally deprotected by hydrogenolysis to give the corresponding trisaccharide **6** (202 mg, 0.342 mmol, 93%) as a colorless solid.

^{1}H-NMR (800 MHz, D_2O): δ = 5.11 (s, 1H, H-1‘), 5.03 (s, 1H, H-1‘‘), 4.74–4.61 (m, 2H, H-6a/H-6b), 4.49 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.09 (d, $J_{H2‘‘,H3‘‘}$ = 3.1 Hz, 1H, H-2‘‘), 4.06–3.98 (m, 4H, H-4/H-5/H-2‘/H-3‘), 3.96 (d, $J_{H6a‘,H6b‘}$ = 12.6 Hz, 1H, H-6a‘), 3.91–3.82 (m, 4H, H-4‘/H-6b‘/H-3‘‘/H-6a‘‘), 3.80–3.67 (m, 4H, H-3/H-5‘/H-5‘‘/H-6b‘‘), 3.61 (t, $J_{H4‘‘,H3‘‘/H5‘‘}$ = 9.9 Hz, 1H, H-4‘‘), 3.58–3.54 (m, 2H, H-2/CH_{Linker}), 3.00 (t, J = 7.6 Hz, 2H, CH_{Linker}), 1.71–1.63 (m, 4H, CH_{Linker}), 1.49–1.42 (m, 2H, CH_{Linker}) ppm.

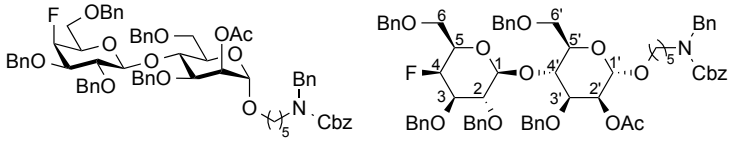
^{13}C-{^{1}H}-NMR (201 MHz, D_2O/CD_3OD): δ = 104.1 (C-1), 103.3 (C-1‘‘), 98.9 (C-1‘), 84.0 (d, $J_{C6,F6}$ = 165.7 Hz, C-6), 78.9 (C-2‘), 78.5 (C-4‘), 74.5 (d, $J_{C5,F6}$ = 19.8 Hz, C-5), 74.3 (C-5‘‘), 73.4 (C-3), 72.3 (C-5‘), 71.8 (C-2), 71.3 (C-3‘‘), 70.9 (C-2‘‘), 70.1 (C-3‘), 69.1 (d, $J_{C4,F6}$ =8.0 Hz, C-4), 68.6 (CH_{Linker}), 67.9 (C-4‘‘), 62.2 (C-6‘‘), 61.4 (C-6‘), 40.4 (CH_{Linker}), 29.0 (CH_{Linker}), 27.5 (CH_{Linker}), 23.4 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, D_2O): δ = − 230.3 (t, $J_{F,H6}$ = 47.1 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{23}H_{43}N_2O_{15}^{+}$ $[M+H]^{+}$: 592.2611, found 592.2604.

5.2.1.9 Synthesis of 5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-4-deoxy-4-fluorogalactopyranosyl)-α-D-mannopyranoside 7

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,6-tri-*O*-benzyl-β-D-4-deoxy-4-fluorogalactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (87)**



According to general procedure (**A**), fluorinated galactosyl imidate **14** (545 mg, 0.913 mmol, 1.3 equiv.) and mannosyl acceptor **12** (500 mg, 0.702 mmol, 1.0 equiv.) were reacted under inverse glycosylation conditions to give the corresponding disaccharide **87** (635 mg, 0.554 mmol, 79%) as a colorless oil.

R_f = 0.42 (^{c}Hex/EtOAc, 2:1);

$[\alpha]_D^{22}$ = + 16.8 (c = 0.5, $CHCl_3$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.41–7.15 (m, 35H, Ar-H), 5.31–5.26 (m, 1H, H-2‘), 5.18 (d, J = 8.0 Hz, 2H, CH_{Cbz}), 4.80 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.79 (dd, $J_{H4,F}$ = 50.1 Hz, $J_{H4,H3/H5}$ = 2.3 Hz, 1H, H-4), 4.77 (br s, 1H, H-1‘), 4.74 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.72–4.61 (m, 4H, CH_{Bn}), 4.58 (d, J = 12.0 Hz, 1H, CH_{Bn}), 4.52–4.49 (m, 2H, NCH_{Bn}), 4.48 (d, J = 12.0 Hz, 1H, CH_{Bn}), 4.45 (d, $J_{H1,H2}$ = 8.1 Hz, 1H, H-1), 4.37 (d, J = 12.0 Hz, 2H, CH_{Bn}), 4.14 (t, $J_{H4‘,H3‘/H5‘}$ = 9.3 Hz, 1H, H-4‘), 3.89–3.81 (m, 2H, H-3‘/H-6a‘), 3.72 (dd, $J_{H5‘,H4‘}$ = 9.9 Hz, $J_{H5‘,H6a‘/b‘}$ = 3.1 Hz, 1H, H-5‘), 3.67–3.58 (m, 4H, H-2/H-6a/H-6b‘/CH_{Linker}), 3.48 (ddd, $J_{H6b,H6a}$ = 9.8 Hz, $J_{H6b,H5}$ = 5.8 Hz, $J_{H6b,F}$ = 1.5 Hz, 1H, H-6b), 3.42–3.29 (m, 3H, H-3/H-5/CH_{Linker}), 3.30–3.17 (m, 2H, CH_{Linker}), 1.98 (s, 3H, CH_3-OAc), 1.63–1.45 (m, 4H, CH_{Linker}), 1.37–1.21 (m, 2H, CH_{Linker}) ppm.

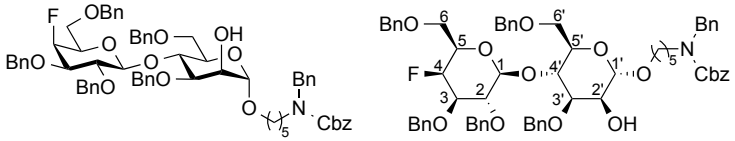
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.6 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 138.9, 138.7, 138.5, 138.1, 138.0, 137.9, 136.9 (7 × C_q), 128.7, 128.6, 128.5, 128.2, 127.8, 127.7, 127.6, 127.5, 127.3 (35 × C_{Ar}), 102.6 (C-1), 97.7 (C-1‘), 85.7 (d, $J_{C4,F}$ = 182.9 Hz, C-4), 79.7 (d, $J_{C3,F}$ = 18.1 Hz, C-3), 79.5 (C-2), 76.1 (C-3‘), 75.5 (CH_{Bn}), 74.6 (C-4‘), 73.7 (CH_{Bn}), 73.3 (CH_{Bn}), 72.4 (CH_{Bn}), 72.3 (d, $J_{C5,F}$ = 18.1 Hz, C-5), 72.2 (CH_{Bn}), 71.4 (C-5‘), 69.8 (C-2‘), 68.6 (C-6‘), 67.9 (CH_{Linker}), 67.8 (C-6), 67.3 (CH_{Cbz}), 50.7/50.4 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.1 (CH_3-OAc) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 217.3 (dt, $J_{F,H4}$ = 53.2 Hz, $J_{F,H3/H5}$ = 27.9 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{69}H_{80}FN_2O_{13}^+$ $[M+NH_4]^+$: 1163.5639, found 1163.5648.

RP-HPLC: t_R = 18.7 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,6-tri-*O*-benzyl-β-D-4-deoxy-4-fluorogalactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (91)**



The acetyl protecting group of fluorinated disaccharide **87** (1.00 g, 0.872 mmol) was removed according to general procedure (**B**) to give the corresponding disaccharide **91** (767 mg, 0.694 mmol, 80%) as a colorless oil.

R_f = 0.33 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 24.6 (c = 1.0, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.40–7.16 (m, 35H, Ar-H), 5.18 (d, J = 15.0 Hz, 2H, CH_{Cbz}), 4.88–4.72 (m, 6H, H-1‘/H-4/4 × CH_{Bn}), 4.68 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.64 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.59 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.52–4.46 (m, 3H, CH_{Bn}/NCH_{Bn}), 4.43–4.40 (m, 2H, 1H, H-1/CH_{Bn}), 4.37 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.15 (t, $J_{H4‘,H3‘/H5‘}$ = 9.4 Hz, 1H, H-4‘), 3.99 (br s, 1H, H-2‘), 3.83 (dd, $J_{H6a‘,H6b‘}$ = 10.8 Hz, $J_{H6a‘,H5‘}$ = 4.2 Hz, 1H, H-6a‘), 3.74 (d, $J_{H3‘,H4‘}$ = 9.3 Hz, 1H, H-3‘), 3.69 (d, $J_{H5‘,H4‘}$ = 9.5 Hz, 1H, H-5‘), 3.66–3.58 (m, 4H, H-2/H-6a/H-6b‘/CH_{Linker}), 3.52–3.48 (m, 1H, H-6b), 3.41–3.30 (m, 3H, H-3/H-5/CH_{Linker}), 3.29–3.17 (m, 2H, CH_{Linker}), 2.50 (d, $J_{OH,H2’}$ = 2.1 Hz, 1H, -OH), 1.62–1.47 (m, 4H, CH_{Linker}), 1.36–1.22 (m, 2H, CH_{Linker}) ppm.

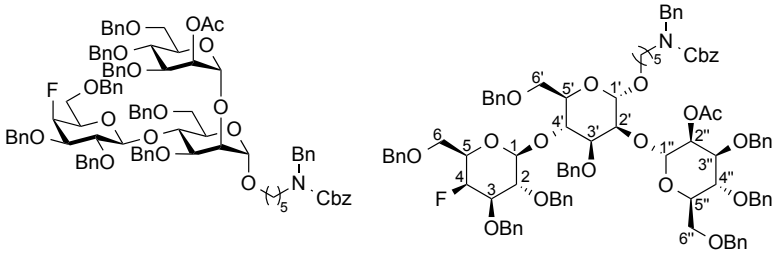
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 138.7, 138.5, 138.1, 138.0, 137.0 (7 × C_q), 128.7, 128.6, 128.4, 128.3, 128.0, 127.9, 127.8, 127.6 (35 × C_{Ar}), 102.7 (C-1), 99.1 (C-1‘), 85.6 (d, $J_{C4,F}$ = 183.1 Hz, C-4), 79.62 (d, $J_{C3,F}$ = 18.0 Hz, C-3), 79.57 (C-2), 78.1 (C-3‘), 75.5 (CH_{Bn}), 74.4 (C-4‘), 73.7 (CH_{Bn}), 73.3 (CH_{Bn}), 73.1 (CH_{Bn}), 72.2 (CH_{Bn}), 72.0 (d, $J_{C5,F}$ = 18.1 Hz, C-5), 71.2 (C-5‘), 69.6 (C-2‘), 68.5 (C-6‘), 67.6 (C-6/CH_{Linker}), 67.5 (C-6/CH_{Linker}), 67.3 (CH_{Cbz}), 50.7/50.4 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 217.5 (dt, $J_{F,H4}$ = 50.1 Hz, $J_{F,H3/H5}$ = 27.8 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{67}H_{78}FN_2O_{12}^+$ $[M+NH_4]^+$: 1121.5533, found 1121.5537.

RP-HPLC: t_R = 13.7 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 30 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-4-deoxy-4-fluorogalactopyranosyl)-α-D-mannopyranoside (97)**



According to general procedure (**C**), fluorinated disaccharide **91** (574 mg, 520 mmol, 1.0 equiv.) was reacted with mannosyl imidate **18** (662 mg, 1.04 mmol, 2.0 equiv.) to give the corresponding trisaccharide **97** (693 mg, 0.438 mmol, 84%) as a colorless oil.

R_f = 0.41 (^{c}Hex/EtOAc, 3:1);

$[\alpha]_D^{22}$ = + 16.0 (c = 0.5, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.39–7.09 (m, 50H, Ar-H), 5.54 (dd, $J_{H2'',H3''}$ = 3.2 Hz, $J_{H2'',H1''}$ = 1.9 Hz, 1H, H-2''), 5.18 (d, J = 14.8 Hz, 2H, CH_{Cbz}), 5.12 (s, 1H, H-1''), 4.89–4.76 (m, 5H, H-1'/H-4/3 × CH_{Bn}), 4.75–4.70 (m, 2H, 2 × CH_{Bn}), 4.68–4.56 (m, 5H, CH_{Bn}), 4.55 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.52–4.36 (m, 7H, 5 × CH_{Bn}/ 2 × NCH_{Bn}), 4.31 (d, J = 10.7 Hz, 1H, CH_{Bn}), 4.16 (t, $J_{H4',H3'/H5'}$ = 8.9 Hz, 1H, H-4'), 4.03 (s, 1H, H-2'), 3.97 (dd, $J_{H3'',H4''}$ = 9.4 Hz, $J_{H3'',H2''}$ = 3.3 Hz, 1H, H-3''), 3.93 (ddd, $J_{H5'',H4''}$ = 9.9 Hz, $J_{H5'',H6a''}$ = 4.5 Hz, $J_{H5'',H6b''}$ = 1.7 Hz, 1H, H-5''), 3.87–3.75 (m, 4H, H-3'/H-6a'/H-4''/H-6a''), 3.74–3.67 (m, 3H, H-5'/H-6b'/H-6b''), 3.65–3.61 (m, 1H, H-2), 3.61–3.47 (m, 3H, H-6a/H-6b/CH_{Linker}), 3.47–3.32 (m, 2H, H-3/H-5), 3.31–3.14 (m, 3H, CH_{Linker}), 2.06 (s, 3H, CH_3-OAc), 1.58–1.44 (m, 4H, CH_{Linker}), 1.32–1.16 (m, 2H, CH_{Linker}) ppm.

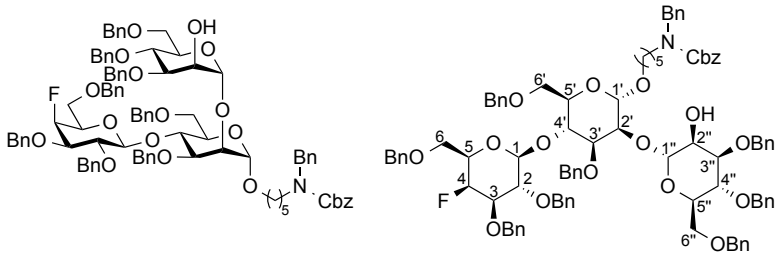
^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 169.9 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 139.1, 138.8, 138.7, 138.6, 138.3, 138.2, 138.0, 137.9, 137.0, 136.9 (10 × C_q), 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 127.3, 127.1, 127.0 (50 × C_{Ar}), 102.9 (C-1), 99.5 (C-1''), 98.7 (C-1'), 85.3 (d, $J_{C4,F}$ = 183.3 Hz, C-4), 79.9 (d, $J_{C3,F}$ = 18.6 Hz, C-3), 79.6 (C-2), 78.6 (C-3''), 77.9 (C-3'), 75.4 (C-4'/CH_{Bn}), 75.2 (CH_{Bn}), 75.0 (C-2'), 74.4 (C-4''), 73.5, 73.1, 72.5, 72.1 (5 × CH_{Bn}), 72.0 (C-5''/CH_{Bn}), 71.8 (C-5'), 71.6 (d, $J_{C5,F}$ = 18.0 Hz, C-5), 69.1 (C-6''), 69.0 (C-6'), 68.5 (C-2''), 67.6 (CH_{Linker}), 67.3 (C-6/CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.2 (CH_3-OAc) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 217.9 (dt, $J_{F,H4}$ = 49.8 Hz, $J_{F,H3/H5}$ = 27.7 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{96}H_{108}FN_2O_{18}^+$ $[M+NH_4]^+$: 1596.7609, found 1596.7636.

RP-HPLC: t_R = 26.4 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-4-deoxy-4-fluorogalactopyranosyl)-α-D-mannopyranoside (101)**



The acetyl protecting group of trisaccharide **97** (573 mg, 0.363 mmol) was removed according to general procedure (**D**) to give the corresponding trisaccharide **101** (522 mg, 0.340 mmol, 94%) as a colorless oil.

R_f = 0.39 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 17.4 (c = 0.5, $CHCl_3$).

^{1}H-NMR (800 MHz, $CDCl_3$): δ = 7.41–7.10 (m, 50H, Ar-H), 5.21–5.14 (m, 3H, H-1‘‘/2 × CH_{Cbz}), 4.84 (s, 1H, H-1‘), 4.84–4.73 (m, 6H, H-4/5 × CH_{Bn}), 4.66 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.64–4.60 (m, 2H, CH_{Bn}), 4.55 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.52–4.40 (m, 8H, 6 × CH_{Bn}/ 2 × NCH_{Bn}), 4.39 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.37 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.17 (t, $J_{H4',H3'/H5'}$ = 7.4 Hz, 1H, H-4‘), 4.04 (s, 1H, H-2‘), 4.01 (q, $J_{H2'',H1''/H3''/OH}$ = 2.6 Hz, 1H, H-2‘‘), 3.92 (ddd, $J_{H5'',H4''}$ = 9.7 Hz, $J_{H5'',H6a''}$ = 4.8 Hz, $J_{H5'',H6b''}$ = 2.1 Hz, 1H, H-5‘‘), 3.86–3.83 (m, 2H, H-3‘/H-6a‘), 3.81 (dd, $J_{H3'',H4''}$ = 9.1 Hz, $J_{H3'',H2''}$ = 3.1 Hz, 1H, H-3‘‘), 3.79–3.75 (m, 1H, H-4‘‘), 3.73–3.67 (m, 3H, H-5‘/H-6a‘‘/H-6b‘‘), 3.67–3.63 (m, 2H, H-2/H-6b‘), 3.59–3.50 (m, 2H, H-6a/CH_{Linker}), 3.46–3.35 (m, 3H, H-3/H-5/H-6b), 3.27–3.14 (m, 3H, CH_{Linker}), 2.21 (s, 1H, -OH), 1.55–1.44 (m, 4H, CH_{Linker}), 1.28–1.17 (m, 2H, CH_{Linker}) ppm.

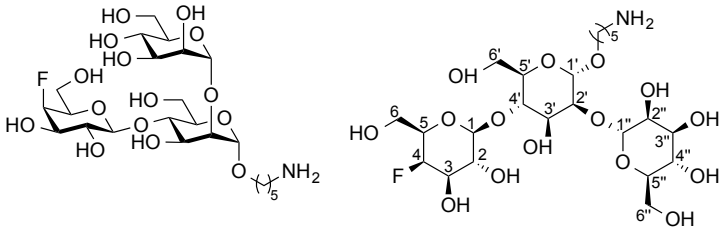
^{13}C-{^{1}H}-NMR (201 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 139.2, 138.8, 138.7, 138.5, 138.4, 138.1, 138.0, 137.9, 137.0, 136.9 (10 × C_q), 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3 (50 × C_{Ar}), 102.7 (C-1), 101.0 (C-1‘‘), 98.9 (C-1‘), 85.7 (d, $J_{C4,F}$ = 183.0 Hz, C-4), 80.2 (C-3‘‘), 79.7 (d, $J_{C3,F}$ = 18.2 Hz, C-3), 79.6 (C-2), 77.9 (C-3‘), 75.5 (CH_{Bn}), 75.4 (C-2‘), 75.1 (C-4‘/CH_{Bn}), 74.5 (C-4‘‘), 73.7, 73.5, 73.2, 72.9, 72.2 (5 × CH_{Bn}), 72.0 (d, $J_{C5,F}$ = 18.7 Hz, C-5), 72.0 (CH_{Bn}), 71.9 (C-5‘), 71.7 (C-5‘‘), 69.3 (C-6‘‘), 68.9 (C-6‘), 68.4 (C-2‘‘), 67.8 (d, $J_{C6,F}$ = 6.1 Hz, C-6), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.4/29.3 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 217.4 (dt, $J_{F,H4}$ = 52.7 Hz, $J_{F,H3/H5}$ = 27.6 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{94}H_{106}FN_2O_{17}^+$ $[M+NH_4]^+$: 1554.7504, found 1554.7511.

RP-HPLC: t_R = 26.8 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-4-deoxy-4-fluorogalactopyranosyl)-α-D-mannopyranoside (7)



According to general procedure (**E**), fluorinated trisaccharide **101** (494 mg, 0.321 mmol) was globally deprotected by hydrogenolysis to give the corresponding trisaccharide **7** (173 mg, 0.292 mmol, 91%) as a colorless solid.

^{1}H-NMR (800 MHz, D_2O): δ = 5.11 (d, $J_{H1',H2'}$ = 1.8 Hz, 1H, H-1'), 5.02 (d, $J_{H1'',H2''}$ = 1.8 Hz, 1H, H-1''), 4.83 (dd, $J_{H4,F}$ = 50.4 Hz, $J_{H4,H3/H5}$ = 2.8 Hz, 1H, H-4), 4.53 (d, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1), 4.08 (dd, $J_{H2'',H3''}$ = 3.4 Hz, $J_{H2'',H1''}$ = 1.8 Hz, 1H, H-2''), 4.02 (dd, $J_{H3',H4'}$ = 9.3 Hz, $J_{H3',H2'}$ = 3.4 Hz, 1H, H-3'), 4.00 (dd, $J_{H2',H3'}$ = 3.5 Hz, $J_{H2',H1'}$ = 1.8 Hz, 1H, H-2'), 3.96 (dd, $J_{H6a',H6b'}$ = 12.3 Hz, $J_{H6a',H5'}$ = 2.3 Hz, 1H, H-6a'), 3.91–3.81 (m, 7H, H-5/H-6a/H-6b/H-4'/H-6b'/H-3''/H-6a''), 3.81–3.69 (m, 5H, H-3/H-5'/H-5''/H-6b''/CH_{Linker}), 3.62–3.56 (m, 2H, H-2/ H-4''), 3.56–3.53 (m, 1H, CH_{Linker}), 2.99 (t, J = 7.7 Hz, 2H, CH_{Linker}), 1.72–1.60 (m, 4H, CH_{Linker}), 1.49–1.39 (m, 2H, CH_{Linker}) ppm.

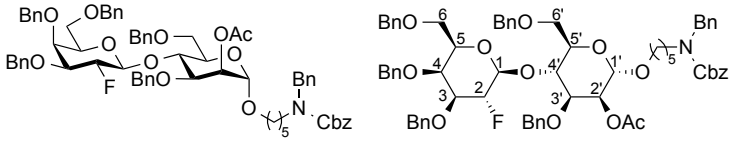
^{13}C-{^{1}H}-NMR (201 MHz, D_2O/CD_3OD): δ = 104.1 (C-1), 103.5 (C-1''), 99.2 (C-1'), 90.3 (d, $J_{C4,F}$ = 179.0 Hz, C-4), 79.2 (C-2'), 78.6 (C-4'), 75.0 (d, $J_{C5,F}$ = 17.9 Hz, C-5), 74.5 (C-5''), 72.7 (C-5'), 72.6 (d, $J_{C3,F}$ = 18.2 Hz, C-3), 72.2 (C-2), 71.6 (C-3''), 71.1 (C-2''), 70.3 (C-3'), 68.5 (CH_{Linker}), 68.1 (C-4''), 62.4 (C-6''), 61.5 (C-6'), 61.0 (d, $J_{C6,F}$ = 6.4 Hz, C-6), 40.4 (CH_{Linker}), 29.24 (CH_{Linker}), 27.7 (CH_{Linker}), 23.7 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, D_2O): δ = − 216.9 (dt, $J_{F,H4}$ = 50.5 Hz, $J_{F,H3/H5}$ = 29.9 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{23}H_{43}N_2O_{15}^+$ $[M+H]^+$: 592.2611, found 592.2607.

5.2.1.10 Synthesis of 5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-2-deoxy-2-fluorogalactopyranosyl)-α-D-mannopyranoside 8

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-β-D-2-deoxy-2-fluorogalactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (88)**



According to general procedure (**A**), fluorinated galactosyl imidate **15** (545 mg, 0.913 mmol, 1.3 equiv.) and mannosyl acceptor **12** (500 mg, 0.702 mmol, 1.0 equiv.) were reacted under inverse glycosylation conditions to give the corresponding disaccharide **88** (598 mg, 0.522 mmol, 74%) as a colorless oil.

R_f = 0.45 (^{c}Hex/EtOAc, 2:1);

$[\alpha]_D^{22}$ = + 14.4 (c = 0.5, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.40–7.17 (m, 35H, Ar-H), 5.26 (br s, 1H, H-2‘), 5.19 (d, J = 19.6 Hz, 2H, CH_{Cbz}), 4.90 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.78–4.76 (m, 1H, H-1‘), 4.69 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.67–4.56 (m, 6H, H-1/H-2/4 × CH_{Bn}), 4.54 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.53 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.50 (d, J = 11.5 Hz, 2H, NCH_{Bn}), 4.31 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.21 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.08 (t, $J_{H4‘,H3‘/H5‘}$ = 9.5 Hz, 1H, H-4‘), 3.94–3.90 (m, 1H, H-3‘), 3.90–3.85 (m, 2H, H-4/H-6a‘), 3.83–3.76 (m, 2H, H-5‘/H-6b‘), 3.65–3.53 (m, 2H, H-6a/CH_{Linker}), 3.46 (ddd, $J_{H3,F}$ = 12.3 Hz, $J_{H3,H2}$ = 9.3 Hz, $J_{H3,H4}$ = 3.0 Hz, 1H, H-3), 3.38–3.27 (m, 3H, H-5/H-6b/CH_{Linker}), 3.29–3.15 (m, 2H, CH_{Linker}), 2.02 (s, 3H, CH_3-OAc), 1.58–1.45 (m, 4H, CH_{Linker}), 1.33–1.18 (m, 2H, CH_{Linker}) ppm.

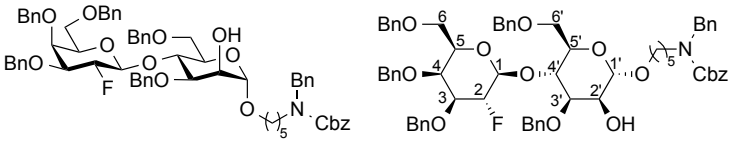
^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.6 (C=O-OAc), 156.9/156.3 (C=O-Cbz), 138.7, 138.6, 138.2, 138.1, 138.0, 137.0, 136.9 (7 × C_q), 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.4 (35 × C_{Ar}), 101.6 (d, $J_{C1,F}$ = 23.7 Hz, C-1), 97.6 (C-1‘), 93.0 (d, $J_{C2,F}$ = 182.2 Hz, C-2), 80.1 (d, $J_{C3,F}$ = 15.4 Hz, C-3), 76.9 (C-3‘), 75.2 (C-4‘), 75.0 (CH_{Bn}), 74.3 (d, $J_{C4,F}$ = 8.4 Hz, C-4), 73.6 (C-5/CH_{Bn}), 73.5 (CH_{Bn}), 72.6 (CH_{Bn}), 71.9 (CH_{Bn}), 70.9 (C-5’), 69.2 (C-2‘), 68.9 (C-6‘), 68.2 (C-6), 67.8 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.7/50.4 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.5 (CH_{Linker}), 21.1 (CH_3-OAc) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 204.7 (dd, $J_{F,H2}$ = 53.1 Hz, $J_{F,H3}$ = 12.3 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{69}H_{80}FN_2O_{13}^+$ $[M+NH_4]^+$: 1163.5639, found 1163.5647.

RP-HPLC: t_R = 19.4 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-β-D-2-deoxy-2-fluorogalactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (92)**



The acetyl protecting group of fluorinated disaccharide **88** (1.00 g, 0.872 mmol) was removed according to general procedure (**B**) to give the corresponding disaccharide **92** (751 mg, 0.680 mmol, 78%) as a colorless oil.

R_f = 0.37 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 20.6 (c = 1.0, $CHCl_3$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.40–7.18 (m, 35H, Ar-H), 5.20 (d, J = 8.9 Hz, 2H, CH_{Cbz}), 4.93 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.86–4.79 (m, 2H, H-1‘/CH_{Bn}), 4.73 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.70–4.54 (m, 6H, H-1/H-2/4 × CH_{Bn}), 4.54–4.48 (m, 3H, CH_{Bn}/2 × NCH_{Bn}), 4.34 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.25 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.11 (t, $J_{H4‘,H3‘/H5‘}$ = 9.4 Hz, 1H, H-4‘), 3.95 (br s, 1H, H-2‘), 3.92 (t, $J_{H4,H3/H5}$ = 2.9 Hz, 1H, H-4), 3.87 (dd, $J_{H6a‘,H6b‘}$ = 10.9 Hz, $J_{H6a‘,H5‘}$ = 4.2 Hz, 1H, H-6a‘), 3.82–3.73 (m, 3H, H-3‘/H-5‘/H-6b‘), 3.69–3.52 (m, 2H, H-6a/CH_{Linker}), 3.50–3.42 (m, 1H, H-3), 3.41–3.33 (m, 3H, H-5/H-6b/CH_{Linker}), 3.32–3.18 (m, 2H, CH_{Linker}), 2.47 (d, $J_{OH,H2‘}$ = 2.4 Hz, 1H, -OH), 1.60–1.47 (m, 4H, CH_{Linker}), 1.36–1.20 (m, 2H, CH_{Linker}) ppm.

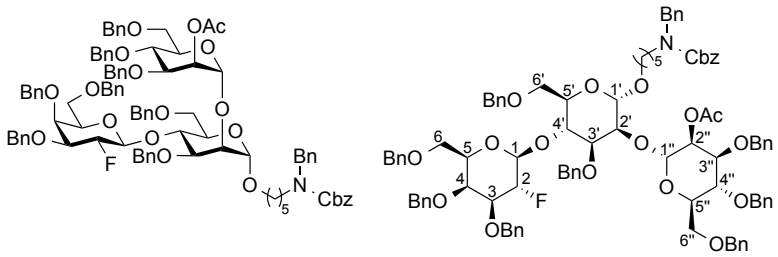
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 156.8/156.28 (C=O-Cbz), 138.7, 138.6, 138.2, 138.1, 138.0, 137.0 (7 × C_q), 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.6, 127.5 (35 × C_{Ar}), 101.5 (d, $J_{C1,F}$ = 23.4 Hz, C-1), 99.1 (C-1‘), 92.7 (d, $J_{C2,F}$ = 182.7 Hz, C-2), 80.2 (d, $J_{C3,F}$ = 15.7 Hz, C-3), 78.6 (C-3‘), 75.0 (C-4‘/CH_{Bn}), 74.4 (d, $J_{C4,F}$ = 8.8 Hz, C-4), 73.6 (CH_{Bn}), 73.5 (CH_{Bn}), 73.4 (C-5), 72.6 (2 × CH_{Bn}), 70.7 (C-5‘), 69.1 (C-2‘), 68.7 (C-6‘), 68.1 (C-6), 67.5 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.7/50.4 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.2 (CH_{Linker}), 28.0/27.6 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 204.4 (dd, $J_{F,H2}$ = 54.1 Hz, $J_{F,H3}$ = 10.9 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{67}H_{78}FN_2O_{12}^+$ $[M+NH_4]^+$: 1121.5533, found 1121.5536.

RP-HPLC: t_R = 14.5 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 30 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-2-deoxy-2-fluorogalactopyranosyl)-α-D-mannopyranoside (98)**



According to general procedure (**C**), fluorinated disaccharide **92** (650 mg, 0.589 mmol, 1.0 equiv.) was reacted with mannosyl imidate **18** (750 mg, 1.18 mmol, 2.0 equiv.) to give the corresponding trisaccharide **98** (734 mg, 0.465 mmol, 79%) as a colorless oil.

R_f = 0.42 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 14.8 (c = 0.5, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.41–7.09 (m, 50H, Ar-H), 5.53 (dd, $J_{H2'',H3''}$ = 3.3 Hz, $J_{H2'',H1''}$ = 1.8 Hz, 1H, H-2''), 5.18 (d, J = 18.7 Hz, 2H, CH_{Cbz}), 5.00 (d, $J_{H1'',H2''}$ = 1.4 Hz, 1H, H-1''), 4.85 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.83–4.78 (m, 3H, H-1'/2 × CH_{Bn}), 4.71–4.67 (m, 2H, H-1/CH_{Bn}), 4.66–4.55 (m, 6H, H-2/5 × CH_{Bn}), 4.55–4.41 (m, 6H, 4 × CH_{Bn}/2 × NCH_{Bn}), 4.36 (d, J = 10.8 Hz, 1H, CH_{Bn}), 4.21 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.15 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.04 (t, $J_{H4',H3'/H5'}$ = 9.4 Hz, 1H, H-4'), 3.97–3.93 (m, 2H, H-2'/H-3''), 3.93–3.88 (m, 2H, H-4/H-5''), 3.88–3.84 (m, 1H, H-3'), 3.84–3.71 (m, 5H, H-5'/H-6a'/H-6b'/H-4''/H-6a''), 3.70–3.65 (m, 1H, H-6b''), 3.59–3.49 (m, 3H, H-3/H-6a/CH_{Linker}), 3.37 (dd, $J_{H5,H6a}$ = 8.7 Hz, $J_{H5,H6b}$ = 5.1 Hz, 1H, H-5), 3.30–3.14 (m, 4H, H-6b/3 × CH_{Linker}), 2.06 (s, 3H, CH_3-OAc), 1.56–1.39 (m, 4H, CH_{Linker}), 1.30–1.13 (m, 2H, CH_{Linker}) ppm.

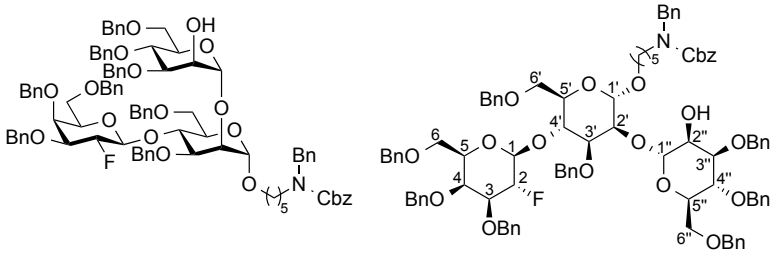
^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.1 (C=O-OAc), 156.9/156.3 (C=O-Cbz), 139.1, 138.9, 138.7, 138.6, 138.3, 138.2, 138.1, 138.0, 137.0 (10 × C_q), 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 126.6 (50 × C_{Ar}), 102.1 (d, $J_{C1,F}$ = 24.0 Hz, C-1), 99.7 (C-1''), 98.7 (C-1'), 93.0 (d, $J_{C2,F}$ = 182.7 Hz, C-2), 80.2 (d, $J_{C3,F}$ = 15.0 Hz, C-3), 78.8 (C-3'), 78.4 (C-3''), 76.1 (C-4'), 75.2 (CH_{Bn}), 74.9 (CH_{Bn}), 74.4 (C-2'/C-4''), 74.1 (d, $J_{C4,F}$ = 8.6 Hz, C-4), 73.5 (CH_{Bn}), 73.4 (2 × CH_{Bn}), 73.1 (C-5), 72.6, 72.1 (2 × CH_{Bn}), 71.9 (C-5''/CH_{Bn}), 71.1 (C-5'), 69.2 (C-6'), 69.1 (C-6''), 68.7 (C-2''), 67.7 (C-6), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.7/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.2 (CH_3-OAc) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 204.7 (dd, $J_{F,H2}$ = 54.2 Hz, $J_{F,H3}$ = 12.0 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{96}H_{108}FN_2O_{18}^+$ $[M+NH_4]^+$: 1596.7609, found 1596.7659.

RP-HPLC: t_R = 27.2 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-2-deoxy-2-fluorogalactopyranosyl)-α-D-mannopyranoside (102)**



The acetyl protecting group of fluorinated trisaccharide **98** (615 mg, 0.390 mmol) was removed according to general procedure (**D**) to give the corresponding trisaccharide **102** (545 mg, 0.355 mmol, 91%) as a colorless oil.

R_f = 0.39 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 18.4 (c = 0.5, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.43–7.11 (m, 50H, Ar-H), 5.19 (d, J = 19.1 Hz, 2H, CH_{Cbz}), 5.08 (d, $J_{H1'',H2''}$ = 1.9 Hz, 1H, H-1''), 4.90 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.85 (s, 1H, H-1'), 4.79 (d, J = 11.0 Hz, 1H, CH_{Bn}), 4.77 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.71 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.70–4.58 (m, 6H, H-1/H-2/4 × CH_{Bn}), 4.56–4.46 (m, 8H, 6 × CH_{Bn}/2 × NCH_{Bn}), 4.28 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.19 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.06 (t, $J_{H4',H3'/H5'}$ = 9.5 Hz, 1H, H-4'), 4.02–4.01 (m, 1H, H-2''), 3.97–3.95 (m, 1H, H-2'), 3.94–3.89 (m, 2H, H-4/H-5''), 3.89–3.84 (m, 2H, H-3'/H-6a'), 3.83 (dd, $J_{H3'',H4''}$ = 9.1 Hz, $J_{H3'',H2''}$ = 3.2 Hz, 1H, H-3''), 3.80–3.73 (m, 3H, H-5'/H-6b'/H-4''), 3.72–3.67 (m, 2H, H-6a''/H-6b''), 3.59–3.48 (m, 3H, H-3/H-6a/CH_{Linker}), 3.39 (ddd, $J_{H5,H6a}$ = 8.1 Hz, $J_{H5,H6a}$ = 5.1 Hz, 1H, H-5), 3.29–3.14 (m, 4H, H-6b/3 × CH_{Linker}), 2.25 (d, $J_{OH,H2'}$ = 2.5 Hz, 1H, -OH), 1.57–1.38 (m, 4H, CH_{Linker}), 1.26–1.14 (m, 2H, CH_{Linker}) ppm.

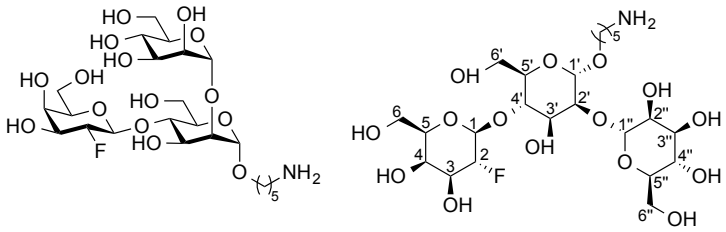
^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 139.0, 138.9, 138.7, 138.5, 138.4, 138.2, 138.1, 138.0, 137.0, 136.9 (10 × C_q), 128.7, 128.6, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 127.3, 127.1 (50 × C_{Ar}), 101.7 (d, $J_{C1,F}$ = 23.7 Hz, C-1), 101.1 (C-1''), 98.8 (C-1'), 93.0 (d, $J_{C2,F}$ = 182.3 Hz, C-2), 80.1 (d, $J_{C3,F}$ = 16.7 Hz, C-3), 80.1 (C-3''), 78.6 (C-3'), 75.8 (C-4'), 75.0 (C-2'/CH_{Bn}), 74.9 (CH_{Bn}), 74.5 (C-4''), 74.2 (d, $J_{C4,F}$ = 8.9 Hz, C-4), 73.5 (2 × CH_{Bn}), 73.4 (C-5), 73.3, 72.5, 72.4, 72.2 (4 × CH_{Bn}), 71.7 (C-5''), 71.3 (C-5'), 69.3 (C-6''), 69.1 (C-6'), 68.6 (C-2''), 68.1 (C-6), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 204.5 (dd, $J_{F,H2}$ = 53.6 Hz, $J_{F,H3}$ = 12.5 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{94}H_{106}FN_2O_{17}^+$ $[M+NH_4]^+$: 1554.7504, found 1554.7506.

RP-HPLC: t_R = 28.1 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-2-deoxy-2-fluorogalactopyranosyl)-α-D-mannopyranoside (8)



According to general procedure (**E**), fluorinated trisaccharide **102** (425 mg, 0.277 mmol) was globally deprotected by hydrogenolysis to give the corresponding trisaccharide **8** (142 mg, 0.240 mmol, 87%) as a colorless solid.

^{1}H-NMR (800 MHz, D_2O): δ = 5.12 (d, $J_{H1‘,H2‘}$ = 1.7 Hz, 1H, H-1‘), 5.02 (d, $J_{H1‘‘,H2‘‘}$ = 1.7 Hz, 1H, H-1‘‘), 4.73 (dd, $J_{H1,H2}$ = 7.8 Hz, $J_{H1,F}$ = 3.2 Hz, 1H, H-1), 4.38 (ddd, $J_{H2,F}$ = 51.9 Hz, $J_{H2,H3}$ = 9.3 Hz, $J_{H2,H1}$ = 7.8 Hz, 1H, H-2), 4.08 (dd, $J_{H2‘‘,H3‘‘}$ = 3.4 Hz, $J_{H2‘‘,H1‘‘}$ = 1.8 Hz, 1H, H-2‘‘), 4.02 (dd, $J_{H3‘,H4‘}$ = 9.4 Hz, $J_{H3‘,H2‘}$ = 3.2 Hz, 1H, H-3‘), 4.01–3.95 (m, 3H, H-3/H-4/H-2‘), 3.93 (t, $J_{H4‘,H3‘/H5‘}$ = 9.8 Hz, 1H, H-4‘), 3.92–3.87 (m, 2H, H-6a‘/H-6a‘‘), 3.85–3.81 (m, 2H, H-6b‘/H-3‘‘), 3.81–3.74 (m, 4H, H-5/H-6a/H-6b/H-5‘‘), 3.74–3.69 (m, 3H, H-5‘/H-6b‘‘/ CH_{Linker}), 3.60 (t, $J_{H4‘‘,H3‘‘/H5‘‘}$ = 9.8 Hz, 1H, H-4‘‘), 3.57–3.52 (m, 1H, CH_{Linker}), 2.99 (t, J = 7.6 Hz, 2H, CH_{Linker}), 1.71–1.61 (m, 4H, CH_{Linker}), 1.50–1.39 (m, 2H, CH_{Linker}) ppm.

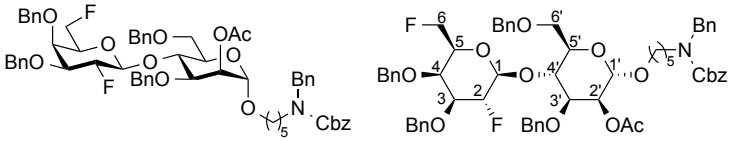
^{13}C-{^{1}H}-NMR (201 MHz, D_2O/CD_3OD): δ = 103.4 (C-1‘‘), 101.8 (d, $J_{C1,F}$ = 23.4 Hz, C-1), 99.0 (C-1‘), 92.8 (d, $J_{C2,F}$ = 180.9 Hz, C-2), 79.5 (C-2‘), 78.1 (C-4‘), 76.6 (C-5), 74.3 (C-5‘‘), 72.5 (C-5‘), 72.2 (d, $J_{C3,F}$ = 17.5 Hz, C-3), 71.4 (C-3‘‘), 71.0 (C-2‘‘), 70.2 (d, $J_{C4,F}$ = 10.6 Hz, C-4), 70.0 (C-3‘), 68.5 (CH_{Linker}), 68.0 (C-4‘‘), 62.2 (C-6‘‘), 61.9 (C-6), 61.0 (C-6‘), 40.4 (CH_{Linker}), 29.0 (CH_{Linker}), 27.6 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, D_2O): δ = − 207.0 (dd, $J_{F,H2}$ = 51.9 Hz, $J_{F,H3}$ = 14.9 Hz) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{23}H_{43}N_2O_{15}^+$ $[M+H]^+$: 592.2611, found 592.2609.

5.2.1.11 Synthesis of 5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-2,6-dideoxy-2,6-difluorogalactopyranosyl)-α-D-mannopyranoside 9

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4-di-*O*-benzyl-β-D-2,6-dideoxy-2,6-difluorogalactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (89)**



According to general procedure (**A**), fluorinated galactosyl imidate **16** (607 mg, 1.19 mmol, 1.4 equiv.) and mannosyl acceptor **12** (598 mg, 0.840 mmol, 1.0 equiv.) were reacted under inverse glycosylation conditions to give the corresponding disaccharide **89** (608 mg, 0.570 mmol, 69%) as a colorless oil.

R_f = 0.29 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 20.0 (c = 0.5, $CHCl_3$).

^{1}H-NMR (800 MHz, $CDCl_3$): δ = 7.41–7.17 (m, 30H, Ar-H), 5.25 (br s, 1H, H-2‘), 5.19 (d, J = 28.0 Hz, 2H, CH_{Cbz}), 4.94 (d, J = 11.3 Hz, 1H, CH_{Bn}), 4.80–4.76 (m, 1H, H-1‘), 4.75 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.68–4.56 (m, 7H, H-1/H-2/5 × CH_{Bn}), 4.53 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.50 (d, J = 19.9 Hz, 2H, NCH_{Bn}), 4.28 (ddd, $J_{H6a,F6}$ = 46.3 Hz, $J_{H6a,H6b}$ = 9.0 Hz, $J_{H6a,H5}$ = 7.1 Hz, 1H, H-6a), 4.20 (ddd, $J_{H6b,F6}$ = 46.7 Hz, $J_{H6b,H6a}$ = 9.0 Hz, $J_{H6b,H5}$ = 5.8 Hz, 1H, H-6b), 4.08 (t, $J_{H4‘,H3‘/H5‘}$ = 9.5 Hz, 1H, H-4‘), 3.96–3.90 (m, 1H, H-3‘), 3.87 (dd, $J_{H6a‘,H6b‘}$ = 10.6 Hz, $J_{H6a‘,H5‘}$ = 3.8 Hz, 1H, H-6a‘), 3.85–3.75 (m, 3H, H-4/H-5‘/H-6b‘), 3.65–3.55 (m, 1H, CH_{Linker}), 3.47 (ddd, $J_{H3,F2}$ = 12.3 Hz, $J_{H3,H2}$ = 9.3 Hz, $J_{H3,H4}$ = 2.9 Hz, 1H, H-3), 3.40–3.36 (m, 1H, H-5), 3.36–3.29 (m, 1H, CH_{Linker}), 3.28–3.17 (m, 2H, CH_{Linker}), 2.09 (s, 3H, CH_3-OAc), 1.57–1.44 (m, 4H, CH_{Linker}), 1.32–1.19 (m, 2H, CH_{Linker}) ppm.

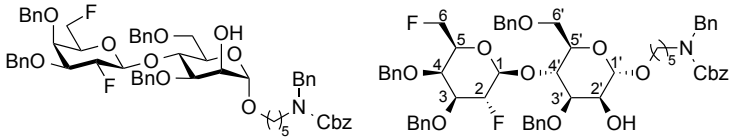
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.5 (C=O-OAc), 156.9/156.3 (C=O-Cbz), 138.7, 138.6, 138.2, 138.1, 138.0, 137.0 (6 × C_q), 128.7, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4 (30 × C_{Ar}), 101.4 (d, $J_{C1,F2}$ = 23.9 Hz, C-1), 97.6 (C-1‘), 92.9 (d, $J_{C2,F2}$ = 182.5 Hz, C-2), 80.9 (d, $J_{C6,F6}$ = 167.5 Hz, C-6), 79.8 (d, $J_{C3,F2}$ = 16.0 Hz, C-3), 76.8 (C-3‘), 75.3 (C-4‘), 75.0 (CH_{Bn}), 73.7 (dd, $J_{C4,F2}$ = 9.0 Hz, $J_{C4,F6}$ = 3.2 Hz, C-4), 73.5, 72.9 (2 × CH_{Bn}), 72.7 (d, $J_{C5,F6}$ = 24.9 Hz, C-5), 71.9 (CH_{Bn}), 70.8 (C-5‘), 69.1 (C-2‘), 68.9 (C-6‘), 67.8 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.7/50.4 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.2 (CH_3-OAc) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 204.9 (d, $J_{F2,H2}$ = 52.9 Hz, F-2), − 230.2 (t, $J_{F6,H6}$ = 45.8 Hz, F-6) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{62}H_{73}F_2N_2O_{12}^+$ $[M+NH_4]^+$: 1075.5126, found 1075.5130.

RP-HPLC: t_R = 15.1 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4-di-*O*-benzyl-β-D-2,6-dideoxy-2,6-difluorogalactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (93)**



The acetyl protecting group of fluorinated disaccharide **89** (620 mg, 0.586 mmol) was removed according to general procedure (**B**) to give the corresponding disaccharide **93** (481 mg, 0.473 mmol, 81%) as a colorless oil.

R_f = 0.20 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 21.6 (c = 1.0, $CHCl_3$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.41–7.21 (m, 30H, Ar-H), 5.19 (d, J = 9.3 Hz, 2H, CH_{Cbz}), 4.96 (d, J = 11.3 Hz, 1H, CH_{Bn}), 4.84 (br s, 1H, H-1'), 4.81–4.75 (m, 2H, CH_{Bn}), 4.72–4.54 (m, 6H, H-1/H-2/4 × CH_{Bn}), 4.54–4.46 (m, 3H, CH_{Bn}/2 × NCH_{Bn}), 4.28 (ddd, $J_{H6a/H6b,F6}$ = 46.6 Hz, $J_{H6a,H5}$ = 6.1 Hz, $J_{H6b,H5}$ = 5.0 Hz, 2H, H-6a/H-6b), 4.11 (t, $J_{H4',H3'/H5'}$ = 9.4 Hz, 1H, H-4'), 3.94 (br s, 1H, H-2'), 3.87 (dd, $J_{H6a',H6b'}$ = 10.8 Hz, $J_{H6a',H5'}$ = 4.1 Hz, 1H, H-6a'), 3.83 (t, $J_{H4,H3/H5}$ = 2.9 Hz, 1H, H-4), 3.81–3.73 (m, 3H, H-3'/H-5'/H-6b'), 3.72–3.55 (m, 1H, CH_{Linker}), 3.47 (dd, $J_{H3,H2}$ = 9.4 Hz, $J_{H3,H4}$ = 3.0 Hz, 1H, H-3), 3.45–3.31 (m, 2H, H-5/CH_{Linker}), 3.31–3.17 (m, 2H, CH_{Linker}), 2.49 (br s, 1H, -OH), 1.62–1.45 (m, 4H, CH_{Linker}), 1.36–1.19 (m, 2H, CH_{Linker}) ppm.

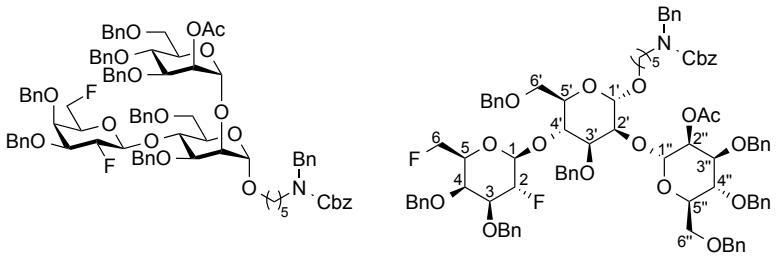
^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 156.9/156.30 (C=O-Cbz), 138.6, 138.2, 138.0 (6 × C_q), 128.7, 128.6, 128.4, 128.3, 128.0, 127.9, 127.6, 127.5 (30 × C_{Ar}), 101.4 (d, $J_{C1,F2}$ = 23.5 Hz, C-1), 99.1 (C-1'), 92.6 (d, $J_{C2,F2}$ = 182.9 Hz, C-2), 80.9 (d, $J_{C6,F6}$ = 167.3 Hz, C-6), 79.9 (d, $J_{C3,F2}$ = 16.1 Hz, C-3), 78.6 (C-3'), 75.2 (C-4'), 75.0 (CH_{Bn}), 73.9 (dd, $J_{C4,F2}$ = 9.1 Hz, $J_{C4,F6}$ = 3.4 Hz, C-4), 73.5 (CH_{Bn}), 72.9 (CH_{Bn}), 72.6 (d, $J_{C5,F6}$ = 25.3 Hz, C-5), 72.6 (CH_{Bn}), 70.7 (C-5'), 69.1 (C-2'), 68.7 (C-6'), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.7/50.4 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 204.5 (d, $J_{F2,H2}$ = 51.1 Hz, F-2), − 230.0 (t, $J_{F6,H6}$ = 48.0 Hz, F-6) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{60}H_{71}F_2N_2O_{11}^+$ $[M+NH_4]^+$: 1033.5020, found 1033.5026.

RP-HPLC: t_R = 10.3 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 30 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4-di-*O*-benzyl-β-D-2,6-dideoxy-2,6-difluorogalactopyranosyl)-α-D-mannopyranoside (99)**



According to general procedure (**C**), fluorinated disaccharide **93** (750 mg, 0.738 mmol, 1.0 equiv.) was reacted with mannosyl imidate **18** (940 mg, 1.48 mmol, 2.0 equiv.) to give the corresponding trisaccharide **99** (906 mg, 0.608 mmol, 82%) as a colorless oil.

R_f = 0.45 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 18.0 (c = 0.5, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.41–7.12 (m, 45H, Ar-H), 5.54 (dd, $J_{H2'',H3''}$ = 3.3 Hz, $J_{H2'',H1''}$ = 1.9 Hz, 1H, H-2''), 5.20 (d, J = 18.7 Hz, 2H, CH_{Cbz}), 5.03 (s, 1H, H-1''), 4.92 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.83 (d, J = 10.9 Hz, 1H, CH_{Bn}), 4.81 (s, 1H, H-1'), 4.80–4.75 (m, 2H, CH_{Bn}), 4.72–4.68 (m, 2H, H-1/CH_{Bn}), 4.68–4.56 (m, 6H, H-2/ 5 × CH_{Bn}), 4.55–4.43 (m, 5H, 3 × CH_{Bn}/2 × NCH_{Bn}), 4.36 (d, J = 10.8 Hz, 1H, CH_{Bn}), 4.24 (ddd, $J_{H6a,F6}$ = 46.2 Hz, $J_{H6a,H6b}$ = 9.0 Hz, $J_{H6a,H5}$ = 7.3 Hz, 1H, H-6a), 4.18 (ddd, $J_{H6b,F6}$ = 46.3 Hz, $J_{H6b,H6a}$ = 9.0 Hz, $J_{H6b,H5}$ = 5.8 Hz, 1H, H-6b), 4.09 (t, $J_{H4',H3'/H5'}$ = 9.4 Hz, 1H, H-4'), 3.98–3.91 (m, 3H, H-2'/H-3''/H-5''), 3.90–3.83 (m, 2H, H-3'/H-6a'), 3.83–3.74 (m, 5H, H-4/H-5'/H-6b'/H-4''/H-6a''), 3.71–3.67 (m, 1H, H-6b''), 3.60–3.51 (m, 2H, H-3/CH_{Linker}), 3.43–3.39 (m, 1H, H-5), 3.30–3.16 (m, 3H, CH_{Linker}), 2.08 (s, 3H, CH_3-OAc), 1.59–1.40 (m, 4H, CH_{Linker}), 1.33–1.14 (m, 2H, CH_{Linker}) ppm.

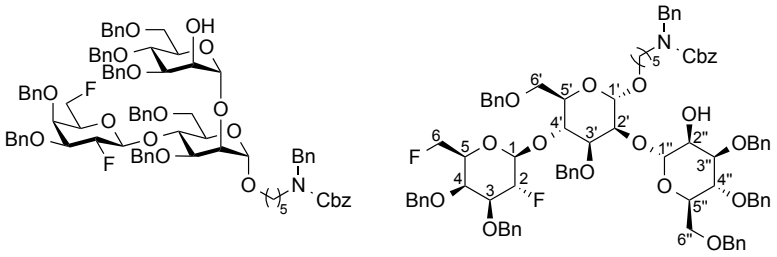
^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.1 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 138.9, 138.8, 138.6, 138.3, 138.2, 138.0, 137.0, 136.9 (9 × C_q), 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 127.3, 126.8 (45 × C_{Ar}), 101.7 (d, $J_{C1,F2}$ = 23.9 Hz, C-1), 99.6 (C-1''), 98.7 (C-1'), 92.9 (d, $J_{C2,F2}$ = 182.5 Hz, C-2), 80.6 (d, $J_{C6,F6}$ = 167.4 Hz, C-6), 79.9 (d, $J_{C3,F2}$ = 15.9 Hz, C-3), 78.5 (C-3'), 78.4 (C-3''), 75.9 (C-4'), 75.1, 74.9 (2 × CH_{Bn}), 74.7 (C-2'), 74.4 (C-4''), 73.7 (d, $J_{C4,F2}$ = 8.0 Hz, C-4), 73.5, 73.3, 72.9 (3 × CH_{Bn}), 72.6 (d, $J_{C5,F6}$ = 25.2 Hz, C-5), 72.1 (2 × CH_{Bn}), 71.9 (C-5''), 71.2 (C-5'), 69.1 (C-6'/C-6''), 68.6 (C-2''), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.5 (CH_{Linker}), 21.2 (CH_3-OAc) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 204.9 (d, $J_{F2,H2}$ = 49.4 Hz, F-2), − 230.40 (t, $J_{F6,H6}$ = 46.9 Hz, F-6) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{89}H_{101}F_2N_2O_{17}^+$ $[M+NH_4]^+$: 1507.7063, found 1507.7090.

RP-HPLC: t_R = 23.3 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4-di-*O*-benzyl-β-D-2,6-dideoxy-2,6-difluorogalactopyranosyl)-α-D-mannopyranoside (103)**



The acetyl protecting group of fluorinated trisaccharide **99** (817 mg, 0.518 mmol) was removed according to general procedure (**D**) to give the corresponding trisaccharide **103** (690 mg, 0.476 mmol, 92%) as a colorless oil.

R_f = 0.29 (cHex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 23.6 (c = 0.5, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.48–7.11 (m, 45H, Ar-H), 5.20 (d, J = 19.2 Hz, 2H, CH_{Cbz}), 5.10 (d, $J_{H1'',H2''}$ = 1.8 Hz, 1H, H-1''), 4.95 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.86 (s, 1H, H-1'), 4.82–4.77 (m, 2H, CH_{Bn}), 4.75 (d, J = 11.8 Hz, 1H, CH_{Bn}), 4.73–4.57 (m, 7H, H-1/H-2/5 × CH_{Bn}), 4.57–4.46 (m, 7H, 5 × CH_{Bn}/2 × NCH_{Bn}), 4.25 (ddd, $J_{H6a,F6}$ = 46.3 Hz, $J_{H6a,H6b}$ = 9.1 Hz, $J_{H6a,H5}$ = 6.7 Hz, 1H, H-6a), 4.18 (ddd, $J_{H6b,F6}$ = 46.4 Hz, $J_{H6b,H6a}$ = 9.1 Hz, $J_{H6b,H5}$ = 6.1 Hz, 1H, H-6b), 4.13–4.07 (m, 2H, H-4'/H-2''), 3.97–3.93 (m, 2H, H-2'/H-5''), 3.90–3.84 (m, 3H, H-3'/H-6a'/H-3''), 3.84–3.73 (m, 4H, H-4/H-5'/H-6b'/H-4''), 3.73–3.68 (m, 2H, H-6a''/H-6b''), 3.61–3.47 (m, 2H, H-3/CH_{Linker}), 3.44–3.39 (m, 1H, H-5), 3.31–3.14 (m, 3H, CH_{Linker}), 2.34 (d, $J_{OH,H2'}$ = 2.4 Hz, 1H, -OH), 1.57–1.39 (m, 4H, CH_{Linker}), 1.31–1.14 (m, 2H, CH_{Linker}) ppm.

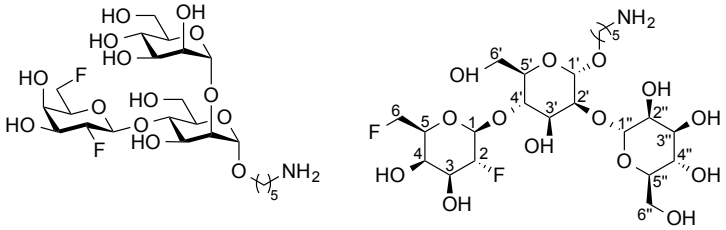
^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 156.8/156.27 (C=O-Cbz), 138.9, 138.5, 138.4, 138.2, 138.1, 138.0, 137.0, 136.9 (9 × C_q), 128.7, 128.5, 128.4, 128.3, 128.0, 127.9, 127.6, 127.4, 127.3, 127.2 (45 × C_{Ar}), 101.3 (d, $J_{C1,F}$ = 23.7 Hz, C-1), 101.1 (C-1''), 98.8 (C-1'), 92.9 (d, $J_{C2,F2}$ = 182.6 Hz, C-2), 81.1 (d, $J_{C6,F6}$ = 167.7 Hz, C-6), 80.1 (C-3''), 79.9 (d, $J_{C3,F2}$ = 15.8 Hz, C-3), 78.4 (C-3'), 75.6 (C-4'), 75.4 (C-2'), 75.0, 74.9 (2 × CH_{Bn}), 74.5 (C-4''), 73.8 (d, $J_{C4,F2}$ = 7.6 Hz, C-4), 73.5, 73.3, 72.9 (3 × CH_{Bn}), 72.8 (d, $J_{C5,F6}$ = 26.1 Hz, C-5), 72.5, 72.2 (2 × CH_{Bn}), 71.6 (C-5''), 71.3 (C-5'), 69.3 (C-6''), 69.1 (C-6'), 68.6 (C-2''), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, $CDCl_3$): δ = − 204.8 (d, $J_{F2,H2}$ = 52.0 Hz, F-2), − 229.9 (t, $J_{F6,H6}$ = 46.7 Hz, F-6) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{87}H_{95}F_2NO_{16}Na^+$ $[M+Na]^+$: 1470.6511, found 1470.6505.

RP-HPLC: t_R = 23.8 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-2,6-dideoxy-2,6-difluorogalactopyranosyl)-α-D-mannopyranoside (9)



According to general procedure (**E**), fluorinated trisaccharide **103** 593 mg, 0.409 mmol) was globally deprotected by hydrogenolysis to give the corresponding trisaccharide **9** (208 mg, 0.350 mmol, 86%) as a colorless solid.

^{1}H-NMR (800 MHz, D_2O): δ = 5.11 (s, 1H, H-1‘), 5.02 (s, 1H, H-1‘‘), 4.80–4.77 (m, 1H, H-1, under solvent peak), 4.73–4.57 (m, 2H, H-6a/H-6b), 4.40 (dt, $J_{H2,F}$ = 51.6 Hz, $J_{H2,H1/H3}$ = 8.8 Hz, 1H, H-2), 4.12–3.96 (m, 6H, H-3/H-4/ H-5/H-2‘/H-3‘/H-2‘‘), 3.95–3.86 (m, 3H, H-4‘/H-6a‘/H-6a‘‘), 3.85–3.80 (m, 2H, H-6b‘/H-3‘‘), 3.79–3.68 (m, 4H, H-5‘/H-5‘‘/H-6b‘‘/CH_{Linker}), 3.60 (t, $J_{H4‘‘,H3‘‘/H5‘‘}$ = 9.8 Hz, 1H, H-4‘‘), 3.57–3.52 (m, 1H, CH_{Linker}), 2.99 (t, J = 7.9 Hz, 2H, CH_{Linker}), 1.73–1.59 (m, 4H, CH_{Linker}), 1.50–1.40 (m, 2H, CH_{Linker}) ppm.

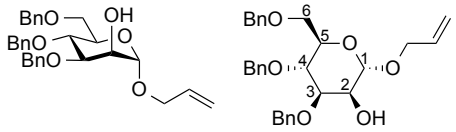
^{13}C-{^{1}H}-NMR (201 MHz, D_2O/CD_3OD): δ = 103.3 (C-1‘‘), 101.6 (d, $J_{C1,F}$ = 23.9 Hz, C-1), 98.9 (C-1‘), 92.5 (d, $J_{C2,F2}$ = 181.0 Hz, C-2), 83.7 (d, $J_{C6,F6}$ = 165.9 Hz, C-6), 79.3 (C-2‘), 78.5 (C-4‘), 74.6 (d, $J_{C5,F6}$ = 20.6 Hz, C-5), 74.3 (C-5‘‘), 72.3 (C-5‘), 71.9 (d, $J_{C3,F2}$ = 18.3 Hz, C-3), 71.3 (C-3‘‘), 70.9 (C-2‘‘), 69.9 (C-3‘), 69.7 (t, $J_{C4,F2/F6}$ = 8.8 Hz, C-4), 68.6 (CH_{Linker}), 67.9 (C-4‘‘), 62.2 (C-6‘‘), 61.0 (C-6‘), 40.3 (CH_{Linker}), 29.0 (CH_{Linker}), 27.5 (CH_{Linker}), 23.4 (CH_{Linker}) ppm.

^{19}F-NMR (376 MHz, D_2O): δ = − 207.2 (d, $J_{F2,H2}$ = 52.1 Hz, F-2), − 230.2 (t, $J_{F6,H6}$ = 47.0 Hz, F-6) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{23}H_{42}F_2NO_{14}^+$ $[M+H]^+$: 594.2568, found 594.2563.

5.2.1.12 Synthesis of 2-*O*-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate 23

Allyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (24)[263]



To a stirred solution of orthoester **46** (9.68 g, 19.1 mmol, 1.0 equiv.) in CH_2Cl_2 (100 mL) under argon were added freshly activated 4Å MS. Allylic alcohol (19.6 mL, 287 mmol, 15 equiv.) was added and the mixture was stirred at room temperature for 30 min. Then the reaction vessel was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (0.94 mL, 7.64mmol, 0.4 equiv.) was added. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Afterwards the mixture was diluted with CH_2Cl_2 (100 mL), filtrated over a pad of celite and washed with sat. aq. $NaHCO_3$ (2 × 100 mL). The organic phase was dried with $MgSO_4$ and solvents were removed under reduced pressure. The oily residue was dried under high vaccum and used for the next step without further purification. To a stirred solution of this compound in MeOH (100 mL) were added catalytic amounts of NaOMe at room temperature. The reaction mixture was stirred for 14 h and then neutralized by the addition of *Amberlite IR120*. The ion-exchange resin was then filtered off and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **24** (8.09 g, 16.5 mmol, 86%) as a colorless oil.

$\boldsymbol{R_f}$ = 0.45 (^{c}Hex/EtOAc, 2:1).

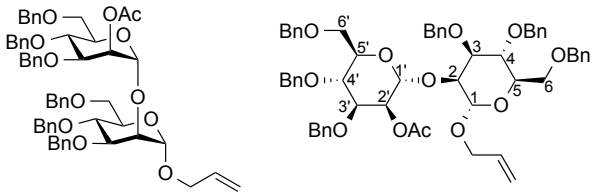
$\boldsymbol{[\alpha]_D^{22}}$ = + 49.6 (c = 1.0, $CHCl_3$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.42–7.19 (m, 15H, Ar-H), 5.92 (dddd, $J_{CH,CH2trans}$ = 17.2 Hz, $J_{CH,CH2cis}$ = 10.4 Hz, $J_{CH,CH2b}$ = 6.1 Hz, $J_{CH,CH2a}$ = 5.2 Hz, 1H, $CH_2C\boldsymbol{H}{=}CH_2$), 5.30 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.6 Hz, 1H, $CH_2CH{=}C\boldsymbol{H}_{2trans}$), 5.21 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.4 Hz, 1H, $CH_2CH{=}C\boldsymbol{H}_{2cis}$), 4.99 (d, $J_{H1,H2}$ = 1.7 Hz, 1H, H-1), 4.87 (d, J = 10.8 Hz, 1H, CH_{Bn}), 4.77–4.67 (m, 3H, CH_{Bn}), 4.60–4.52 (m, 2H, CH_{Bn}), 4.22 (ddt, $J_{CH2a,CH2b}$ = 12.9 Hz, $J_{CH2a,CH}$ = 5.2 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.5 Hz, 1H, $C\boldsymbol{H}_{2a}CH{=}CH_2$), 4.10 (td, $J_{H2,H3}$ = 2.8 Hz, $J_{H2,H1}$ = 1.7 Hz, 1H, H-2), 4.03 (ddt, $J_{CH2b,CH2a}$ = 12.9 Hz, $J_{CH2a,CH}$ = 6.1 Hz, $J_{CH2b,CH2cis/CH2trans}$ = 1.4 Hz, 1H, $C\boldsymbol{H}_{2b}CH{=}CH_2$), 3.95 (dd, $J_{H3,H4}$ = 8.6 Hz, $J_{H3,H2}$ = 3.2 Hz, 1H; H-3), 3.91 (t, $J_{H4,H3}$ = 8.9 Hz, 1H, H-4), 3.87–3.82 (m, 1H, H-5), 3.80–3.72 (m, 2H, H-6a/H-6b), 2.58 (d, $J_{OH,H2}$ = 2.7 Hz, 1H, -OH) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 138.4, 138.0 (3 × C_q), 133.8 ($CH_2\boldsymbol{C}H{=}CH_2$), 128.6, 128.4, 128.0, 127.9, 127.7, 127.6 (15 × C_{Ar}), 117.5 ($CH_2CH{=}\boldsymbol{C}H_2$), 98.5 (C-1), 80.3 (C-3), 75.2 (CH_{Bn}), 74.4 (C-4), 73.5, 72.1 (2 × CH_{Bn}), 71.2 (C-5), 69.0 (C-6), 68.5 (C-2), 68.0 ($\boldsymbol{C}H_2CH{=}CH_2$) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{30}H_{38}NO_6^+$ $[M+NH_4]^+$: 508.2699, found 508.2697.

Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (108)



According to general procedure (**C**), mannosyl acceptor **24** (4.00 g, 8.15 mmol, 1.0 equiv.) was reacted with mannosyl imidate **18** (6.75 g, 10.6 mmol, 1.3 equiv.) to give the corresponding disaccharide **108** (6.88 g, 7.13 mmol, 87%) as a colorless oil.

R_f = 0.62 (^{c}Hex/EtOAc, 3:1).

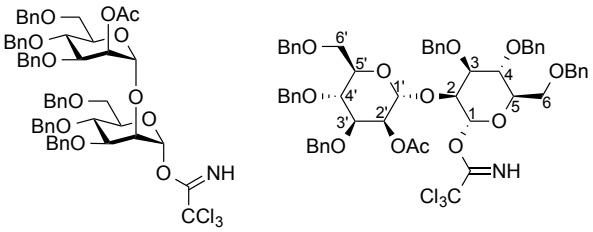
$[\alpha]_D^{22}$ = + 19.8 (c = 1.0, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.40–7.16 (m, 30H, Ar-H), 5.85 (dddd, $J_{CH,CH2trans}$ = 17.2 Hz, $J_{CH,CH2cis}$ = 10.4 Hz, $J_{CH,CH2b}$ = 6.0 Hz, $J_{CH,CH2a}$ = 5.1 Hz, 1H, CH_2CH=CH_2), 5.58 (dd, $J_{H2',H3'}$ = 3.3 Hz, $J_{H2',H1'}$ = 1.9 Hz, 1H, H-2'), 5.23 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.7 Hz, 1H, CH_2CH=CH_{2trans}), 5.15 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.4 Hz, 1H, CH_2CH=CH_{2cis}), 5.12 (d, $J_{H1',H2'}$ = 1.8 Hz, 1H, H-1'), 4.96 (d, $J_{H1,H2}$ = 1.9 Hz, 1H, H-1), 4.91–4.86 (m, 2H, CH_{Bn}), 4.73–4.67 (m, 5H, CH_{Bn}), 4.61–4.54 (m, 2H, CH_{Bn}), 4.54–4.48 (m, 2H, CH_{Bn}), 4.44 (d, J = 10.9 Hz, 1H, CH_{Bn}), 4.12 (ddt, $J_{CH2a,CH2b}$ = 13.0 Hz, $J_{CH2a,CH}$ = 5.1 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.6 Hz, 1H, $CH_{2a}CH$=CH_2), 4.07 (dd, $J_{H2,H3}$ = 3.1 Hz, $J_{H2,H1}$ = 1.9 Hz, 1H, H-2), 4.02 (dd, $J_{H3',H4'}$ = 9.4 Hz, $J_{H3',H2'}$ = 3.3 Hz, 1H, H-3'), 4.00–3.96 (m, 2H, H-3/H-5'), 3.91–3.85 (m, 3H, H-4/H-4'/$CH_{2b}CH$=CH_2), 3.83–3.77 (m, 3H, H-5/H-6a/H-6a'), 3.76–3.71 (m, 2H, H-6b/H-6b'), 2.15 (s, 3H, CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.1 (C=O-OAc), 138.5, 138.4, 138.2, 138.0 (6 × C_q), 133.8 (CH_2CH=CH_2), 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.6, 127.5, 127.4 (30 × C_{Ar}), 117.2 (CH_2CH=CH_2), 99.6 (C-1'), 97.9 (C-1), 79.7 (C-3), 78.2 (C-3'), 75.2, 75.1 (2 × CH_{Bn}), 74.8 (C-2), 74.7 (C-4), 74.4 (C-4'), 73.4, 73.3, 72.1 (3 × CH_{Bn}), 71.9 (C-5/CH_{Bn}), 71.8 (C-5'), 69.3 (C-6), 69.0 (C-6'), 68.8 (C-2'), 67.9 (CH_2CH=CH_2), 21.2 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{59}H_{68}NO_{12}^+$ $[M+NH_4]^+$: 982.4742, found 982.4741.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (23)[161, 180]



Dimannoside **108** (6.08 g, 6.30 mmol, 1.0 equiv.) was mixed with NaOAc (1.89 g, 23.1 mmol, 3.7 equiv.) and $PdCl_2$ (1.90 g, 10.7 mmol, 1.7 equiv.). The mixture was then dissolved in AcOH (47.5 mL) and H_2O dest. (2.5 mL) and it was stirred at room temperature for 20 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and carefully neutralized with sat. aq. $NaHCO_3$ (150 mL) and solid $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic layers were washed with brine (2 × 100 mL). The organic phase was dried with $MgSO_4$ and solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give the lactol (4.01 g mixture of anomers, 4.33 mmol, 69%) as a colorless oil. To a solution of this compound (3.80 g, 4.17 mmol, 1.0 equiv.) in CH_2Cl_2 (50 mL) at 0 °C was added trichloroacetonitrile (2.09 mL, 20.8 mmol, 5.0 equiv.) and DBU (0.154 mL, 1.04 mmol, 0.25 equiv.) in sequential order. The mixture was allowed to warm to room temperature and stirred for 21 h. Then sat. aq. NH_4Cl (30 mL) was added and the aqeous phase was extracted with CH_2Cl_2 (80 mL). The combined organic layers were washed with brine (2 × 50 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 5:1, 1% NEt_3) to give **23** (2.83 g, 2.65 mmol, 64%) as a colorless oil.

R_f = 0.58 (^{c}Hex/EtOAc, 3:1).

$[\alpha]_D^{22}$ = + 37.4 (c = 1.0, $CHCl_3$).

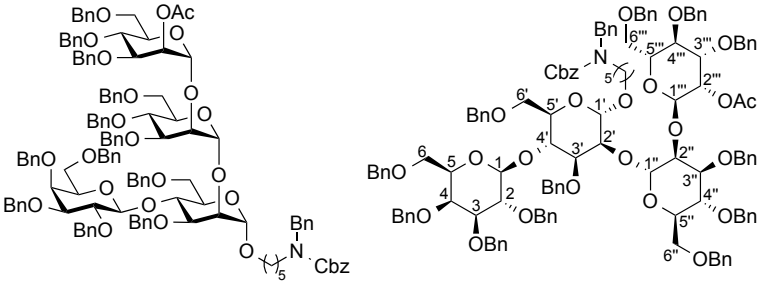
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.50 (s, 1H, C=NH), 7.39–7.11 (m, 30H, Ar-H), 6.32 (d, $J_{H1,H2}$ = 2.1 Hz, 1H, H-1), 5.56 (dd, $J_{H2',H3'}$ = 3.1 Hz, $J_{H2',H1'}$ = 1.8 Hz, 1H, H-2‘), 5.14 (d, $J_{H1',H2'}$ = 1.8 Hz, 1H, H-1‘), 4.88 (d, J = 10.7 Hz, 1H, CH_{Bn}), 4.84 (d, J = 10.9 Hz, 1H, CH_{Bn}), 4.78–4.55 (m, 6H, CH_{Bn}), 4.52–4.44 (m, 3H, CH_{Bn}), 4.40 (d, J = 10.9 Hz, 1H, CH_{Bn}), 4.09 (t, $J_{H2,H1/H3}$ = 2.5 Hz, 1H, H-2), 4.07–3.89 (m, 6H, H-3/H-4/H-5/H-3‘/H-4‘/H-5‘), 3.86–3.78 (m, 2H, H-6a/H-6a‘), 3.75–3.67 (m, 2H, H-6b/H-6b‘), 2.14 (s, 3H, CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.3 (C=O-OAc), 160.1 (C=NH), 138.6, 138.5, 138.4, 138.3, 138.1 (6 × C_q), 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.5 (30 × C_{Ar}), 99.7 (C-1‘), 96.9 (C-1), 91.0 (CCl_3), 78.7 (C-3), 78.3 (C-3‘), 75.5, 75.2 (2 × CH_{Bn}), 74.9 (C-4), 74.3 (C-4‘), 74.0 (C-5), 73.5, 73.4 (2 × CH_{Bn}), 73.3 (C-2), 72.6 (CH_{Bn}), 72.1 (C-5‘/CH_{Bn}), 68.8 (C-6/C-2‘/C-6‘), 21.2 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{58}H_{64}Cl_3N_2O_{12}^+$ $[M+NH_4]^+$: 1085.3525, found 1085.3523.

5.2.1.13 Synthesis of 5-Aminopentyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside 5

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (25)**[264]



Disaccharide acceptor **22** (460 mg, 0.390 mmol, 1.0 equiv.) and dimannosyl donor **23** (631 mg, 0.590 mmol, 1.5 equiv.) were combined and co-evaporated with toluene (3 × 8 mL) and CH_2Cl_2 (8 mL). The mixture was dried under high vacuum and then dissolved in Et_2O (6.5 mL) under argon. The solution was stirred with freshly activated 4Å MS (0.5 g) at room temperature for 30 min. The mixture was cooled to −25 °C, TMSOTf (21.2 µL, 0.117 mmol, 0.3 equiv.) was added dropwise and the mixture was stirred for 15 min at the same temperature. The reaction mixture was diluted with CH_2Cl_2 (20 mL), quenched by the addition of NEt_3 and filtered through a pad of celite. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 10:1, 1% NEt_3) to give **25** (429 mg, 0.204 mmol, 52%) as a colorless oil.

R_f = 0.45 (^{c}Hex/EtOAc = 3:1).

$[\alpha]_D^{25}$ = + 16.5 (c = 0.5, $CHCl_3$).

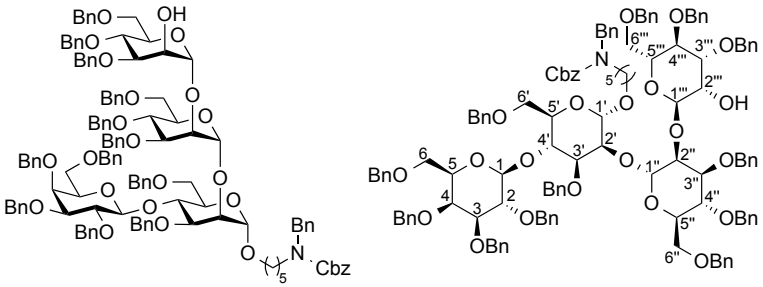
^{1}H-NMR (800 MHz, $CDCl_3$): δ = 7.43–7.08 (m, 70H, Ar-H), 5.50 (dd, $J_{H2'''',H3''''}$ = 3.2 Hz, $J_{H2'''',H1''''}$ = 2.0 Hz, 1H, H-2‘‘‘), 5.18 (d, J = 19.7 Hz, 2H, CH_{Cbz}), 5.12 (d, $J_{H1'''',H2''''}$ = 1.9 Hz, 1H, H-1‘‘), 4.95 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.92 (d, $J_{H1'''',H2''''}$ = 1.9 Hz, 1H, H-1‘‘‘), 4.91 (s, 1H, H-1‘), 4.87–4.79 (m, 3H, CH_{Bn}), 4.77–4.71 (m, 2H, CH_{Bn}), 4.71–4.62 (m, 4H, CH_{Bn}), 4.61–4.55 (m, 5H, H-1/4 × CH_{Bn}), 4.54 (d, J = 10.9 Hz, 1H, CH_{Bn}), 4.52–4.42 (m, 7H, 5 × CH_{Bn}/2 × NCH_{Bn}), 4.41 (d, J = 11.1 Hz, 1H, CH_{Bn}), 4.28 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.26 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.20 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.13–4.08 (m, 1H, H-4‘), 4.04–4.02 (m, 1H, H-2‘‘), 4.01–3.94 (m, 3H, H-2‘/H-5‘‘/H-3‘‘‘), 3.94–3.85 (m, 4H, H-4/H-3‘‘/H-4‘‘‘/H-5‘‘‘), 3.85–3.77 (m, 3H, H-3‘/H-6a‘/H-4‘‘), 3.77–3.69 (m, 5H, H-2/H-5‘/H-6b‘/H-6a‘‘/ H-6b‘‘), 3.64–3.59 (m, 2H, H-6a/H-6a‘‘‘), 3.58–3.49 (m, 1H, CH_{Linker}), 3.47–3.42 (m, 2H, H-3/H-6b‘‘‘), 3.39–3.33 (m, 2H, H-5/H-6b), 3.26–3.14 (m, 3H, CH_{Linker}), 2.11 (s, 3H, CH_3-OAc), 1.57–1.42 (m, 4H, CH_{Linker}), 1.26–1.15 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (201 MHz, $CDCl_3$): δ = 170.1 (C=O-OAc), 156.8/156.23 (C=O-Cbz), 139.4, 139.1, 138.9, 138.8, 138.6, 138.2, 138.1, 138.0, 137.0, 136.9 (14 × C_q), 128.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.1 (70 × C_{Ar}), 103.0 (C-1), 101.1 (C-1‘‘), 99.5 (C-1‘‘‘), 98.7 (C-1‘), 82.9 (C-3), 80.2 (C-2), 79.7 (C-3‘‘), 78.2 (C-3‘‘‘), 77.7 (C-3‘), 76.8 (C-2‘), 75.3 (C-2‘‘), 75.2, 75.1 (3 × CH_{Bn}), 74.9 (C-4‘), 74.7 (C-4‘‘/CH_{Bn}), 74.3 (C-4‘‘‘), 73.4 (C-4/CH_{Bn}), 73.3, 73.1 (3 × CH_{Bn}), 72.8 (C-5), 72.6 (CH_{Bn}), 72.3 (C-5‘‘/CH_{Bn}), 72.0 (C-5‘‘‘/2 × CH_{Bn}), 71.7 (C-5‘), 69.7 (C-6‘‘), 69.1 (C-6‘), 68.8 (C-2‘‘‘/C-6‘‘‘), 68.3 (C-6), 67.6 (CH_{Linker}), 67.2 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.4 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.3 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{130}H_{147}N_3O_{24}^{2+}$ $[M+2NH_4]^{2+}$: 1067.5199, found 1067.5199.

RP-HPLC: t_R = 42.6 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (109)**



The acetyl protecting group of tetrasaccharide **25** (348 mg, 0.166 mmol) was removed according to general procedure (**D**) to give the corresponding tetrasaccharide **109** (239 mg, 0.116 mmol, 70%) as a colorless oil.

R_f = 0.14 (^{c}Hex/EtOAc = 3:1).

$[\alpha]_D^{25}$ = + 22.0 (c = 0.5, $CHCl_3$).

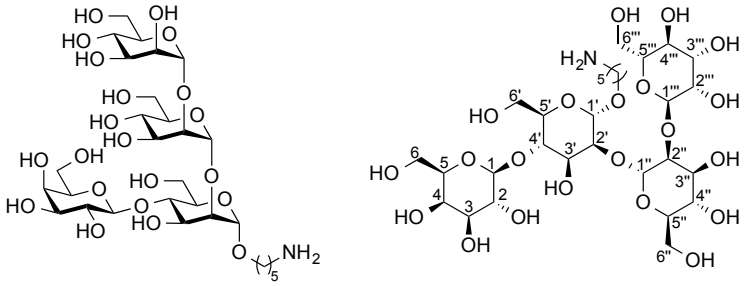
^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.39–7.10 (m, 70H, Ar-H), 5.20–5.15 (m, 3H, H-1‘‘/2 × CH_{Cbz}), 4.97 (d, $J_{H1‘‘‘,H2‘‘‘}$ = 1.8 Hz, 1H, H-1‘‘‘), 4.94 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.90 (d, $J_{H1‘,H2‘}$ = 2.0 Hz, 1H, H-1‘), 4.83–4.78 (m, 3H, CH_{Bn}), 4.76–4.63 (m, 5H, CH_{Bn}), 4.60–4.53 (m, 7H, H-1/6 × CH_{Bn}), 4.53–4.44 (m, 5H, 3 × CH_{Bn}/2 × NCH_{Bn}), 4.44–4.38 (m, 3H, CH_{Bn}), 4.30 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.25 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.19 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.10 (d, $J_{H4‘,H3‘/H5‘}$ = 9.3 Hz, 1H, H-4‘), 4.07–4.05 (m, 1H, H-2‘‘‘), 4.04–4.02 (m, 1H, H-2‘‘), 3.99–3.94 (m, 2H, H-2‘/H-5‘‘), 3.93–3.89 (m, 2H, H-4/H-3‘‘), 3.89–3.83 (m, 3H, H-3‘‘‘/H-4‘‘‘/H-5‘‘‘), 3.83–3.73 (m, 5H, H-2/H-3‘/H-6a‘/H-4‘‘/ H-6a‘‘), 3.73–3.68 (m, 3H, H-5‘/H-6b‘/H-6b‘‘), 3.63–3.43 (m, 5H, H-3/H-6a/H-6a‘‘‘/H-6b‘‘‘/CH_{Linker}), 3.41–3.34 (m, 2H, H-5/H-6b), 3.26–3.12 (m, 3H, CH_{Linker}), 2.26 (s, 1H, -OH), 1.57–1.41 (m, 4H, CH_{Linker}), 1.28–1.13 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 139.4, 139.1, 139.0, 138.9, 138.7, 138.6, 138.5, 138.4, 138.2, 138.1, 137.0, 136.9 (14 × C_q), 128.7, 128.5, 128.5, 128.4, 128.3, 128.0, 127.8, 127.6, 127.5, 127.3, 127.3, 127.1 (70 × C_{Ar}), 103.1 (C-1), 101.2 (C-1‘‘), 101.1 (C-1‘‘‘), 98.7 (C-1‘), 82.9 (C-3), 80.2 (C-2), 80.0 (C-3‘‘‘), 79.8 (C-3‘‘), 77.7 (C-3‘), 77.0 (C-2‘), 75.4 (C-2‘‘), 75.3, 75.1 (3 × CH_{Bn}), 74.9 (C-4‘/C-4‘‘), 74.7 (CH_{Bn}), 74.4 (C-4‘‘‘), 73.4 (C-4/CH_{Bn}), 73.3, 73.1 (3 × CH_{Bn}), 72.8 (C-5), 72.6, 72.4 (3 × CH_{Bn}), 72.3 (C-5‘‘), 72.2 (CH_{Bn}), 71.7 (C-5‘/C-5‘‘‘), 69.7 (C-6‘‘), 69.1 (C-6‘), 69.0 (C-6‘‘‘), 68.7 (C-2‘‘‘), 68.4 (C-6), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.4 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{128}H_{145}N_3O_{23}^{2+}$ $[M+2NH_4]^{2+}$: 1046.5151, found 1046.5152.

RP-HPLC: t_R = 26.5 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (90:10) 5 min, →(100:0) 20 min, →(100:0) 35 min.

5-Aminopentyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside (5)



According to general procedure (**E**), tetrasaccharide **109** (185 mg, 89.9 μmol) was globally deprotected by hydrogenolysis to give the corresponding tetrasaccharide **5** (63.0 mg, 83.8 μmol, 93%) as a colorless solid.

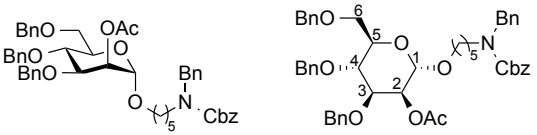
^{1}H-NMR (800 MHz, D_2O): δ = 5.29 (d, $J_{H1'',H2''}$ = 1.9 Hz, 1H, H-1''), 5.09 (d, $J_{H1',H2'}$ = 1.8 Hz, 1H, H-1'), 5.02 (d, $J_{H1''',H2'''}$ = 1.8 Hz, 1H, H-1'''), 4.43 (d, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1), 4.11 (dd, $J_{H2'',H3''}$ = 3.3 Hz, $J_{H2'',H1''}$ = 1.9 Hz, 1H, H-2''), 4.05 (dd, $J_{H2''',H3'''}$ = 3.4 Hz, $J_{H2''',H1'''}$ = 1.9 Hz, 1H, H-2'''), 4.00 (dd, $J_{H3',H4'}$ = 9.3 Hz, $J_{H3',H2'}$ = 3.3 Hz, 1H, H-3'), 3.97 (dd, $J_{H2',H3'}$ = 3.4 Hz, $J_{H2',H1'}$ = 1.8 Hz, 1H, H-2'), 3.96–3.92 (m, 2H, H-6a'/H-3''), 3.91 (d, $J_{H4,H3/H5}$ = 3.4 Hz, 1H, H-4), 3.90–3.83 (m, 3H, H-6a/H-4'/H-6b'), 3.82 (dd, $J_{H3''',H4'''}$ = 9.7 Hz, $J_{H3''',H2'''}$ = 3.4 Hz, 1H, H-3'''), 3.81–3.75 (m, 3H, H-6b/H-5''/H-5'''), 3.75–3.70 (m, 7H, H-5/H-5'/H-6a''/ H-6b''/H-6a'''/H-6b'''/CH_{Linker}), 3.67–3.63 (m, 2H, H-3/H-4''), 3.60 (t, $J_{H4''',H3'''/H5'''}$ = 9.8 Hz, 1H, H-4'''), 3.55–3.51 (m, 2H, H-2/CH_{Linker}), 2.99 (t, J = 7.7 Hz, 2H, CH_{Linker}), 1.71–1.61 (m, 4H, CH_{Linker}), 1.48–1.39 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, D_2O/CD_3OD): δ = 104.2 (C-1), 103.3 (C-1'''), 101.7 (C-1''), 99.0 (C-1'), 79.8 (C-2''), 79.3 (C-2'), 78.0 (C-4'), 76.4 (C-5), 74.4 (C-5''/C-5'''), 74.3 (C-5''/C-5'''), 73.8 (C-3), 72.5 (C-5'), 72.1 (C-2), 71.5 (C-3'''), 71.1 (C-3''/C-2'''), 70.2 (C-3'), 69.7 (C-4), 68.5 (CH_{Linker}), 68.3 (C-4''), 68.0 (C-4'''), 62.3 (C-6''/C-6'''), 62.1 (C-6), 61.4 (C-6'), 40.4 (CH_{Linker}), 29.1 (CH_{Linker}), 27.6 (CH_{Linker}), 23.6 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{29}H_{54}N_2O_{21}^{+}$ $[M+H]^{+}$: 752.3187, found 752.3187.

5.2.1.14 Synthesis of 5-Aminopentyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside 4

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (104)**[160]



Mannosyl imidate **18** (2.60 g, 4.08 mmol, 1.0 equiv.) and *N*-Benzyl-*N*-benzyloxycarbonyl-5-aminopentanol **55** (1.93 g, 5.89 mmol, 1.4 equiv.) were combined and co-evaporated with toluene (3 × 10 mL) and CH_2Cl_2 (10 mL). The mixture was dried under high vacuum and then dissolved in CH_2Cl_2 (40 mL) under argon. The solution was stirred with freshly activated 4Å MS (2.5 g) at room temperature for 30 min. The reaction vessel was cooled to 0 °C, TMSOTf (0.94 mL, 5.21 mmol, 1.3 equiv.) was added dropwise and the mixture was stirred for 15 min at the same temperature. The reaction mixture was diluted with CH_2Cl_2 (40 mL), quenched by the addition of NEt_3 and filtered through a pad of celite. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1, 1% NEt_3) to give **104** (2.94 g, 3.67 mmol, 90%) as a colorless oil.

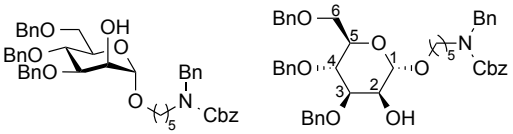
R_f = 0.53 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.45–7.18 (m, 25H, Ar-H), 5.40 (s, 1H, H-2), 5.22 (d, J = 20.5 Hz, 2H, CH_{Cbz}), 4.91 (d, J = 10.7 Hz, 1H, CH_{Bn}), 4.88–4.84 (m, 1H, H-1), 4.75 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.73 (d, J = 12.1 Hz, 1H, CH_{Bn}), 4.60–4.51 (m, 5H, 3 × CH_{Bn}/2 × NCH_{Bn}), 4.05–3.99 (m, 1H, H-3), 3.94 (t, $J_{H4,H3/H5}$ = 9.5 Hz, 1H, H-4), 3.88–3.78 (m, 2H, H-5/H-6a), 3.77–3.73 (m, 1H, H-6b), 3.72–3.60 (m, 1H, CH_{Linker}), 3.45–3.34 (m, 1H, CH_{Linker}), 3.34–3.20 (m, 2H, CH_{Linker}), 2.19 (s, 3H, CH_3-OAc), 1.66–1.49 (m, 4H, CH_{Linker}), 1.39–1.22 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.5 (C=O-OAc), 156.7/156.2 (C=O-Cbz), 138.4, 138.3, 138.0, 136.9, 136.8 (5 × C_q), 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.6, 127.3, 127.2 (25 × C_{Ar}), 97.8 (C-1), 78.3 (C-3), 75.3 (CH_{Bn}), 74.4 (C-4), 73.5, 71.8 (2 × CH_{Bn}), 71.4 (C-5), 68.9 (C-2/C-6), 67.7 (CH_{Linker}), 67.2 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.1/46.2 (CH_{Linker}), 29.1 (CH_{Linker}), 28.0/27.5 (CH_{Linker}), 23.4 (CH_{Linker}), 21.2 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{49}H_{59}N_2O_{19}^+$ $[M+NH_4]^+$: 819.4221, found 819.4217.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (19)**



The acetyl protecting group of mannoside **104** (2.78 g, 3.47 mmol) was removed according to general procedure (**D**) to give the corresponding mannosyl acceptor **19** (2.05 mg, 2.70 mmol, 79%) as a colorless oil.

R_f = 0.32 (^{c}Hex/EtOAc, 2:1).

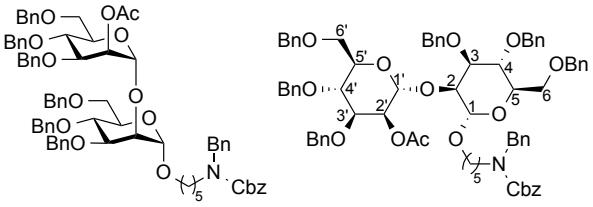
$[\alpha]_D^{22}$ = + 29.4 (c = 1.0, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.40–7.16 (m, 25H, Ar-H), 5.19 (d, J = 18.0 Hz, 2H, CH_{Cbz}), 4.89–4.85 (m, 1H, H-1), 4.83 (d, J = 10.8 Hz, 1H, CH_{Bn}), 4.74–4.63 (m, 3H, CH_{Bn}), 4.56–4.46 (m, 4H, 2 × CH_{Bn}/2 × NCH_{Bn}), 4.02 (s, 1H, H-2), 3.91–3.82 (m, 2H, H-3/H-4), 3.79–3.73 (m, 2H, H-5/H-6a), 3.73–3.57 (m, 2H, H-6b/CH_{Linker}), 3.43–3.15 (m, 3H, CH_{Linker}), 2.47 (d, $J_{OH,H2}$ = 2.6 Hz, 1H, -OH), 1.60–1.46 (m, 4H, CH_{Linker}), 1.33–1.21 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 138.4, 138.1, 138.0, 137.0, 136.9 (5 × C_q), 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.4, 127.3 (25 × C_{Ar}), 99.2 (C-1), 80.4 (C-3), 75.3 (CH_{Bn}), 74.5 (C-4), 73.6, 72.1 (2 × CH_{Bn}), 71.2 (C-5), 69.1 (C-6), 68.5 (C-2), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.2 (CH_{Linker}), 28.0/27.6 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{49}H_{59}N_2O_{19}^+$ $[M+NH_4]^+$: 777.4115, found 777.4110.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (105)**



According to general procedure (**C**), mannosyln acceptor **19** (1.90 g, 2.50 mmol, 1.0 equiv.) was reacted with mannosyl imidate **18** (2.39 g, 3.75 mmol, 1.5 equiv.) to give the corresponding disaccharide **105** (2.73 g, 2.21 mmol, 88%) as a colorless oil.

R_f = 0.63 (^{c}Hex/EtOAc, 2:1).

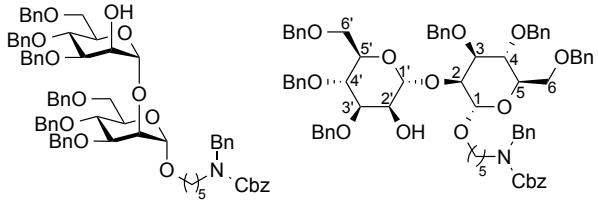
$[\alpha]_D^{22}$ = + 17.4 (c = 1.0, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.40–7.16 (m, 40H, Ar-H), 5.57 (dd, $J_{H2',H3'}$ = 3.3 Hz, $J_{H2',H1'}$ = 1.9 Hz, 1H, H-2'), 5.20 (d, J = 17.6 Hz, 2H, CH_{Cbz}), 5.11 (d, $J_{H1',H2'}$ = 1.8 Hz, 1H, H-1'), 4.90–4.85 (m, 3H, H-1/2 × CH_{Bn}), 4.72–4.65 (m, 5H, 5 × CH_{Bn}), 4.58 (d, J = 10.8 Hz, 1H, CH_{Bn}), 4.56 (d, J = 12.0 Hz, 1H, CH_{Bn}), 4.54–4.47 (m, 4H, 2 × CH_{Bn}/ 2 × NCH_{Bn}), 4.43 (d, J = 10.9 Hz, 1H, CH_{Bn}), 4.03–3.97 (m, 3H, H-2/H-3'/H-5'), 3.94–3.89 (m, 1H, H-3), 3.88–3.83 (m, 2H, H-4/H-4'), 3.82–3.76 (m, 2H, H-6a/H-6a'), 3.75–3.69 (m, 3H, H-5/H-6b/H6b'), 3.63–3.51 (m, 1H, CH_{Linker}), 3.31–3.15 (m, 3H, CH_{Linker}), 2.14 (s, 3H, CH_3-OAc), 1.57–1.43 (m, 4H, CH_{Linker}), 1.29–1.15 (m, 3H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.2 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 138.6, 138.5, 138.3, 138.1, 138.0, 137.0, 136.9 (8 × C_q), 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3 (40 × C_{Ar}), 99.7 (C-1'), 98.8 (C-1), 79.9 (C-3), 78.2 (C-3'), 75.3, 75.2 (2 × CH_{Bn}), 75.1 (C-2), 74.8 (C-4), 74.5 (C-4'), 73.5, 73.4, 72.2, 72.0 (4 × CH_{Bn}), 71.9 (C-5/C-5'), 69.4 (C-6), 69.2 (C-6'), 68.8 (C-2'), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.6, (CH_{Linker}) 23.5 (CH_{Linker}), 21.3 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{76}H_{87}N_2O_{14}^+$ $[M+NH_4]^+$: 1251.6157, found 1251.6149.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (20)**



The acetyl protecting group of disaccharide **105** (2.73 g, 2.21 mmol) was removed according to general procedure (**D**) to give the corresponding disaccharide **20** (1.76 g, 1.48 mmol, 67%) as a colorless oil.

R_f = 0.40 (^{c}Hex/EtOAc, 2:1).

$[\alpha]_D^{22}$ = + 26.4 (c = 0.5, $CHCl_3$).

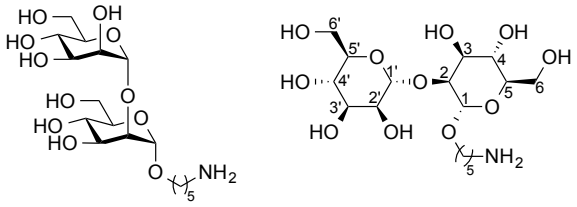
^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.41–7.14 (m, 40H, Ar-H), 5.18 (d, J = 17.4 Hz, 2H, CH_{Cbz}), 5.15 (d, $J_{H1',H2'}$ = 1.7 Hz, 1H, H-1'), 4.89 (d, $J_{H1,H2}$ = 1.9 Hz, 1H, H-1), 4.87–4.81 (m, 2H, CH_{Bn}), 4.72–4.66 (m, 3H, CH_{Bn}), 4.66–4.62 (m, 1H, CH_{Bn}), 4.60 (d, J = 11.2 Hz, 1H, CH_{Bn}), 4.58–4.46 (m, 7H, 5 × CH_{Bn}/2 × NCH_{Bn}), 4.14 (dd, $J_{H2',H3'}$ = 3.1 Hz, $J_{H2',H1'}$ = 1.8 Hz, 1H, H-2'), 4.02 (d, $J_{H2,H3}$ = 3.3 Hz, 1H, H-2), 3.98 (ddd, $J_{H5',H4'}$ = 9.9 Hz, $J_{H5',H6a'}$ = 4.8 Hz, $J_{H5',H6b'}$ = 2.3 Hz, 1H, H-5'), 3.94–3.87 (m, 2H, H-3/H-3'), 3.86–3.78 (m, 3H, H-4/H-6a/H-4'), 3.77–3.69 (m, 4H, H-5/H-6b/H-6a'/H-6b'), 3.61–3.47 (m, 1H, CH_{Linker}), 3.29–3.14 (m, 3H, CH_{Linker}), 2.40 (s, 1H, -OH), 1.56–1.38 (m, 4H, CH_{Linker}), 1.28–1.13 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 138.8, 138.6, 138.5, 138.4, 138.1, 138.0, 137.0, 136.9 (8 × C_q), 128.9, 128.7, 128.6, 128.4, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3

(40 × C_{Ar}), 101.2 (C-1‘), 98.9 (C-1), 80.1 (C-3‘), 80.0 (C-3), 75.3 (CH_{Bn}), 75.2 (C-2), 75.1 (CH_{Bn}), 75.0 (C-4), 74.6 (C-4‘), 73.5, 73.4, 72.4, 72.3 (4 × CH_{Bn}), 72.0 (C-5), 71.7 (C-5‘), 69.5 (C-6), 69.4 (C-6‘), 68.7 (C-2‘), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.4 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{74}H_{85}N_2O_{13}^+$ $[M+NH_4]^+$: 1209.6052, found 1209.6043.

5-Aminopentyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (4)



According to general procedure (**E**), disaccharide **20** (330 mg, 0.277 mmol) was globally deprotected by hydrogenolysis to give the corresponding disaccharide **4** (118 mg, 0.264 mmol, 95%) as a colorless solid.

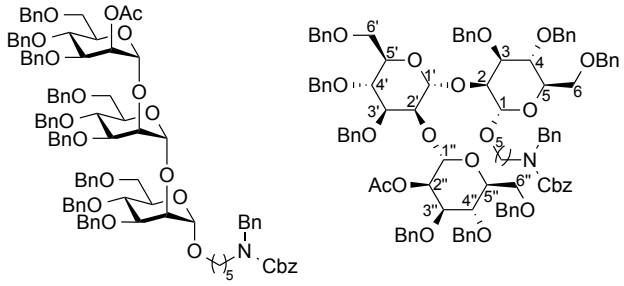
^{1}H-NMR (800 MHz, D_2O): δ = 5.09 (s, 1H, H-1), 5.01 (s, 1H, H-1‘), 4.06 (s, 1H, H-2‘), 3.94 (s, 1H, H-2), 3.92–3.85 (m, 3H, H-3/H-6a/H-6a‘), 3.85–3.81 (m, 1H, H-3‘), 3.79–3.64 (m, 5H, H-4/H-6b/H-5‘/H-6b‘/CH_{Linker}), 3.63–3.57 (m, 2H, H-5/H-4‘), 3.56–3.51 (m, 1H, CH_{Linker}), 3.03–2.95 (m, 2H, CH_{Linker}), 1.73–1.59 (m, 4H, CH_{Linker}), 1.50–1.38 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (201 MHz, D_2O/CD_3OD): δ = 103.4 (C-1‘), 99.1 (C-1), 79.8 (C-2), 74.3 (C-5‘), 73.8 (C-5), 71.4 (C-3/C-3‘), 71.3 (C-3/C-3‘), 71.0 (C-2‘), 68.4 (CH_{Linker}), 68.1 (C-4), 68.0 (C-4‘), 62.2 (C-6‘), 62.0 (C-6), 40.4 (CH_{Linker}), 29.0 (CH_{Linker}), 27.6 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{17}H_{34}NO_{11}^+$ $[M+H]^+$: 428.2126, found 428.2123.

5.2.1.15 Synthesis of 5-Aminopentyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside 3

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (21)**



According to general procedure (**C**), disaccharide **20** (1.37 mg, 1.15 mmol, 1.0 equiv.) was reacted with mannosyl imidate **18** (1.10 mg, 1.72 mmol, 1.5 equiv.) to give the corresponding trisaccharide **21** (1.71 g, 1.03 mmol, 90%) as a colorless oil.

R_f = 0.38 (^{c}Hex/EtOAc, 3:1).

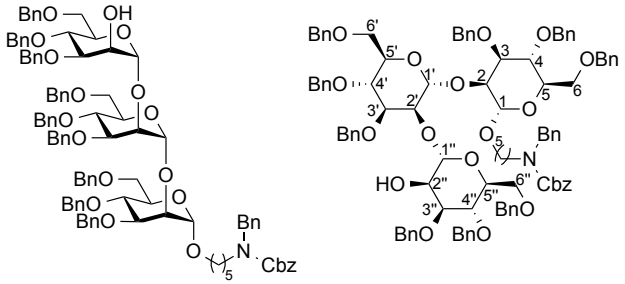
$[\alpha]_D^{22}$ = + 18.4 (c = 0.5, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.41–7.12 (m, 55H, Ar-H), 5.55 (dd, $J_{H2'',H3''}$ = 3.3 Hz, $J_{H2'',H1''}$ = 1.8 Hz, 1H, H-2''), 5.21–5.14 (m, 3H, H-1'/CH_{Cbz}), 5.06 (d, $J_{H1'',H2''}$ = 1.8 Hz, 1H), 4.89 (s, 1H, H-1), 4.88–4.81 (m, 3H, 3 × CH_{Bn}), 4.71–4.66 (m, 2H, 2 × CH_{Bn}), 4.66–4.59 (m, 3H, 3 × CH_{Bn}), 4.58–4.44 (m, 10H, 8 × CH_{Bn}/2 × NCH_{Bn}), 4.43 (d, J = 11.0 Hz, 1H, CH_{Bn}), 4.32 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.11 (t, $J_{H2',H1'/H3'}$ = 2.5 Hz, 1H, H-2'), 4.02–3.98 (m, 1H, H-3''), 3.98–3.94 (m, 2H, H-2/H-5'), 3.94–3.88 (m, 3H, H-3'/H-4''/H-5''), 3.88–3.75 (m, 4H, H-3/H-4/H-6a/H-4'), 3.75–3.65 (m, 5H, H-5/H-6b/H-6a'/H-6b'/H-6a''), 3.57–3.46 (m, 2H, H-6b''/CH_{Linker}), 3.27–3.12 (m, 3H, CH_{Linker}), 2.14 (s, 3H, CH_3-OAc), 1.55–1.37 (m, 4H, CH_{Linker}), 1.25–1.12 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 170.2 (C=O-OAc), 156.8/156.3 (C=O-Cbz), 138.7, 138.6, 138.5, 138.4, 138.2, 138.0, 137.0, 136.9 (11 × C_q), 128.7, 128.7, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3 (55 × C_{Ar}), 100.8 (C-1'), 99.5 (C-1''), 98.9 (C-1), 79.7 (C-3), 79.4 (C-3'), 78.3 (C-3''), 75.3 (C-2/CH_{Bn}), 75.2 (CH_{Bn}), 75.1 (C-2'/CH_{Bn}), 75.0 (C-4), 74.9 (C-4'), 74.4 (C-4''), 73.5 (2 × CH_{Bn}), 73.4 (CH_{Bn}), 72.3 (C-5'), 72.2 (CH_{Bn}), 72.0 (C-5/C-5''/2 × CH_{Bn}), 69.7 (C-6'), 69.5 (C-6), 68.9 (C-2''/C-6''), 67.6 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.4 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}), 21.3 (CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{103}H_{115}N_2O_{19}^+$ $[M+NH_4]^+$: 1684.8128, found 1684.8123.

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (106)**



The acetyl protecting group of trisaccharide **21** (1.53 mg, 0.918 mmol) was removed according to general procedure (**D**) to give the corresponding trisaccharide **106** (1.06 g, 0.653 mmol, 71%) as a colorless oil.

R_f = 0.44 (^{c}Hex/EtOAc, 2:1).

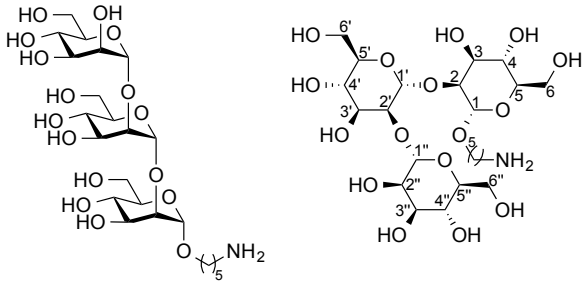
$[\alpha]_D^{22}$ = + 27.2 (c = 0.5, $CHCl_3$).

^{1}H-NMR (800 MHz, $CDCl_3$): δ = 7.44–7.11 (m, 55H, Ar-H), 5.22 (d, $J_{H1‘,H2‘}$ = 2.0 Hz, 1H, H-1‘), 5.17 (d, J = 22.6 Hz, 2H, CH_{Cbz}), 5.13 (d, $J_{H1‘‘,H2‘‘}$ = 1.7 Hz, 1H, H-1‘‘), 4.90 (s, 1H, H-1), 4.87–4.79 (m, 3H, CH_{Bn}), 4.70–4.65 (m, 2H, CH_{Bn}), 4.64–4.43 (m, 14H, 12 × CH_{Bn}/2 × NCH_{Bn}), 4.33 (d, J = 12.2 Hz, 1H, CH_{Bn}), 4.14–4.11 (m, 2H, H-2‘/H-2‘‘), 3.98–3.94 (m, 2H, H-2/H-5‘), 3.94–3.87 (m, 4H, H-3‘/H-3‘‘/H-4‘‘/H-5‘‘), 3.87–3.82 (m, 1H, H-3), 3.82–3.71 (m, 4H, H-4/H-6a/H-6a‘/H-6b‘), 3.71–3.66 (m, 2H, H-5/H-6b), 3.64 (dd, $J_{H6a‘‘,H6b‘‘}$ = 10.7 Hz, $J_{H6a‘‘,H5‘‘}$ = 3.8 Hz, 1H, H-6a‘‘), 3.58–3.47 (m, 2H, H-6b‘‘/CH_{Linker}), 3.27–3.11 (m, 3H, CH_{Linker}), 2.36 (s, 1H, -OH), 1.54–1.37 (m, 4H, CH_{Linker}), 1.25–1.10 (m, 2H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (201 MHz, $CDCl_3$): δ = 156.8/156.3 (C=O-Cbz), 138.7, 138.6, 138.5, 138.4, 138.2, 138.1, 138.0, 137.0, 136.9 (11 × C_q), 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3 (55 × C_{Ar}), 101.1 (C-1‘‘), 101.0 (C-1‘), 98.9 (C-1), 80.1 (C-3‘‘), 79.6 (C-3/C-3‘), 75.5 (C-2), 75.2 (C-2‘/2 × CH_{Bn}), 75.1 (C-4‘), 75.0 (C-4/CH_{Bn}), 74.4 (C-4‘‘), 73.4, 72.4 (4 × CH_{Bn}), 72.3 (C-5‘), 72.2 (CH_{Bn}), 71.9 (C-5/CH_{Bn}), 71.7 (C-5‘‘), 69.7 (C-6‘), 69.5 (C-6), 69.0 (C-6‘‘), 68.7 (C-2‘‘), 67.6 (CH_{Linker}), 67.2 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.2 (CH_{Linker}), 29.4 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{101}H_{113}N_2O_{18}^+$ $[M+NH_4]^+$: 1642.8022, found 1642.7977.

5-Aminopentyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (3)



According to general procedure (**E**), trisaccharide **106** (370 mg, 0.229 mmol) was globally deprotected by hydrogenolysis to give the corresponding trisaccharide **3** (124 mg, 0.210 mmol, 92%) as a colorless solid.

^{1}H-NMR (800 MHz, D_2O): δ = 5.28 (d, $J_{H1',H2'}$ = 1.9 Hz, 1H, H-1'), 5.09 (d, $J_{H1,H2}$ = 1.8 Hz, 1H, H-1), 5.04 (d, $J_{H1'',H2''}$ = 1.8 Hz, 1H, H-1''), 4.10 (dd, $J_{H2',H3'}$ = 3.4 Hz, $J_{H2',H1'}$ = 1.8 Hz, 1H, H-2'), 4.06 (dd, $J_{H2'',H3''}$ = 3.5 Hz, $J_{H2'',H1''}$ = 1.8 Hz, 1H, H-2''), 3.94 (dd, $J_{H3',H4'}$ = 9.7 Hz, $J_{H3',H2'}$ = 3.3 Hz, 1H, H-3'), 3.92 (dd, $J_{H2,H3}$ = 3.5 Hz, $J_{H2,H1}$ = 1.7 Hz, 1H, H-2), 3.90–3.86 (m, 4H, H-3/H-6a/H-6a'/H-6a''), 3.83 (dd, $J_{H3'',H4''}$ = 9.7 Hz, $J_{H3'',H2''}$ = 3.4 Hz, 1H, H-3''), 3.78–3.70 (m, 6H, H-6b/H-5'/H-6b'/H-5''/H-6b''/CH_{Linker}), 3.69–3.59 (m, 4H, H-4/H-5/H-4'/H-4''), 3.56–3.51 (m, 1H, CH_{Linker}), 2.99 (t, J = 7.6 Hz, 2H, CH_{Linker}), 1.73–1.61 (m, 4H, CH_{Linker}), 1.50–1.39 (m, 2H, CH_{Linker}) ppm.

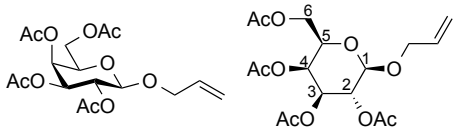
^{13}C-{^{1}H}-NMR (201 MHz, D_2O/CD_3OD): δ = 103.2 (C-1''), 101.7 (C-1'), 99.0 (C-1), 80.0 (C-2), 79.6 (C-2'), 74.3 (C-5'/C-5''), 73.8 (C-5), 71.4 (C-3/C-3''), 71.3 (C-3/C-3''), 71.0 (C-3'/C-2''), 68.5 (CH_{Linker}), 68.2 (C-4), 68.0 (C-4'), 67.9 (C-4''), 62.2 (C-6'/C-6''), 62.0 (C-6), 40.4 (CH_{Linker}), 29.0 (CH_{Linker}), 27.5 (CH_{Linker}), 23.5 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{23}H_{44}NO_{16}^+$ $[M+H]^+$: 590.2655, found 590.2646.

5.2.2 Synthesis of α-GalCer derivative 11

5.2.2.1 Synthesis of Galactosyl donor 36

Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (110)[199, 240]



A solution of penta-*O*-acetyl-α/β-D-galactopyranoside **62** (10.1 g, 25.9 mmol, 1.0 equiv.) and allylic alcohol (5.30 mL, 77.6 mmol, 3.0 equiv.) in CH_2Cl_2 (80 mL) under an argon atmosphere was cooled to 0 °C and $BF_3 \cdot Et_2O$ (4.16 mL, 33.7 mmol, 1.3 equiv.) was added slowly. After the addition, the solution was allowed to warm to room temperature and it was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), carefully neutralized with sat. aq. $NaHCO_3$ (100 ml) and solid $NaHCO_3$. The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was redissolved in pyridine (25 mL) and cooled to 0 °C. Ac_2O (15 mL) was added dropwise and the reaction mixture was stirred for 16 h. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to furnish **110** (7.03 g, 18.1 mmol, 70%) as a colorless oil.

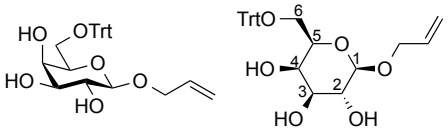
$\boldsymbol{R_f}$ = 0.38 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.83 (dddd, $J_{CH,CH2trans}$ = 17.3 Hz, $J_{CH,CH2cis}$ = 10.4 Hz, $J_{CH,CH2b}$ = 6.1 Hz, $J_{CH,CH2a}$ = 4.9 Hz, 1H, CH_2CH=CH_2), 5.36 (dd, $J_{H4,H3}$ = 3.5 Hz, $J_{H4,H5}$ = 1.2 Hz, 1H, H-4), 5.28–5.15 (m, 3H, CH_2CH=CH_{2trans}/CH_2CH=CH_{2cis}/H-2), 4.99 (dd, $J_{H3,H2}$ = 10.5 Hz, $J_{H3,H4}$ = 3.4 Hz, 1H, H-3), 4.50 (d, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1), 4.32 (ddt, $J_{CH2a,CH2b}$ = 13.2 Hz, $J_{CH2a,CH}$ = 4.9 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.6 Hz, 1H, $CH_{2a}CH$=CH_2), 4.19–4.04 (m, 3H, $CH_{2b}CH$=CH_2/H-6a/H-6b), 3.87 (td, $J_{H5,H6a/b}$ = 6.7 Hz, $J_{H5,H4}$ = 1.2 Hz, 1H, H-5), 2.12, 2.03, 2.02, 1.95 (4s, 12H, 4 × CH_3-OAc) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.2, 169.5 (4 × C=O-OAc), 133.4 (CH_2CH=CH_2), 117.6 (CH_2CH=CH_2), 100.2 (C-1), 71.0 (C-3), 70.7 (C-5), 70.1 (CH_2CH=CH_2), 68.9 (C-2), 67.2 (C-4), 61.4 (C-6), 20.8, 20.7 (4 × CH_3-OAc) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{17}H_{28}NO_{10}^+$ $[M+NH_4]^+$: 406.1708, found 406.1708.

Allyl 6-*O*-trityl-β-D-galactopyranoside (111)[89]



To a stirred solution of allyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **110** (4.51 g, 11.6 mmol, 1.0 equiv.) in MeOH (150 mL) were added catalytic amounts of NaOMe. After 20 h the reaction mixture was neutralized by adding *Amberlite IR120*. The ion-exchange resin was filtered off and the solvents were removed under reduced pressure. The crude prodruct was dissolved in pyridine (80 mL) and trityl chloride (4.20 g, 15.1 mmol, 1.3 equiv.) was added. The mixture was stirred at room temperature for 20 h. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 10:1) to furnish **111** (4.78 g, 10.3 mmol, 89% over two steps) as a yellow oil.

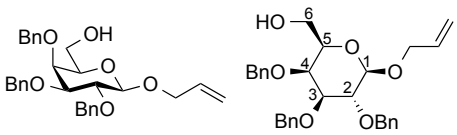
$\boldsymbol{R_f}$ = 0.66 (CH_2Cl_2/MeOH, 5:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.51–7.23 (m, 15H, Ar-H), 5.97 (dddd, $J_{CH,CH2trans}$ = 17.0 Hz, $J_{CH,CH2cis}$ = 10.3 Hz, $J_{CH,CH2b}$ = 6.5 Hz, $J_{CH,CH2a}$ = 5.3 Hz, 1H, CH_2CH=CH_2), 5.34 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.6 Hz, 1H, CH_2CH=CH_{2trans}), 5.23 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.3 Hz, 1H, CH_2CH=CH_{2cis}), 4.41 (ddt, $J_{CH2a,CH2b}$ = 12.6 Hz, $J_{CH2a,CH}$ = 5.3 Hz, $J_{CH2a,CH2cis/CH2trans}$ = 1.5 Hz, 1H, $CH_{2a}CH$=CH_2), 4.30 (d, $J_{H1,H2}$ = 7.6 Hz, 1H, H-1), 4.17 (ddt, $J_{CH2b,CH2a}$ = 12.6 Hz, $J_{CH2b,CH}$ = 6.5 Hz, $J_{CH2b,CH2cis/CH2trans}$ = 1.3 Hz, 1H, $CH_{2b}CH$=CH_2), 4.03–4.00 (m, 1H, H-4), 3.68 (dd, $J_{H2,H3}$ = 9.5 Hz, $J_{H2,H1}$ = 7.6 Hz, 1H, H-2), 3.61–3.53 (m, 2H, H-3/H-5), 3.48 (dd, $J_{H6a,H6b}$ = 9.6 Hz, $J_{H6a,H5}$ = 5.7 Hz, 1H, H-6a), 3.39 (dd, $J_{H6b,H6a}$ = 9.6 Hz, $J_{H6b,H5}$ = 6.1 Hz, 1H, H-6b) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 143.8 (3 × C_q), 133.9 (CH_2CH=CH_2), 128.8, 128.0, 127.28 (15 × C_{Ar}), 118.2 (CH_2CH=CH_2), 102.0 (C-1), 87.1 (C_q), 73.8 (C-3), 73.7 (C-5), 72.2 (C-2), 70.2 (CH_2CH=CH_2), 69.2 (C-4), 62.9 (C-6) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{28}H_{34}NO_6^+$ $[M+NH_4]^+$: 480.2381, found 480.2380.

Allyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (112)[89]



Allyl 6-*O*-trityl-β-D-galactopyranoside **111** (5.04 g, 10.9 mmol, 1.0 equiv.) was dissolved in DMF (HPLC grade, 150 mL) under an argon atmosphere and cooled to 0 °C. Sodium hydride (60% dispersion in oil, 2.04 g, 49.0 mmol, 4.5 equiv.) was added portionwise and the mixture was stirred for 1 h at 0 °C. After the addition of benzylbromide (5.60 mL, 47.0 mmol, 4.5 equiv.) the mixture was allowed to warm to room temperature and left to stir for 20 h. The reaction mixture was then quenched by the addition of MeOH (7.0 mL) and diluted with Et_2O (50 mL). The organic phase was washed with sat. aq. $NaHCO_3$ (2 × 20 mL), water (3 × 100 mL) and brine (50 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced

pressure and the crude product was filtered over a pad of silica (^{c}Hex/EtOAc, 2:1, 1% NEt_3). The obtained yellow oil (6.30 g, 8.60 mmol, 1.0 equiv.) and triethylsilane (6.87 mL, 43.0 mmol, 5.0 equiv.) were dissolved in CH_2Cl_2 (90 mL) and cooled to 0 °C. Trifluoroacetic acid (3.32 mL, 43.0 mmol, 5.0 equiv.) was added dropwise to the solution. After 20 min, the reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$ (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 10:1→1:1) to furnish **112** (3.73 g, 7.60 mmol, 70% over two steps) as a pale yellow oil.

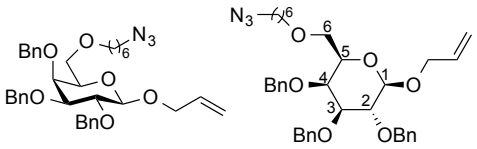
$\boldsymbol{R_f}$ = 0.20 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.42–7.25 (m, 15H, Ar-H), 5.95 (dddd, $J_{CH,CH2trans}$ = 17.3 Hz, $J_{CH,CH2cis}$ = 10.5 Hz, $J_{CH,CH2b}$ = 5.9 Hz, $J_{CH,CH2a}$ = 5.2 Hz, 1H, CH_2CH=CH_2), 5.33 (dq, $J_{CH2trans,CH}$ = 17.8 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.9 Hz, 1H, CH_2CH=CH_{2trans}), 5.19 (dq, $J_{CH2cis,CH}$= 10.4 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.4 Hz, 1H, CH_2CH=CH_{2cis}), 4.99–4.93 (m, 2H, CH_{Bn}), 4.85–4.65 (m, 4H, CH_{Bn}), 4.45–4.38 (m, 2H, H-1/CH_{2a}CH=CH_2), 4.14 (ddt, $J_{CH2b,CH2a}$ = 13.0 Hz, $J_{CH2b,CH}$ = 5.9 Hz, $J_{CH2b,CH2cis/CH2trans}$ = 1.5 Hz, 1H, CH_{2b}CH=CH_2), 3.88 (dd, $J_{H2,H1}$ = 9.7 Hz, $J_{H2,H3}$ = 7.7 Hz, 1H, H-2), 3.81–3.73 (m, 2H, H-4/H-6a), 3.56–3.46 (m, 2H, H-3/H-6b), 3.40–3.35 (m, 1H, H-5) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 138.8, 138.6, 138.4 (3 × C_q), 134.4 (CH_2CH=CH_2), 128.8, 128.6, 128.4, 128.3, 128.1, 127.8, 127.7 (15 × C_{Ar}), 117.2 (CH_2CH=CH_2), 103.3 (C-1), 82.4 (C-3), 79.8 (C-2), 75.4 (CH_{Bn}), 74.7 (C-5), 74.3, 73.6 (2 × CH_{Bn}), 73.0 (C-4), 70.5 (CH_2CH=CH_2), 62.2 (C-6) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{30}H_{38}NO_6^+$ $[M+NH_4]^+$: 508.2694, found 508.2696.

Allyl 6-(6′-azidohexyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (114)[89]



Allyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside **112** (2.75 g, 5.61 mmol, 1.0 equiv.) was dissolved in DMF (HPLC grade, 20 mL) under argon and cooled to 0 °C. Sodium hydride (60% dispersion in oil, 325 mg, 8.40 mmol, 1.5 equiv.) was added portionwise and the mixture was stirred for 1 h at 0 °C. After the addition of 6-azidohexyl 4-methylbenzenesulfonate **113**[89] (2.50 g, 8.4 mmol, 1.5 equiv.) the mixture was left to stir for 21 h at room temperature. The reaction mixture was then quenched by the addition of MeOH (5 mL) and diluted with CH_2Cl_2 (20 mL). Sat. aq. NH_4Cl (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phase was washed with water (2 × 30 mL) and brine (30 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 1:0→1:1) to give **114** (2.51 g, 4.08 mmol, 73%) as a colorless oil.

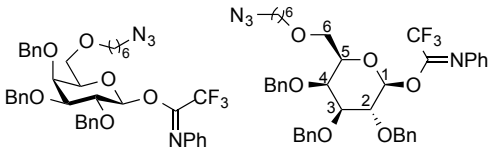
R_f = 0.62 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.47–7.29 (m, 15H, Ar-H), 6.01 (dddd, $J_{CH,CH2trans}$ = 17.3 Hz, $J_{CH,CH2cis}$ = 10.8 Hz, $J_{CH,CH2b}$ = 6.0 Hz, $J_{CH,CH2a}$ = 5.1 Hz, 1H, CH_2CH=CH_2), 5.39 (dq, $J_{CH2trans,CH}$ = 17.2 Hz, $J_{CH2trans,CH2cis/CH2a/CH2b}$ = 1.7 Hz, 1H, CH_2CH=CH_{2trans}), 5.23 (dq, $J_{CH2cis,CH}$= 10.5 Hz, $J_{CH2cis,CH2trans/CH2a/CH2b}$ = 1.4 Hz, 1H, CH_2CH=CH_{2cis}), 5.05–4.99 (m, 2H, CH_{Bn}), 4.85–4.81 (m, 2H, CH_{Bn}), 4.78 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.72 (d, J = 11.7 Hz, 1H, CH_{Bn}), 4.50–4.45 (m, 2H, H-1/CH_{2a}CH=CH_2), 4.18 (ddt, $J_{CH2b,CH2a}$ = 13.0 Hz, $J_{CH2b,CH}$ = 6.0 Hz, $J_{CH2b,CH2cis/CH2trans}$ = 1.5 Hz, 1H, CH_{2b}CH=CH_2), 3.95–3.90 (m, 2H, H-2/H-4), 3.62–3.58 (m, 3H, H-3/H-6a/H-6b), 3.58–3.54 (m, 1H, H-5), 3.47 (dt, J = 9.4 Hz, J = 6.6 Hz, 1H, CH_{Linker}), 3.38 (dt, J = 9.5 Hz, J = 6.6 Hz, 1H, CH_{Linker}), 3.27 (t, J = 6.9 Hz, 2H, CH_{Linker}), 1.71–1.53 (m, 4H, CH_{Linker}), 1.46–1.31 (m, 4H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 138.7, 138.7, 138.5 (3 × C_q), 134.2 (CH_2*C*H=CH_2), 129.8, 128.3, 128.2, 128.1, 127.8, 127.5 (15 × C_{Ar}), 116.9 (CH_2CH=*C*H_2), 102.9 (C-1), 82.2 (C-3), 79.6 (C-2), 75.2, 74.4 (2 × CH_{Bn}), 73.6 (C-4), 73.4 (C-5), 73.0 (CH_{Bn}), 71.3 (CH_{Linker}), 70.1 (*C*H_2CH=CH_2), 69.1 (C-6), 51.3 (CH_{Linker}), 29.5 (CH_{Linker}), 28.7 (CH_{Linker}), 26.5 (CH_{Linker}), 25.7 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{36}H_{49}N_4O_6^+$ $[M+NH_4]^+$: 633.3647, found 633.3656.

6-(6´-Azidohexyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl *N*-phenyltrifluoroacetimidate (36)[89]



A mixture of allyl 6-(6´-azidohexyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside **114** (899 mg, 1.46 mmol, 1.0 equiv.) and $PdCl_2$ (52 mg, 0,292 mmol, 0.2 equiv.) in MeOH/H_2O (10:1, 44 mL) was stirred at room temperature for 14 h. The reaction mixture was filtered over a pad of celite and the solvents were removed under reduced pressure. The residue was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to yield a colorless oil (526 mg, 0.914 mmol, 63%). The obtained lactol (462 mg, 0.803 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (7 mL) and cesium carbonate (392 mg, 1.20 mmol, 1.5 equiv.) was added. After the addition of 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride **115** (250 mg, 1.20 mmol, 1.5 equiv.), the reaction mixture was left to stir at room temperature for 3 h and subsequently filtered over a pad of celite and washed with CH_2Cl_2 (15 mL). The solvents were removed under reduced pressure and the crude prodruct was purified by flash chromatography on silica (^{c}Hex/EtOAc, 7:1) to yield **36** (535 mg, 0.716 mmol, 89%) as a colorless oil.

R_f = 0.64 (^{c}Hex/EtOAc, 2:1).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.39–7.25 (m, 17H, Ar-H), 7.09 (t, J = 7.4 Hz, 1H, Ar-H), 6.79 (d, J = 7.7 Hz, 2H, Ar-H), 5.65 (s, 1H, H-1), 4.98 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.86–4.81 (m, 2H, CH_{Bn}), 4.79–4.73 (d, 2H, CH_{Bn}), 4.66 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.08 (s, 1H, H-2), 3.92 (s, 1H, H-4), 3.65–3.50 (m, 4H,

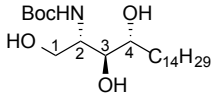
H-3/H-5/H-6a/H-6b), 3.45–3.29 (m, 2H, CH_{Linker}), 3.23 (t, J = 6.9 Hz, 2H, CH_{Linker}), 1.59–1.47 (m, 4H, CH_{Linker}), 1.41–1.20 (m, 4H, CH_{Linker}) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 143.7, 138.6, 138.3, 138.2 (4 × C_q), 128.8, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 124.3, 119.4 (20 × C_{Ar}), 97.5 (C-1), 82.3 (C-3), 78.3 (C-2), 75.7, 74.9 (2 × CH_{Bn}), 74.6 (C-5), 73.4 (C-4), 73.2 (CH_{Bn}), 71.4 (CH_{Linker}), 68.7 (C-6), 51.5 (CH_{Linker}), 29.7 (CH_{Linker}), 28.9 (CH_{Linker}), 26.7 (CH_{Linker}), 25.9 (CH_{Linker}) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{41}H_{59}F_3N_5O_6^+$ $[M+NH_4]^+$: 764.3629, found 764.3654.

5.2.2.2 Synthesis of Sphingolipid 35

(*2S*,*3S*,*4R*)-2-[(*N-tert*-Butoxycarbonyl)amino]octadecane-1,3,4-triol (117)[276]



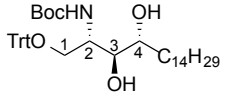
To a suspension of phytosphingosine **116** (4.50 g, 14.2 mmol, 1.0 equiv.) in THF (100 mL) were added NEt_3 (2.36 mL, 14.9 mmol, 1.05 equiv.) and Boc_2O (3.25 g, 17.0 mmol, 1.2 equiv.) sequentially. The reaction mixture was stirred at room temperature for 19 h and was then concentrated under reduced pressure. Recrystallization of the residue from EtOAc (50 mL) gave **117** (5.09 g, 12.2 mmol, 86%) as colorless crystals.

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 5.43 (d, $J_{NH,H2}$ = 8.3 Hz, 1H, NH), 3.87 (dd, $J_{H1a,H1b}$ = 11.2 Hz, $J_{H1a,H2}$ = 3.1 Hz, 1H, H-1a), 3.84–3.80 (m, 1H, H-2), 3.72 (dd, $J_{H1b,H1a}$ = 11.2 Hz, $J_{H1b,H2}$ = 5.4 Hz, 1H, H-1b), 3.68–3.64 (m, 1H, H-4), 3.64–3.61 (m, 1H, H-3), 1.72–1.64 (m, 1H, -CH_2-), 1.56–1.41 (m, 11H, -CH_2-, 3 × CH_3-Boc), 1.36–1.21 (m, 23H, 11.5 × -CH_2-), 0.87 (t, J = 7.0 Hz, 3H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 156.6 (C=O-Boc), 80.2 (C_q-Boc), 76.3 (C-3), 73.1 (C-4), 62.1 (C-1), 53.1 (C-2), 33.2, 32.1, 29.9, 29.8, 29.5 (11 × -CH_2-), 28.5 (4 × CH_3-Boc), 26.1, 22.8 (2 × -CH_2-), 14.3 (-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{23}H_{48}NO_5^+$ $[M+H]^+$: 418.3527, found 418.3528.

(*2S*,*3S*,*4R*)-1-*O*-Triphenylmethyl-2-[(*N-tert*-butoxycarbonyl)amino]octadecane-3,4-diol (118)[277]



To a solution of carbamate **117** (4.28 g, 10.2 mmol, 1.0 equiv.) in pyridine (50 mL) were added trityl chloride (3.43 g, 12.3 mmol, 1.2 equiv.) and catalytic amounts of 4-dimethylaminopyridine (DMAP), and the mixture was stirred at 80 °C for 10 h. The reaction mixture was concentrated under reduced pressure and the oily

residue was purified by flash chromatography on silica (^{c}Hex/EtOAc, 4:1) to give **118** (6.43 g, 9.74 mmol, 95%) as a colorless oil.

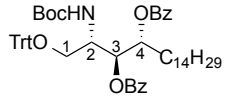
R_f = 0.41 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.45–7.22 (m, 15H, Ar-H), 5.19 (d, $J_{NH,H2}$ = 8.9 Hz, 1H, NH), 4.00–3.90 (m, 1H, H-2), 3.65–3.55 (m, 1H, H-3), 3.45–3.34 (m, 3H, H-1a/H-1b/H-4), 2.85 (d, $J_{OH,H3}$ = 8.0 Hz, 1H, -OH), 2.10 (d, $J_{OH,H4}$ = 7.4 Hz, 1H, -OH), 1.73–1.60 (m, 1H, -CH_2-), 1.50–1.37 (m, 10H, -CH_2-, 3 × CH_3-Boc), 1.35–1.22 (m, 24H, 12 × -CH_2-), 0.89 (t, J = 6.8 Hz, 3H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 155.8 (C=O-Boc), 143.4 (3 × C_q-Ar), 128.6, 128.2, 127.4 (15 × C_{Ar}), 87.7 (C_q-Trt), 79.8 (C_q-Boc), 76.0 (C-3), 73.3 (C-4), 63.4 (C-1), 51.3 (C-2), 33.2, 32.1, 29.9, 29.80, 29.7, 29.5 (11 × -CH_2-), 28.6 (3 × CH_3-Boc), 26.0, 22.8 (2 × -CH_2-), 14.3 (-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{43}H_{52}NO_7^-$ $[M+FA-H]^-$: 704.4532, found 704.4538.

(*2S*,*3S*,*4R*)-1-*O*-Triphenylmethyl-2-(*N*-*tert*-butoxycarbonyl)amino-3,4-di-*O*-benzoyl-octadecane (119)[277]



To a solution of **118** (6.42 g, 9.73 mmol, 1.0 equiv.) in pyridine (50 mL) were added benzoyl chloride (4.48 mL, 38.9 mmol, 4.0 equiv.) and catalytic amounts of DMAP. The reaction mixture was stirred at room temperature for 24 h and was then quenched by the addition of cooled water (100 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 80 mL) and the combined organic phase was washed with sat. aq. $NaHCO_3$ (2 × 60 mL) and brine (60 mL). The organic phase was dried with $MgSO_4$, the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 5:1) to give **119** (7.10 g, 8.18 mmol, 84%) as a colorless oil.

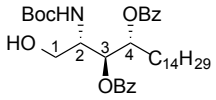
R_f = 0.75 (^{c}Hex/EtOAc, 3:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.96–7.82 (m, 4H, Ar-H), 7.60–7.09 (m, 21H, Ar-H), 5.73 (dd, J = 8.9 Hz, J = 2.9 Hz, 1H, H-3), 5.53–5.46 (m, 1H, H-4), 5.06 (d, $J_{NH,H2}$ = 9.9 Hz, 1H, NH), 4.35–4.25 (m, 1H, H-2), 3.33 (dd, $J_{H1a,H1b}$ = 9.7 Hz, $J_{H1a,H2}$ = 3.1 Hz, 1H, H-1a), 3.21 (dd, $J_{H1b,H1a}$ = 9.6 Hz, $J_{H1b,H2}$ = 4.3 Hz, 1H, H-1b), 1.96–1.79 (m, 2H, -CH_2-), 1.49 (s, 9H, 3 × CH_3-Boc), 1.40–1.19 (m, 24H, 12 × -CH_2-), 0.89 (t, J = 7.0 Hz, 3H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 166.3, 165.2 (2 × C=O-OBz), 155.5 (C=O-Boc), 147.0, 143.6 (5 × C_q-Ar), 133.1, 133.0, 130.3, 130.0, 129.9, 128.7, 128.5, 128.4, 128.1, 128.0, 127.9, 127.4, 127.1 (25 × C_{Ar}), 86.9 (C_q-Trt), 79.9 (C_q-Boc), 73.9 (C-4), 73.0 (C-3), 62.3 (C-1), 50.6 (C-2), 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 28.8 (11 × -CH_2-), 28.5 (3 × CH_3-Boc), 25.7, 22.8 (2 × -CH_2-), 14.3 (-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{56}H_{73}N_2O_7^+$ $[M+NH_4]^+$: 885.5412, found 885.5437.

(*2S*,*3S*,*4R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-3,4-di-*O*-benzoyl-octadecane-1-ol (35)[277]



A solution of **119** (6.30 g, 7.25 mmol, 1.0 equiv.) and *p*-toluenesulfonic acid monohydrate (1.25 g, 7.25 mmol, 1.0 equiv.) in CH_2Cl_2/MeOH (1:1, 200 mL) was stirred at room temperature for 3 h. Then the mixture was neutralized with sat. aq. $NaHCO_3$ (150 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 × 80 mL) and the organic phase was dried with $MgSO_4$. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 4:1) to furnish **35** (3.03 g, 4.84 mmol, 67%) as a colorless oil.

R_f = 0.51 (^{c}Hex/EtOAc, 3:1).

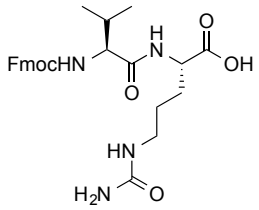
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.07–7.92 (m, 4H, Ar-H), 7.65–7.34 (m, 6H, Ar-H), 5.53–5.48 (m, 1H, H-4), 5.42 (dd, J = 9.4 Hz, J = 2.7 Hz, 1H, H-3), 5.35 (d, $J_{NH,H2}$ = 9.7 Hz, 1H, NH), 4.08–4.00 (m, 1H, H-2), 3.66 (d, $J_{H1a/H1b,H2}$ = 2.9 Hz, 2H, H-1a/H-1b), 2.10–1.96 (m, 2H, -CH_2-), 1.48 (s, 9H, 3 × CH_3-Boc), 1.40–1.19 (m, 24H, 12 × -CH_2-), 0.90–0.85 (t, J = 6.8 Hz, 3H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $CDCl_3$): δ = 167.0, 166.1 (2 × C=O-OBz), 155.5 (C=O-Boc), 133.7, 133.0, 130.0, 129.7 (6 × C_{Ar}), 129.2 (2 × C_q-Ar), 128.6, 128.4 (4 × C_{Ar}), 80.0 (C_q-Boc), 73.9 (C-3), 73.8 (C-4), 61.7 (C-1), 51.5 (C-2), 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.6 (11 × -CH_2-), 28.3 (3 × CH_3-Boc), 25.8, 22.7 (2 × -CH_2-), 14.1 (-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{37}H_{56}NO_7^+$ $[M+H]^+$: 626.4051, found 626.4065.

5.2.2.3 Synthesis of the Peptidelinkers 28 and 132

Fmoc-Val-Cit (123)[182]



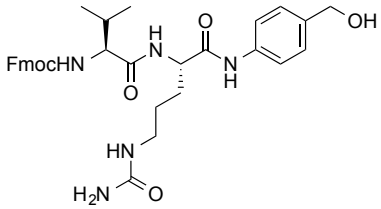
A solution of Fmoc-Val **122** (5.06 g, 14.9 mmol, 1.0 equiv.) and HOSu (1.72 g, 14.9 mmol, 1.0 equiv.) in THF (50 mL) was cooled to 0 °C and treated with DCC (3.08 g, 14.9 mmol, 1.0 equiv.). The reaction mixture was stirred at room temperature for 14 h. All solids were filtered off and washed with THF (50 mL). The solvents were removed under reduced pressure to yield Fmoc-Val-OSu quantitatively as a colorless solid, which was used without further purification. To a solution of Citrulline (2.74 mg, 15.6 mmol, 1.05 equiv.) and $NaHCO_3$ (1.32 g, 15.6 mmol, 1.05 equiv.) in water (40 mL) was added a solution of Fmoc-Val-OSu in DME (40 mL). THF (40 mL) was added until all solids were dissolved and the reaction mixture was stirred at room temperature for 17 h. Then aqueous citric acid (15%, 75 mL) was added and the mixture was extracted with 2-propanol/EtOAc (1:9, 2 × 100 mL). The suspension was washed with water (2 × 150 mL) and solvents were removed under reduced pressure. The colorless solid was treated with Et_2O (80 mL) and after short sonication and trituration, the colorless solid was collected by filtration to give **123** (6.74 g, 13.6 mmol, 91%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ = 8.15 (d, $J_{NH,H\alpha}$ = 7.3 Hz, 1H, NH-Cit), 7.89 (d, $J_{H4,H3} = J_{H5,H6}$ = 7.5 Hz, 2H, H4-Fmoc/H5-Fmoc), 7.78–7.71 (m, 2H, H1-Fmoc/H8-Fmoc), 7.45–7.28 (m, 5H, NH-Val/H2-Fmoc/H3-Fmoc/ H6-Fmoc/H7-Fmoc), 5.93 (t, $J_{H\varepsilon,H\delta2/H\delta3}$ = 5.7 Hz, 1H, Hε-Cit), 5.37 (s, 2H, Hη1-Cit/Hη2-Cit), 4.37–4.12 (m, 4H, Hα-Cit/H9-Fmoc/CH_2-Fmoc), 3.93 (dd, J = 9.2 Hz, J = 7.0 Hz, 1H, Hα-Val), 2.99–2.91 (m, 2H, Hδ2-Cit/ Hδ3-Cit), 2.04–1.93 (m, 1H, Hβ-Val), 1.77–1.51 (m, 2H, Hβ2-Cit/Hβ3-Cit), 1.46–1.35 (m, 2H, Hγ2-Cit/Hγ3-Cit), 0.89 (d, $J_{H\gamma1,H\beta}$ = 6.9 Hz, 3H, Hγ1-Val), 0.86 (d, $J_{H\gamma2,H\beta}$ = 6.8 Hz, 3H, Hγ2-Val) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $(CD_3)_2SO$): δ = 173.4 (C=O-Cit), 171.3 (C=O-Val), 158.7 (Cζ-Cit), 156.0 (C=O-Fmoc), 143.9 (C1a-Fmoc), 143.8 (C8a-Fmoc), 140.7 (C4a-Fmoc/C5a-Fmoc), 127.6 (C3-Fmoc/C6-Fmoc), 127.1 (C2-Fmoc/C7-Fmoc), 125.4 (C1-Fmoc/C8-Fmoc), 120.1 (C4-Fmoc/C5-Fmoc), 65.7 (CH_2-Fmoc), 59.8 (Cα-Val), 51.9 (Cα-Cit), 46.7 (C9-Fmoc), 38.8 (Cδ-Cit), 30.6 (Cβ-Val), 28.4 (Cβ-Cit), 26.7 (Cγ-Cit), 19.2 (Cγ1-Val), 18.2 (Cγ2-Val) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{26}H_{33}N_4O_6^+$ $[M+H]^+$: 497.2395, found 497.2403.

Fmoc-Val-Cit-4-aminobenzyl alcohol (124)[182]



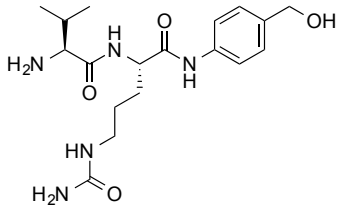
Fmoc-Val-Cit **123** (1.04 g, 2.09 mmol, 1.0 equiv.) and 4-aminobenzyl alcohol (518 mg, 4.18 mmol, 2.0 equiv.) were dissolved in CH_2Cl_2/MeOH (2:1, 35 mL) and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (1.04 g, 4.18 mmol, 2.0 equiv.) was added. The mixture was stirred for 1.5 d in the dark. The solvents were removed under reduced pressure and the solid residue was redissolved in Et_2O (75 mL). The suspension was sonicated for 5 min and then left to stand for 1 h at room temperature. The colorless solid was collected by filtration to furnish **124** (1.06 g, 1.76 mmol, 84%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ = 9.97 (s, 1H, -NH-Ar), 8.10 (d, $J_{NH,H\alpha}$ = 7.6 Hz, 1H, NH-Cit), 7.92–7.86 (m, 2H, H4-Fmoc/H5-Fmoc), 7.77–7.71 (m, 2H, H1-Fmoc/H8-Fmoc), 7.57–7.52 (m, 2H, Ar-H), 7.45–7.38 (m, 3H, NH-Val/H3-Fmoc/ H6-Fmoc), 7.35–7.29 (m, 2H, H2-Fmoc/H7-Fmoc), 7.26–7.21 (m, 2H, Ar-H), 5.97 (t, $J_{H\varepsilon,H\delta2/H\delta3}$ = 5.9 Hz, 1H, Hε-Cit), 5.40 (s, 2H, Hη1-Cit/Hη2-Cit), 4.47–4.40 (m, 3H, Hα-Cit/Ar-CH_2-OH), 4.34–4.20 (m, 3H, H9-Fmoc/CH_2-Fmoc), 3.94 (dd, J = 9.0 Hz, J = 6.9 Hz, 1H, Hα-Val), 3.08–2.89 (m, 2H, Hδ2-Cit/ Hδ3-Cit), 2.05–1.94 (m, 1H, Hβ-Val), 1.77–1.54 (m, 2H, Hβ2-Cit/Hβ3-Cit), 1.50–1.33 (m, 2H, Hγ2-Cit/Hγ3-Cit), 0.88 (d, $J_{H\gamma1,H\beta}$ = 6.8 Hz, 3H, Hγ1-Val), 0.86 (d, $J_{H\gamma2,H\beta}$ = 6.8 Hz, 3H, Hγ2-Val) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $(CD_3)_2SO$): δ = 171.2 (C=O-Val), 170.4 (C=O-Cit), 158.9 (Cζ-Cit), 156.1 (C=O-Fmoc), 143.9 (C1a-Fmoc), 143.8 (C8a-Fmoc), 140.7 (C4a-Fmoc/C5a-Fmoc), 137.5, 137.4 (2 × C_q), 127.6 (C3-Fmoc/C6-Fmoc), 127.1 (C2-Fmoc/C7-Fmoc), 126.9 (2 × C_{Ar}), 125.34 (C1-Fmoc/C8-Fmoc), 120.1 (C4-Fmoc/C5-Fmoc), 118.9 (2 × C_{Ar}), 65.7 (CH_2-Fmoc), 62.6 (Ar-*C*H_2-OH), 60.1 (Cα-Val), 53.1 (Cα-Cit), 46.7 (C9-Fmoc), 38.6 (Cδ-Cit), 30.4 (Cβ-Val), 29.5 (Cβ-Cit), 26.8 (Cγ-Cit), 19.2 (Cγ1-Val), 18.3 (Cγ2-Val) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{33}H_{40}N_5O_6^+$ $[M+H]^+$: 602.2973, found 602.2979.

Val-Cit-4-aminobenzyl alcohol (31)[182]



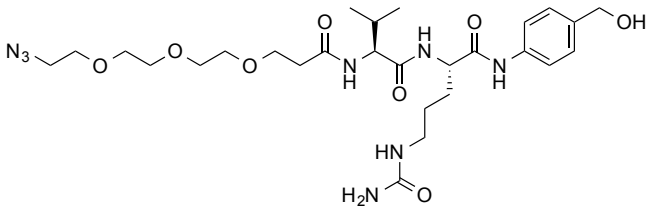
Fmoc-Val-Cit-PABOH **124** (1.70 g, 2.83 mmol) in DMF (80 mL) was treated with piperidine (20 mL). The mixture was stirred for 30 min and then the solvents were removed under reduced pressure. The solid residue was dissolved in MeOH (1.5 mL) and was added dropwise to icecold Et_2O (40 mL). The precipitate was collected by centrifugation. After repeated trituration with Et_2O and centrifugation (3 × 20 mL), the colorless solid was dried under high vacuum to give **31** (0.934 g, 2.46 mmol, 87%).

^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ = 10.03 (s, 1H, -NH-Ar), 8.14 (d, $J_{NH,H\alpha}$ = 8.1 Hz, 1H, NH-Cit), 7.54 (d, J = 8.5 Hz, 2H, Ar-H), 7.24 (d, J = 8.5 Hz, 2H, Ar-H), 5.98 (t, $J_{H\varepsilon,H\delta2/H\delta3}$ = 5.8 Hz, 1H, Hε-Cit), 5.40 (s, 2H, Hη1-Cit/Hη2-Cit), 4.51–4.40 (m, 3H, Hα-Cit/Ar-CH_2-OH), 3.07–2.89 (m, 3H, Hα-Val/Hδ2-Cit/Hδ3-Cit), 1.99–1.85 (m, 1H, Hβ-Val), 1.78–1.52 (m, 2H, Hβ2-Cit/Hβ3-Cit), 1.49–1.27 (m, 2H, Hγ2-Cit/Hγ3-Cit), 0.89 (d, $J_{H\gamma1,H\beta}$ = 6.8 Hz, 3H, Hγ1-Val), 0.79 (d, $J_{H\gamma2,H\beta}$ = 6.8 Hz, 3H, Hγ2-Val) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $(CD_3)_2SO$): δ = 174.3 (C=O-Val), 170.5 (C=O-Cit), 158.8 (Cζ-Cit), 137.5, 137.4 (2 × C_q), 126.9, 118.9 (4 × C_{Ar}), 62.6 (Ar-*C*H_2-OH), 59.6 (Cα-Val), 52.5 (Cα-Cit), 38.6 (Cδ-Cit), 31.3 (Cβ-Val), 30.1 (Cβ-Cit), 26.7 (Cγ-Cit), 19.5 (Cγ1-Val), 16.9 (Cγ2-Val) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{18}H_{30}N_5O_4^+$ $[M+H]^+$: 380.2292, found 380.2292.

***N*-(12-Azido-4,7,10-trioxadodecanoyl)-Val-Cit-4-aminobenzyl alcohol (125)**[94]



To a solution of azide **30**[291] (428 mg, 1.73 mmol, 1.25 equiv.) in CH_2Cl_2 (8 mL) at 0 °C was added Et_3N (0.25 mL, 1.81 mmol, 1.30 equiv.) and isobutylchloroformate (0.22 mL, 1.67 mmol, 1.20 equiv.). The mixture was added after 1 h to a solution of **31** (526 mg, 1.39 mmol, 1.0 equiv.) in CH_2Cl_2/MeOH (3:1, 8 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred for 3 h. The solvents were removed under reduced pressure and the solid residue was dissolved in MeOH (1.5 mL) and was added dropwise to icecold Et_2O (40 mL). The precipitate was collected by centrifugation. After repeated trituration with Et_2O and centrifugation (3 × 20 mL), the colorless solid was dried under high vaccum to give **125** (779 mg, 1.28 mmol, 92%).

R_f = 0.35 (CH_2Cl_2/MeOH, 7:1).

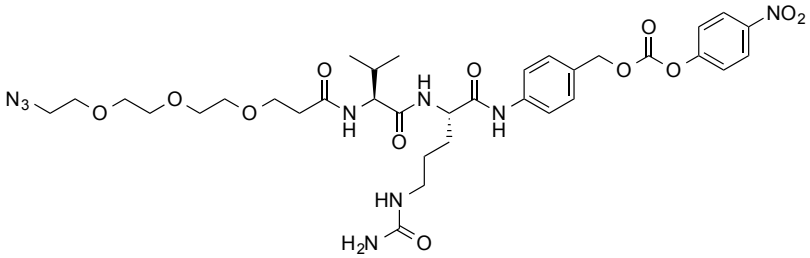
^{1}H-NMR (599 MHz, CD_3OD): δ = 7.59 (d, J = 8.5 Hz, 2H, Ar-H), 7.32 (d, J = 8.8 Hz, 2H, Ar-H), 4.57 (s, 2H, Ar-CH_2-OH), 4.52 (dd, J = 9.1 Hz, J = 5.1 Hz, 1H, Hα-Cit), 4.24 (d, J = 7.0 Hz, 1H, Hα-Val), 3.79–3.75 (m, 2H, -C*H*2-CH2-CONH-), 3.68–3.59 (m, 10H, -CH_2-O-), 3.40–3.36 (m, 2H, -CH_2-N_3), 3.24–3.10 (m, 2H, Hδ2-Cit/Hδ3-Cit), 2.62–2.52 (m, 2H, -CH_2-CONH-), 2.17–2.09 (m, 1H, Hβ-Val), 1.97–1.73 (m, 2H, Hβ2-Cit/Hβ3-Cit), 1.67–1.53 (m, 1H, Hγ2-Cit/Hγ3-Cit), 1.00 (d, $J_{H\gamma1,H\beta}$ = 6.9 Hz, 3H, Hγ1-Val), 0.99 (d, $J_{H\gamma2,H\beta}$ = 6.9 Hz, 3H, Hγ2-Val) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, CD_3OD): δ = 174.4 (C=O-Spacer), 173.9 (C=O-Val), 172.2 (C=O-Cit), 162.3 (Cζ-Cit), 138.7, 138.7 (2 × C_q), 128.6, 121.2 (4 × C_{Ar}), 71.6, 71.5, 71.3, 71.1, 68.3 (6 × -CH_2-O-), 64.8 (Ar-*C*H_2-OH), 60.5 (Cα-Val), 55.0 (Cα-Cit), 51.8 (-CH_2-N_3), 40.4 (Cδ-Cit), 37.4 (-*C*H_2-CONH-), 31.8 (Cβ-Val), 30.4 (Cβ-Cit), 27.8 (Cγ-Cit), 19.8 (Cγ1-Val), 18.8 (Cγ2-Val) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{27}H_{45}N_8O_8^+$ $[M+H]^+$: 609.3355, found 609.3368.

RP-HPLC: t_R = 43.8 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(30:70) 60 min, →(80:20) 90 min.

***N*-(12-Azido-4,7,10-trioxadodecanoyl)-Val-Cit-4-aminobenzyl 4-nitrophenyl carbonate (28)**[94]



To a solution of **125** (768 mg, 1.26 mmol, 1.0 equiv.) in anhydrous DMF (10 mL) was added *N*,*N*-diisopropylethylamine (0.25 mL, 1.58 mmol, 1.25 equiv.) and *bis*(4-nitrophenyl) carbonate (481 mg, 1.58 mmol, 1.25 equiv.). The mixture was stirred at room temperature for 45 h. Then the solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 10:1) to give **28** (607 mg, 0.784 mmol, 62%) as a colorless solid.

R_f = 0.42 (CH_2Cl_2/MeOH, 7:1).

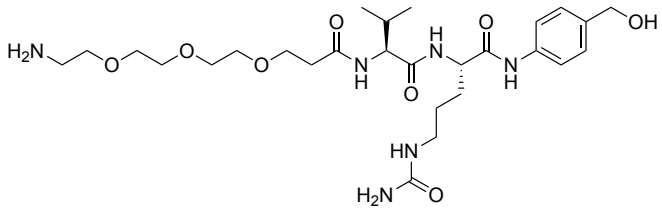
^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ = 10.06 (s, 1H, -NH-Ar), 8.34–8.29 (m, 2H, Ar-H), 8.12 (d, $J_{NH,H\alpha}$ = 7.5 Hz, 1H, NH-Cit), 7.86 (d, $J_{NH,H\alpha}$ = 8.6 Hz, 1H, NH-Val), 7.66 (d, J = 8.6 Hz, 2H, Ar-H), 7.59–7.54 (m, 2H, Ar-H), 7.41 (d, J = 8.7 Hz, 2H, Ar-H), 5.99 (t, $J_{H\varepsilon,H\delta2/H\delta3}$ = 5.8 Hz, 1H, Hε-Cit), 5.24 (s, 2H, Ar-CH_2-O-), 4.43–4.36 (m, 1H, Hα-Cit), 4.24 (dd, J = 8.7 Hz, J = 6.6 Hz, 1H, Hα-Val), 3.65–3.45 (m, 12H, -CH_2-O-), 3.40–3.36 (m, 2H, -CH_2-N_3), 3.10–2.90 (m, 2H, Hδ2-Cit/Hδ3-Cit), 2.49–2.34 (m, 2H, -CH_2-CONH-), 2.03–1.93 (m, 1H, Hβ-Val), 1.77–1.54 (m, 2H, Hβ2-Cit/Hβ3-Cit), 1.51–1.32 (m, 1H, Hγ2-Cit/Hγ3-Cit), 0.87 (d, $J_{H\gamma1,H\beta}$ = 6.8 Hz, 3H, Hγ1-Val), 0.84 (d, $J_{H\gamma2,H\beta}$ = 6.8 Hz, 3H, Hγ2-Val) ppm.

^{13}C-{^{1}H}-NMR (101 MHz, $(CD_3)_2SO$): δ = 171.1 (C=O-Val), 170.7 (C=O-Cit), 170.3 (C=O-Spacer), 158.9 (Cζ-Cit), 155.3 (C_q), 151.9 (O-CO-O), 145.2, 139.4 (2 × C_q), 129.4 (C_{Ar}), 129.3 (C_q), 125.4, 122.6, 119.0 (3 × C_{Ar}), 70.2 (Ar-*C*H_2-O-), 69.8, 69.7, 69.5, 69.2, 66.9 (6 × -CH_2-O-), 57.5 (Cα-Val), 53.2 (Cα-Cit), 50.0 (-CH_2-N_3), 38.5 (Cδ-Cit), 35.9 (-*C*H_2-CONH-), 30.6 (Cβ-Val), 29.2 (Cβ-Cit), 26.8 (Cγ-Cit), 19.2 (Cγ1-Val), 18.1 (Cγ2-Val) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{34}H_{48}N_9O_{12}^+$ $[M+H]^+$: 774.3417, found 774.3433.

RP-HPLC: t_R = 73.9 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(30:70) 60 min, →(80:20) 90 min.

***N*-(12-Amino-4,7,10-trioxadodecanoyl)-Val-Cit-4-aminobenzyl alcohol (132)**



Dipeptide **31** (656 mg, 1.73 mmol, 1.0 equiv.) was dissolved in DMF (10 mL) and the pH was adjusted to 8-9 by addition of DIPEA. Fmoc-protected PEG spacer **133**[307] (923 mg, 2.08 mmol, 1.2 equiv.) and PyBOP (1.08 g, 2.08 mmol, 1.2 equiv.) were added and the solution was stirred at room temperature for 2 h. The solvents were removed under reduced pressure and the crude product was used in the next step without further purification. The yellow solid was dissolved in DMF (8 mL) and was treated with piperidine (2 mL). The mixture was stirred for 30 min and then the solvents were removed under reduced pressure. The solid residue was dissolved in MeOH (2 mL) and was added dropwise to icecold Et_2O (40 mL). The precipitate was collected by centrifugation. After repeated trituration with Et_2O and centrifugation (3 ×), the colorless solid was dried *in vacuo* to give **132** (716 mg, 1.23 mmol, 71%).

^{1}H-NMR (400 MHz, CD_3OD): δ = 7.63–7.56 (m, 2H, Ar-H), 7.37–7.31 (m, 2H, Ar-H), 4.59 (s, 2H, Ar-C*H*$_2$-OH), 4.55 (dd, *J* = 9.1 Hz, *J* = 5.1 Hz, 1H, Hα-Cit), 4.26 (d, *J* = 7.0 Hz, 1H, Hα-Val), 3.79 (t, *J* = 6.0 Hz, 2H, -C*H*2-CH2-CONH-), 3.69–3.60 (m, 10H, -CH_2-O-), 3.28–3.10 (m, 2H, Hδ2-Cit/Hδ3-Cit), 2.96 (t, *J* = 5.2 Hz, 2H, -CH_2-NH_2), 2.61 (t, *J* = 5.9 Hz, 2H, -C*H*$_2$-CONH-), 2.20–2.10 (m, 1H, Hβ-Val), 2.01–1.90 (m, 1H, Hβ2-Cit), 1.85–1.73 (m, 1H, Hβ3-Cit), 1.71–1.54 (m, 2H, Hγ2-Cit/Hγ3-Cit), 1.02 (t, *J* = 6.4 Hz, 6H, Hγ1-Val/Hγ2-Val) ppm.

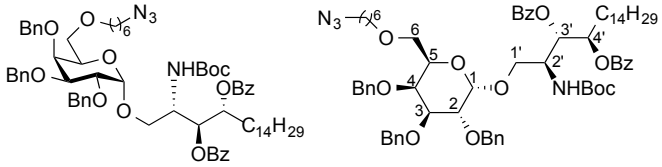
^{13}C-{^{1}H}-NMR (101 MHz, CD_3OD): δ = 174.4 (C=O-Spacer), 173.8 (C=O-Val), 172.2 (C=O-Cit), 162.3 (Cζ-Cit), 138.8, 138.7 (2 × C_q), 128.6, 121.2 (4 × C_{Ar}), 71.5, 71.4, 71.3, 71.2, 71.0, 68.3 (6 × -CH_2-O-), 64.8 (Ar-*C*H_2-OH), 60.6 (Cα-Val), 55.0 (Cα-Cit), 41.5 (-CH_2-NH_2), 40.3 (Cδ-Cit), 37.3 (-*C*H_2-CONH-), 31.8 (Cβ-Val), 30.4 (Cβ-Cit), 27.9 (Cγ-Cit), 19.8 (Cγ1-Val), 18.8 (Cγ2-Val) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{27}H_{47}N_6O_8^+$ $[M+H]^+$: 583.3450, found 583.3445.

RP-HPLC: t_R = 11.2 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

5.2.2.4 Assembly of the α-GalCer derivative (11)

(*2S*,*3S*,*4R*)-1-(6-(6'-Azidohexyl)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2-(*N*-*tert*-butoxycarbonyl)amino-3,4-di-*O*-benzoyl-octadecane (34)[89]



6-(6′-Azidohexyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl *N*-phenyltrifluoroacetimidate **36** (510 mg, 0.683 mmol, 1.3 equiv.) and acceptor **35** (329 mg, 0.525 mmol, 1.0 equiv.) were combined and co-evaporated with toluene (3 × 5 mL) and with THF (1 × 5 mL), dried under high vacuum and then dissolved in THF (15 mL) under argon. The mixture was stirred with freshly activated 4Å MS at room temperature for 30 min, before the reaction vessel was cooled to −40 °C. TMSOTf (29 µL, 0.158 mmol, 0.3 equiv.) was added and the reaction mixture was allowed to warm to −20 °C over a period of 1.5 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL), quenched by the addition of NEt_3 and filtered through a pad of celite. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 7:1) to give **34** (598 mg, 0.505 mmol, 74%) as a colorless oil.

R_f = 0.40 (^{c}Hex/EtOAc, 5:1).

$[\alpha]_D^{22}$ = + 17.2 (c = 0.5, $CHCl_3$).

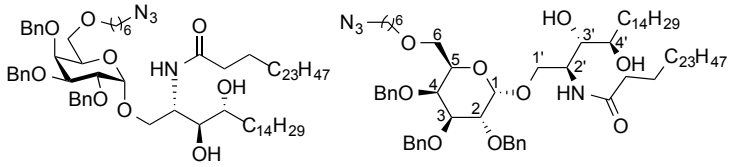
^{1}H-NMR (599 MHz, $CDCl_3$): δ = 8.05–7.92 (m, 4H, Ar-H), 7.62–7.13 (m, 21H, Ar-H), 5.64 (dd, J = 9.2 Hz, J = 3.0 Hz, 1H, H-3'), 5.57 (d, $J_{NH,H2'}$ = 9.8 Hz, 1H, NH), 5.48 (dt, J = 9.9 Hz, J = 2.9 Hz, 1H, H-4'), 4.92 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.79–4.75 (m, 2H, H-1/CH_{Bn}), 4.68 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.66 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.61 (d, J = 11.9 Hz, 1H, CH_{Bn}), 4.57 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.25–4.19 (m, 1H, H-2'), 4.00–3.95 (m, 2H, H-2/H-5), 3.92–3.90 (m, 1H, H-4), 3.87 (dd, $J_{H3,H2}$ = 10.1 Hz, $J_{H3,H4}$ = 2.8 Hz, 1H, H-3), 3.81 (dd, $J_{H1a',H1b'}$ = 11.5 Hz, $J_{H1a',H2'}$ = 3.9 Hz, 1H, H-1a'), 3.69 (dd, $J_{H1b',H1a'}$ = 11.4 Hz, $J_{H1b',H2'}$ = 3.1 Hz, 1H, H-1b'), 3.45–3.35 (m, 3H, H-6a/H-6b/CH_{Linker}), 3.31–3.26 (m, 1H, CH_{Linker}), 3.23 (t, J = 6.9 Hz, 2H, CH_{Linker}), 1.98–1.77 (m, 2H, CH_{Linker}), 1.60–1.17 (m, 34H, 8 × CH_{Linker}/13 × -CH_2-), 0.88 (t, J = 7.0 Hz, 3H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 166.1, 165.4 (2 × C=O-OBz), 155.6 (C=O-Boc), 139.1, 138.8, 138.7, 135.2 (5 × C_q-Ar), 133.3, 133.0, 130.3, 130.1, 129.9, 129.5, 128.6, 128.4, 128.3, 128.2, 127.7, 127.6, 126.5 (25 × C_{Ar}), 99.6 (C-1), 79.9 (C_q-Boc), 78.9 (C-3), 76.8 (C-2), 75.2 (C-4), 74.9 (CH_{Bn}), 73.9 (C-4'), 73.3, 73.2 (2 × CH_{Bn}), 73.0 (C-3'), 71.5 (CH_{Linker}), 69.9 (C-5), 69.4 (C-6/C-1'), 51.5 (CH_{Linker}), 50.6 (C-2'), 32.1, 29.8, 29.7, 29.5, 28.9 (CH_{Linker}/11 × -CH_2-), 28.5 (3 × CH_3-Boc), 26.7, 25.8 (3 × CH_{Linker}), 22.8 (-CH_2-), 14.3 (-CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{70}H_{98}N_5O_{12}^+$ $[M+NH_4]^+$: 1200.7207, found 1200.7249.

RP-HPLC: t_R = 17.2 min, Phenomenex Luna C18, λ = 254 nm, Grad.: MeCN:H_2O + 0.1% TFA (30:70) →(90:10) 40 min.

(*2S*,*3S*,*4R*)-1-(6-(6'-Azidohexyl)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2-hexacosanoylamino-octadecane-3,4-diol (33)[89, 276]



To a solution of **34** (890 mg, 0.752 mmol, 1.0 equiv.) in CH_2Cl_2 (4 mL) was added TFA (1 mL) and the reaction mixture was stirred for 2 h at room temperature. The solvents were removed under reduced pressure and the crude product was dissolved in dry CH_2Cl_2 (8 mL) under an argon atmosphere. To the solution was added cerotic acid (447 mg, 1.13 mmol, 1.5 equiv.), HATU (430 mg, 1.13 mmol, 1.5 equiv.) and DIPEA (1.92 mL, 11.3 mmol, 15 equiv.) and the reaction mixture was stirred for 4.5 h at room temperature. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 6:1). The colorless oil was dissolved in MeOH/CH_2Cl_2 (2:1, 30 mL) and catalytic amounts of NaOMe were added. The solution was stirred over night. The reaction mixture was neutralized by adding *Amberlite IR120*. The ion-exchange resin was filtered off and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography on silica (^{c}Hex/EtOAc, 3:1) to give **33** (527 mg, 0.420 mmol, 56% over three steps) as a colorless solid.

R_f = 0.21 (^{c}Hex/EtOAc, 3:1).

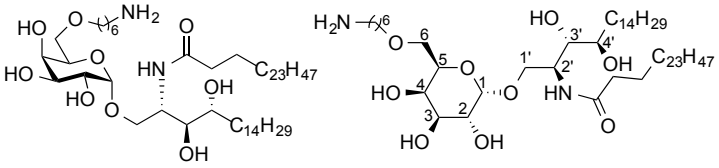
$[\alpha]_D^{22}$ = + 23.2 (c = 0.25, $CHCl_3$).

^{1}H-NMR (599 MHz, $CDCl_3$): δ = 7.40–7.25 (m, 15H, Ar-H), 6.39 (d, $J_{NH,H2'}$ = 8.4 Hz, 1H, NH), 4.94 (d, J = 11.4 Hz, 1H, CH_{Bn}), 4.88 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.85 (d, $J_{H1,H2}$ = 3.8 Hz, 1H, H-1), 4.80–4.75 (m, 2H, CH_{Bn}), 4.68 (d, J = 11.6 Hz, 1H, CH_{Bn}), 4.60 (d, J = 11.5 Hz, 1H, CH_{Bn}), 4.24–4.20 (m, 1H, H-2'), 4.05 (dd, $J_{H2,H3}$ = 10.0 Hz, $J_{H2,H1}$ = 3.8 Hz, 1H, H-2), 3.97–3.94 (m, 1H, H-4), 3.89–3.85 (m, 3H, H-3/H-1a'/H-1b'), 3.84–3.80 (m, 1H, H-5), 3.53–3.37 (m, 5H, H-6a/H-6b/H-3'/H–4'/CH_{Linker}), 3.34–3.28 (m, 1H, CH_{Linker}), 3.25 (t, J = 6.9 Hz, 2H, CH_{Linker}), 2.19–2.12 (m, 2H, -CH_2-), 1.70–1.20 (m, 80H, 8 × CH_{Linker} /36 × -CH_2-), 0.90–0.86 (m, 6H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (151 MHz, $CDCl_3$): δ = 173.2 (C=O), 138.6, 138.5, 138.0 (3 × C_q), 128.6, 128.4, 128.3, 128.1, 127.8, 127.6 (15 × C_{Ar}), 99.3 (C-1), 79.5 (C-3), 76.4 (C-3'), 76.2 (C-2), 74.9 (CH_{Bn}), 74.6 (C-4), 74.4 (CH_{Bn}), 73.5 (C-4'), 72.9 (CH_{Bn}), 71.6 (CH_{Linker}), 70.1 (C-1'), 70.0 (C-5), 69.4 (C-6), 51.5 (CH_{Linker}), 49.6 (C-2'), 36.9, 33.5, 32.1, 29.9, 29.7, 29.6, 29.5, 28.9, 27.1, 26.7, 26.1, 25.9, 25.9, 22.9 (4 × CH_{Linker}/37 × -CH_2-), 14.3 (2 × -CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{77}H_{129}N_4O_9^+$ $[M+H]^+$: 1253.9754, found 1253.9767.

(*2S*,*3S*,*4R*)-1-(6-(6'-Aminohexyl)-α-D-galactopyranosyl)-2-hexacosanoylamino-octadecane-3,4-diol (29)[89]



Compound **33** (106 mg, 84.4 μmol) was dissolved in a mixture of EtOH/$CHCl_3$ (3:1, 12 mL) and purged with argon. Then 10% $Pd(OH)_2$/C (53 mg) was added and the reaction mixture was purged with H_2 four times and then stirred under an H_2 atmosphere at room temperature for 20 h. The reaction mixture was filtered over a pad of celite and it was washed with EtOH/$CHCl_3$ (3:1, 3 × 10 mL). The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 5:1) to give **29** (75 mg, 78.3 μmol, 93%) as a colorless solid.

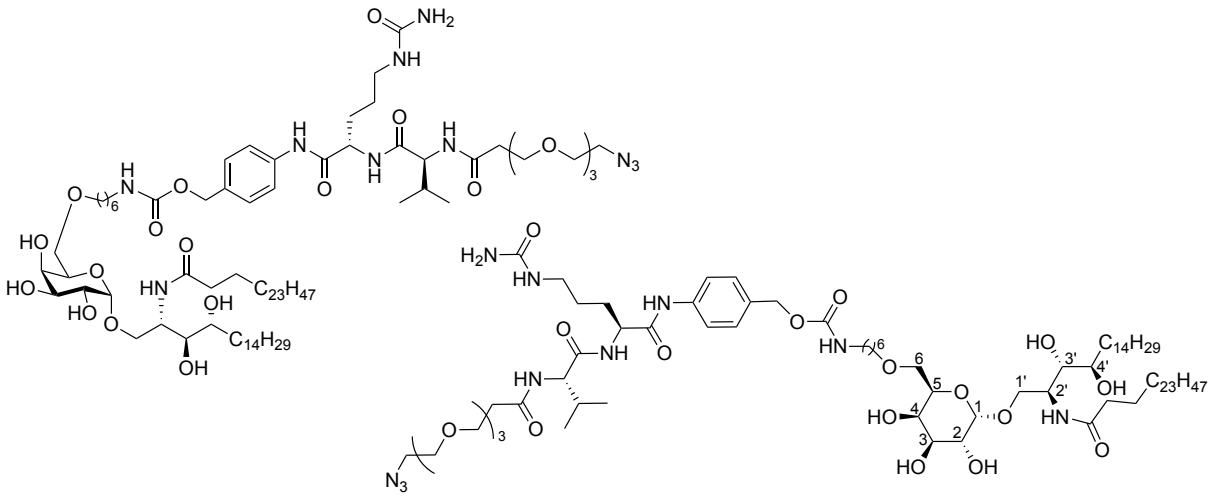
$[\alpha]_D^{22}$ = + 34.4 (c = 0.25, C_5H_5N).

^{1}H-NMR (800 MHz, C_5D_5N): δ = 8.51 (d, $J_{NH,H2'}$ = 8.5 Hz, 1H, NH), 5.55 (d, $J_{H1,H2}$ = 3.9 Hz, 1H, H-1), 5.30–5.22 (m, 1H, H-2'), 4.69–4.66 (m, 1H, H-1a'), 4.63 (dd, $J_{H2,H3}$ = 9.4 Hz, $J_{H2,H1}$ = 3.5 Hz, 1H, H-2), 4.49 (t, $J_{H5,H6a/H6b}$ = 6.2 Hz, 1H, H-5), 4.43–4.39 (m, 2H, H-3/H-4), 4.38 (dd, J = 10.8 Hz, J = 4.6 Hz, 1H, H-1b'), 4.35–4.30 (m, 2H, H-3'/H–4'), 4.09 (dd, $J_{H6a,H6b}$ = 9.9 Hz, $J_{H6a,H5}$ = 5.7 Hz, 1H, H-6a), 4.02 (dd, $J_{H6b,H6a}$ = 9.7 Hz, $J_{H6b,H5}$ = 6.6 Hz, 1H, H-6b), 3.58–3.48 (m, 2H, CH_{Linker}), 2.82–2.77 (m, 2H, CH_{Linker}), 2.50–2.44 (m, 2H, -CH_2-CONH-), 2.33–2.25 (m, 1H, -CH_2-), 1.97–1.88 (m, 2H, -CH_2-), 1.87–1.81 (m, 2H, CH_{Linker}), 1.73–1.20 (m, 77H, 8 × CH_{Linker} /34.5 × -CH_2-), 0.90–0.85 (m, 6H, -CH_3) ppm.

^{13}C-{^{1}H}-NMR (201 MHz, C_5D_5N): δ = 173.5 (C=O), 101.8 (C-1), 77.0 (C-3'), 72.8 (C-4'), 71.8 (C-3/CH_{Linker}), 71.4 (C-6), 71.2 (C-5), 71.1 (C-4), 70.5 (C-2), 69.0 (C-1'), 51.7 (C-2'), 42.5 (CH_{Linker}), 37.2 (-CH_2-CONH-), 34.7, 33.6, 32.5, 30.8, 30.5, 30.5, 30.4, 30.3, 30.2, 30.0, 27.3, 26.9, 26.8, 26.7, 23.3 (4 × CH_{Linker}/36 × -CH_2-), 14.7 (2 × -CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{56}H_{113}N_2O_9^+$ $[M+H]^+$: 957.8441, found 957.8449.

(*2S*,*3S*,*4R*)-1-(6-(6‘-(*N*-(12-Azido-4,7,10-trioxadodecanoyl)-Val-Cit-4-aminobenzyloxycarbonylamino)-hexyl)-α-D-galactopyranosyl)-2-hexacosanoylamino-octadecane-3,4-diol (11)[94]



KRN7000 derivative **29** (58 mg, 60.6 µmol, 1.0 equiv.) and dipeptide linker **28** (52 mg, 66.6 µmol, 1.1 equiv.) were dissolved in dry pyridine (5 mL) and dry Et_3N (17 µL, 121 µmol, 2.0 equiv.) was added. The reaction mixture was stirred for 3 h at room temperature. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography on silica (CH_2Cl_2/MeOH, 9:1) to give **11** (85 mg, 53.4 µmol, 88%) as a colorless solid.

$[\alpha]_D^{22}$ = + 16.0 (c = 0.1, C_5H_5N).

^{1}H-NMR (400 MHz, C_5D_5N): δ = 10.86 (s, 1H, -NH-Ar), 9.49 (d, $J_{NH,H\alpha}$ = 8.2 Hz, 1H, NH-Cit), 8.88 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH-Val), 8.43 (d, $J_{NH,H2‘}$ = 8.6 Hz, 1H, NH-Ceramide), 8.10 (d, J = 8.4 Hz, 2H, Ar-H), 7.88 (t, J = 5.7 Hz, 1H, NH-Linker), 7.43 (d, J = 8.4 Hz, 2H, Ar-H), 6.96 (t, $J_{H\varepsilon,H\delta2/H\delta3}$ = 6.1 Hz, 1H, Hε-Cit), 5.54 (d, $J_{H1,H2}$ = 3.8 Hz, 1H, H-1), 5.44–5.37 (m, 1H, Hα-Cit), 5.29 (s, 2H, Ar-CH_2-O-), 5.27–5.22 (m, 1H, H-2‘), 5.05 (dd, J = 8.6 Hz, J = 7.2 Hz, 1H, Hα-Val), 4.69–4.65 (m, 1H, H-1a‘), 4.63 (dd, $J_{H2,H3}$ = 9.5 Hz, $J_{H2,H1}$ = 3.9 Hz, 1H, H-2), 4.48 (t, $J_{H5,H4/H6a/H6b}$ = 6.2 Hz, 1H, H-5), 4.45–4.30 (m, 5H, H-3/H-4/H-1b‘/H-3‘/ H-4‘), 4.09 (dd, $J_{H6a,H6b}$ = 9.8 Hz, $J_{H6a,H5}$ = 6.1 Hz, 1H, H-6a), 4.01–3.94 (m, 2H, H-6b/-CH_2-O-Spacer), 3.91–3.84 (m, 1H, -CH_2-O-Spacer), 3.80–3.69 (m, 1H, Hδ2-Cit), 3.68–3.59 (m, 10H, 5 × -CH_2-O-Spacer), 3.55–3.44 (m, 2H, -CH_2-O-Linker), 3.42–3.36 (m, 2H, -CH_2-NH-Linker), 3.36–3.32 (m, 2H, -CH_2-N_3), 3.29–3.18 (m, 1H, Hδ3-Cit), 2.89–2.68 (m, 2H, -CH_2-CONH-Spacer), 2.50–2.37 (m, 3H, Hβ-Val/-CH_2-CONH-Ceramide), 2.35–2.21 (m, 2H, Hβ2-Cit/-CH_2-), 2.00–1.77 (m, 5H, Hβ3-Cit/2 × -CH_2-), 1.76–1.53 (m, 6H, Hγ2-Cit/Hγ3-Cit/ 2 × -CH_2-), 1.51–1.20 (m, 70H, 35 × -CH_2-), 1.15 (d, $J_{H\gamma1,H\beta}$ = 6.8 Hz, 3H, Hγ1-Val), 1.10 (d, $J_{H\gamma2,H\beta}$ = 6.8 Hz, 3H, Hγ2-Val), 0.89 (t, J = 6.6 Hz, 6H, 2 × -CH_3) ppm.

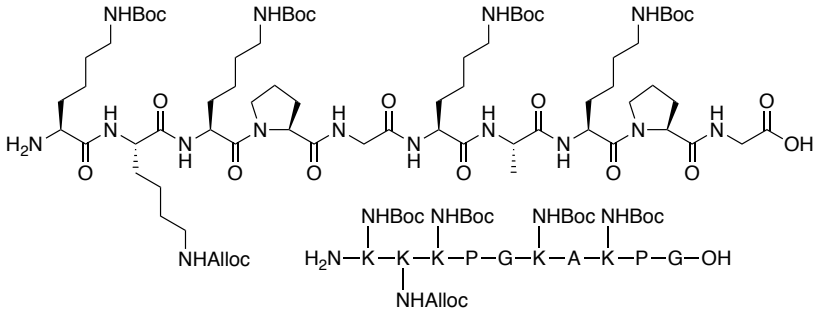
^{13}C-{^{1}H}-NMR (201 MHz, C_5D_5N): δ = 173.6 (C=O-Ceramide), 173.1 (C=O-Val), 172.4 (C=O-Cit), 171.9 (C=O-PEG), 161.4 (Cζ-Cit), 157.7 (C=O-Carbamate), 140.3, 133.4 (2 × C_q), 129.4, 120.4 (4 × C_{Ar}), 101.9 (C-1), 77.0 (C-3‘), 72.9 (C-4‘), 71.9 (CH_{Linker}), 71.8 (C-3), 71.2, 71.1 (C-5/C-6/5 × -CH_2-O-Spacer), 71.0

(C-4), 70.5 (C-2), 69.1 (C-1‘), 68.3 (-CH_2-O-Spacer), 66.4 (Ar-*C*H_2-O-), 59.6 (Cα-Val), 53.7 (Cα-Cit), 51.7 (C-2‘), 51.3 (-CH_2-N_3), 41.8 (CH_{Linker}), 39.1 (Cδ-Cit), 37.7 (-*C*H_2-CONH-Spacer), 37.2 (-*C*H_2-CONH-Ceramide), 34.7, 32.5, 32.1, 30.8, 30.6, 30.4, 30.3, 30.2, 30.0, 28.3, 27.4, 26.9, 26.8, 26.6 (Cβ-Val/Cβ-Cit/Cγ-Cit/4 × CH_{Linker}/ 36 × -CH_2-Ceramid), 20.2 (Cγ2-Val), 19.3 (Cγ1-Val), 14.7 (2 × -CH_3) ppm.

HRMS (ESI-MS): m/z calcd. for $C_{84}H_{155}N_{10}O_{18}^+$ $[M+H]^+$: 1592.1515, found 1592.1550.

5.2.3 Synthesis of cyclic decapeptides 39 and 129

Lys(Boc)-Lys(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly (40)[183]

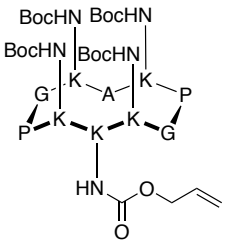


The solid-phase synthesis of linear peptide **40** was carried out on a *CEM Liberty Blue* peptide synthesizer (standard program 0.50 mmol) using a pre-loaded Fmoc-Gly-SASRIN resin (loading: 0.79 mmol/g). In every coupling cycle, the *N*-terminal Fmoc group was removed by treatment of the resin with a solution of piperidine (20%) in DMF for at least 2 × 5 min at room temperature. The couplings of the amino acids (2.5 equiv. based on the loaded resin) were carried out with a cocktail containing PyBOP (2.5 equiv.), and DIPEA (5 equiv.) in DMF (60 min, room temperature). The resin was treated with 1% trifluoroacetic acid in dichloromethane for three times until the resin beads became dark purple. The combined washings were immediately neutralized with a mixture of pyridine in methanol and then concentrated under reduced pressure. The white solid peptide was obtained by precipitation from diethyl ether in 80% (611 mg, 0.40 mmol) yield.

HRMS (ESI-MS): m/z calcd. for $C_{71}H_{128}N_{16}O_{21}^{2+}$ $[M+H+NH_4]^{2+}$: 770.4714, found 770.4737.

RP-HPLC: t_R = 26.3 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

c[Lys(Boc)-Lys(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (41)[183]

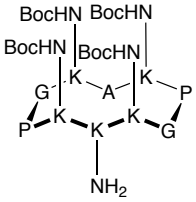


Linear peptide **40** (200 mg, 131 μmol, 1.0 equiv.) was dissolved in DMF (200 mL) and the pH was adjusted to 8-9 by addition of DIPEA. PyBOP (82 mg, 158 μmol, 1.2 equiv.) was added and the solution was stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure and the residue was co-evaporated with toluene (2 × 10 mL) and CH_2Cl_2 (10 mL). The residue was dissolved in 5 mL of methanol and added to cold Et_2O (20 mL). The precipitate was triturated and washed with cold Et_2O (3 × 10 mL) to furnish cyclic peptide **41** (173 mg, 115 μmol, 88%) as a colorless solid.

HRMS (ESI-MS): m/z calcd. for $C_{71}H_{125}N_{16}O_{20}^{+}$ $[M+NH_4]^{+}$: 1521.9251, found 1521.9294.

RP-HPLC: t_R = 32.3 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

c[Lys(Boc)-Lys-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (126)[183]

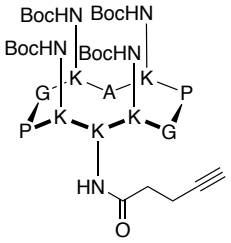


Cyclic peptide **41** (184 mg, 122 μmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (30 mL) under an argon atmosphere. Phenylsilane (1.03 mL, 8.36 mmol, 68.5 eqiuv.) was added and the mixture was stirred for 5 min before $Pd(PPh_3)_4$ (28.0 mg, 24 μmol, 0.2 equiv.) was added. The solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the crude product was dissolved in a mixture of CH_2Cl_2/methanol (1:1, 1 mL). The solution was added dropwise to cold Et_2O (20 mL) and the precipitate was triturated and washed with Et_2O (3 × 10 mL). The colorless solid was dissolved in a mixture of MeCN/H_2O (1:1, 1 mL) and subjected to lyophilization to give the desired product **126** (134 mg, 94.3 μmol, 77%) as a colorless powder.

HRMS (ESI-MS): m/z calcd. for $C_{67}H_{118}N_{15}O_{18}^+$ $[M+H]^+$: 1420.8774, found 1420.8839.

RP-HPLC: t_R = 27.4 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O+ 0.1% TFA (5:95) →(20:80) 40 min.

c[Lys(Boc)-Lys(4-Pentynoyl)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (127)[183]

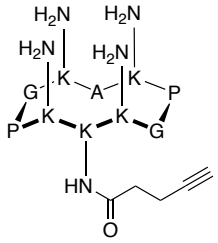


Compound **126** (266 mg, 187 μmol, 1.0 equiv.) was dissolved in DMF (30 mL) and the pH was adjusted to 8-9 by addition of DIPEA. 4-Pentynoic acid (22 mg, 225 μmol, 1.2 equiv.) and PyBOP (117 mg, 225 μmol, 1.2 equiv.) were added and the solution was stirred at room temperature for 20 h. The solvents were removed under reduced pressure and the crude product was dissolved in CH_2Cl_2/MeOH (1:1, 2 mL). The product was precipitated by adding the solution dropwise to cold Et_2O (20 mL) and the precipitate was triturated and washed with Et_2O (3 × 10 mL). The colorless solid was purified by preparative HPLC (Phenomenex Aeris) before it was subjected to lyophilization to furnish the desired product **127** (257 mg, 171 μmol, 91%) as a colorless powder.

HRMS (ESI-MS): m/z calcd. for $C_{72}H_{122}N_{15}O_{19}^{2+}$ $[M+H+Na]^{2+}$: 761.9464, found 761.9410.

RP-HPLC: t_R = 30.0 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

c[Lys-Lys(4-Pentynoyl)-Lys-Pro-Gly-Lys-Ala-Lys-Pro-Gly] (39)[183]

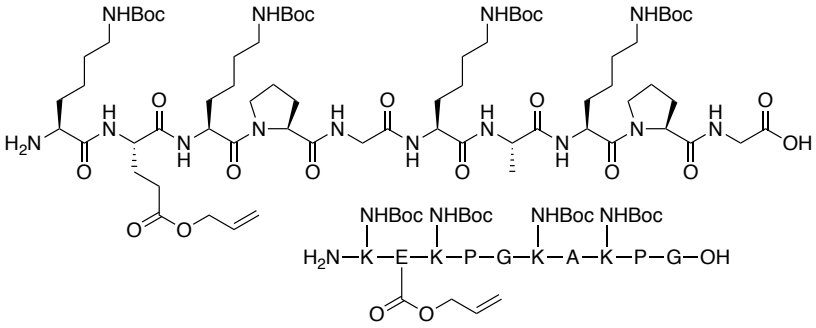


Peptide **127** (152 mg, 101 μmol) was dissolved in CH_2Cl_2 (4 mL), and H_2O (0.5 mL) and TFA (1 mL) were added. The reaction mixture was stirred at room temperature for 1 h. The solvents were removed under reduced pressure and the peptide was dissolved in MeCN/H_2O (1:1, 4 mL) and subjected to lyophilization to furnish the desired product **39** (102 mg, 92.7 μmol, 92%) as a colorless powder.

HRMS (ESI-MS): m/z calcd. for $C_{52}H_{91}N_{15}O_{11}^{2+}$ $[M+2H]^{2+}$: 550.8506, found 550.8513.

RP-HPLC: t_R = 26.0 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(10:90) 20 min, →(75:25) 30 min, →(50:50) 40 min, →(100:0) 50 min.

Lys(Boc)-Glu(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly (128)

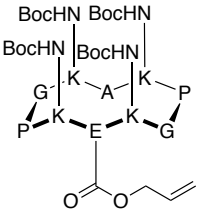


The solid-phase synthesis of linear peptide **128** was carried out on a *CEM Liberty Blue* peptide synthesizer (standard program 0.50 mmol) using a pre-loaded Fmoc-Gly-SASRIN resin (loading: 0.79 mmol/g). In every coupling cycle, the *N*-terminal Fmoc group was removed by treatment of the resin with a solution of piperidine (20%) in DMF for at least 2 × 5 min at room temperature. The couplings of the amino acids (2.5 equiv. based on the loaded resin) were carried out with a cocktail containing PyBOP (2.5 equiv.), and DIPEA (5 equiv.) in DMF (60 min, room temperature). The resin was treated with 1% trifluoroacetic acid in dichloromethane for three times until the resin beads became dark purple. The combined washings were immediately neutralized with a mixture of pyridine in methanol and then concentrated under reduced pressure. The white solid peptide was obtained by precipitation from diethyl ether in 72% (531 mg, 0.36 mmol) yield.

HRMS (ESI-MS): m/z calcd. for $C_{69}H_{119}N_{14}O_{21}^{+}$ $[M+H]^{+}$: 1479.8669, found 1479.8655.

RP-HPLC: t_R = 26.0 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

c[Lys(Boc)-Glu(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (130)

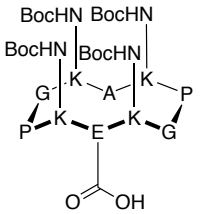


Linear peptide **128** (525 mg, 365 μmol, 1.0 equiv.) was dissolved in DMF (300 mL) and the pH was adjusted to 8-9 by addition of DIPEA. PyBOP (222 mg, 426 μmol, 1.2 equiv.) was added and the solution was stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure and the residue was co-evaporated with toluene (2 × 10 mL) and CH_2Cl_2 (10 mL). The residue was dissolved in 5 mL of methanol and added to cold Et_2O (20 mL). The precipitate was triturated and washed with cold Et_2O (3 × 10 mL) to furnish cyclic peptide **130** (445 mg, 304 μmol, 83%) as a colorless solid.

HRMS (ESI-MS): m/z calcd. for $C_{69}H_{120}N_{15}O_{20}^+$ $[M+NH_4]^+$: 1478.8829, found 1478.8835.

RP-HPLC: t_R = 32.6 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

c[Lys(Boc)-Glu-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (131)

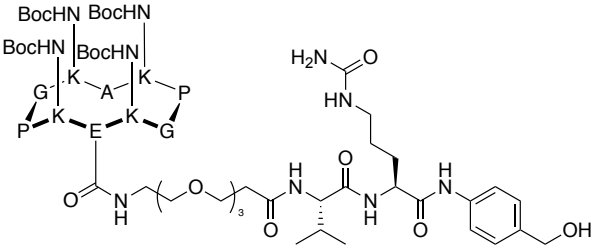


Cyclic peptide **130** (439 mg, 300 μmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (50 mL) under an argon atmosphere. Phenylsilane (82 μL, 660 μmol, 2.2 eqiuv.) was added and the mixture was stirred for 5 min before $Pd(PPh_3)_4$ (7.0 mg, 6 μmol, 0.02 equiv.) was added. The solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the crude product was dissolved in a mixture of CH_2Cl_2/methanol (1:1, 1 mL). The solution was added dropwise to cold Et_2O (20 mL) and the precipitate was triturated and washed with Et_2O (3 × 10 mL). The crude product was purified by preparative HPLC (Phenomenex Aeris) to give the desired compound **131** (337 mg, 237 μmol, 79%) as a colorless powder.

HRMS (ESI-MS): m/z calcd. for $C_{66}H_{116}N_{15}O_{20}^+$ $[M+NH_4]^+$: 1438.8516, found 1438.8515.

RP-HPLC: t_R = 29.2 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

c[Lys(Boc)-Glu(PEG-Val-Cit-4-aminobenzyl alcohol)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (134)

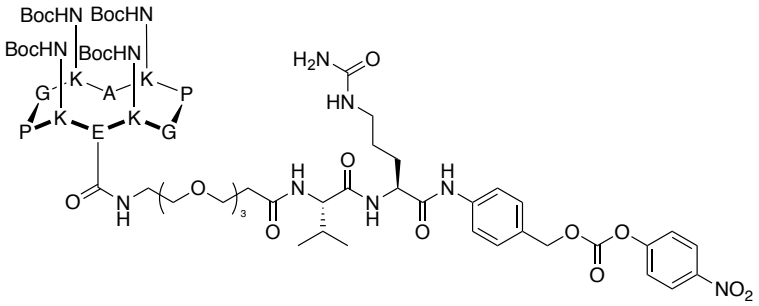


Compound **131** (231 mg, 163 μmol, 1.2 equiv.) was dissolved in DMF (10 mL) and the pH was adjusted to 8-9 by addition of DIPEA. Dipeptide linker **132** (79 mg, 136 μmol, 1.0 equiv.) and PyBOP (106 mg, 204 μmol, 1.5 equiv.) were added and the solution was stirred at room temperature for 20 h. The solvents were removed under reduced pressure and the crude product was dissolved in MeOH (2 mL). The product was precipitated by adding the solution dropwise to cold Et_2O (20 mL) and the precipitate was triturated and washed with Et_2O (3 × 10 mL). The colorless solid was purified by preparative HPLC (Phenomenex Aeris) before it was subjected to lyophilization to furnish the desired product **134** (200 mg, 101 μmol, 74%) as a colorless powder.

HRMS (ESI-MS): m/z calcd. for $C_{93}H_{158}N_{20}O_{27}^{2+}$ $[M+2H]^{2+}$: 994.0814, found 994.0804.

RP-HPLC: t_R = 27.2 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

c[Lys(Boc)-Glu(PEG-Val-Cit-4-aminobenzyl 4-nitrophenyl carbonate)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (129)



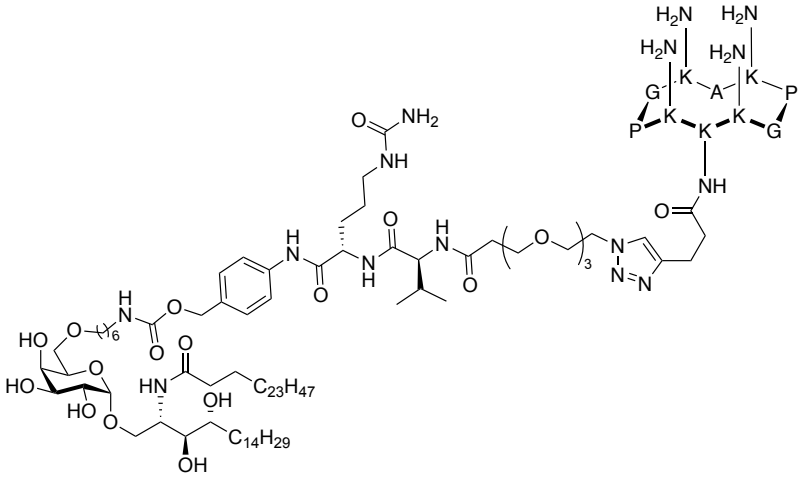
To a solution of **134** (20 mg, 10 μmol, 1.0 equiv.) in anhydrous DMF (1.5 mL) was added *N*,*N*-diisopropylethylamine (21 μL, 12.5 μmol, 1.25 equiv.) and bis(4-nitrophenyl) carbonate (3.8 mg, 12.5 μmol, 1.25 equiv.). The mixture was stirred at room temperature for 25 h. Then the solvents were removed under reduced pressure and the crude product was dissolved in MeOH (2 mL). The product was precipitated by adding the solution dropwise to cold Et_2O (20 mL) and the precipitate was triturated and washed with Et_2O (3 × 10 mL). The colorless solid was purified by preparative HPLC (Phenomenex Aeris) before it was subjected to lyophilization to furnish the desired product **129** (15 mg, 7.0 μmol, 70%) as a colorless powder.

HRMS (ESI-MS): m/z calcd. for $C_{100}H_{164}N_{22}O_{31}^{2+}$ $[M+H+NH_4]^{2+}$: 1085.0978, found 1085.0977.

RP-HPLC: t_R = 32.5 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

5.2.4 Studies toward a multivalent fully synthetic vaccine candidate and synthesis of a monovalent fully synthetic vaccine candidate

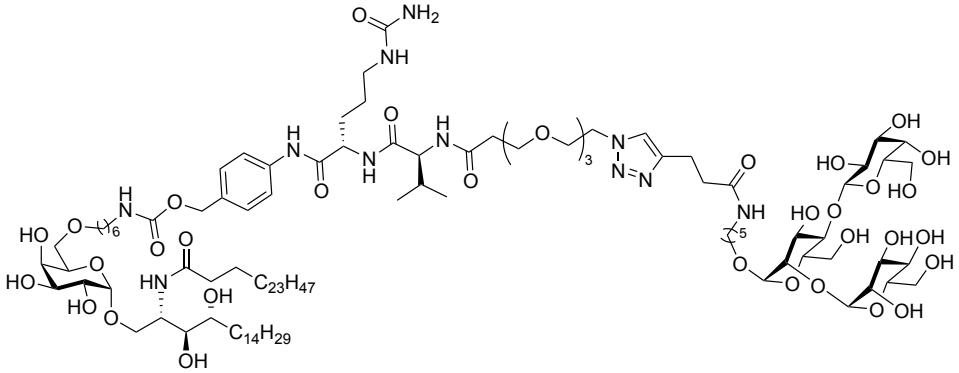
CuAAC Conjugate 38[94]



KRN7000 derivative **11** (3.0 mg, 1.9 μmol, 1.0 equiv.) and cyclic decapeptide **39** (4.2 mg, 3.8 μmol, 2.0 equiv.) were dissolved in a mixture of DMSO (300 μL) and MeOH (300 μL). TBTA (1.0 mg, 1.9 μmol, 1.0 equiv.) in $CHCl_3$ (300 μL) was added followed by the addition of a piece of copper foil (5 mm × 5 mm). The reaction mixture was stirred for 20 h at room temperature before all volatiles were removed under reduced pressure. The residue was added to aq. EDTA (0.05 M, 10 mL) and the formed precipitate was separated by centrifugation. The pellet was washed with further EDTA (10 mL) and water (3 × 10 mL). However, the pure compound could not be obtained *via* HPLC, reversed-phase flash column chromatography, precipitation, size exclusion chromatography or MWCO spin filters.

HRMS (ESI-MS): m/z calcd. for $C_{136}H_{245}N_{25}O_{29}^{2+}$ $[M+2H]^{2+}$: 1346.9244, found 1346.9265.

CuAAC conjugate 26[94]

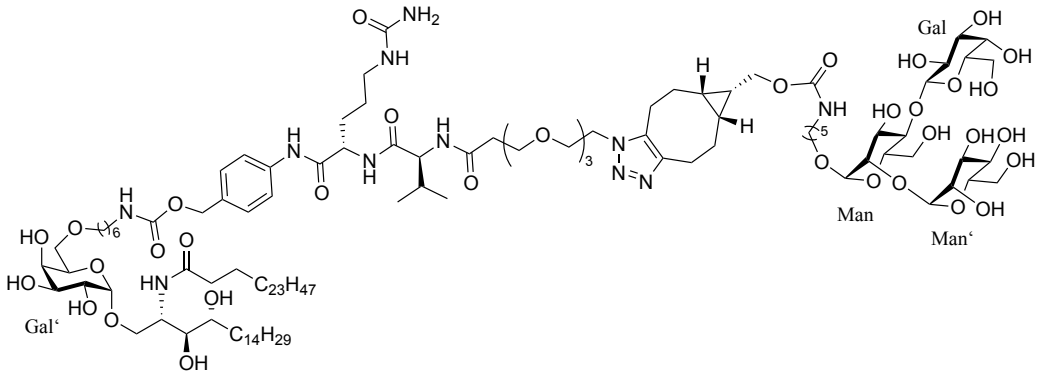


Trisaccharide **1** (15 mg, 17.0 μmol, 1.0 equiv.), pentynoic acid **27** (2.0 mg, 20.0 μmol, 1.2 equiv.) and PyBOP (13 mg, 25.0 μmol, 1.5 equiv.) were dissoved in DMF (1 mL) and DIPEA (14.5 μL, 85.0 μmol, 5.0 equiv.) was added. The solution was stirred at room temperature for 2.5 h before the solvent was removed under reduced pressure. The oily residue was redissolved in MeOH (1 mL) and the product was precipitated by

adding the solution dropwise to cold Et_2O (10 mL). After centrifugation, the precipitate was triturated and washed with Et_2O (3 × 10 mL) to furnish the alkyne (11 mg, 16.4 μmol, 96%) as a colorless solid. This compound (3.2 mg, 4.8 μmol, 1.5 equiv.) and KRN7000 derivative **11** (5.0 mg, 3.2 μmol, 1.0 equiv.) were dissolved in a mixture of DMSO (300 μL) and MeOH (300 μL). TBTA (1.7 mg, 3.2 μmol, 1.0 equiv.) in $CHCl_3$ (300 μL) was added followed by the addition of a piece of copper foil (5 mm × 5 mm). The reaction mixture was stirred for 20 h at room temperature before all volatiles were removed under reduced pressure. The residue was added to aq. EDTA (0.05 M, 10 mL) and the formed precipitate was separated by centrifugation. The pellet was washed with further EDTA (10 mL) and water (3 × 10 mL). However, the pure compound could not be obtained *via* HPLC, reversed-phase flash column chromatography, precipitation, size exclusion chromatography or MWCO spin filters.

HRMS (ESI-MS): m/z calcd. for $C_{112}H_{203}N_{11}O_{35}^{2+}$ $[M+2H]^{2+}$: 1131.7233, found 1131.7224.

SPAAC conjugate 138[95]



Trisaccharide **1** (16 mg, 27 μmol, 1.0 equiv.) and BCN-NHS **136** (10 mg, 34 μmol, 1.26 equiv.) were dissolved in DMF (500 μL) and NEt_3 (7.6 μL, 54 μmol, 2.0 equiv.) was added. The solution was stirred at room temperature for 20 h before the solvent was removed under reduced pressure. The oily residue was redissolved in MeOH (1 mL) and the product was precipitated by adding the solution dropwise to cold Et_2O (10 mL). After centrifugation, the precipitate was triturated and washed with Et_2O (3 × 10 mL) to furnish cyclic alkyne **137** (15 mg, 20 μmol, 74%) as a colorless solid. This compound (15 mg, 20 μmol, 4.0 equiv.) and KRN7000 derivative **11** (8.0 mg, 5.0 μmol, 1.0 equiv.) were dissolved in DMSO (500 μL) and the solution was stirred at room temperature for 4 d. The solvent was removed under reduced pressure and the oily residue was taken up in MeOH (500 μL). The product was precipitated by adding the solution dropwise to cold water (10 mL). After centrifugation, the obtained pellet was triturated and washed with water (4 × 10 mL) to furnish vaccine candidate **138** (11 mg, 4.7 μmol, 94%) as a colorless solid.

^{1}H-NMR (800 MHz, $(CD_3)_2SO$): characteristic signals δ = 9.97 (s, 1H, -NH-Ar), 8.10 (d, $J_{NH,H\alpha}$ = 7.6 Hz, 1H, NH-Cit), 7.86 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH-Val), 7.60–7.55 (m, 3H, NH-Ceramide/2 × Ar-H), 7.28–7.24 (m, 2H, Ar-H), 7.15 (t, J = 5.7 Hz, 1H, NH-Carbamate), 7.08 (t, J = 5.6 Hz, 1H, NH-Carbamate), 5.97 (t, $J_{H\varepsilon,H\delta2/H\delta3}$ = 5.4 Hz, 1H, Hε-Cit), 5.40 (s, 2H, Hη-Cit), 4.92 (s, 2H, Ar-CH_2-), 4.84 (s, 1H, H-1-Man‘), 4.81 (s, 1H, H-1-Man), 4.66 (d, J = 3.5 Hz, 1H, H-1-Gal‘), 4.19 (d, J = 7.2 Hz, 1H, H-1-Gal) ppm.

^{13}C-{^{1}H}-NMR (201 MHz, $(CD_3)_2SO$): δ = 171.6 (C=O-Ceramide), 171.1 (C=O-Val), 170.5 (C=O-Cit), 170.2 (C=O-PEG), 158.9 (Cζ-Cit), 156.4 (C=O-Carbamate-BCN), 156.1 (C=O-Carbamate-dipeptide), 143.2 ($C_{qTriazole}$), 138.5 (C_{qAr}), 133.8 ($C_{qTriazole}$), 131.9 (C_{qAr}), 128.5, 118.9 (4 × C_{Ar}), 103.6 (C-1-Gal), 102.2 (C-1-Man‘), 99.3 (C-1-Gal‘), 98.1 (C-1-Man), 78.2, 76.1, 75.3, 74.3, 73.6, 73.2, 71.6, 70.7, 70.6, 70.5, 70.4, 70.1, 69.7, 69.5, 69.3, 69.2, 69.1, 68.4, 68.0, 67.0, 66.9, 66.6, 64.9 (Ar-*C*H_2-), 61.3, 60.6, 60.2, 57.4 (Cα-Val), 53.1 (Cα-Cit), 49.7, 47.2, 40.2, 40.1, 35.9, 35.4, 31.3, 30.6 (Cβ-Val), 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 28.7, 28.6, 26.8, 26.2, 25.4, 23.0, 22.2, 22.1, 22.0, 21.3, 19.2 (Cγ2-Val), 18.6 (Cγ1-Val), 18.1, 17.3, 13.9 (2 × -*C*H_3-Ceramide) ppm.

The assignment of all protons and carbons was not possible due to signal overlap.

HRMS (ESI-MS): m/z calcd. for $C_{118}H_{211}N_{11}O_{36}^{2+}$ $[M+2H]^{2+}$: 1179.7520, found 1179.7510.
m/z calcd. for $C_{120}H_{211}N_{11}O_{40}^{2-}$ $[M+2FA-2H]^{2-}$: 1223.7430, found 1223.7490.

RP-HPLC: t_R = 19.4 min, Phenomenex Luna C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

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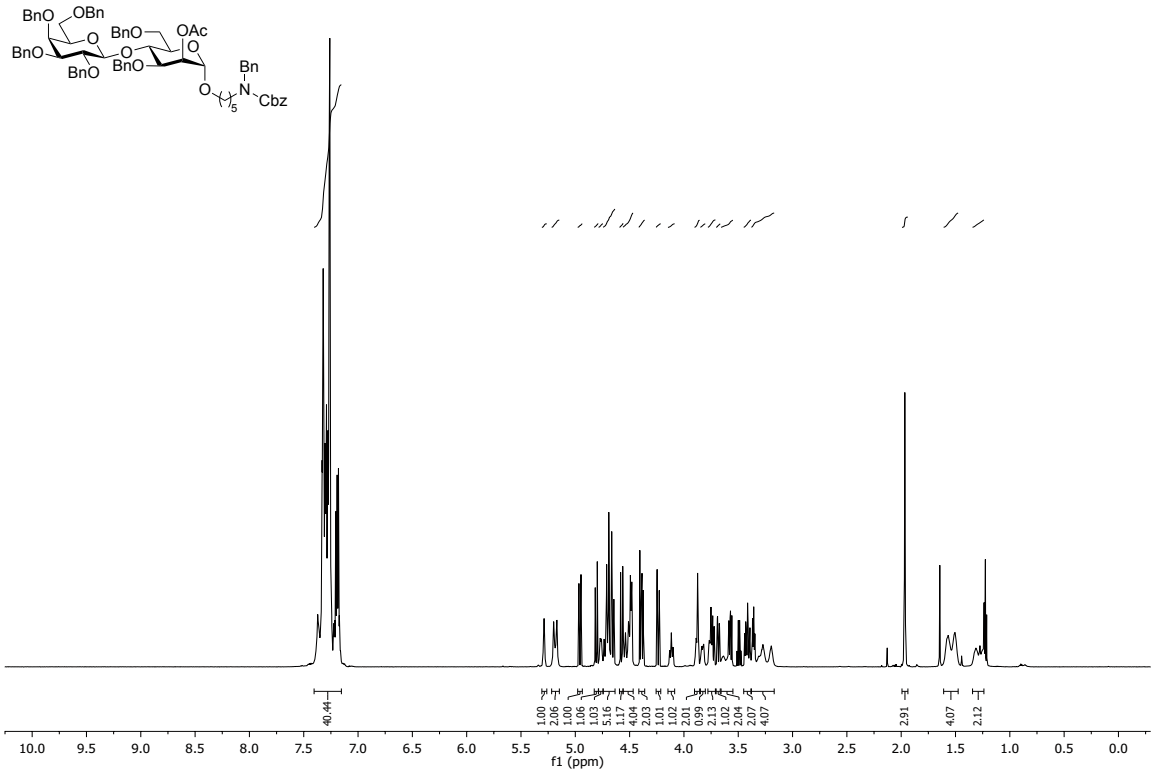
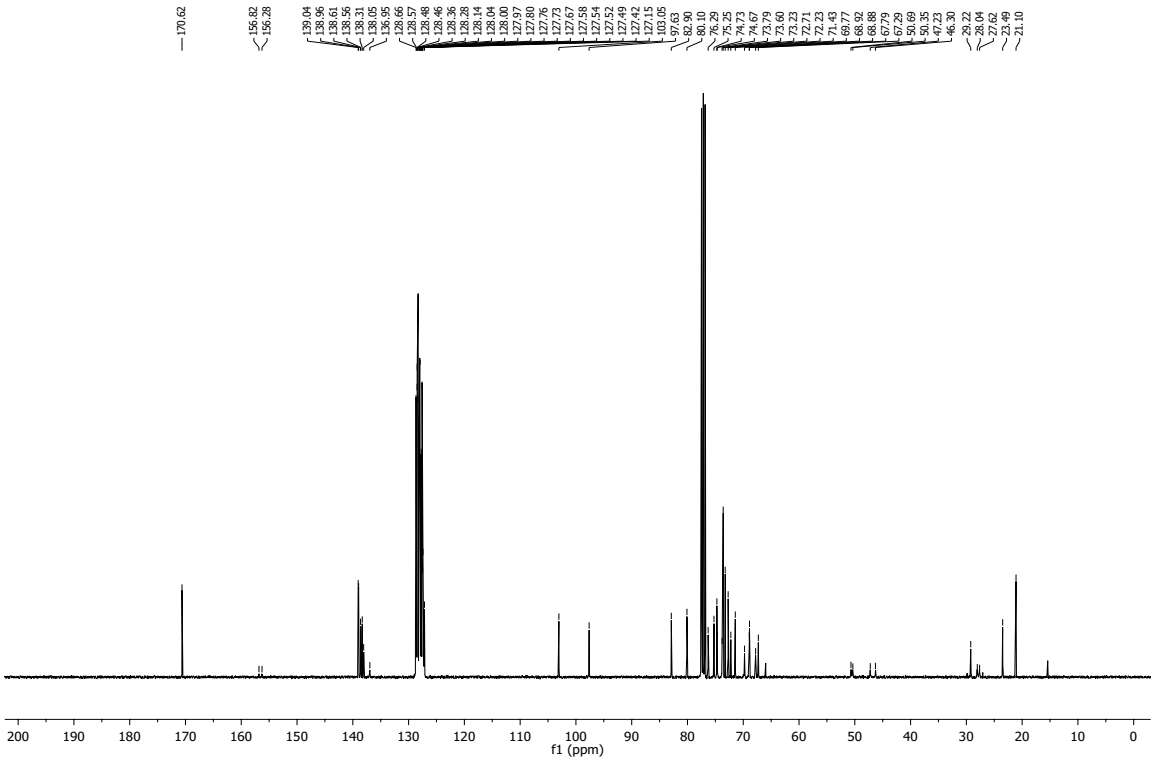
7 APPENDIX

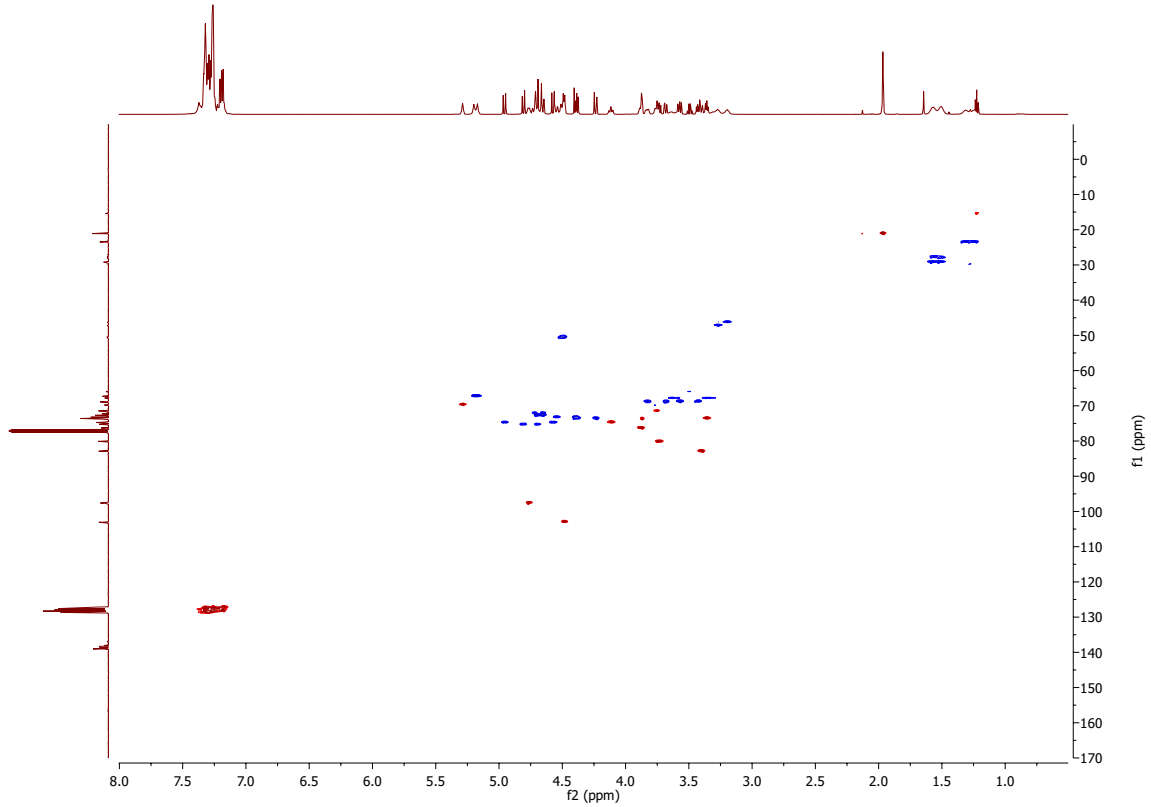
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (85)**



^{1}H NMR (599 MHz, $CDCl_3$)

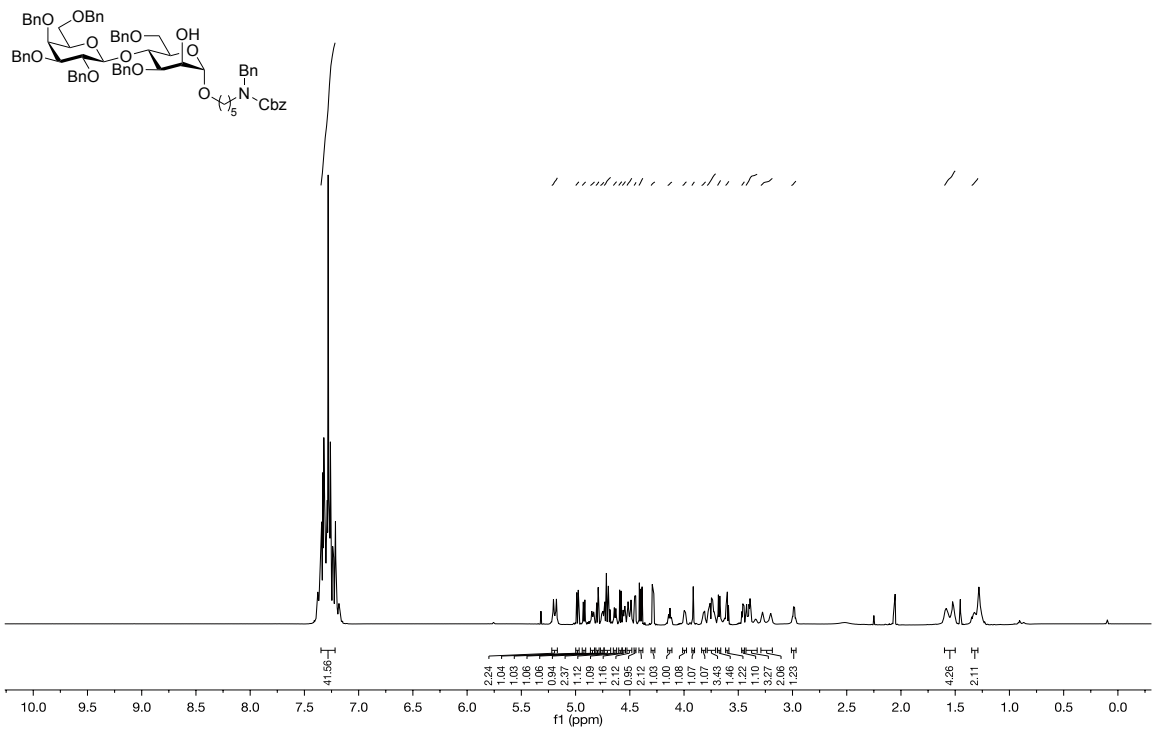


^{13}C NMR (151 MHz, $CDCl_3$)

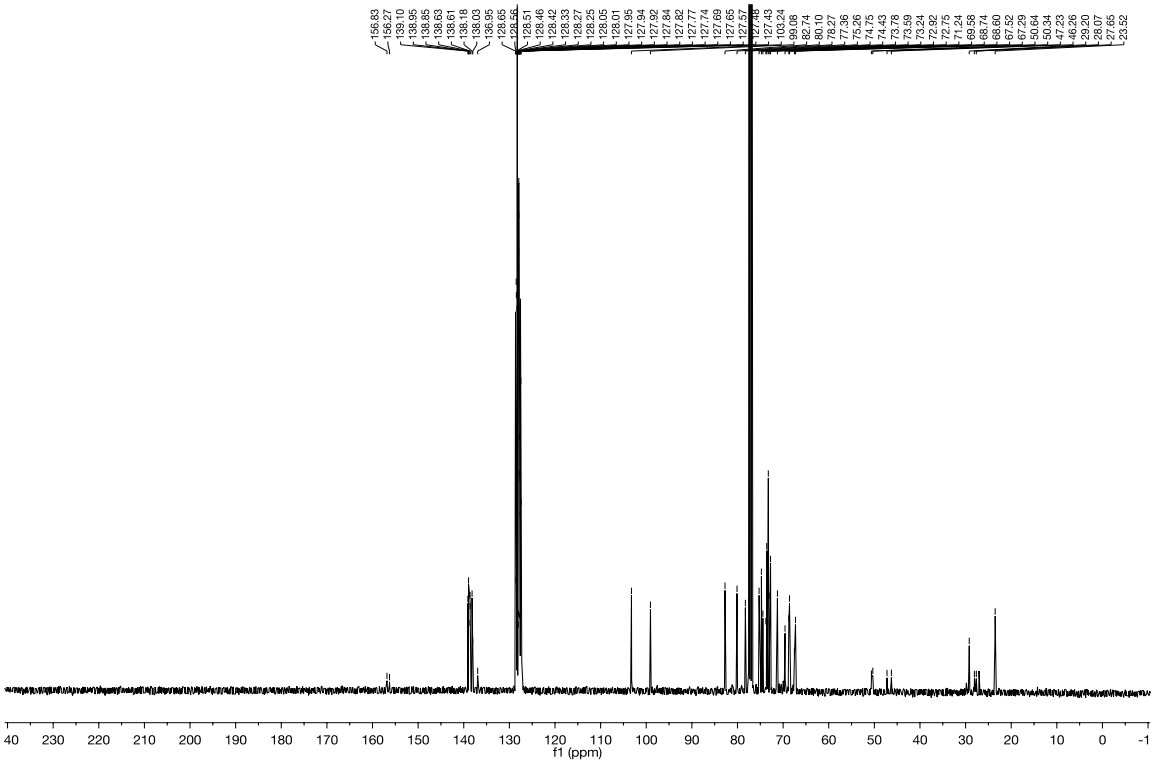


HSQC (599 MHz/151 MHz, $CDCl_3$)

***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (22)**



^{1}H NMR (400 MHz, $CDCl_3$)



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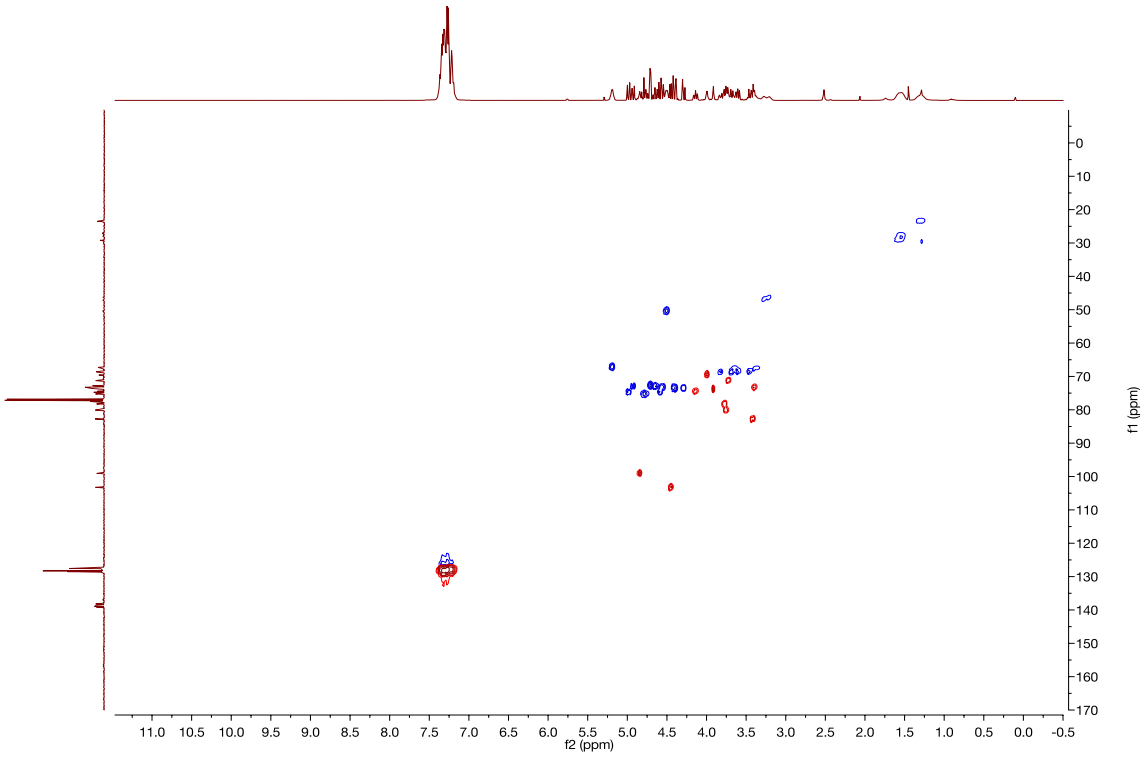
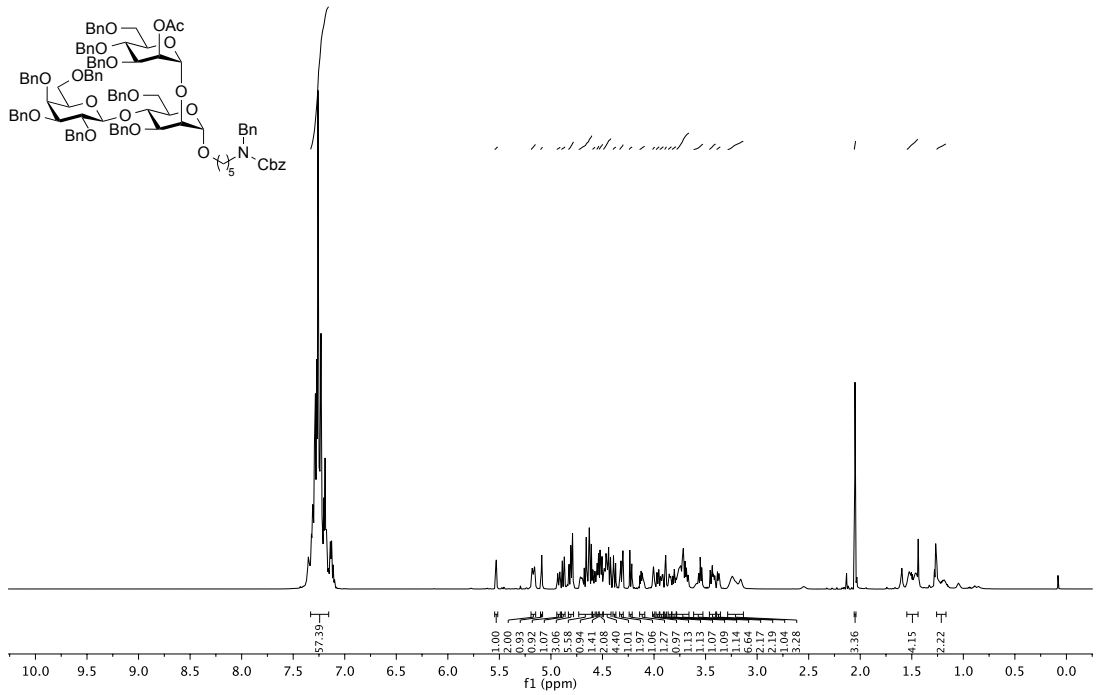
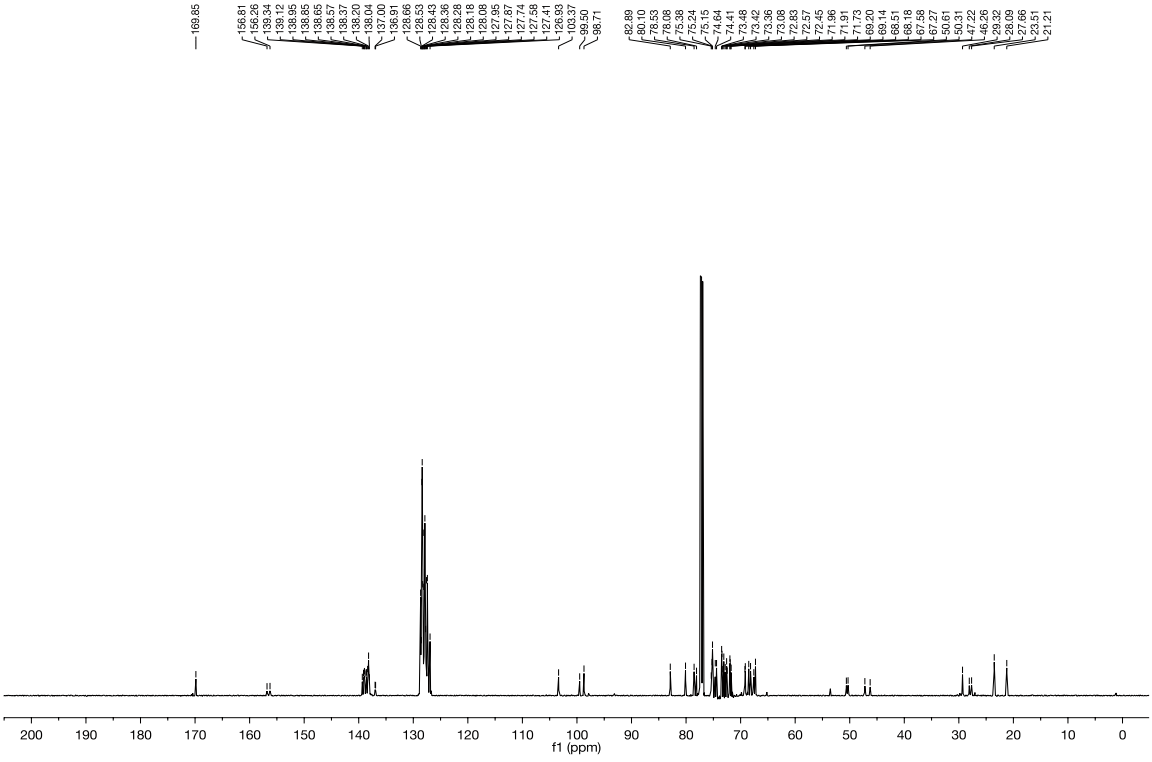


HSQC (400 MHz/101 MHz, $CDCl_3$)

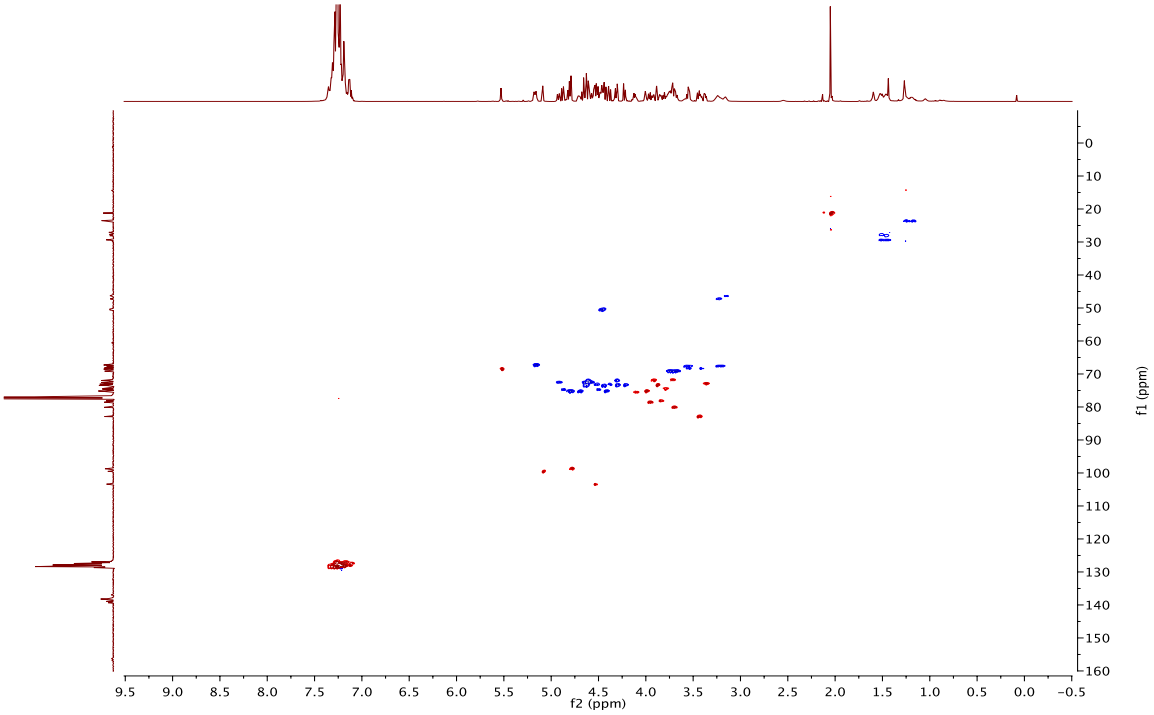
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (94)**



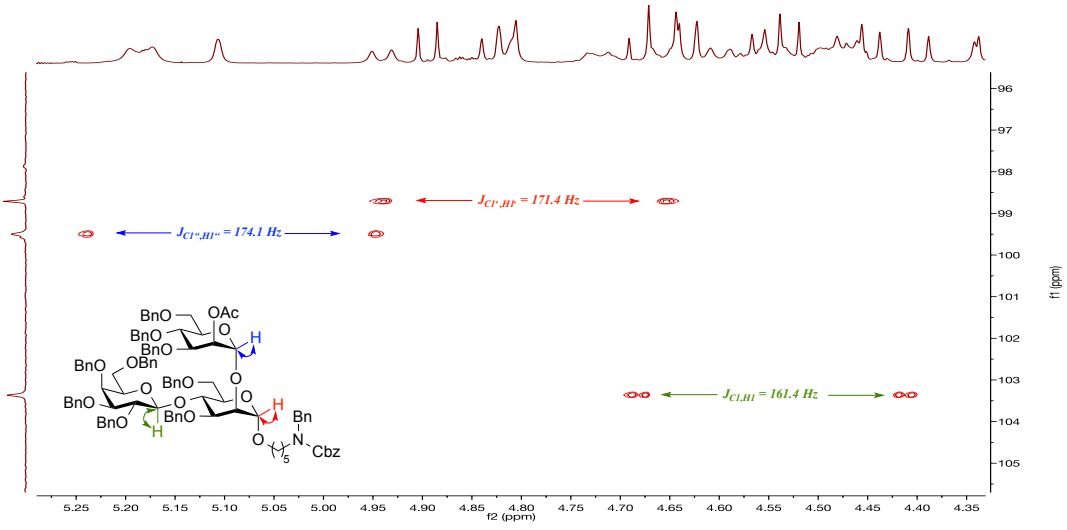
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

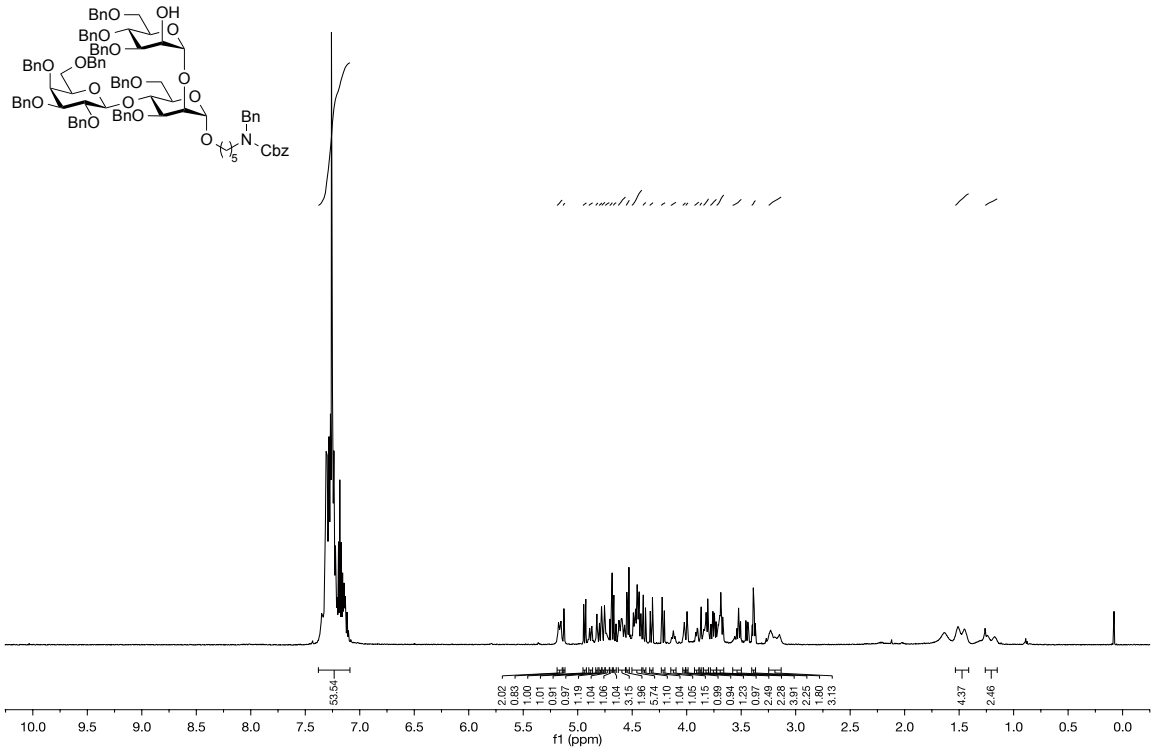


HSQC (599 MHz/151 MHz, $CDCl_3$)

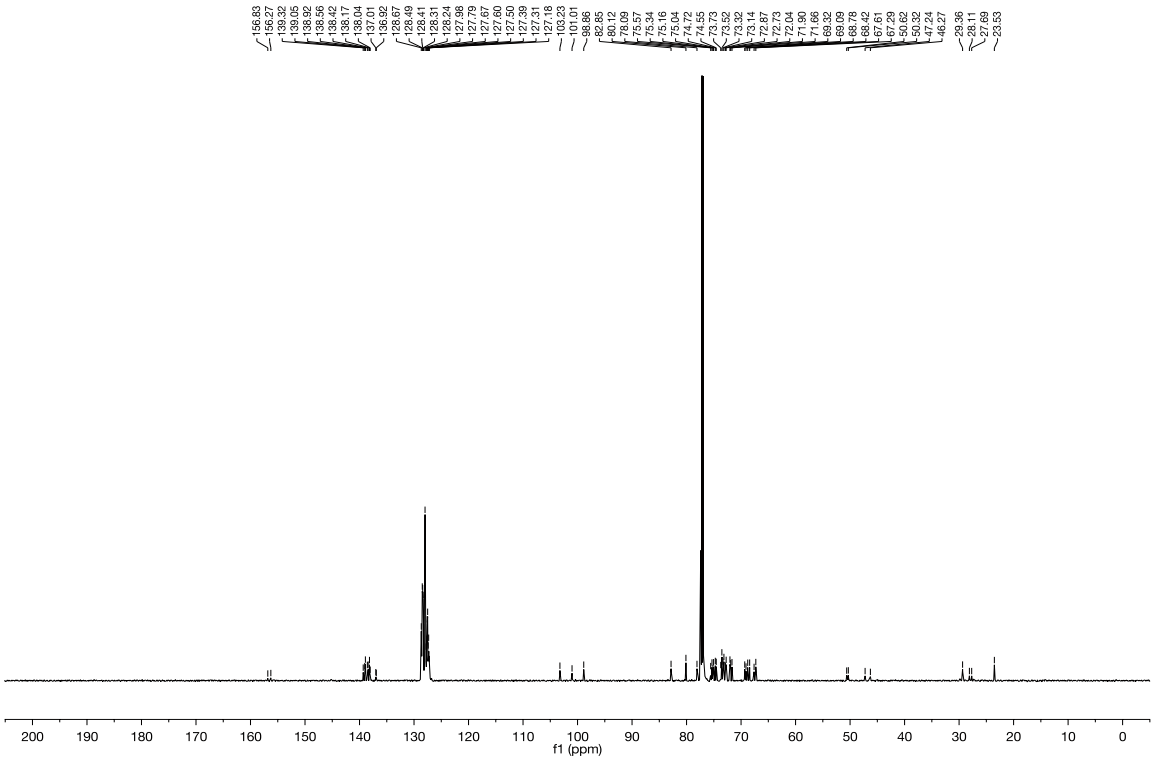


^{1}H-^{13}C coupled HSQC-NMR

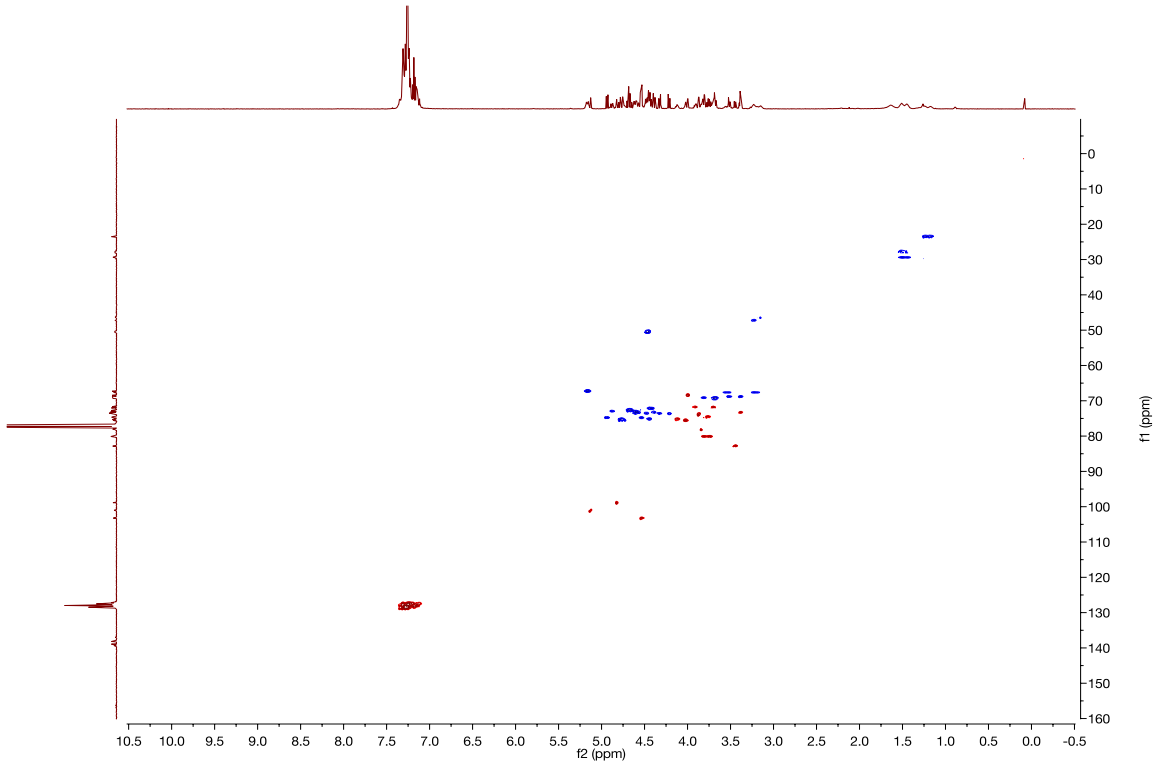
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (95)**



^{1}H NMR (599 MHz, $CDCl_3$)

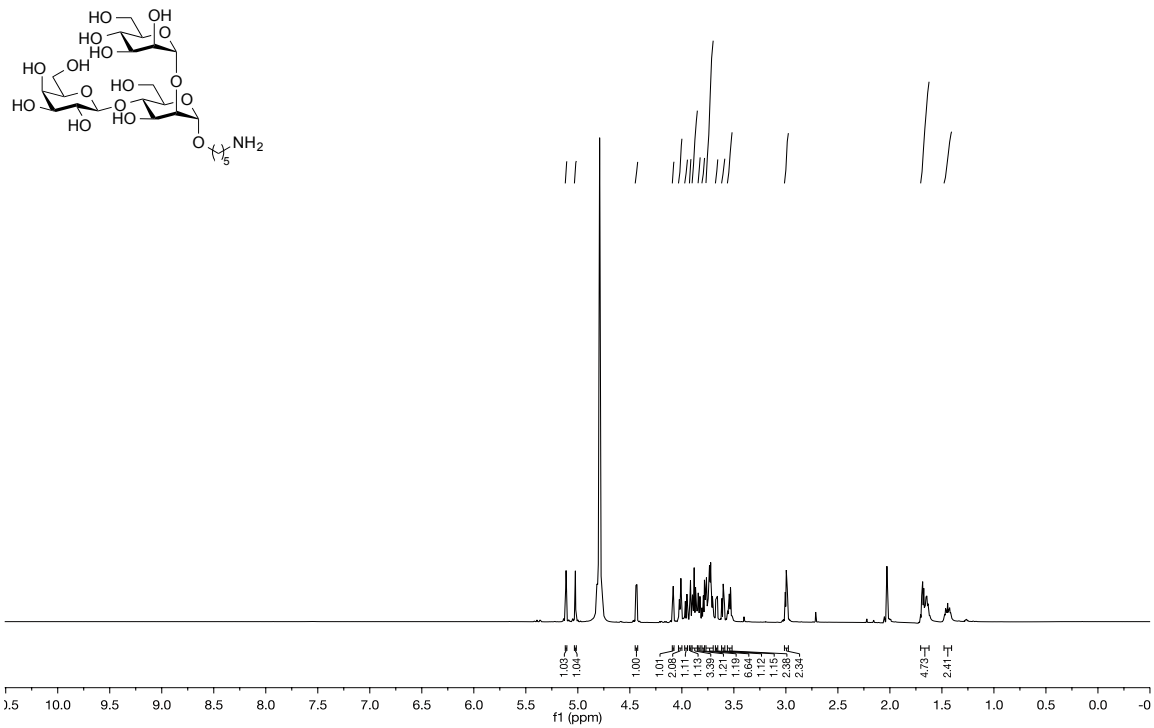


^{13}C NMR (151 MHz, $CDCl_3$)

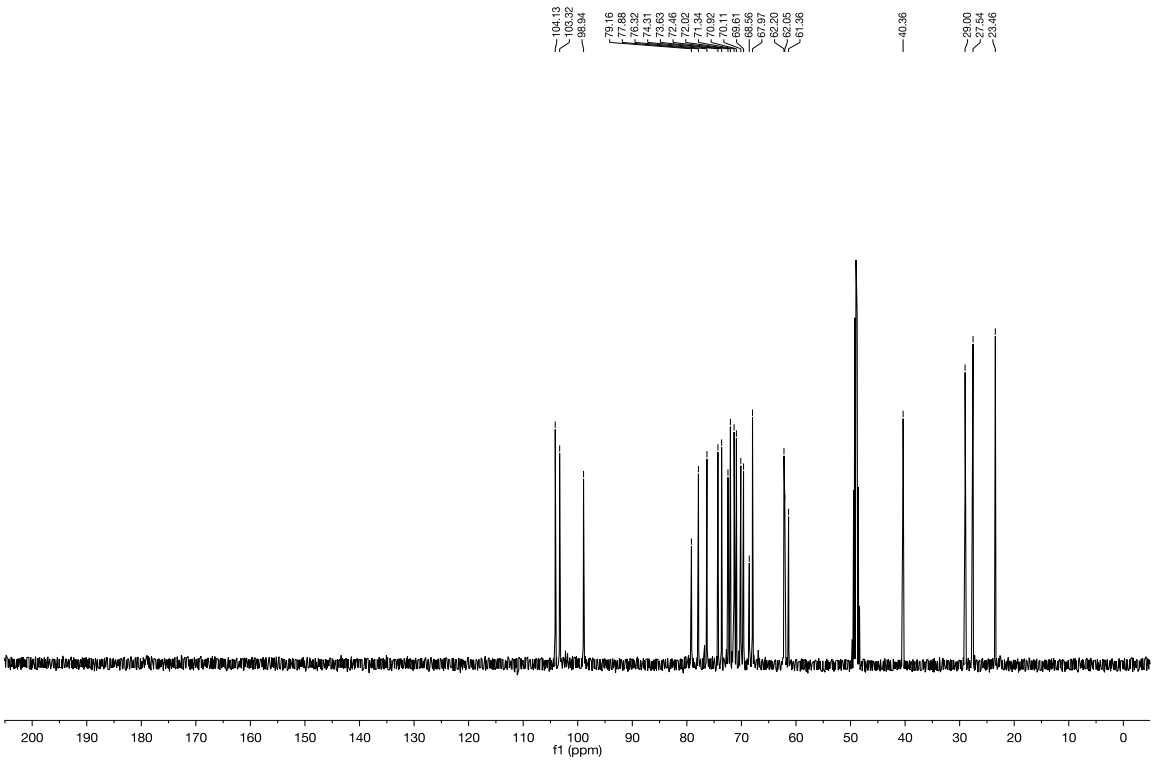


HSQC (599 MHz/151 MHz, $CDCl_3$)

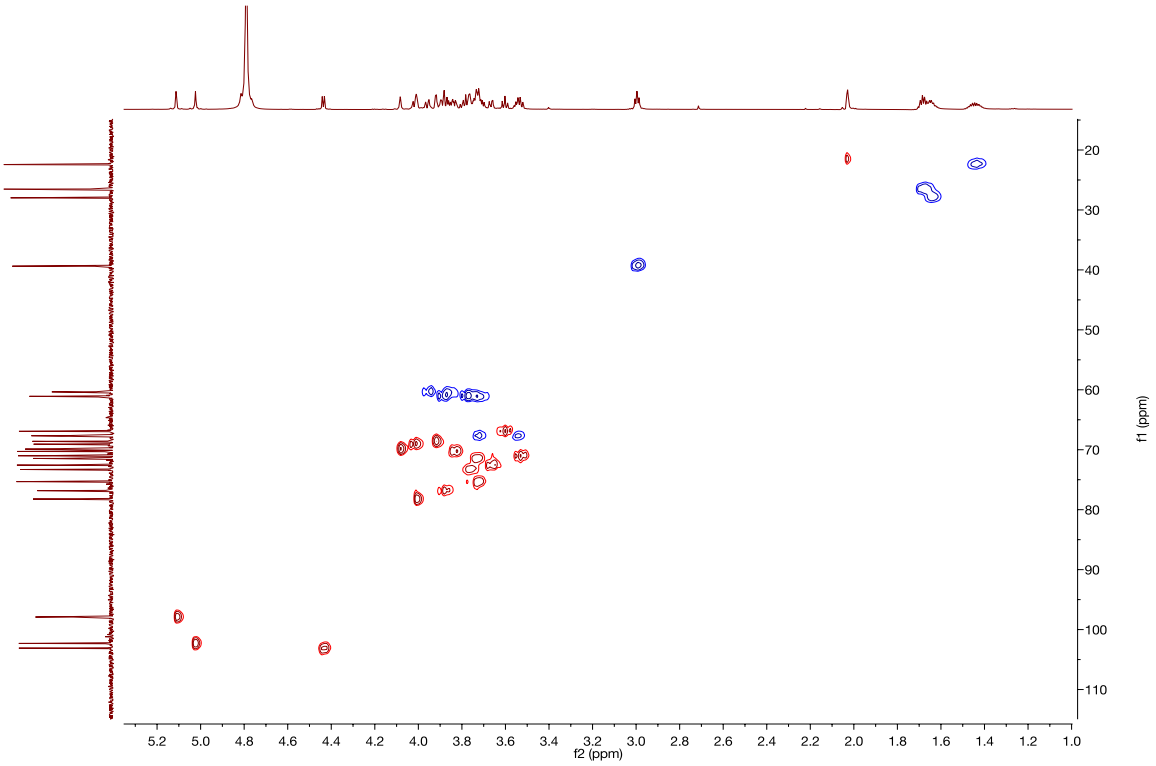
5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside (1)



^{1}H NMR (800 MHz, D_2O)

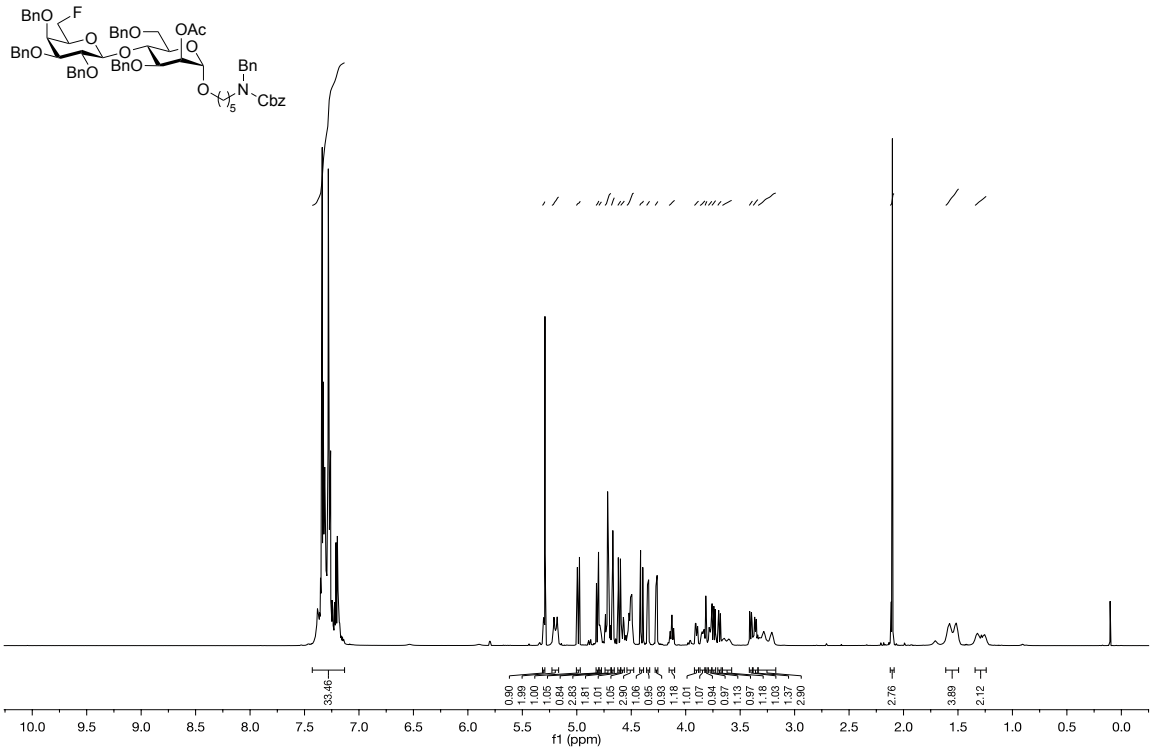


^{13}C NMR (101 MHz, D_2O/CD_3OD)

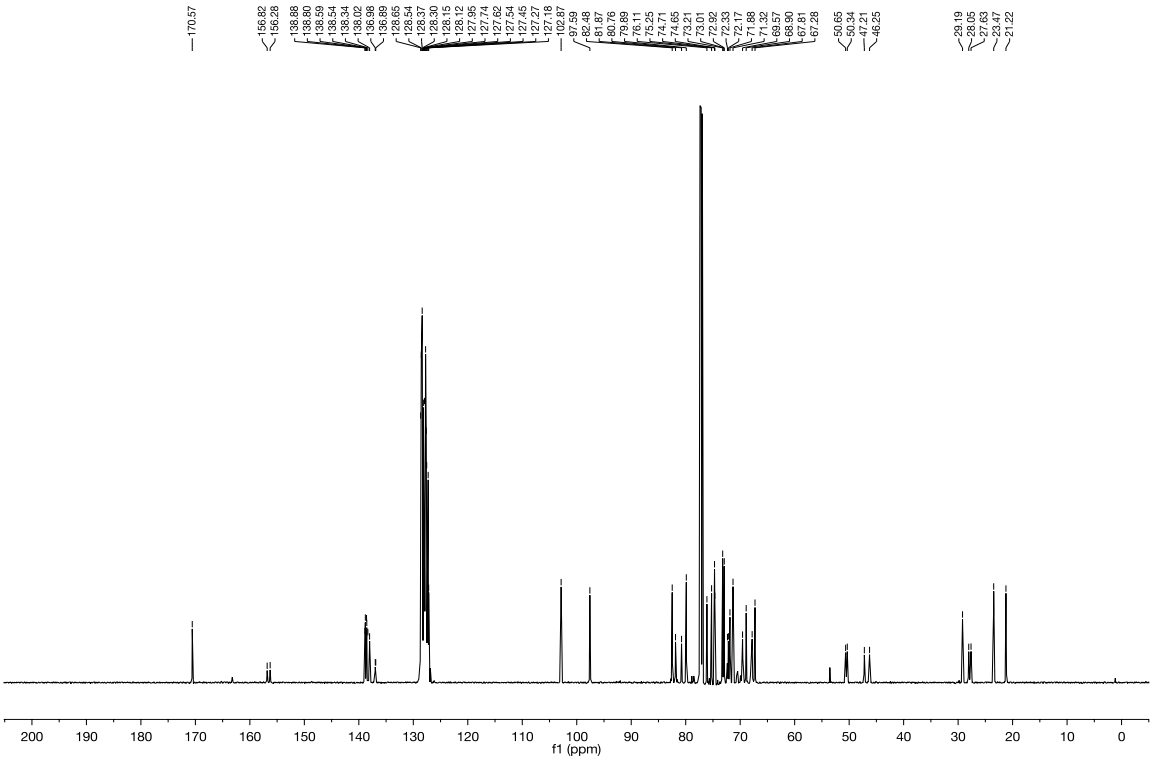


HSQC (400 MHz/101 MHz, D_2O)

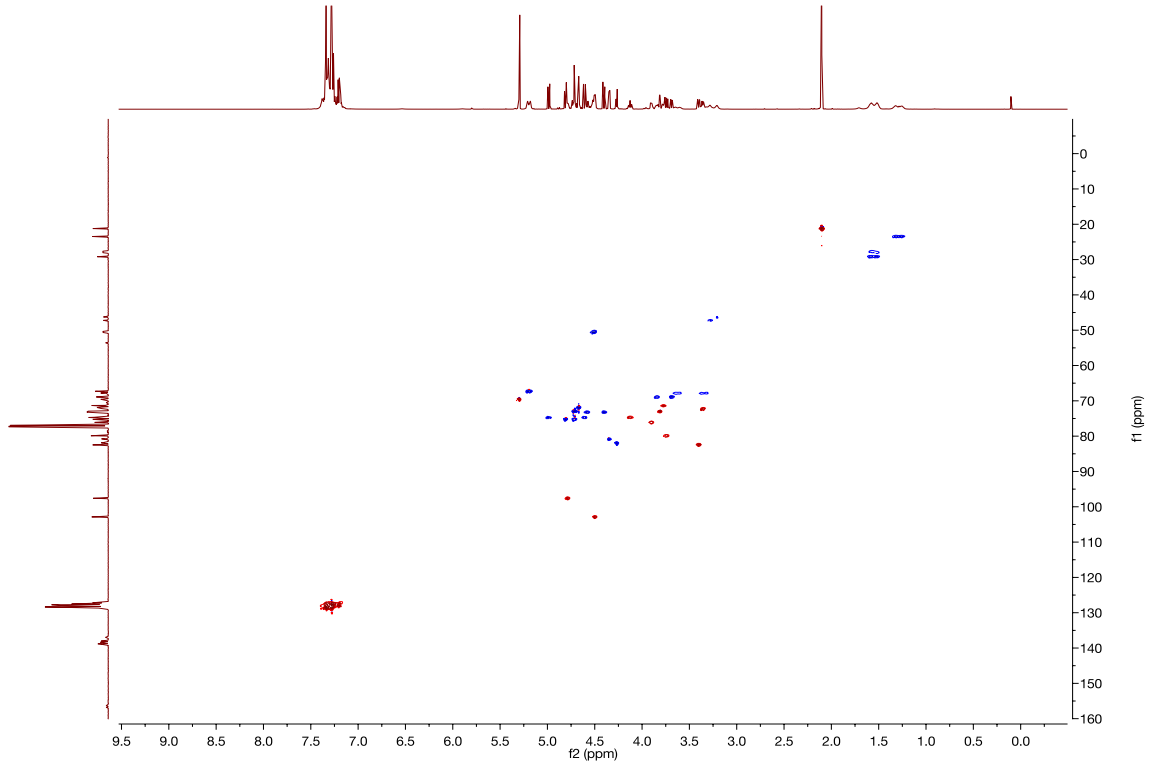
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3,4-tri-_O_-benzyl-β-D-6-deoxy-6-fluoro-galactopyranosyl-(1→4)-2-_O_-acetyl-3,6-di-_O_-benzyl-α-D-mannopyranoside (86)



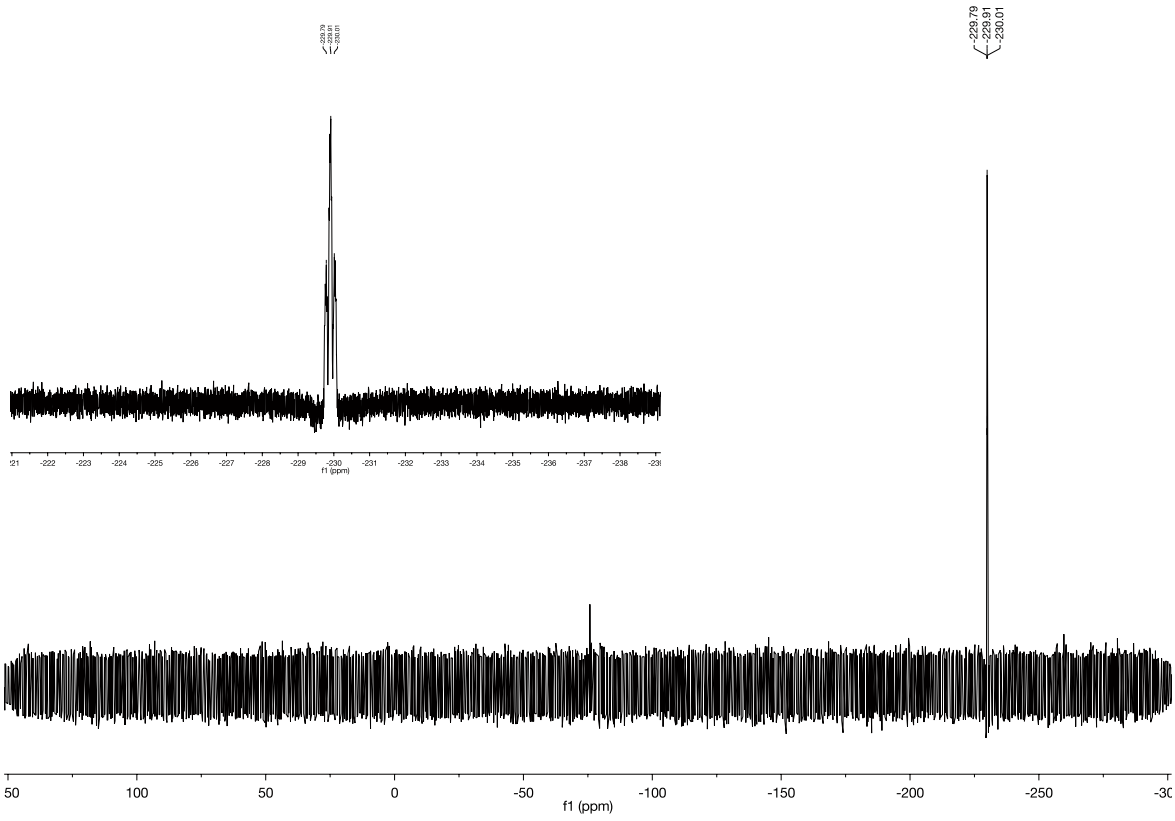
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

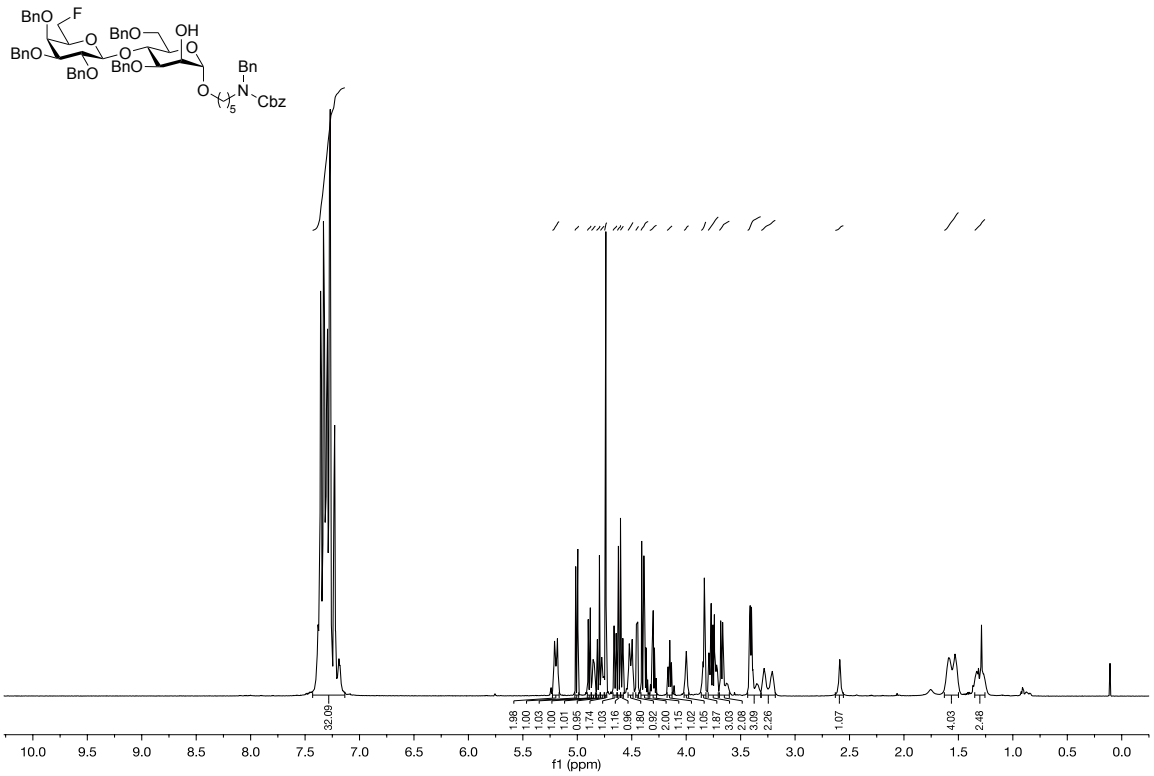


HSQC (599 MHz/151 MHz, $CDCl_3$)

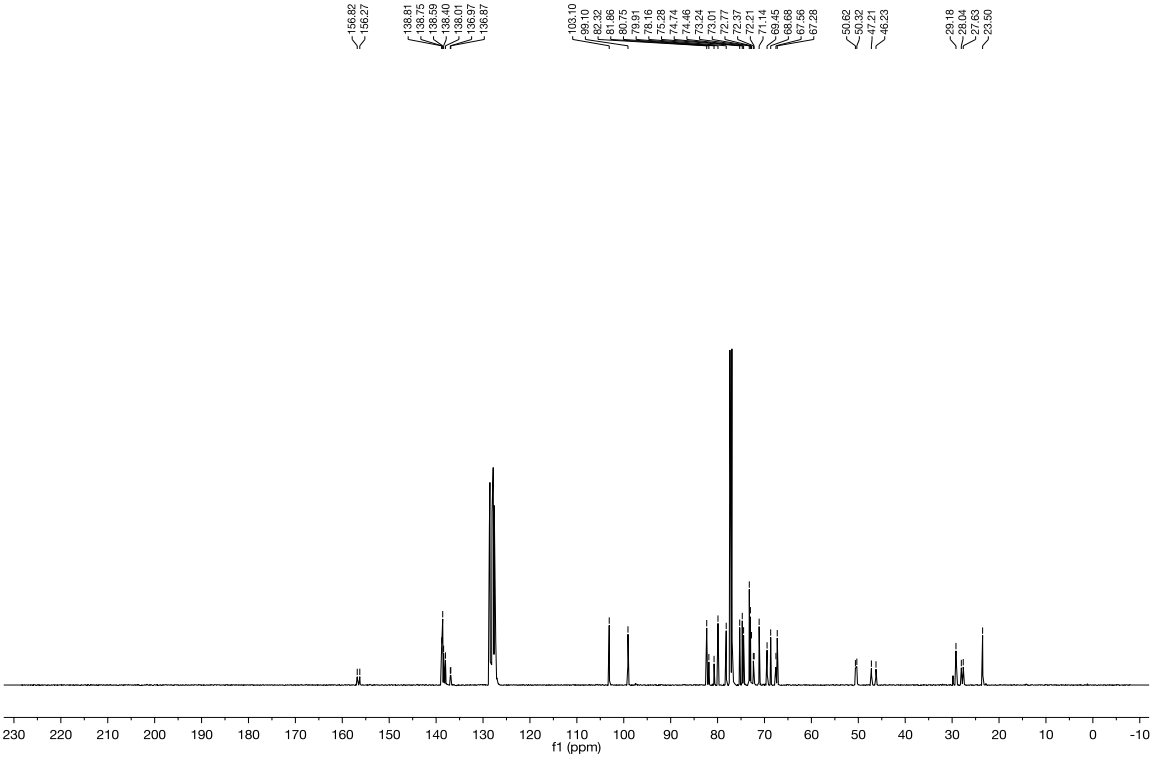


^{19}F NMR (376 MHz, $CDCl_3$)

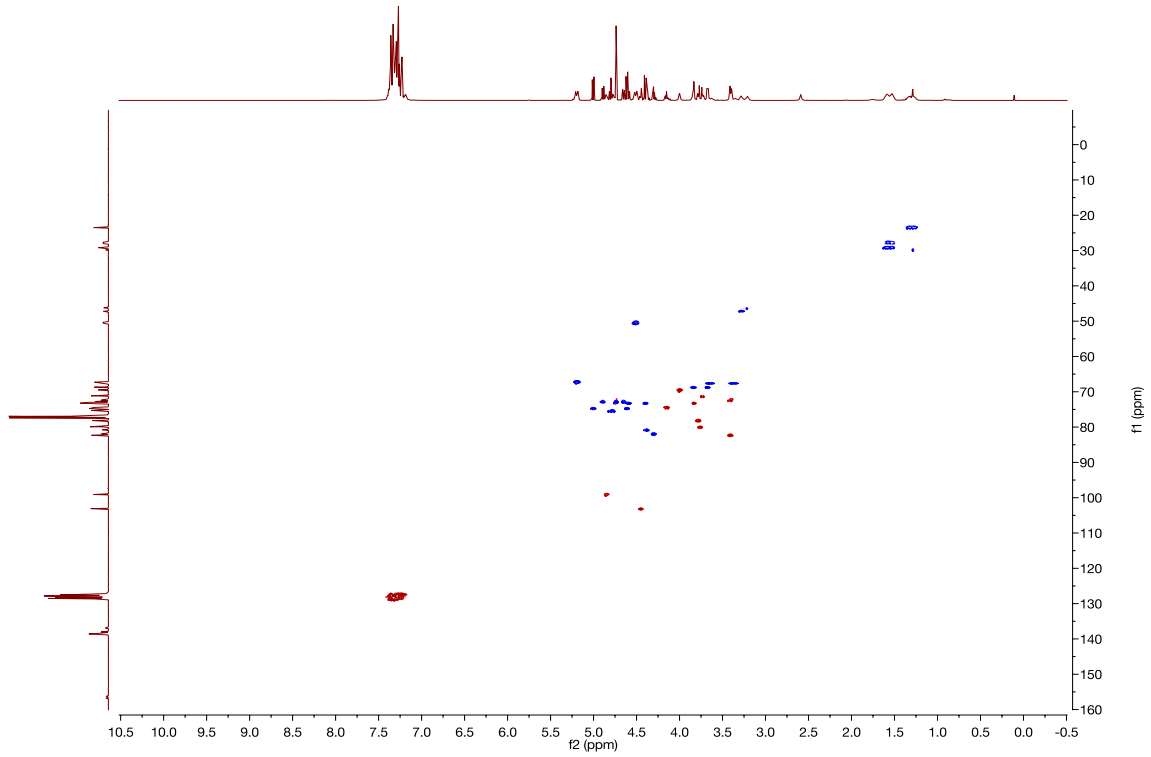
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,4-tri-*O*-benzyl-β-D-6-deoxy-6-fluoro-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (90)**



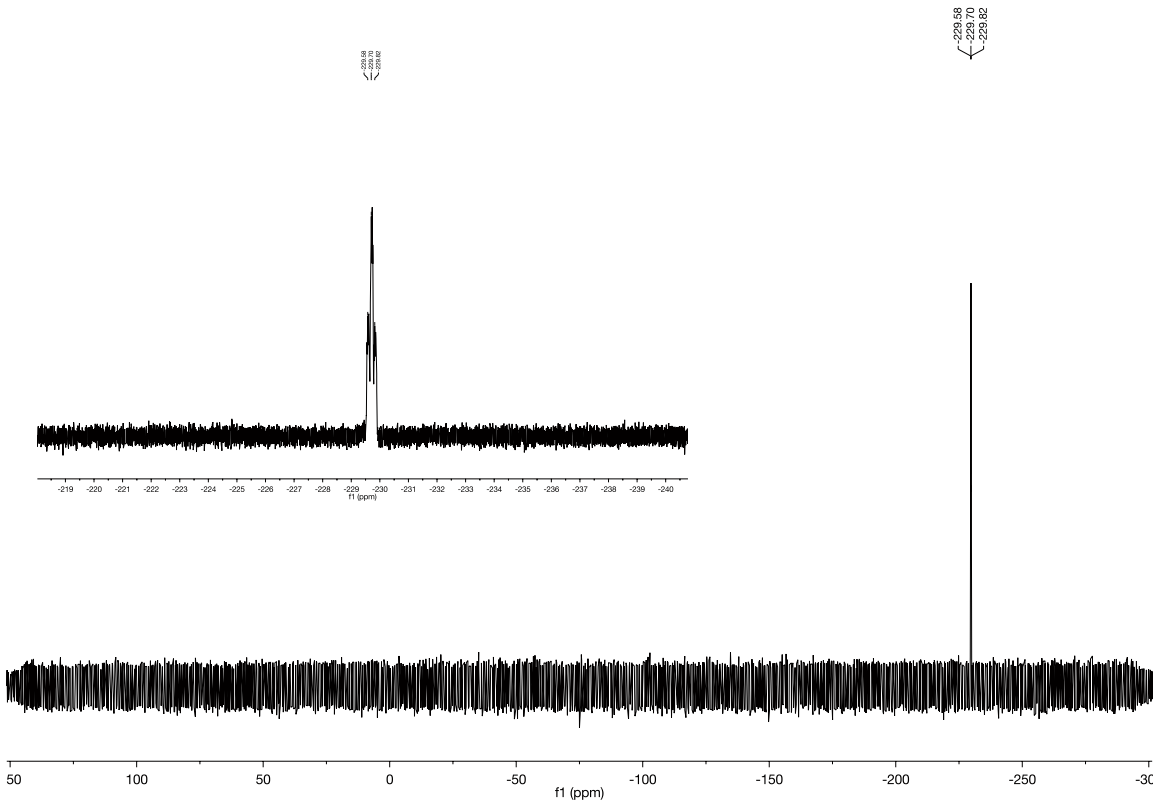
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

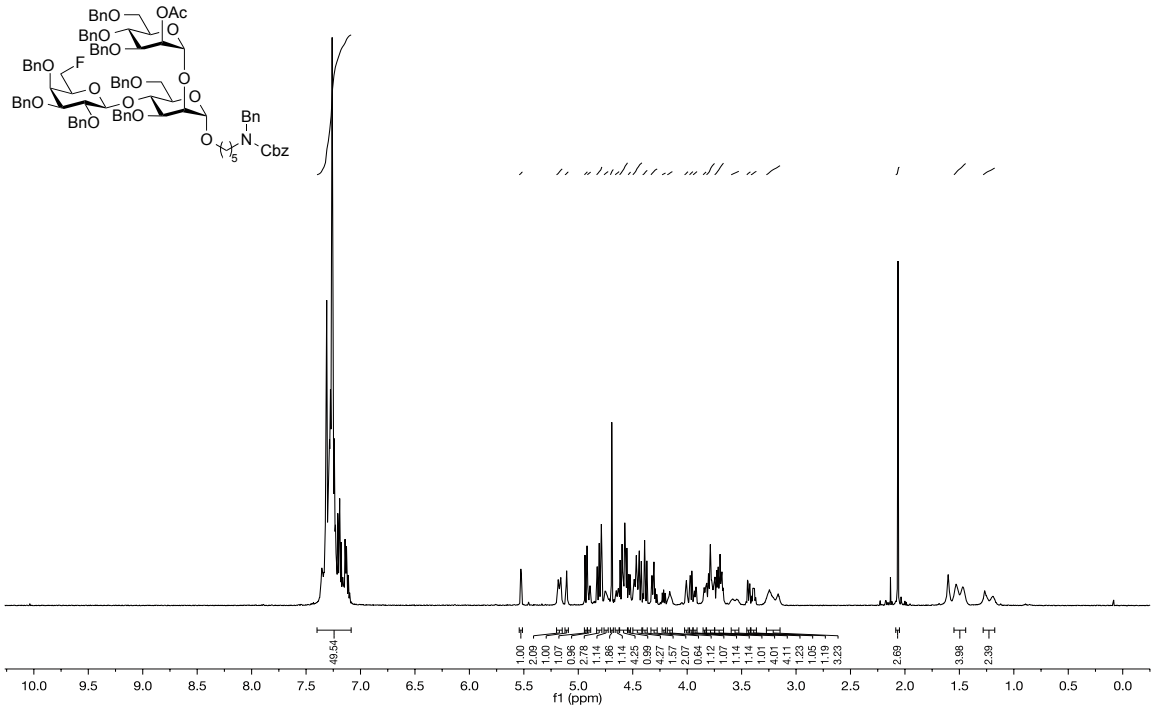


HSQC (599 MHz/151 MHz, $CDCl_3$)

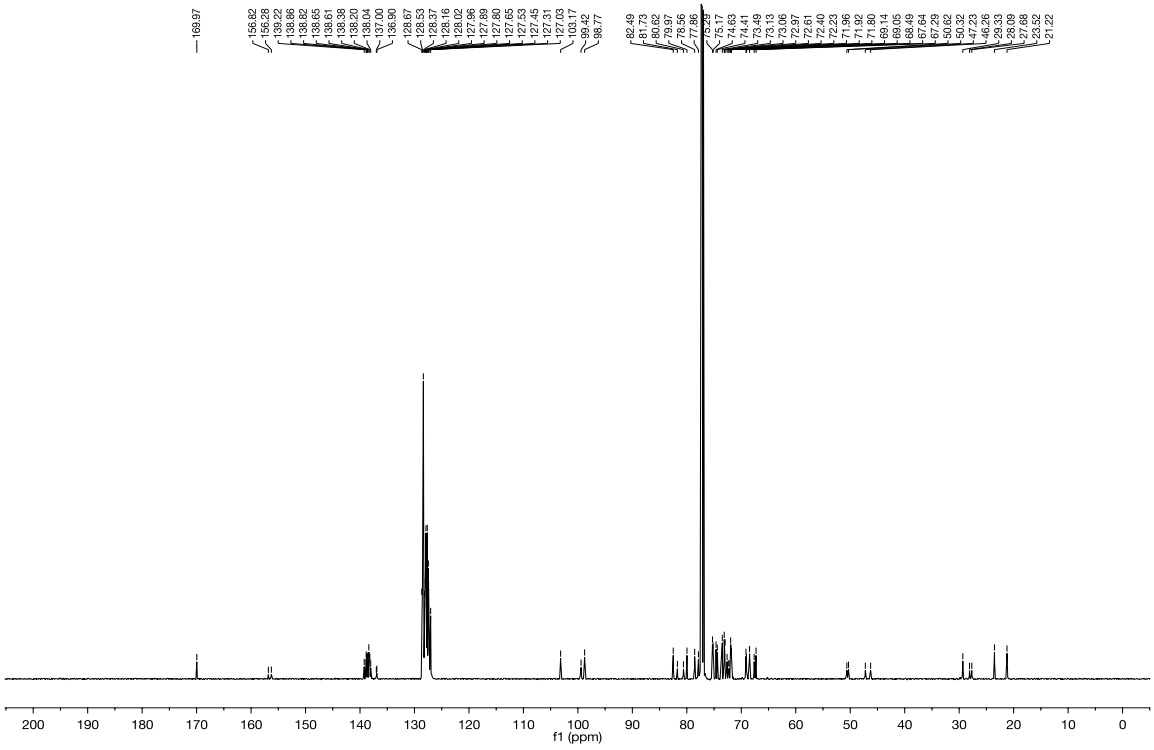


^{19}F NMR (376 MHz, $CDCl_3$)

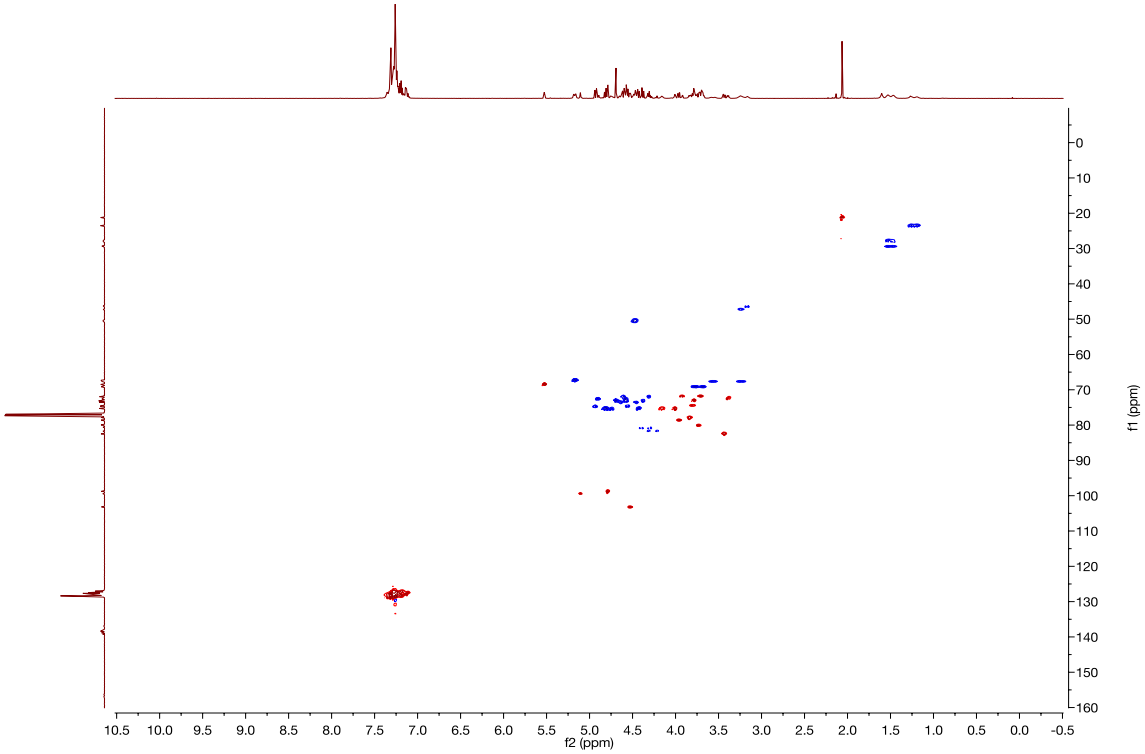
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-b-D-6-deoxy-6-fluoro-galactopyranosyl)-α-D-mannopyranoside (96)**



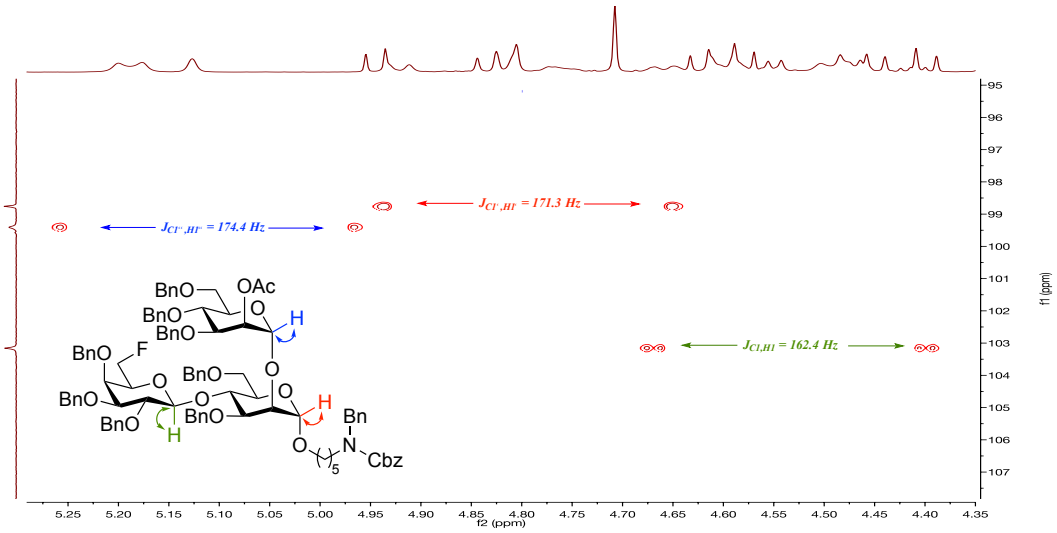
^{1}H NMR (599 MHz, $CDCl_3$)



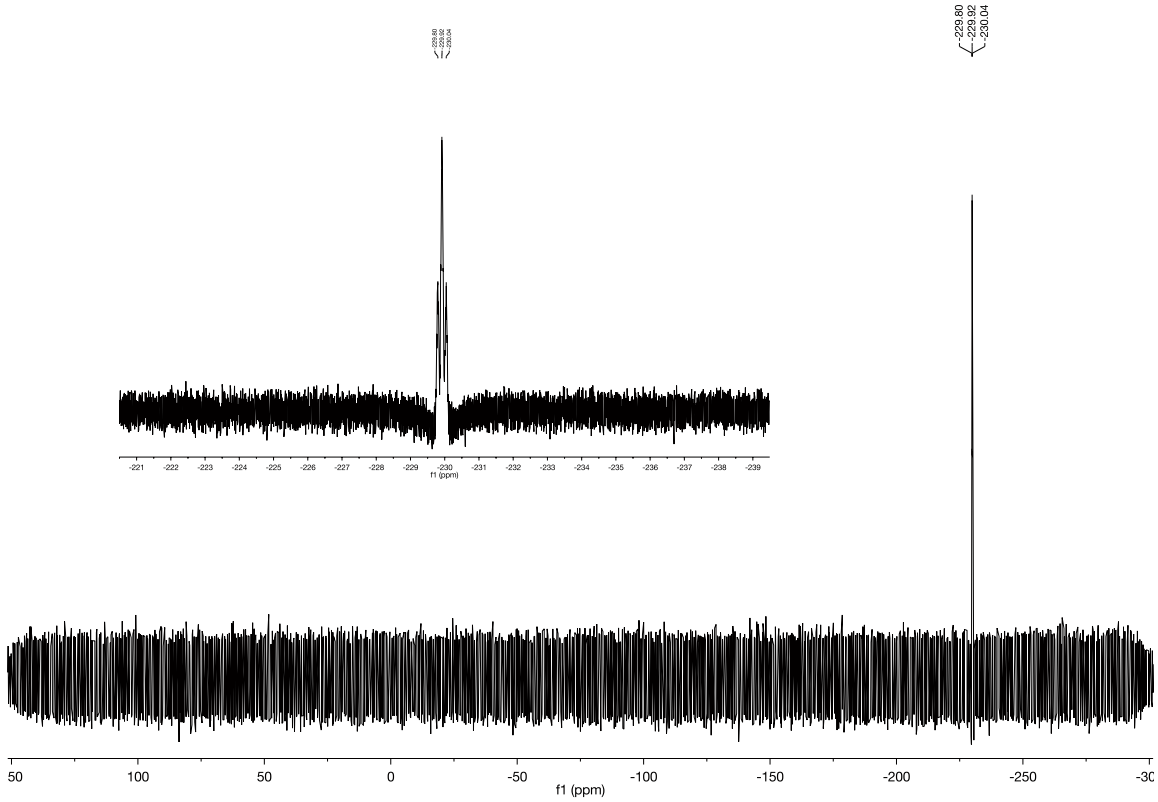
^{13}C NMR (151 MHz, $CDCl_3$)



HSQC (599 MHz/151 MHz, $CDCl_3$)

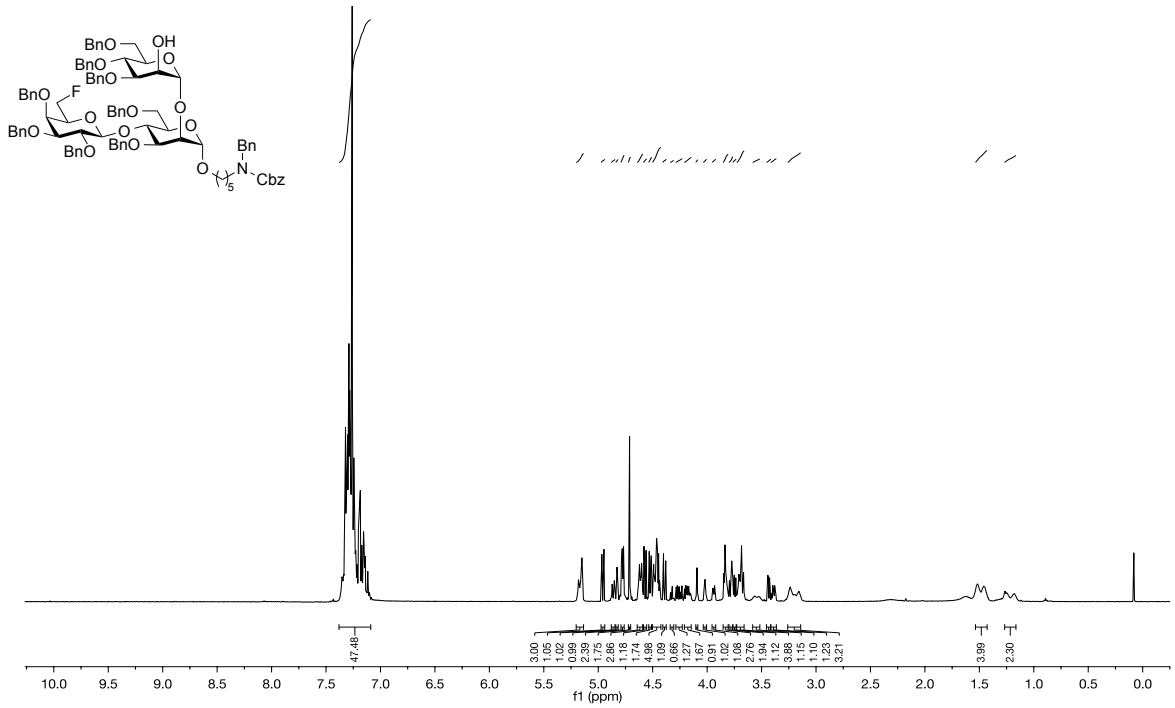


^{1}H-^{13}C coupled HSQC-NMR

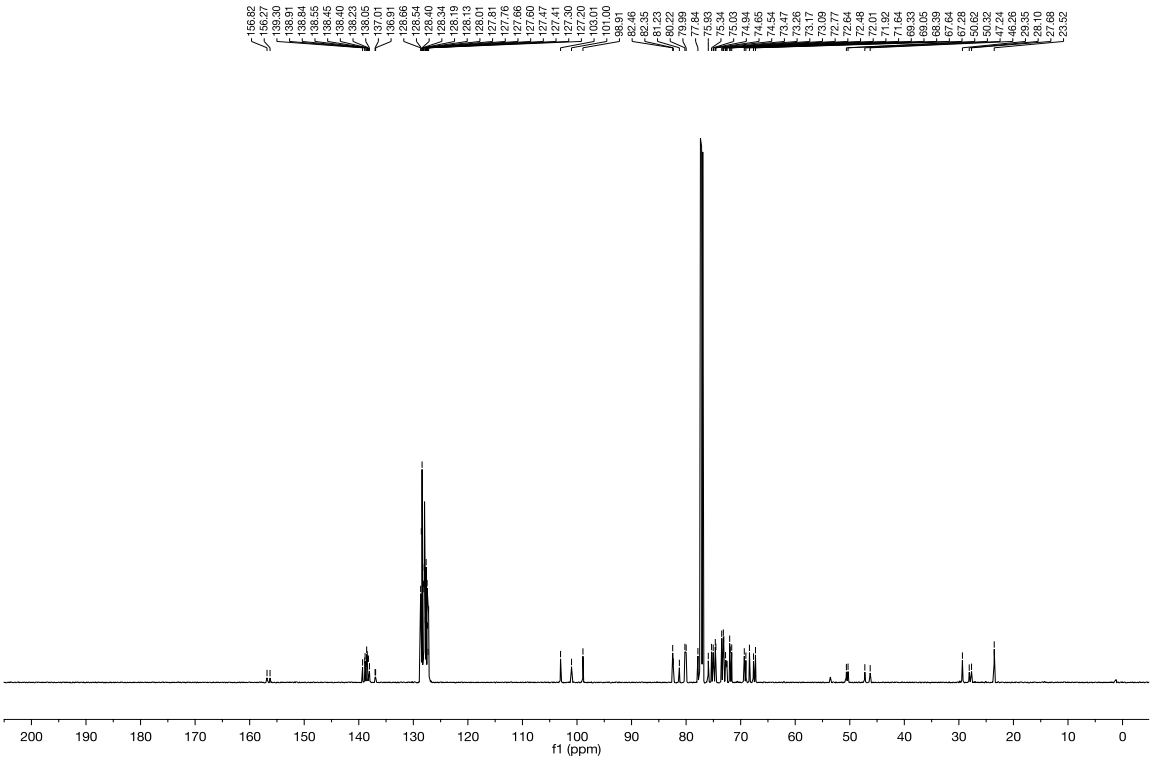


^{19}F NMR (376 MHz, $CDCl_3$)

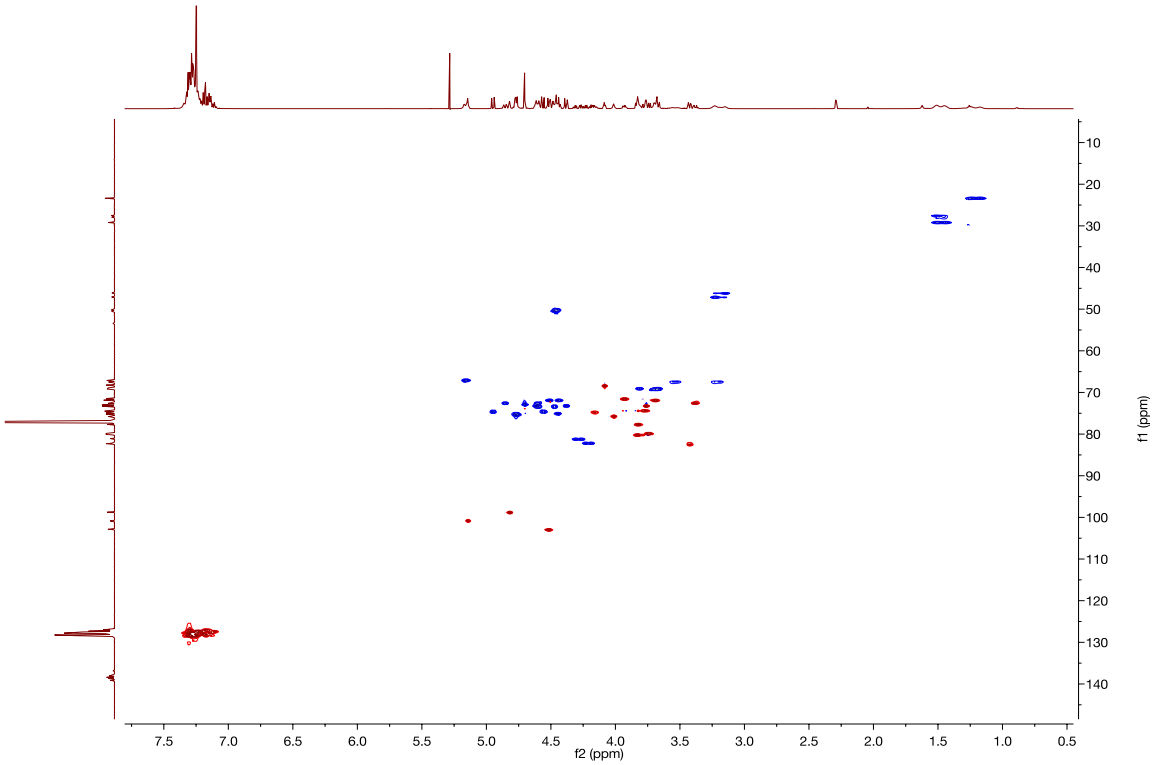
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-_O_-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-_O_-benzyl-4-_O_-(2,3,4-tri-_O_-benzyl-β-D-6-deoxy-6-fluoro-galactopyranosyl)-α-D-mannopyranoside (100)



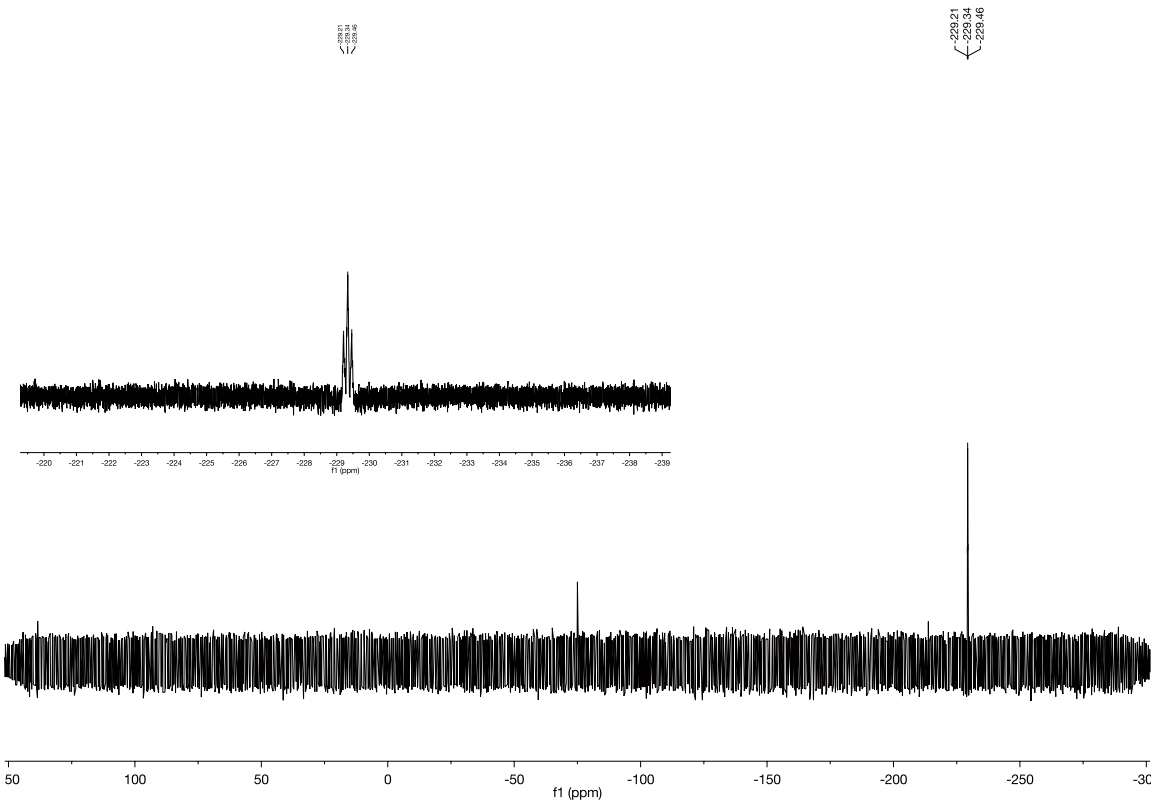
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

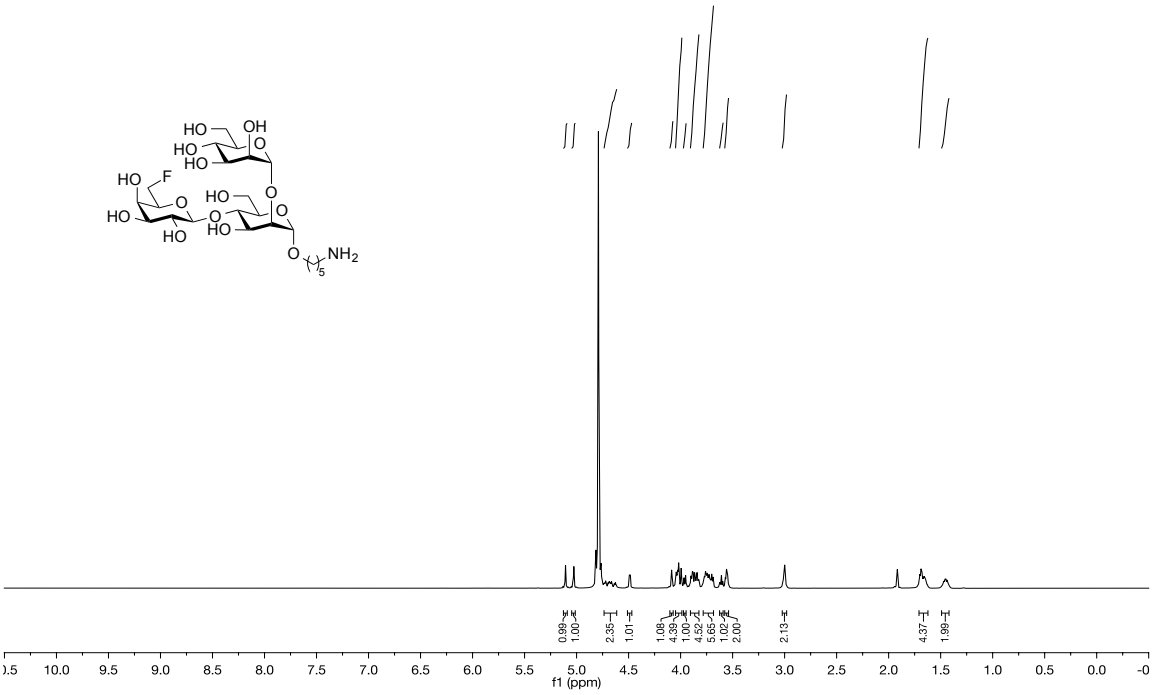


HSQC (599 MHz/151 MHz, $CDCl_3$)

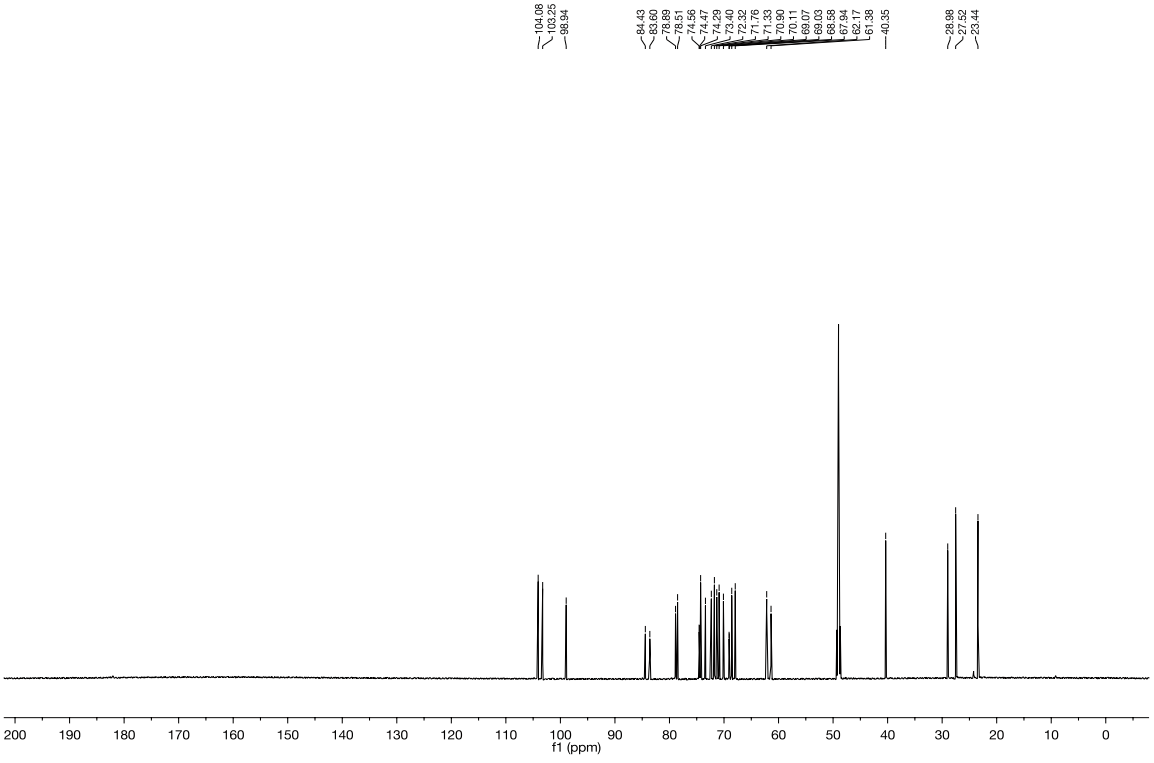


^{19}F NMR (376 MHz, $CDCl_3$)

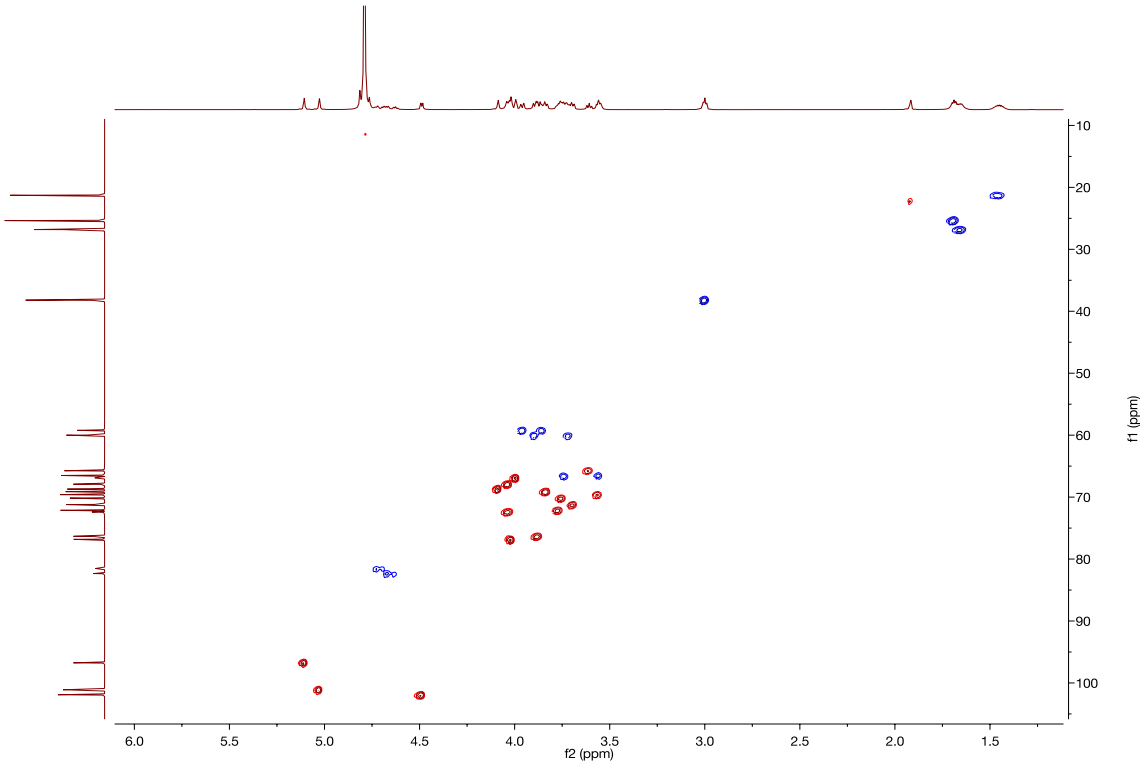
5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-6-deoxy-6-fluoro-galactopyranosyl)-α-D-mannopyranoside (6)



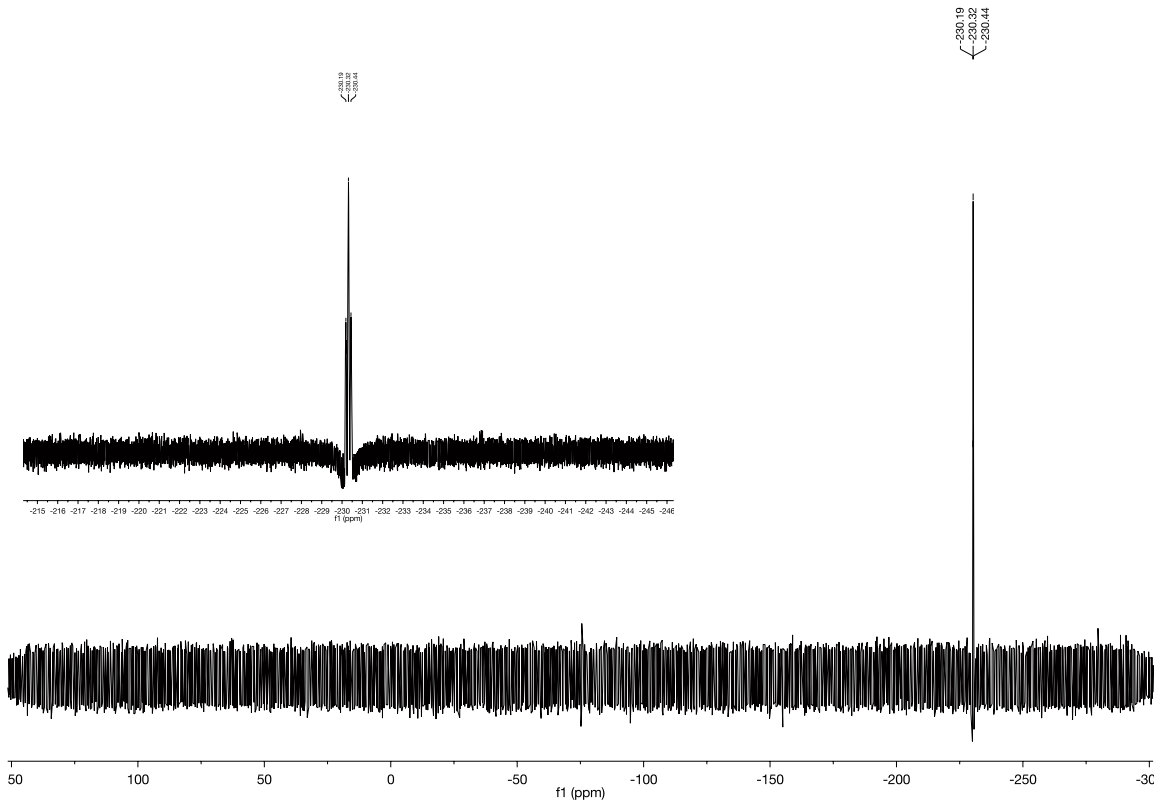
^{1}H NMR (800 MHz, D_2O)



^{13}C NMR (201 MHz, D_2O/CD_3OD)

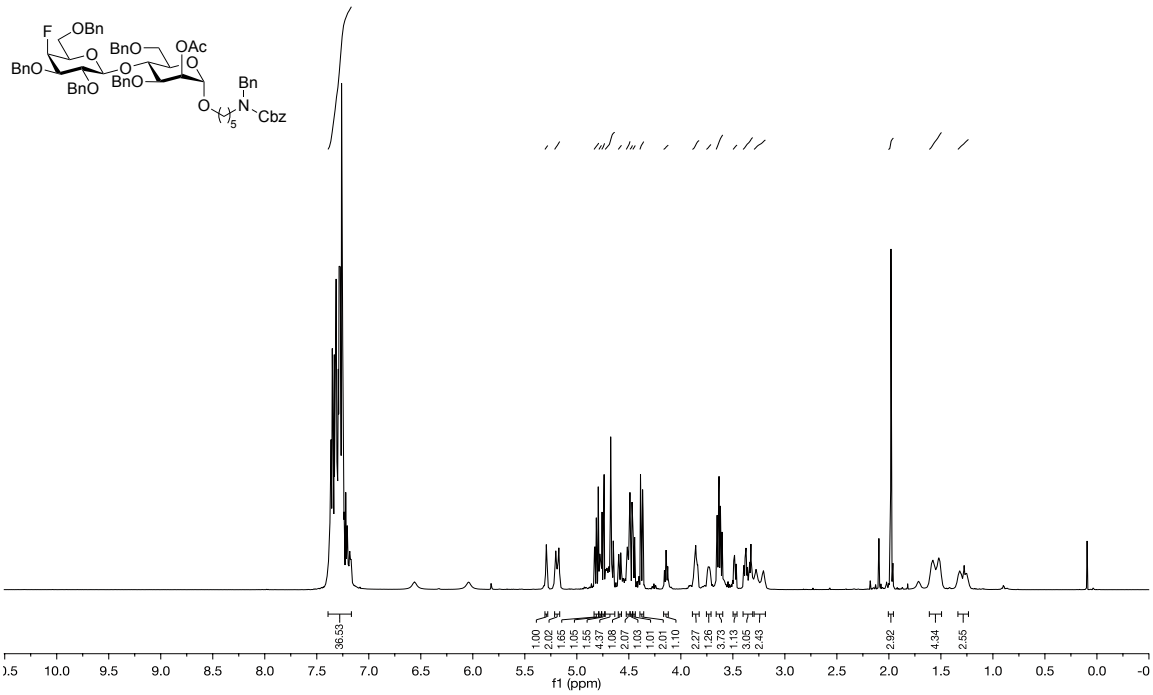


HSQC (800 MHz/201 MHz, D_2O)

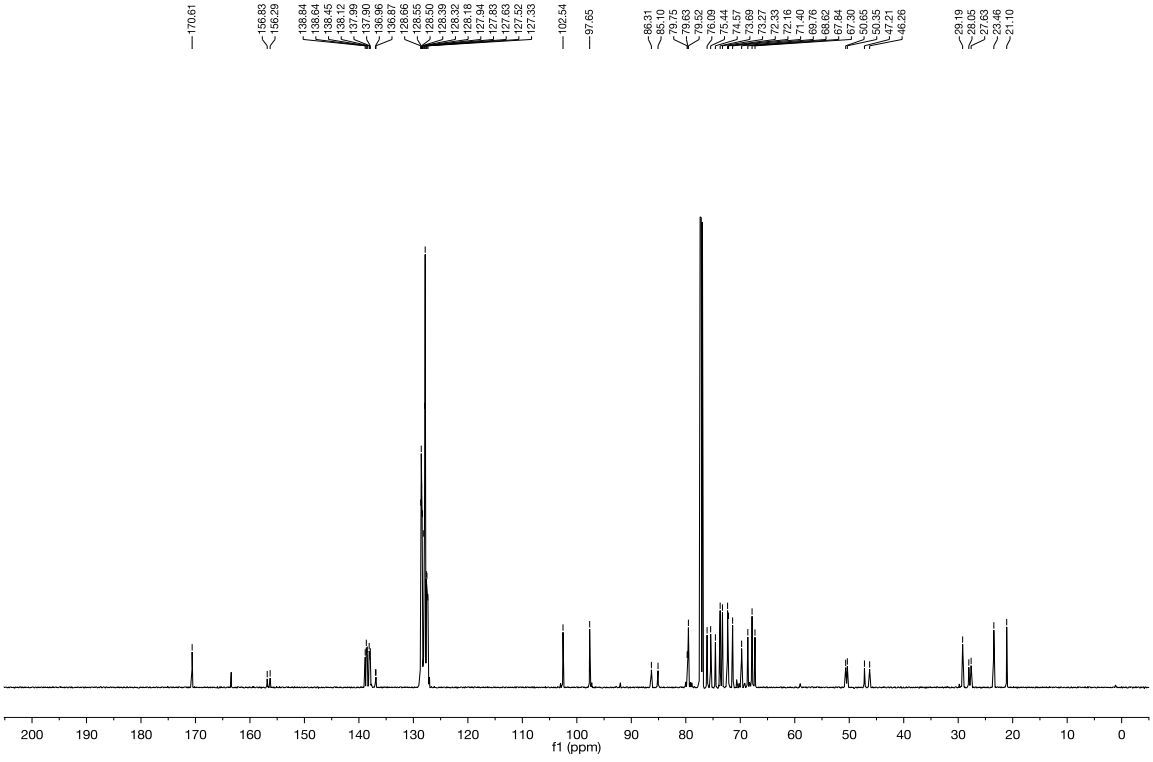


^{19}F NMR (376 MHz, D_2O)

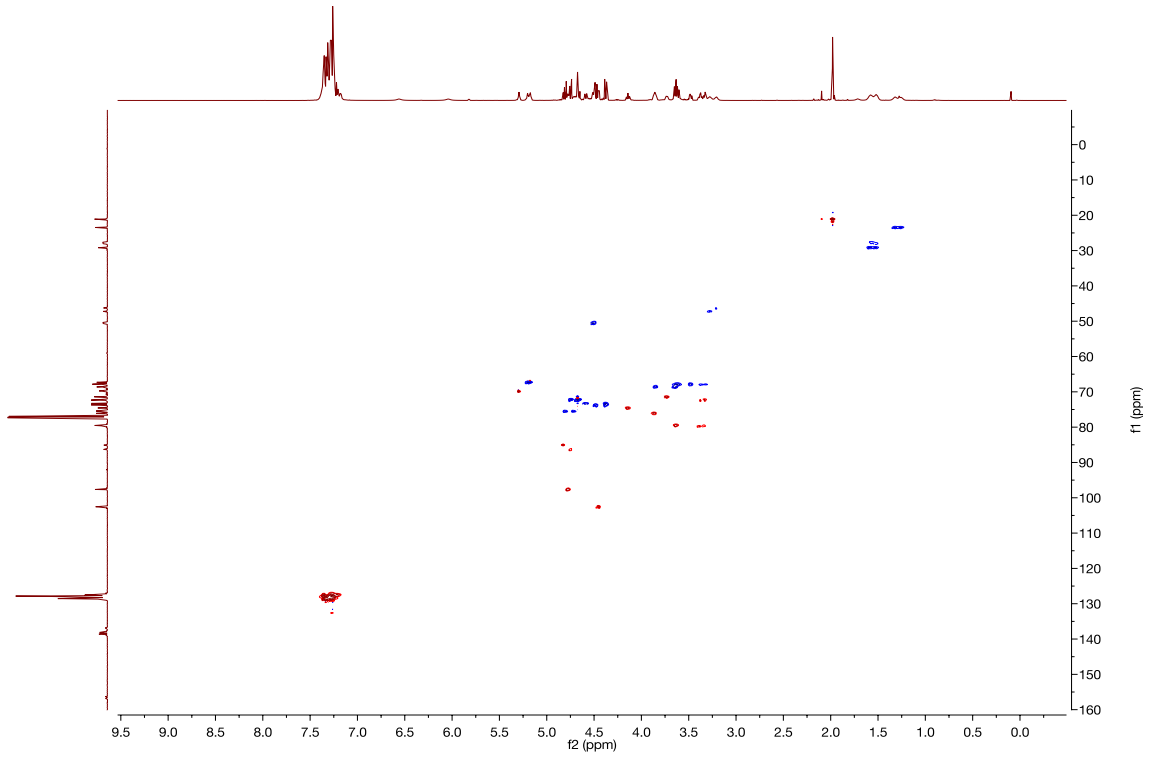
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,6-tri-*O*-benzyl-β-D-4-deoxy-4-fluoro-galactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (87)**



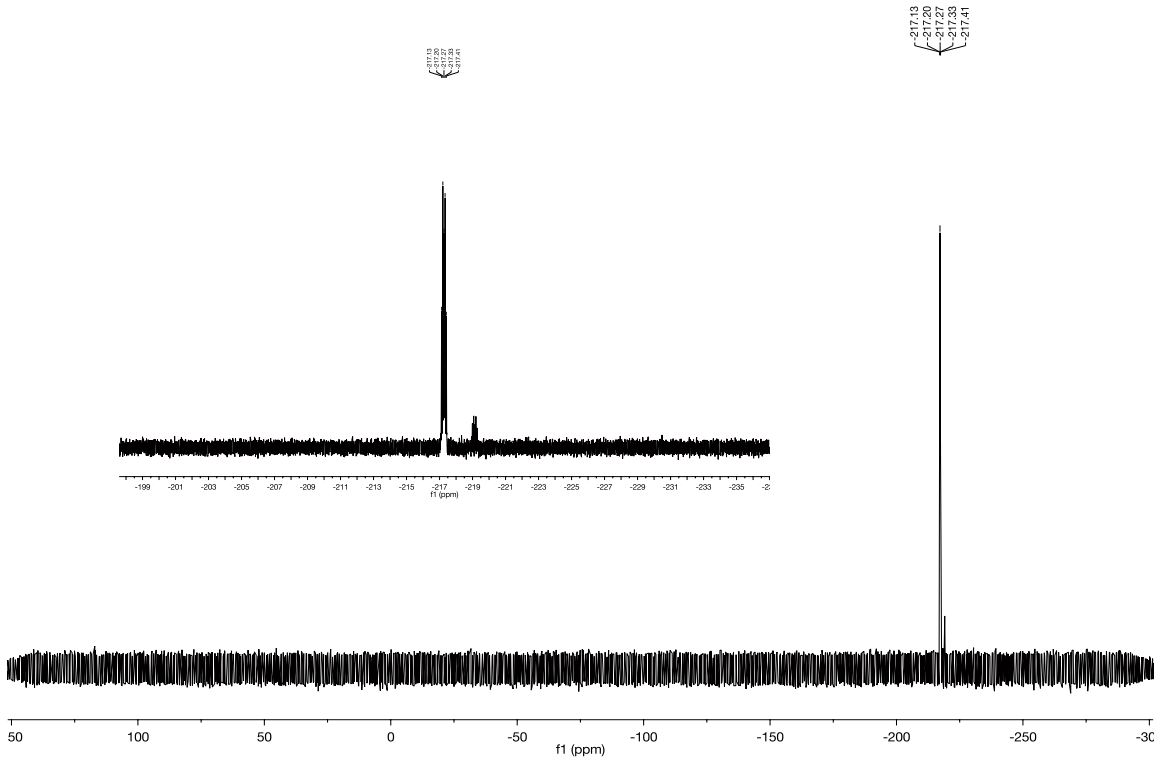
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

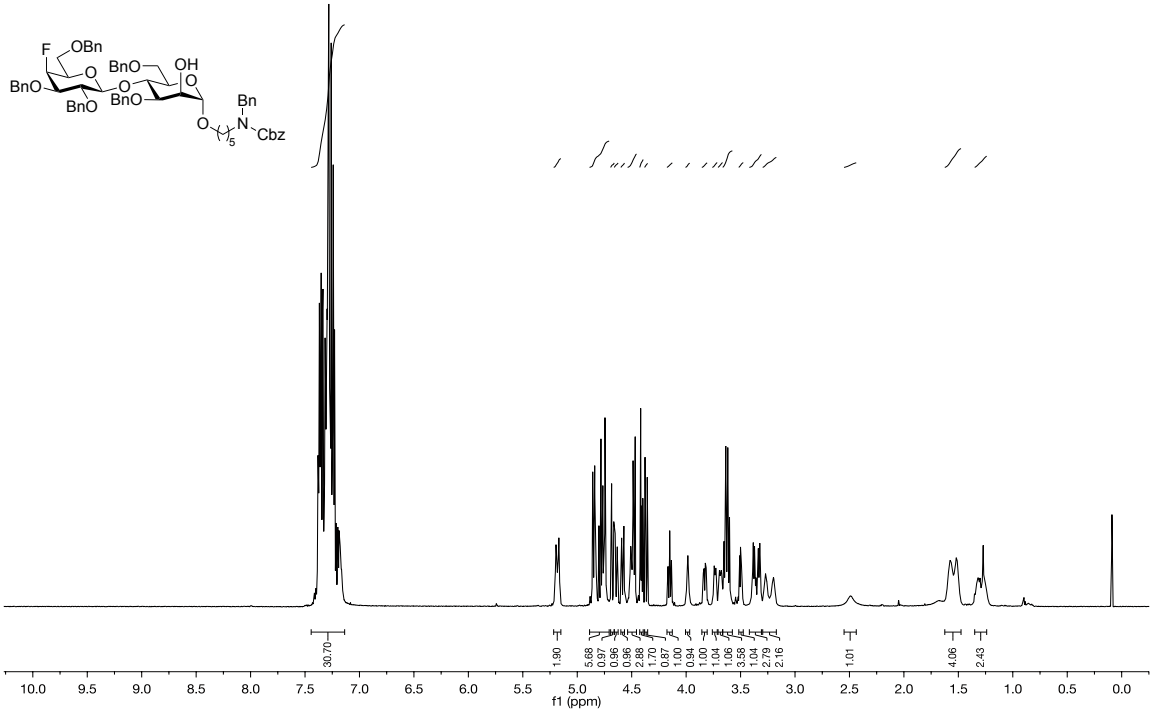


HSQC (599 MHz/151 MHz, $CDCl_3$)

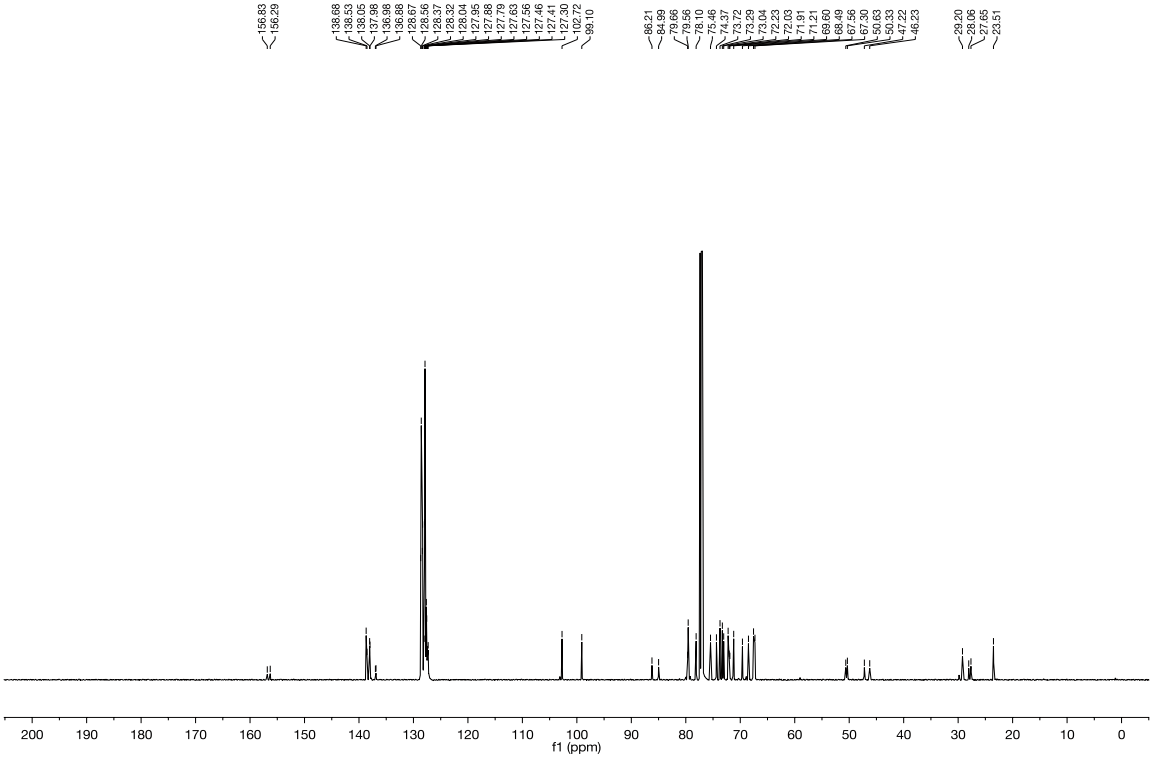


^{19}F NMR (376 MHz, $CDCl_3$)

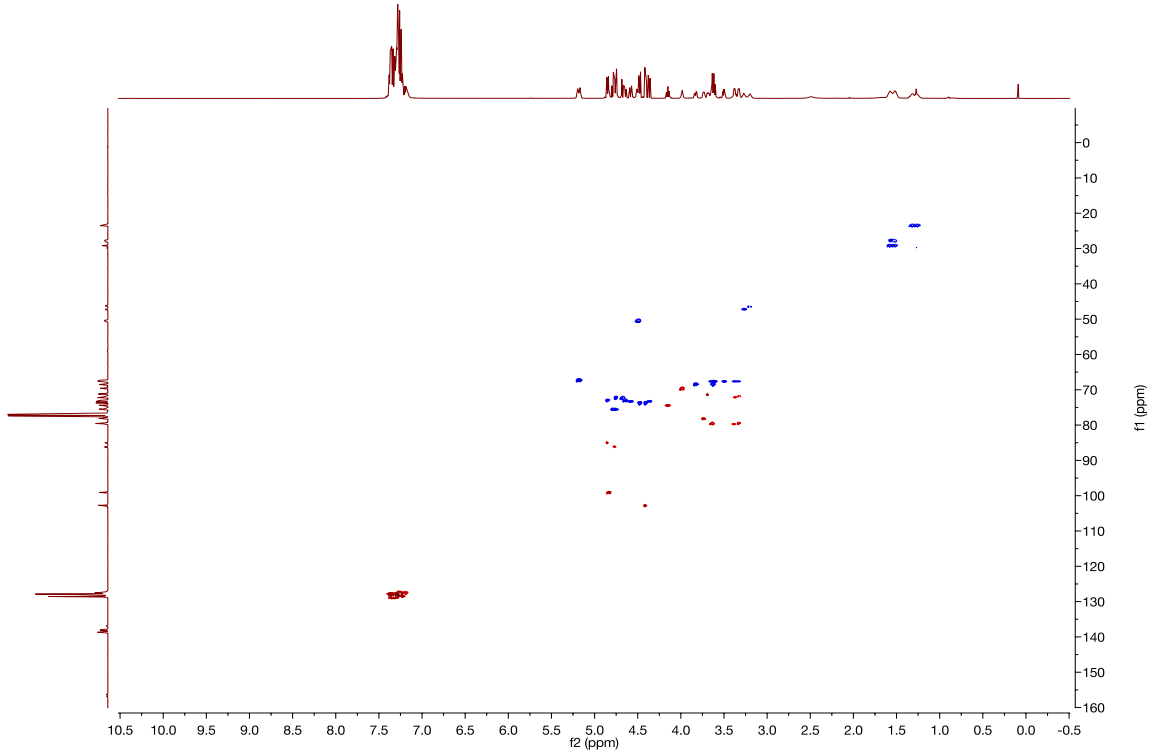
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2,3,6-tri-_O_-benzyl-β-D-4-deoxy-4-fluoro-galactopyranosyl-(1→4)-3,6-di-_O_-benzyl-α-D-mannopyranoside (91)



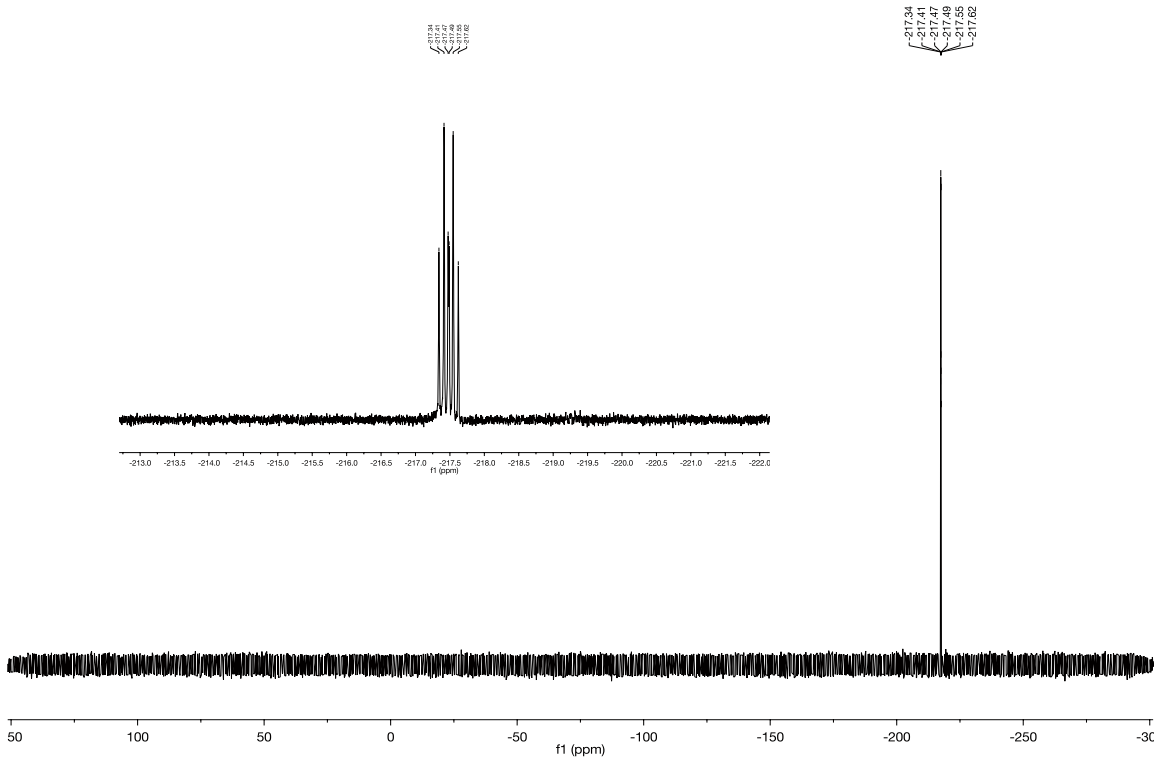
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

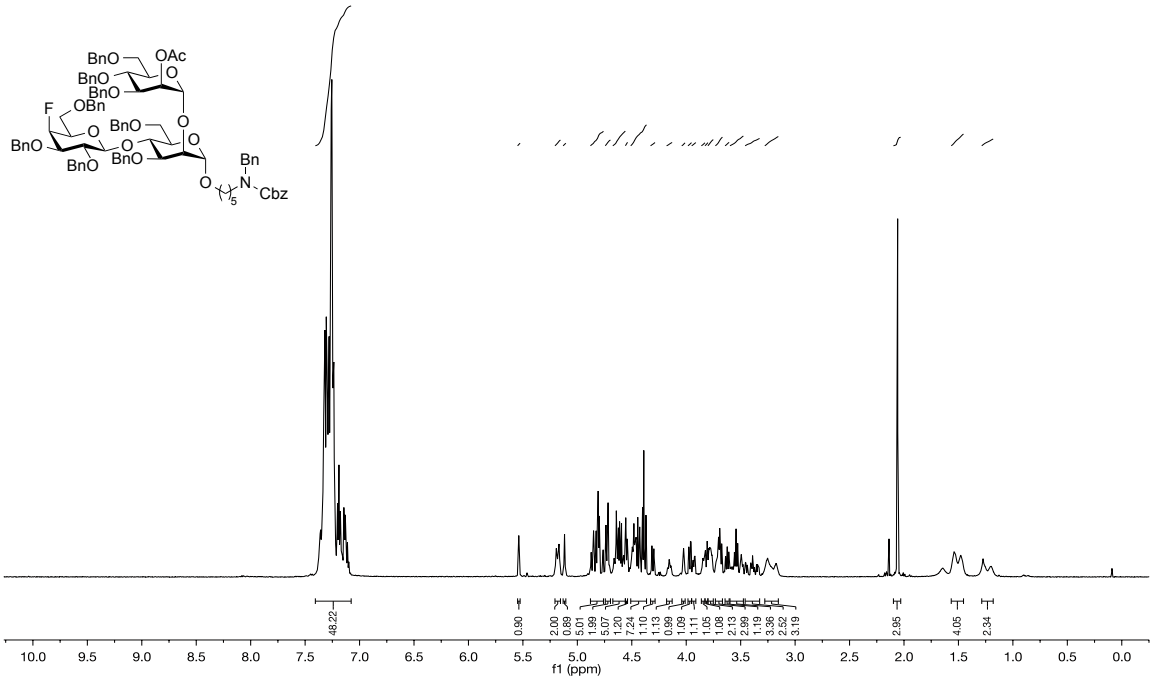


HSQC (599 MHz/151 MHz, $CDCl_3$)

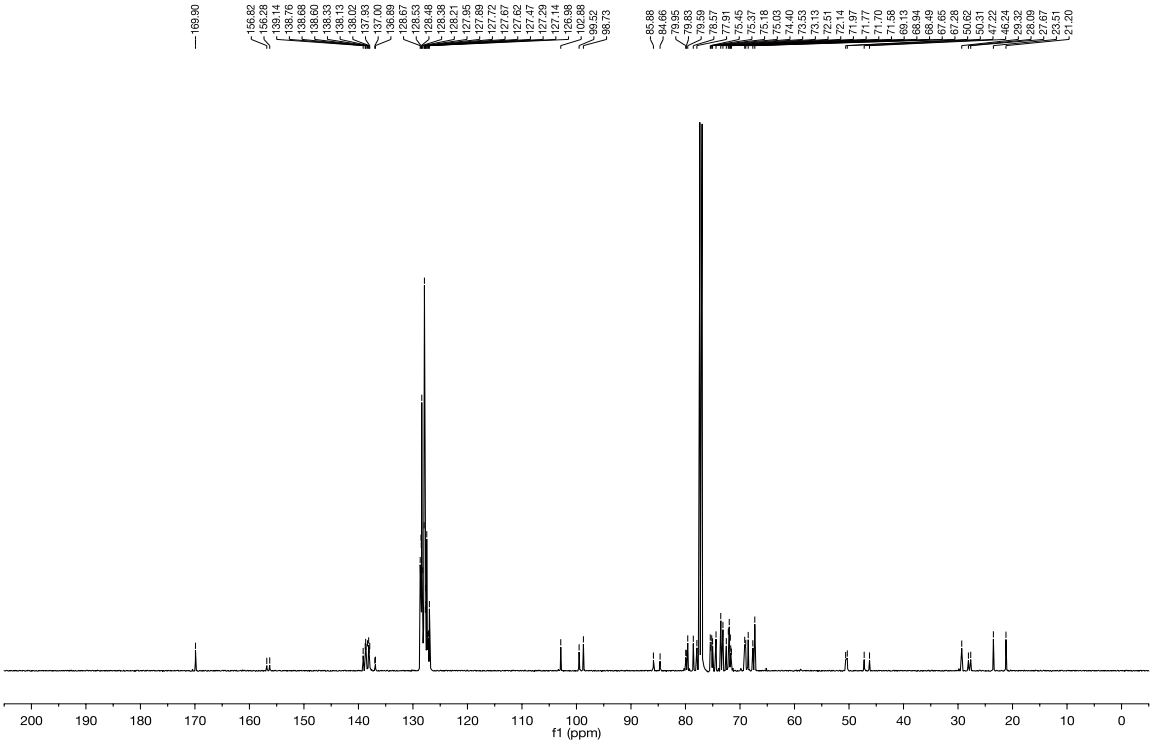


^{19}F NMR (376 MHz, $CDCl_3$)

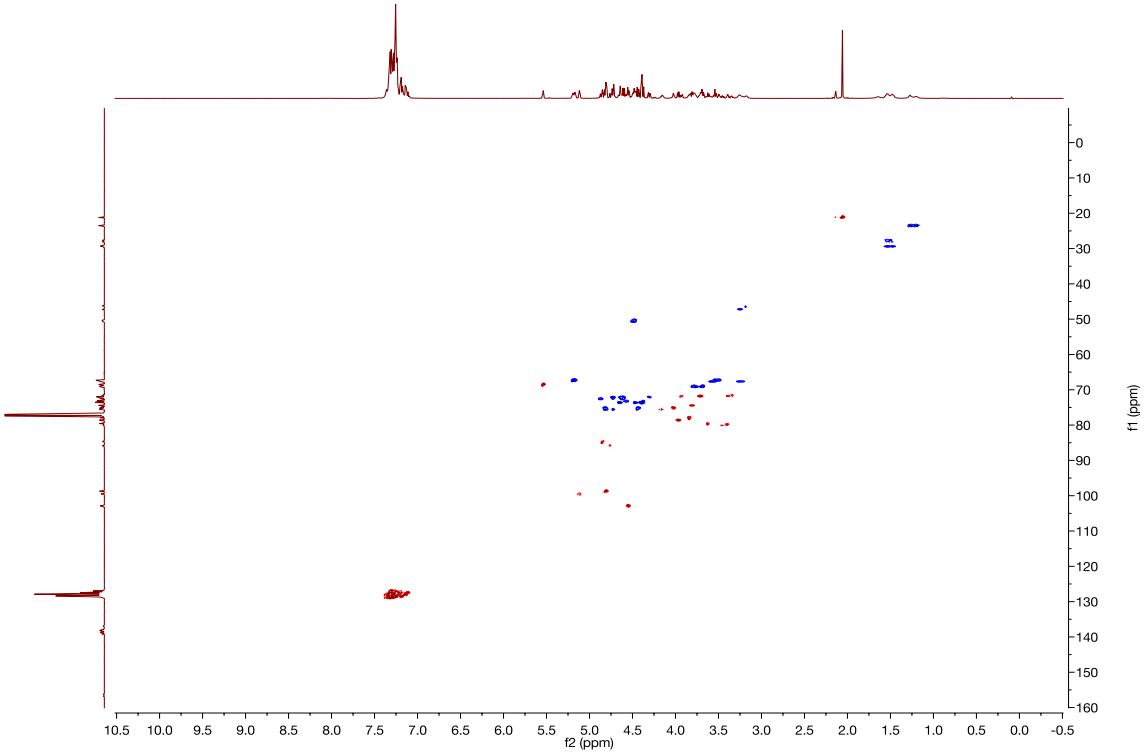
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-_O_-acetyl-3,4,6-tri-_O_-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-_O_-benzyl-4-_O_-(2,3,6-tri-_O_-benzyl-β-D-4-deoxy-4-fluoro-galactopyranosyl)-α-D-mannopyranoside (97)



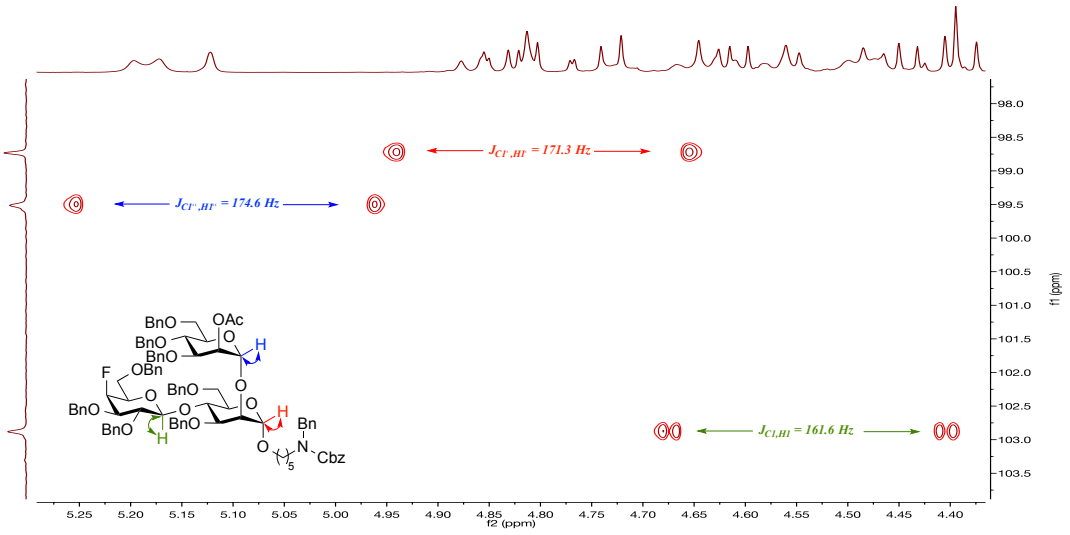
^{1}H NMR (599 MHz, $CDCl_3$)



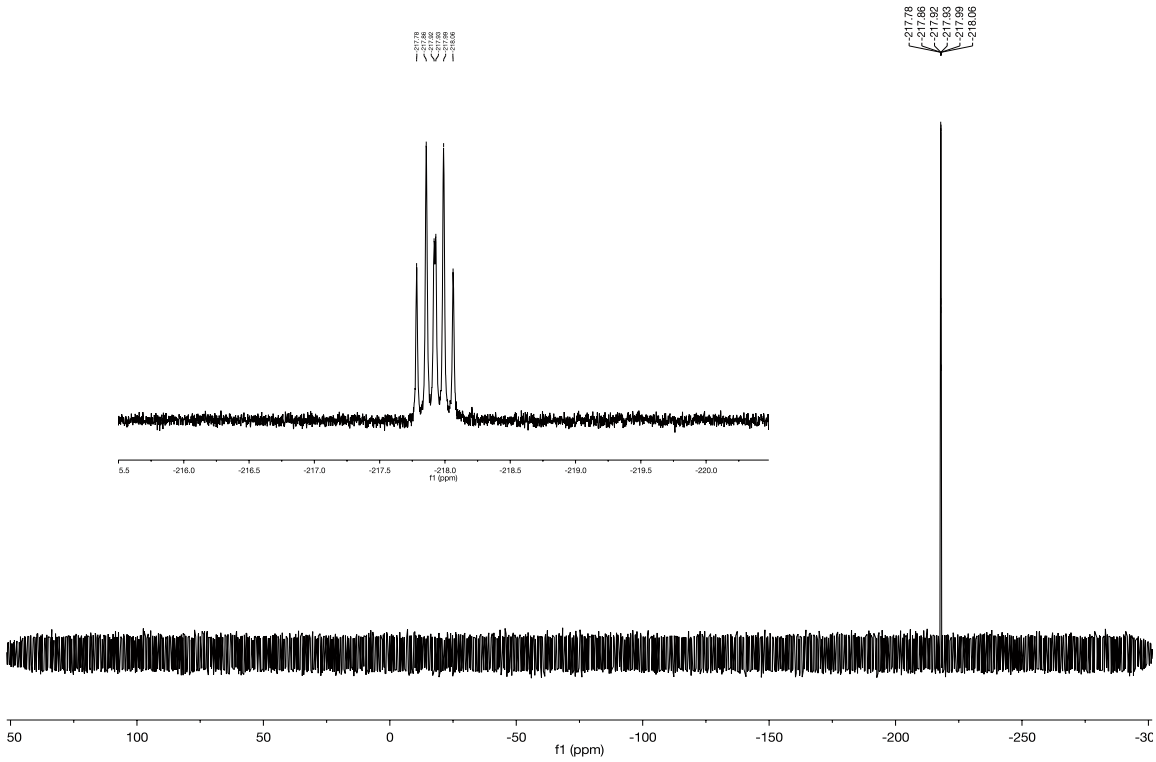
^{13}C NMR (151 MHz, $CDCl_3$)



HSQC (599 MHz/151 MHz, $CDCl_3$)

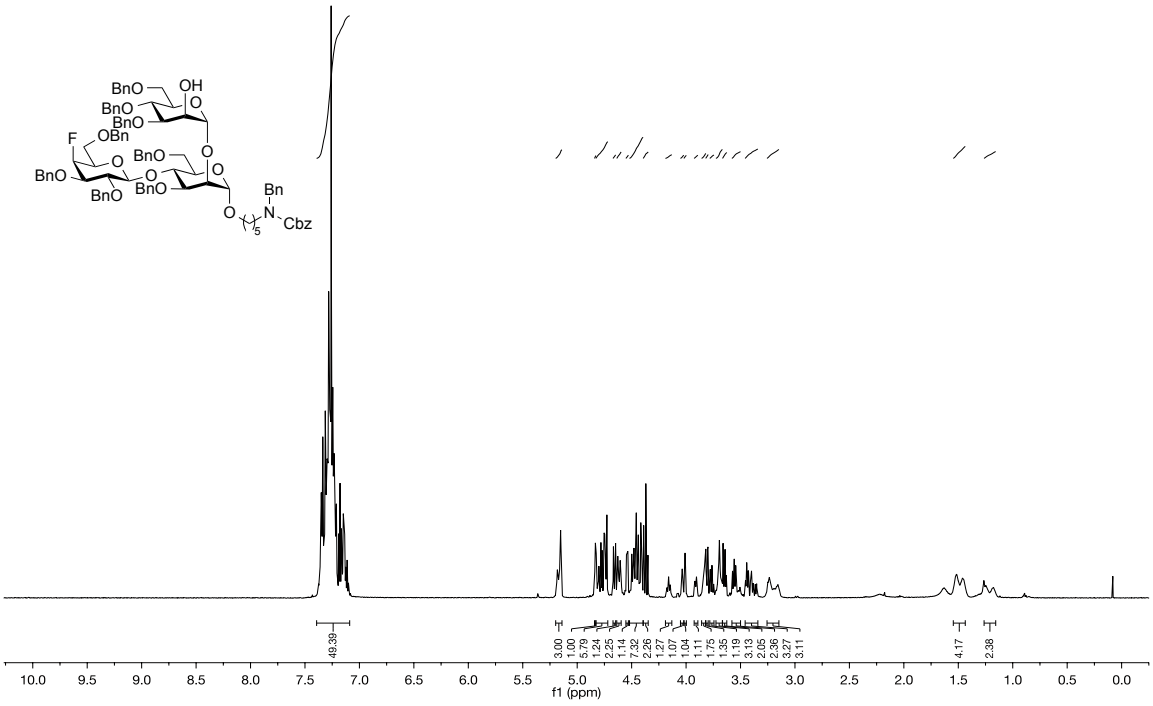


^{1}H-^{13}C coupled HSQC-NMR

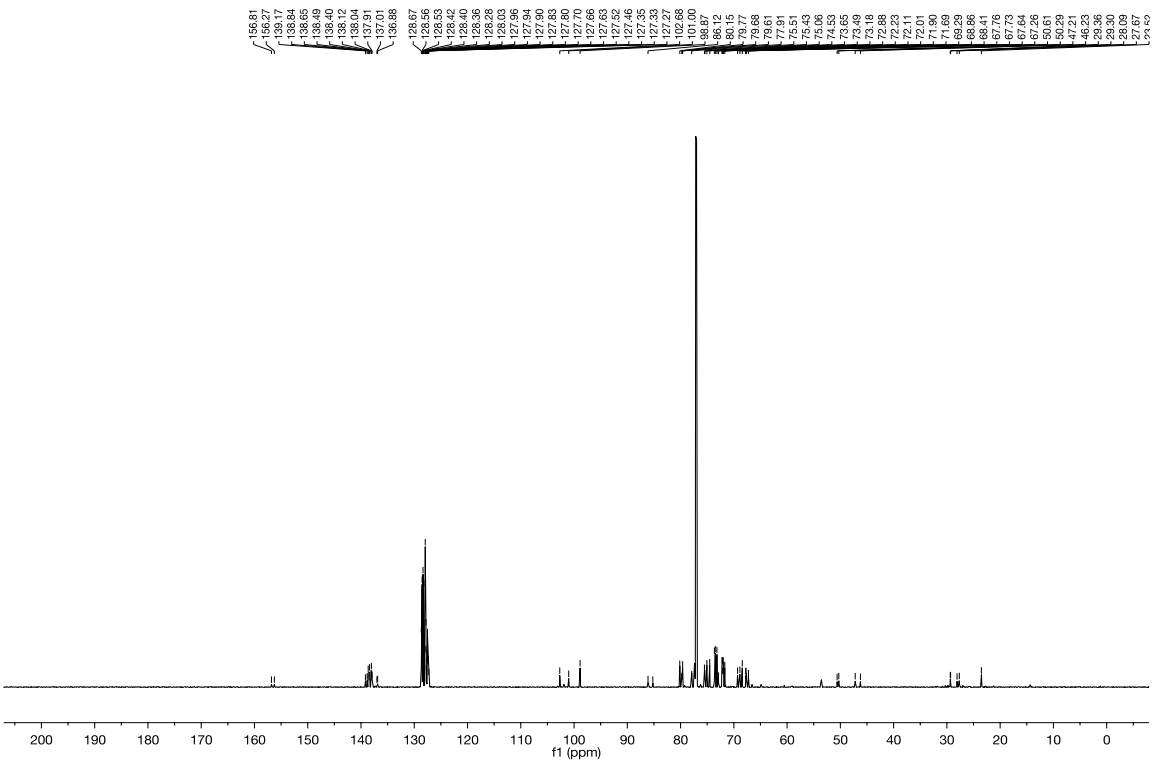


^{19}F NMR (376 MHz, $CDCl_3$)

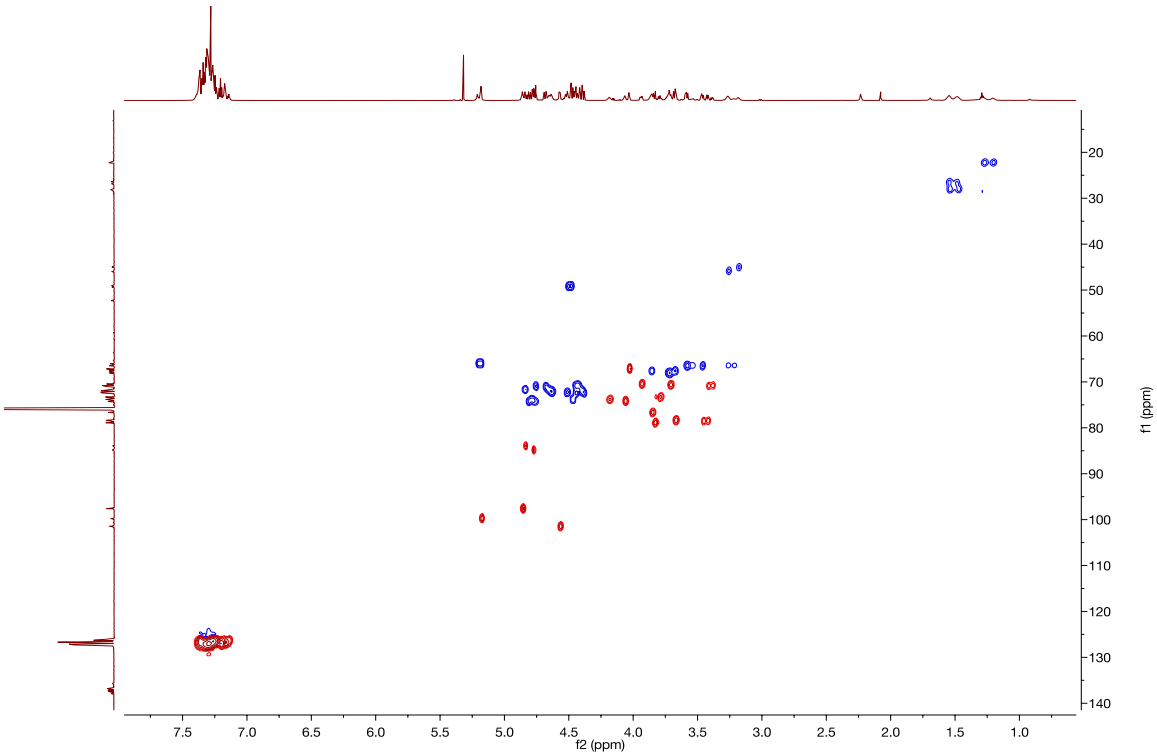
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-4-deoxy-4-fluoro-galactopyranosyl)-α-D-mannopyranoside (101)**



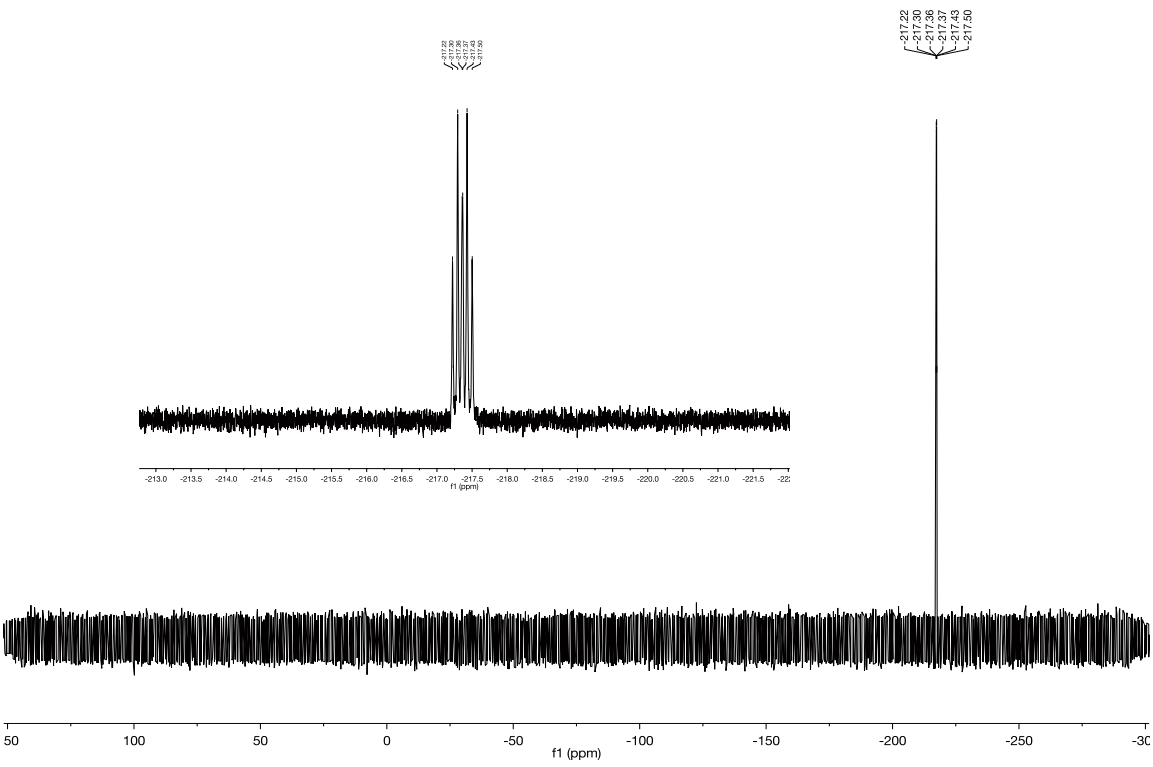
^{1}H NMR (800 MHz, $CDCl_3$)



^{13}C NMR (201 MHz, $CDCl_3$)

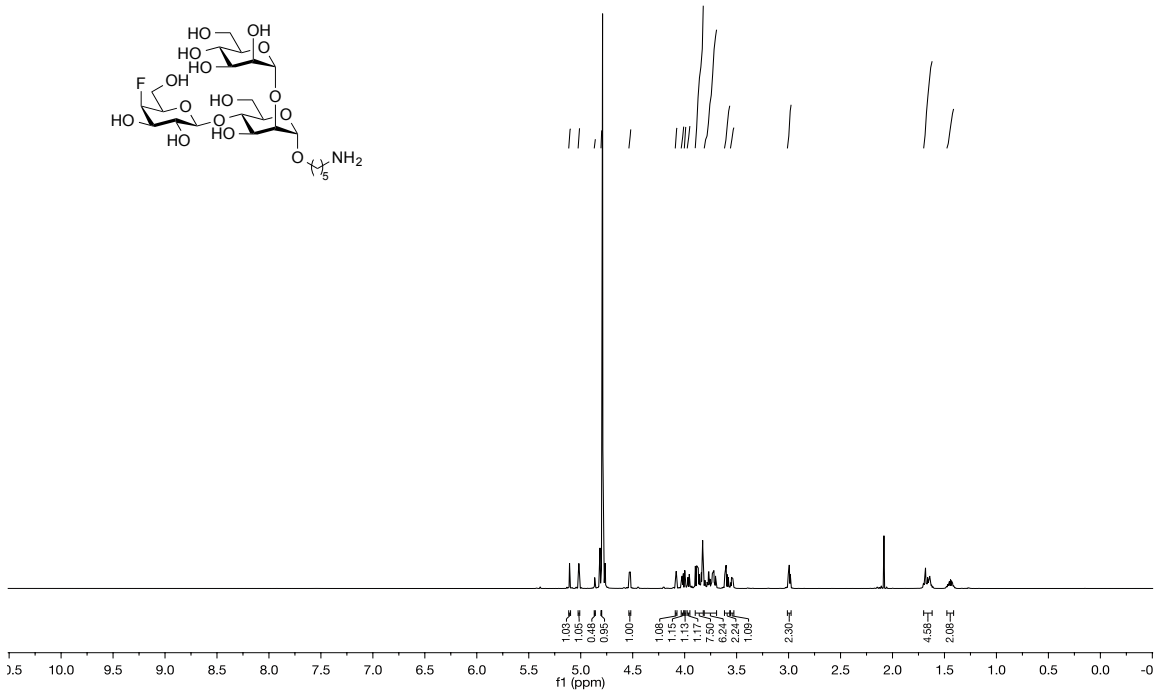


HSQC (800 MHz/201 MHz, $CDCl_3$)

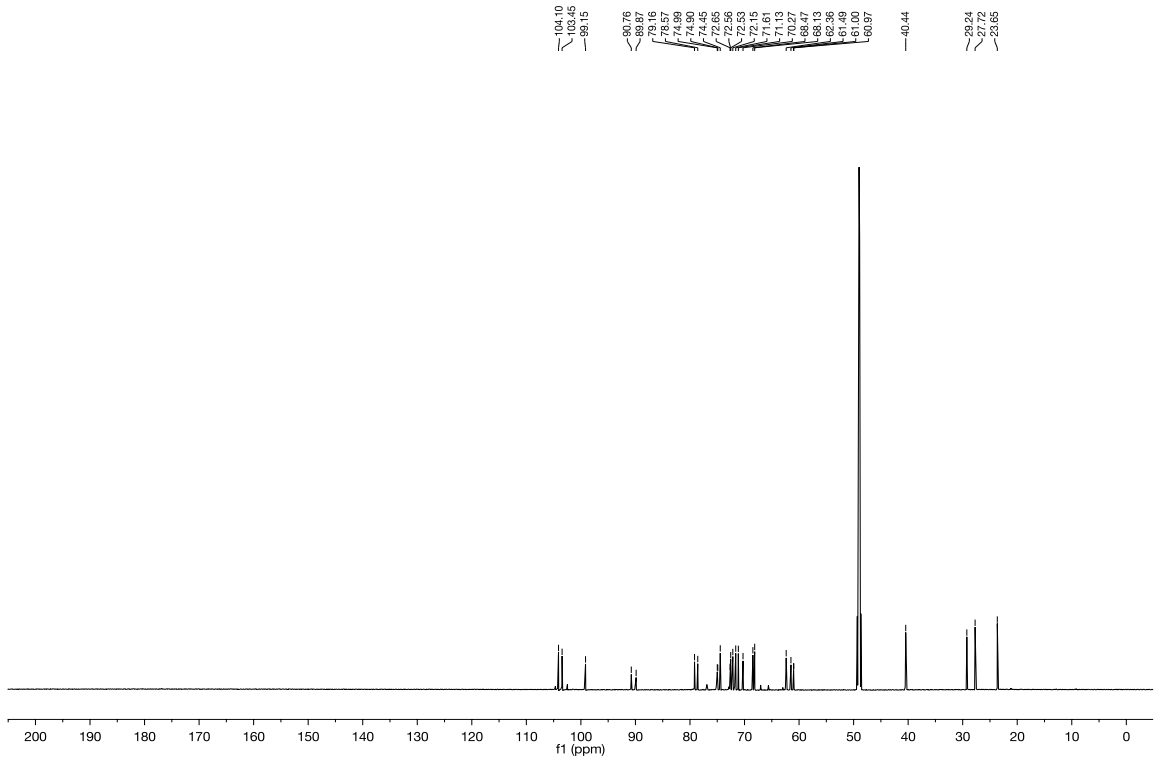


^{19}F NMR (376 MHz, $CDCl_3$)

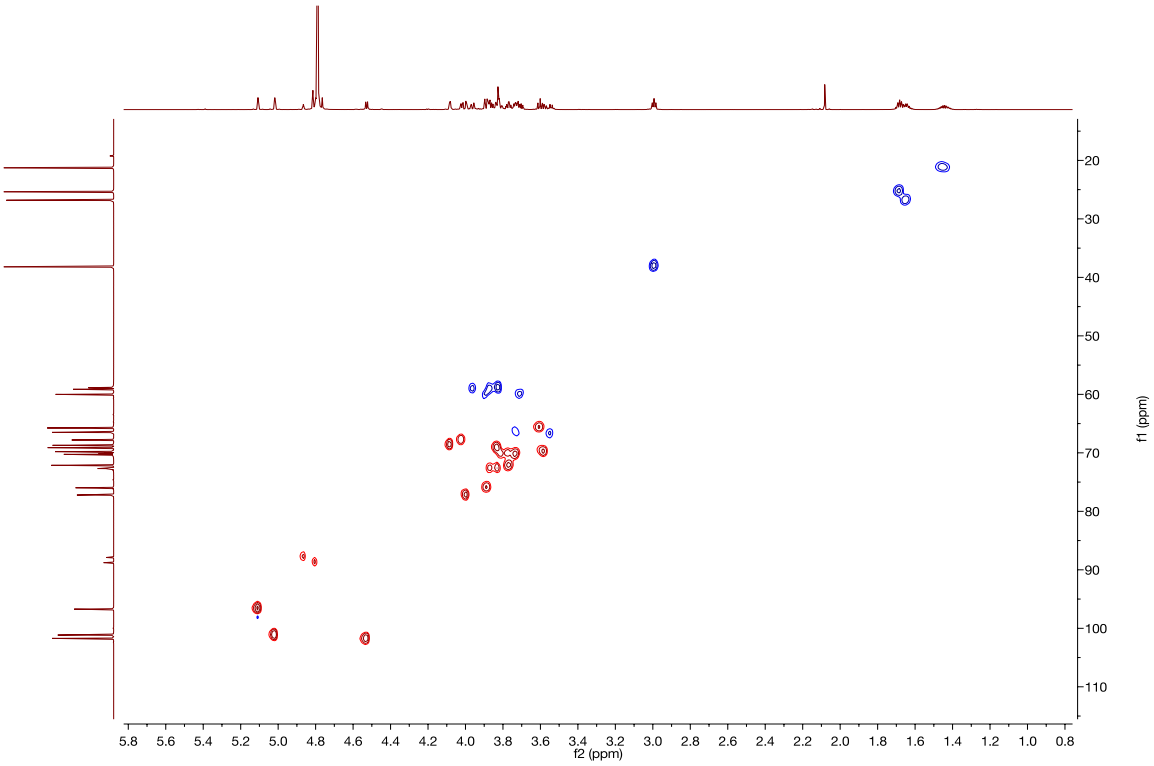
5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-4-deoxy-4-fluoro-galactopyranosyl)-α-D-mannopyranoside (7)



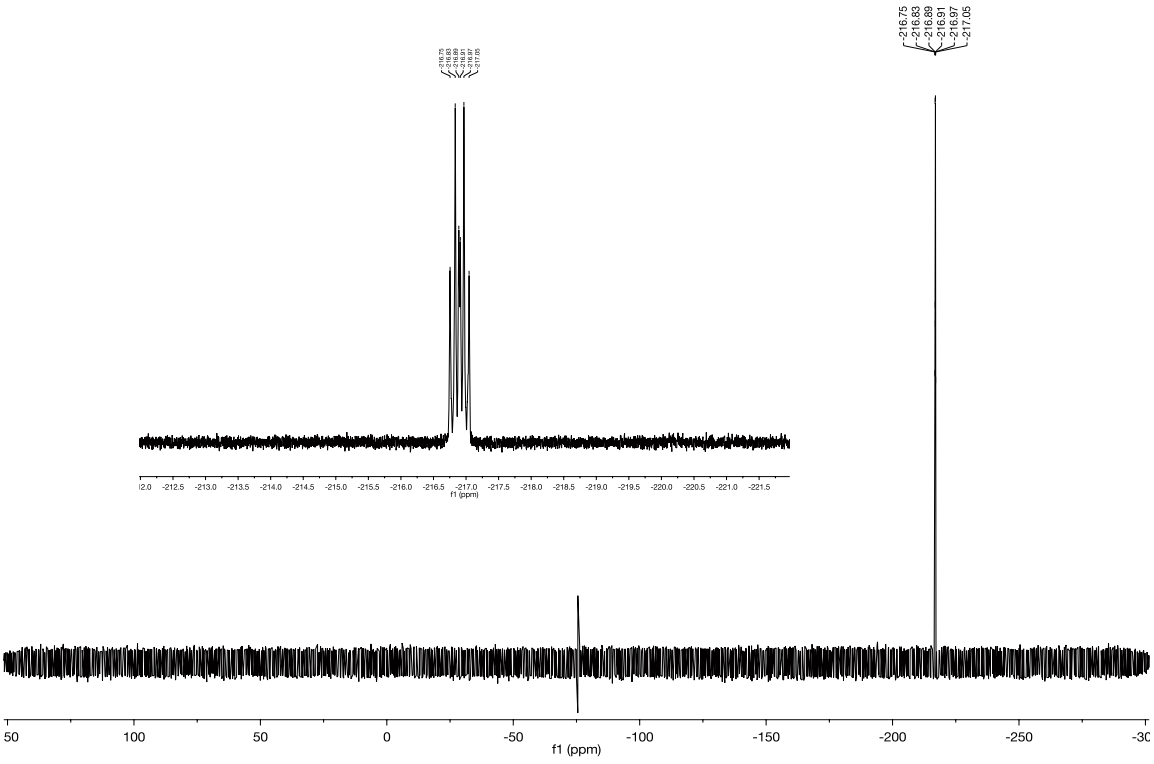
^{1}H NMR (800 MHz, D_2O)



^{13}C NMR (201 MHz, D_2O/CD_3OD)

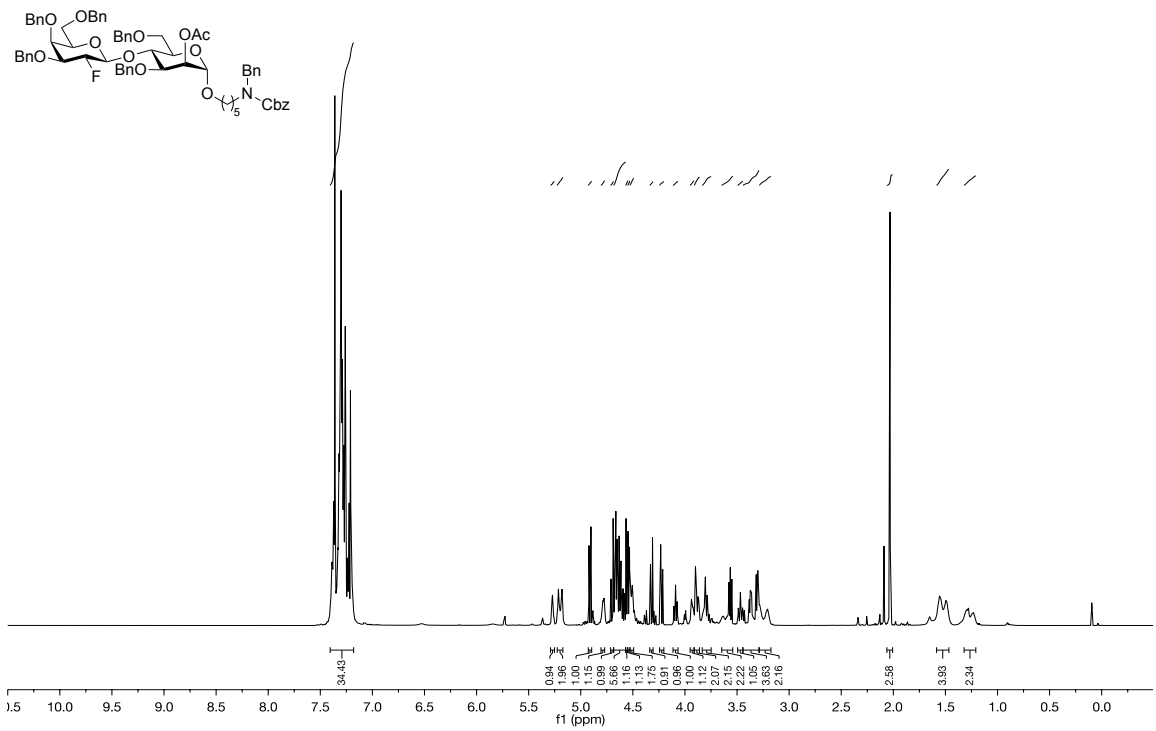


HSQC (800 MHz/201 MHz, D_2O)

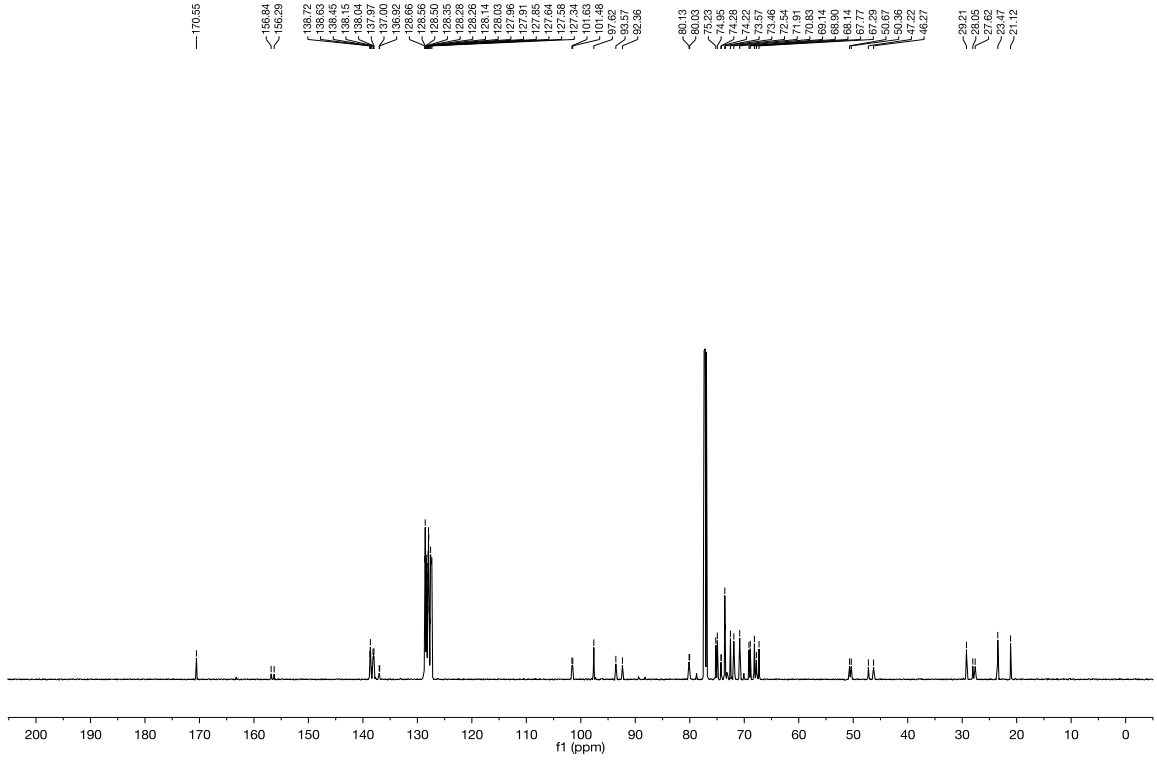


^{19}F NMR (376 MHz, D_2O)

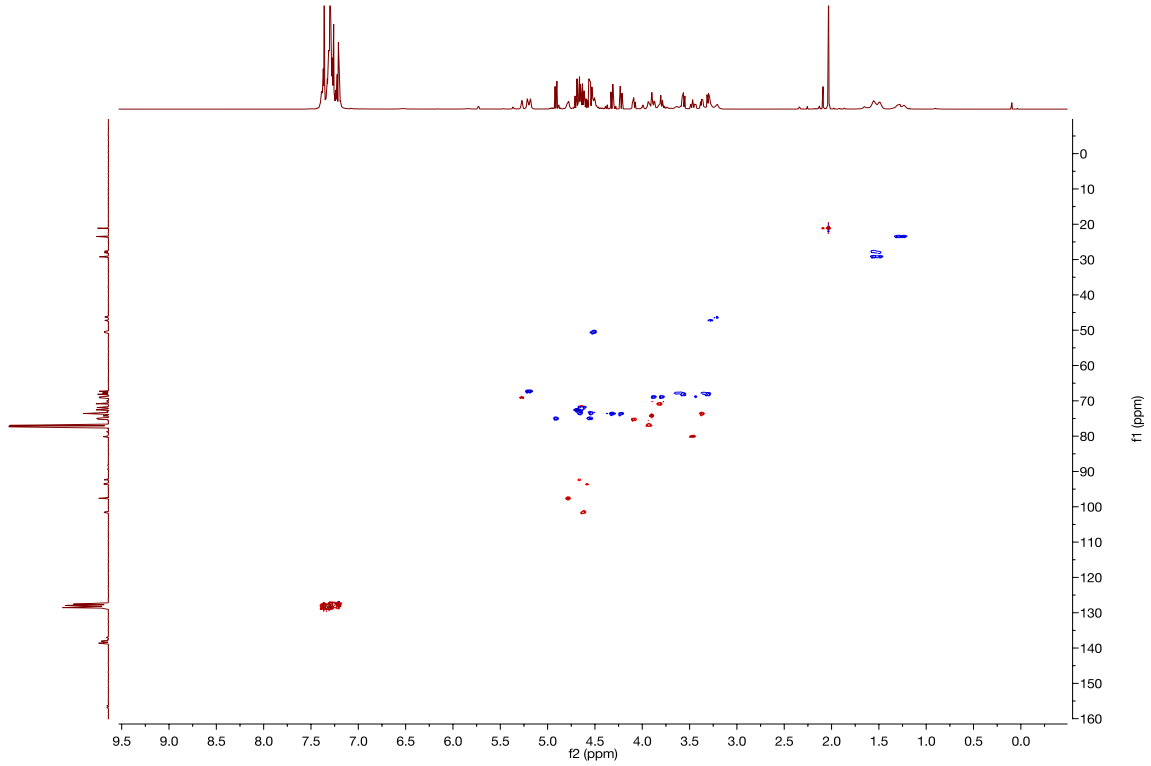
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-β-D-2-deoxy-2-fluoro-galactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (88)**



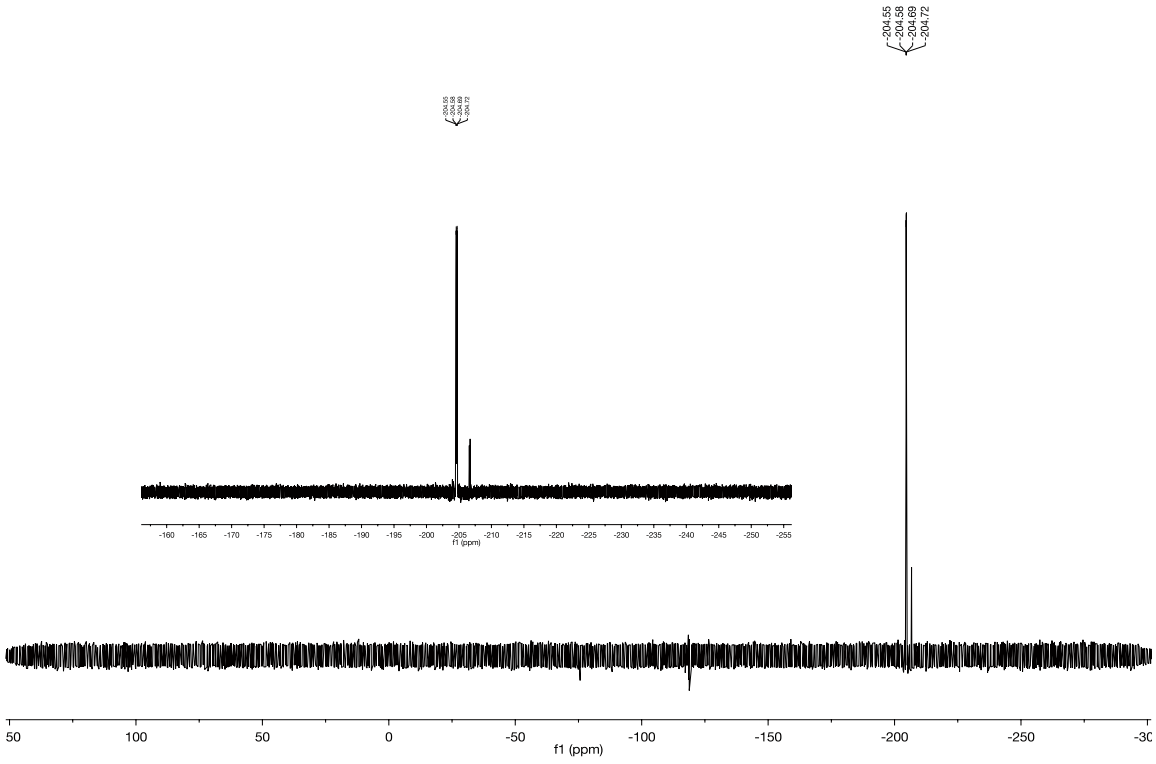
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

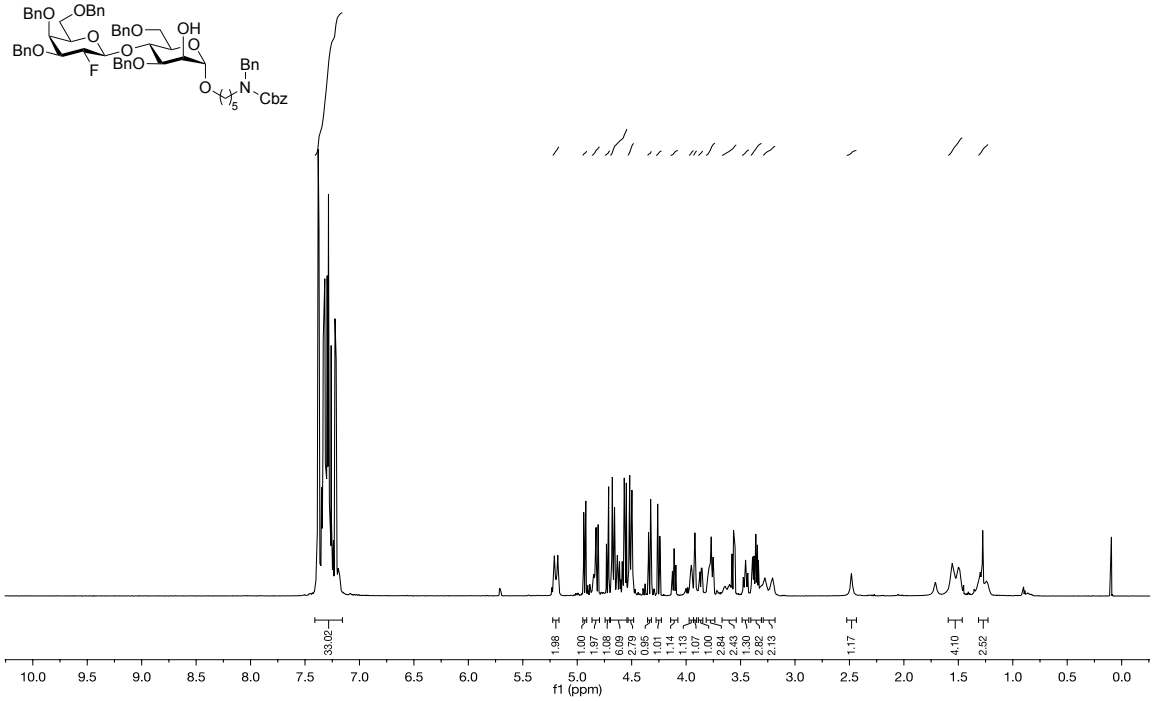


HSQC (599 MHz/151 MHz, $CDCl_3$)

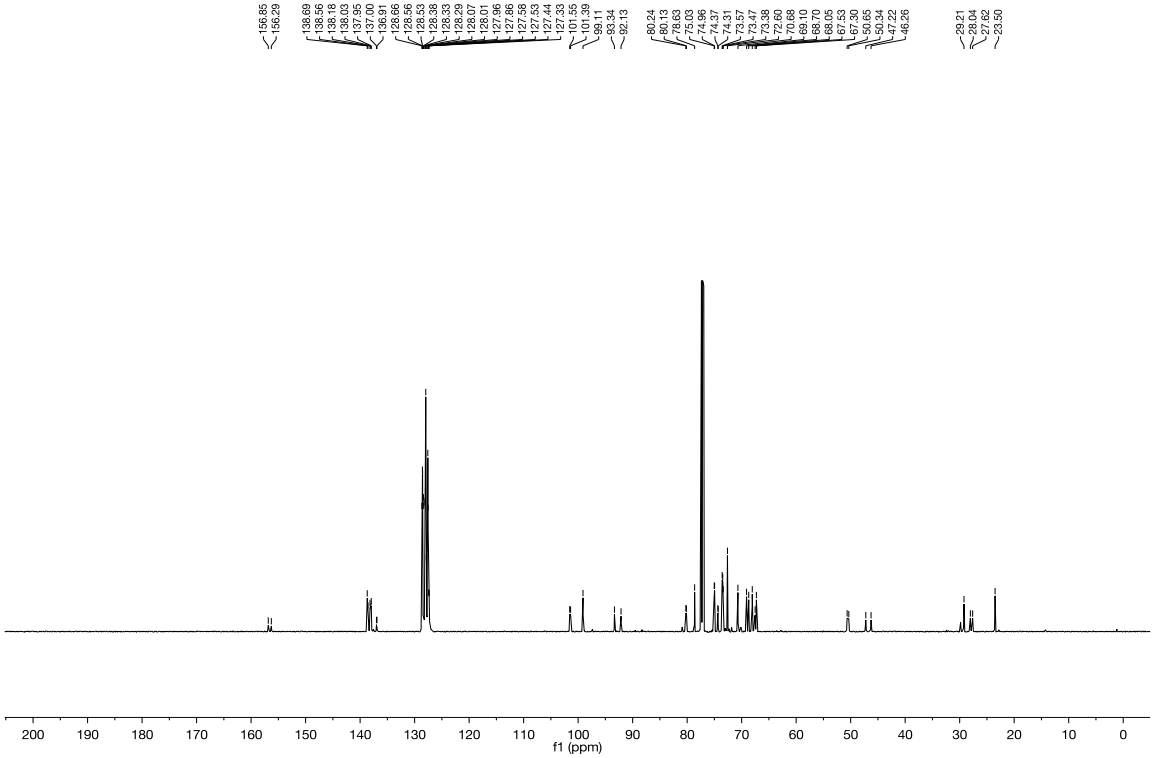


^{19}F NMR (376 MHz, $CDCl_3$)

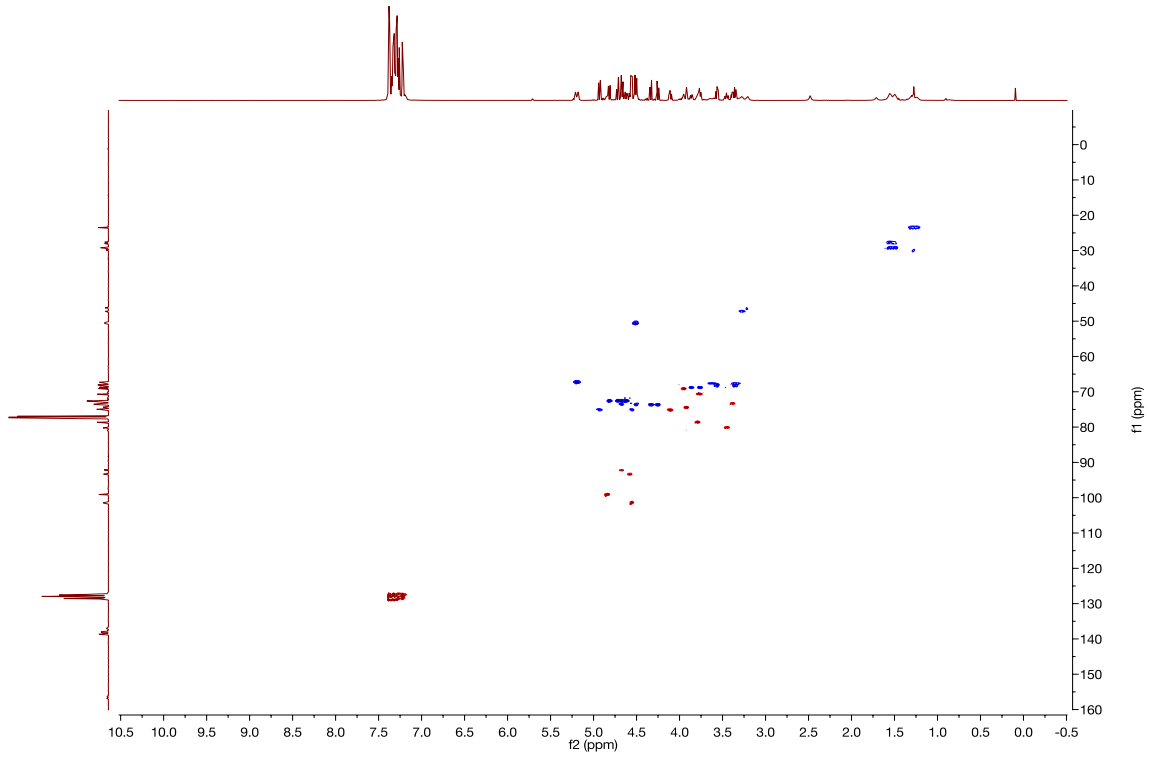
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-β-D-2-deoxy-2-fluoro-galactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (92)**



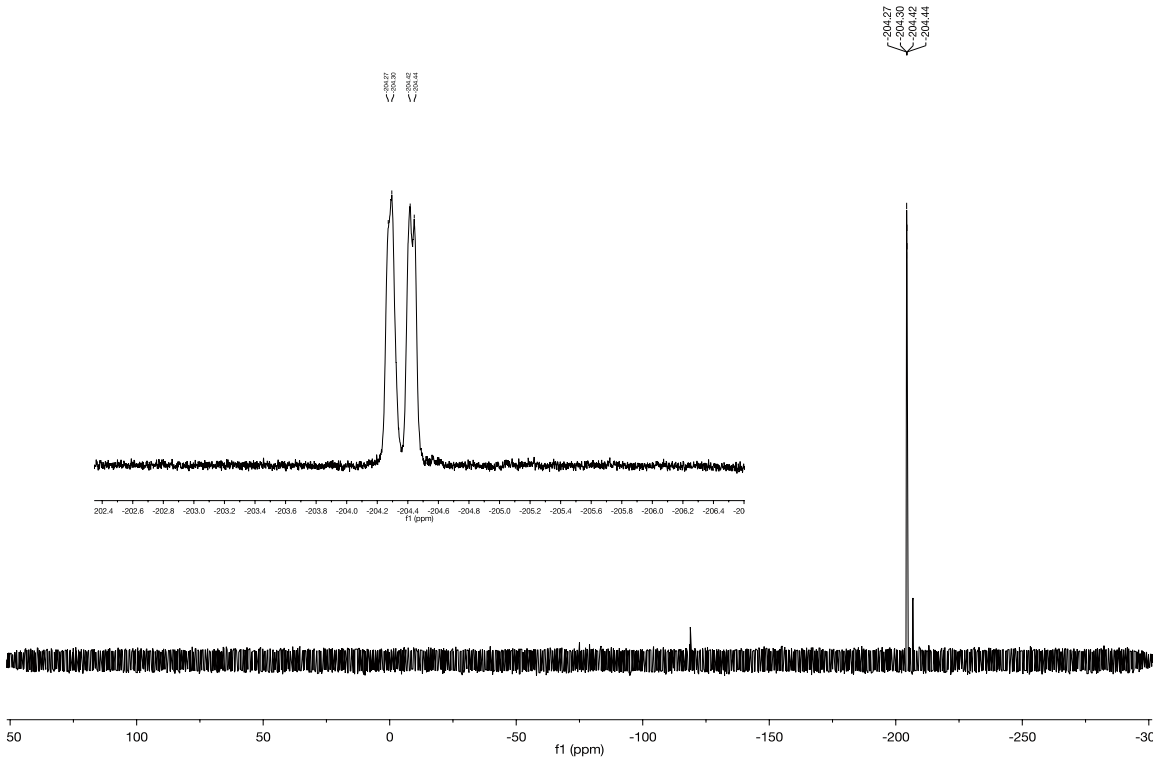
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

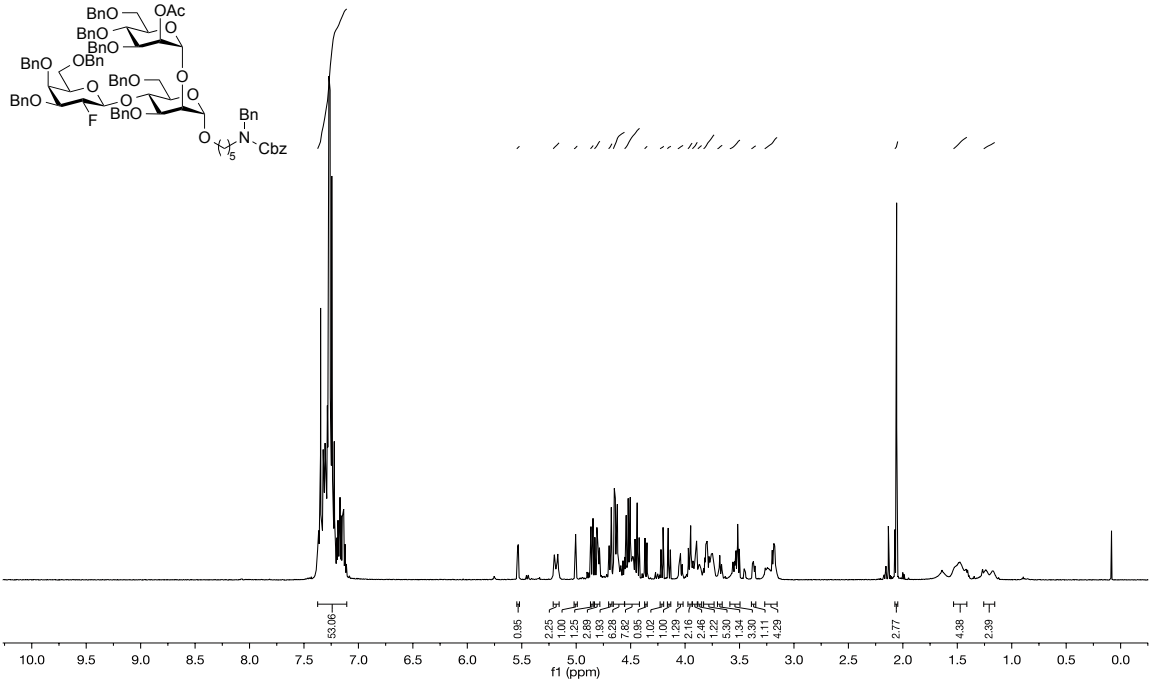


HSQC (599 MHz/151 MHz, $CDCl_3$)

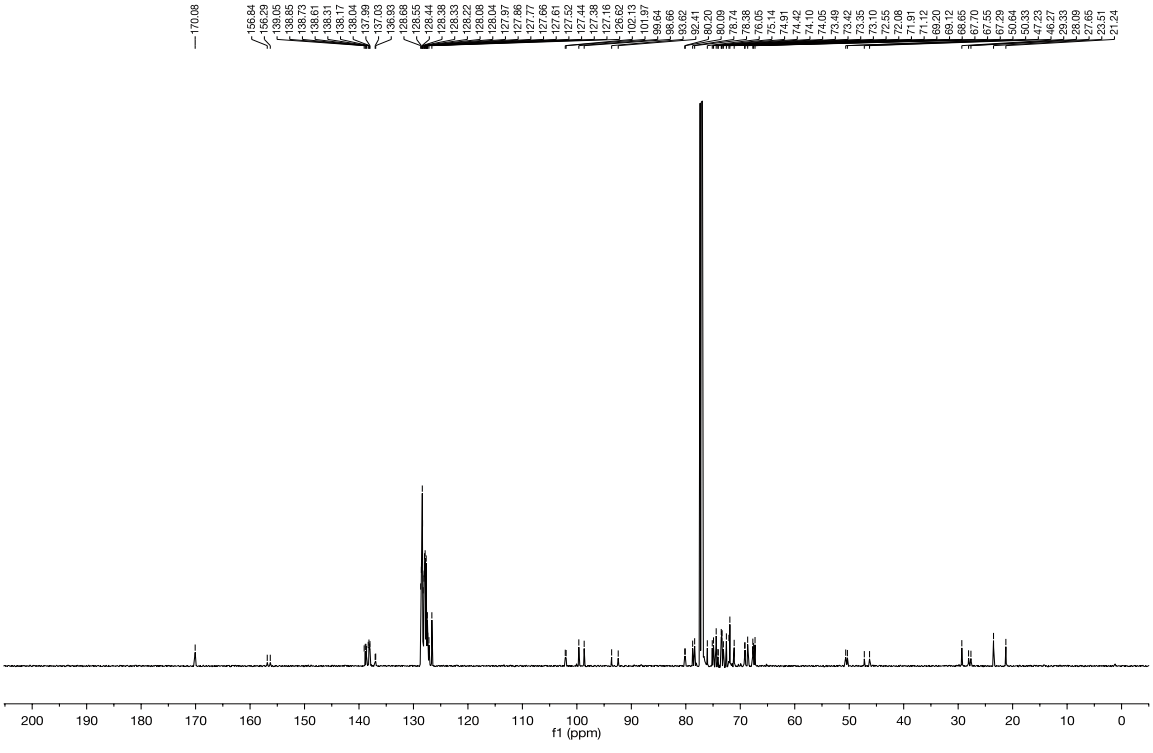


^{19}F NMR (376 MHz, $CDCl_3$)

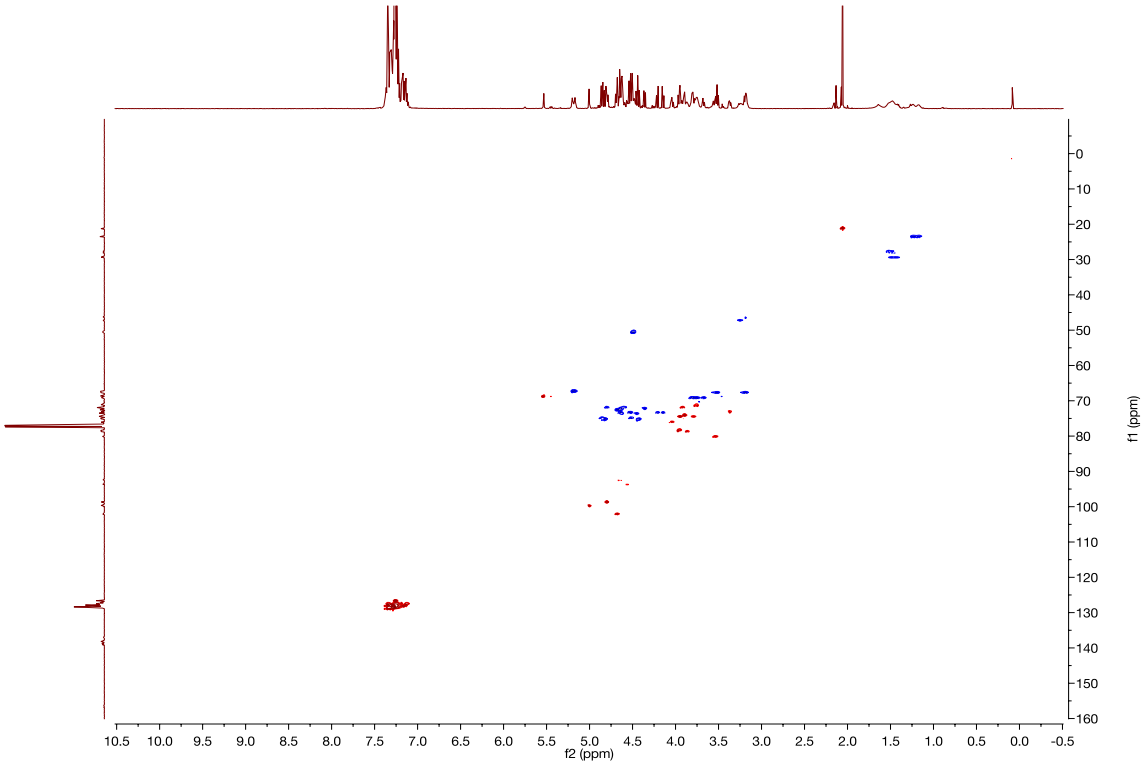
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-2-deoxy-2-fluoro-galactopyranosyl)-α-D-mannopyranoside (98)**



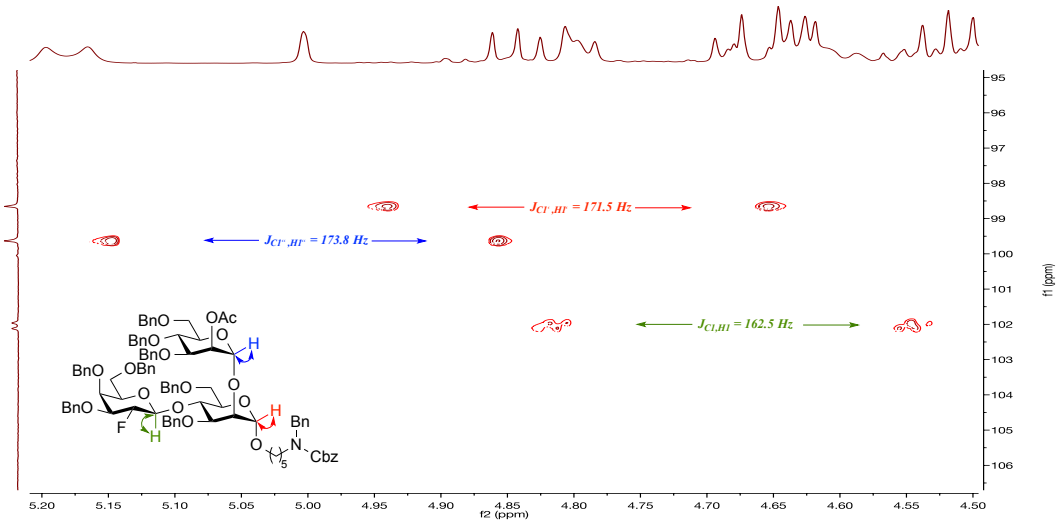
^{1}H NMR (599 MHz, $CDCl_3$)



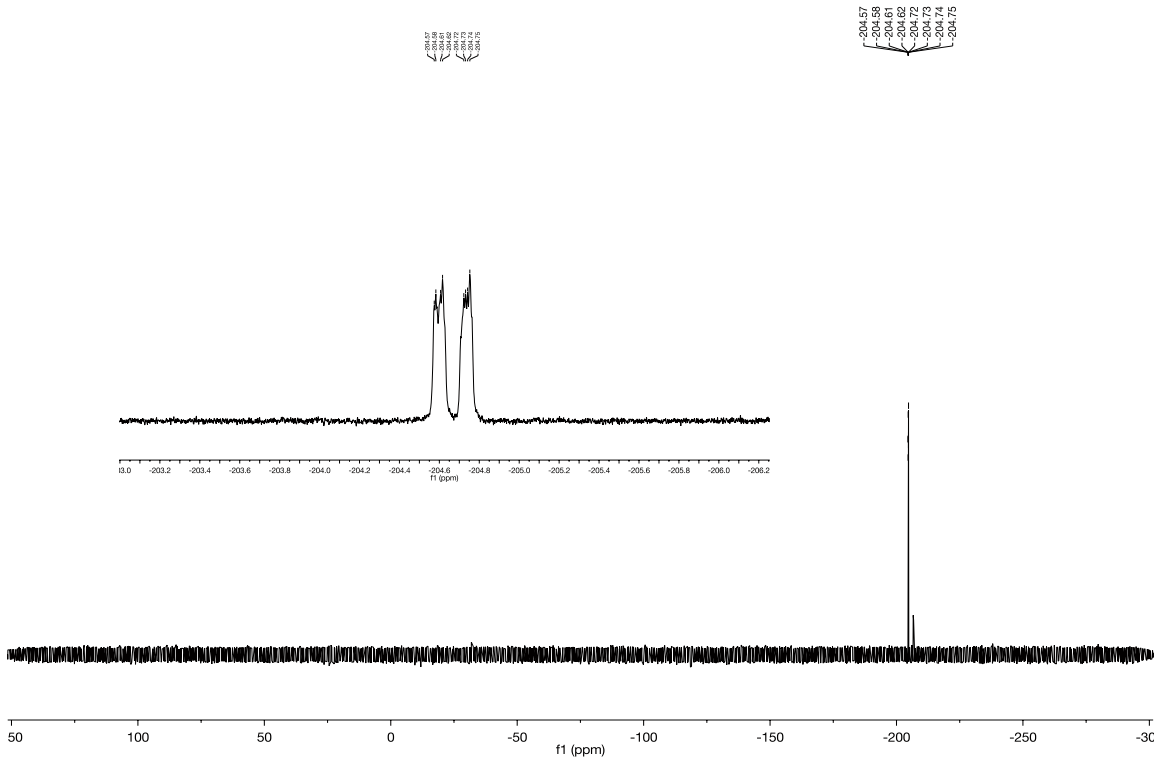
^{13}C NMR (151 MHz, $CDCl_3$)



HSQC (599 MHz/151 MHz, $CDCl_3$)

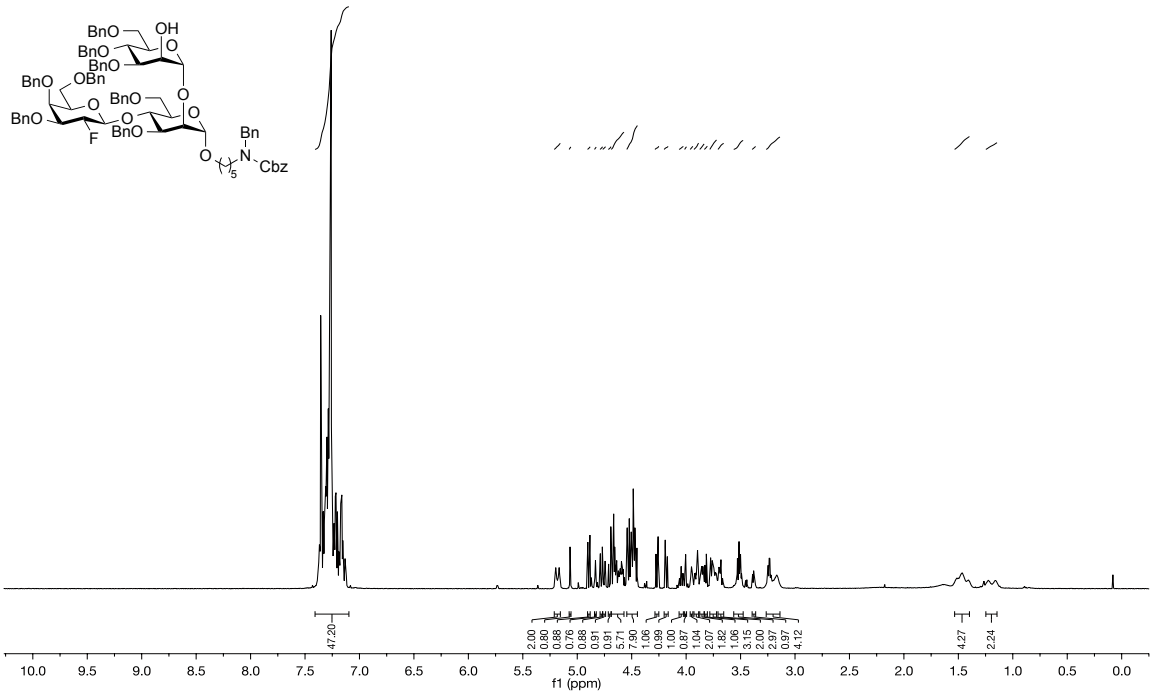


^{1}H-^{13}C coupled HSQC-NMR

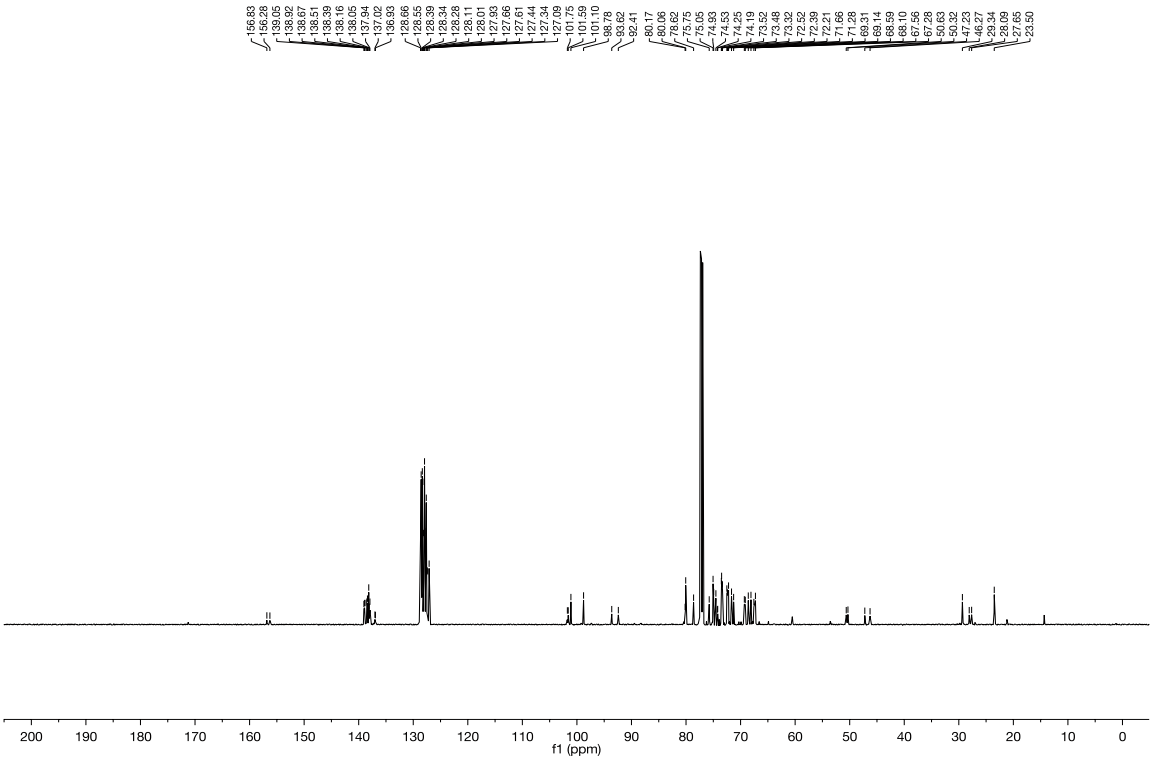


^{19}F NMR (376 MHz, $CDCl_3$)

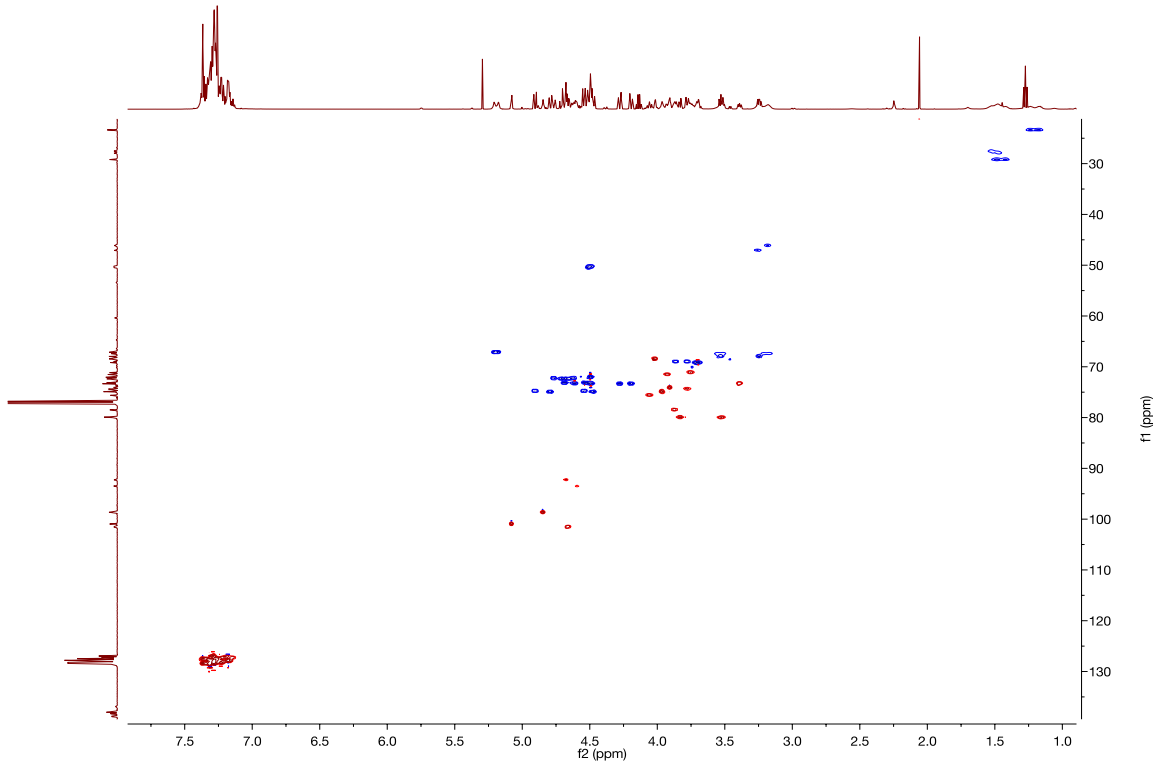
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-_O_-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-_O_-benzyl-4-_O_-(3,4,6-tri-_O_-benzyl-β-D-2-deoxy-2-fluoro-galactopyranosyl)-α-D-mannopyranoside (102)



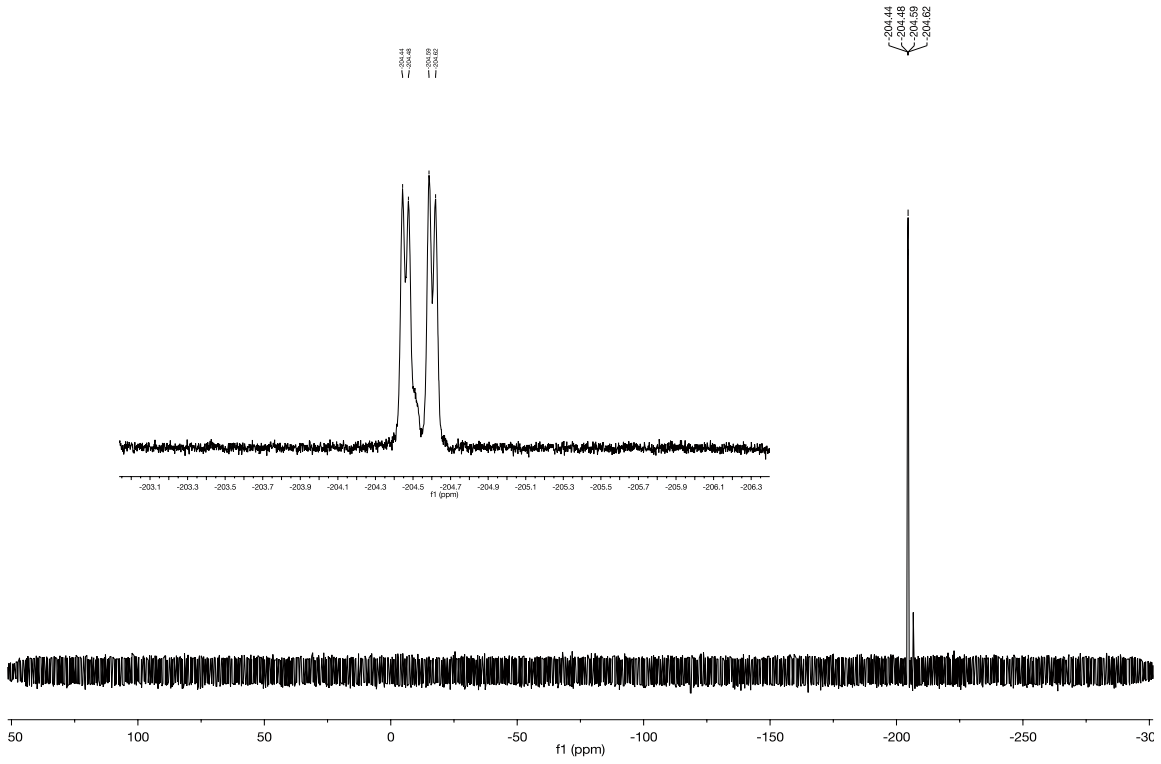
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

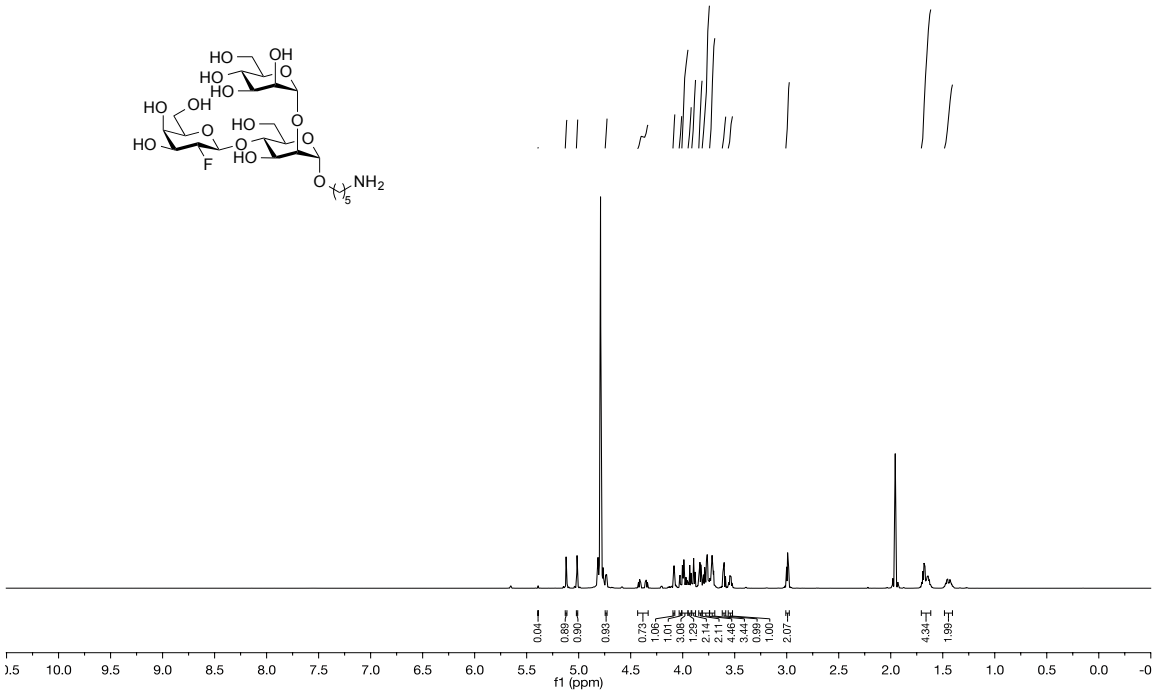


HSQC (599 MHz/151 MHz, $CDCl_3$)

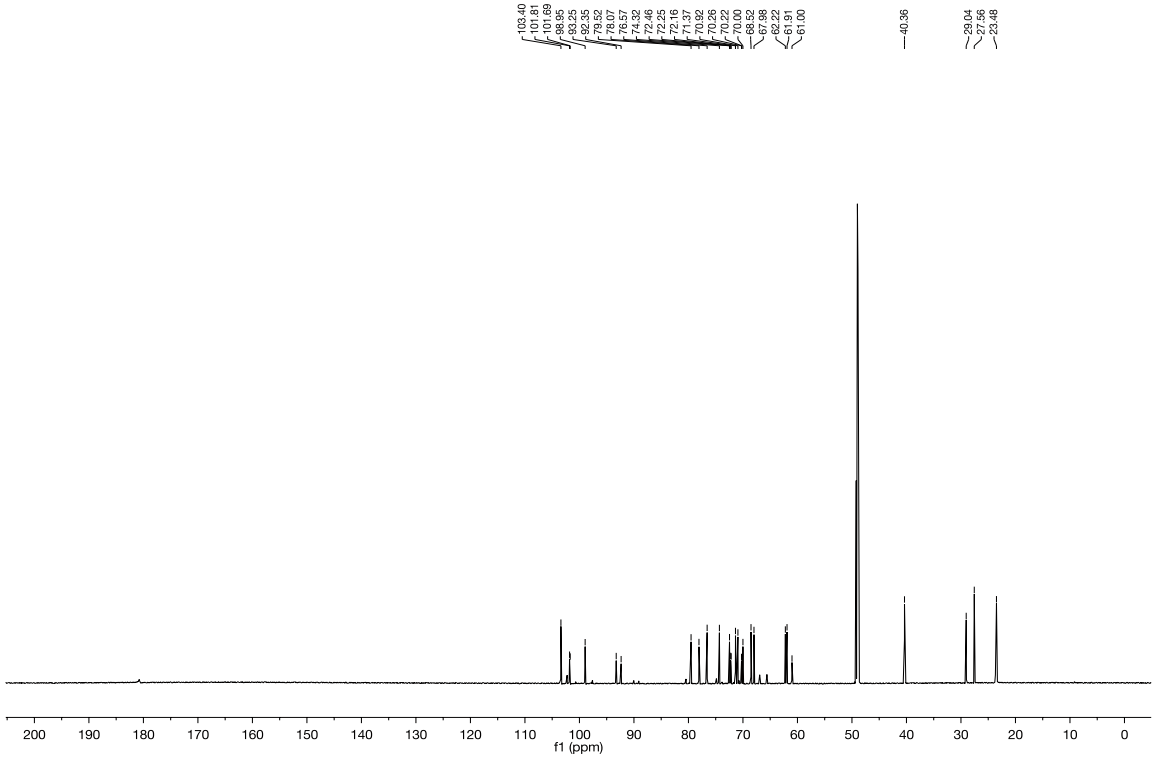


^{19}F NMR (376 MHz, $CDCl_3$)

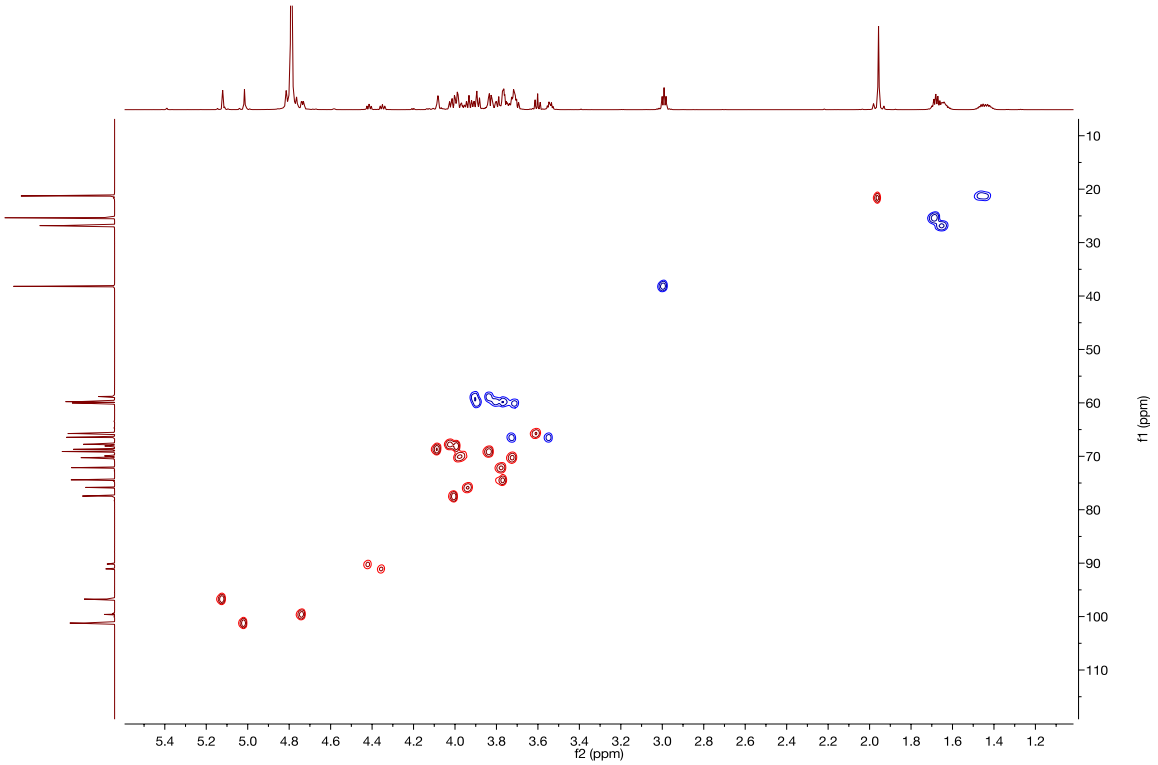
5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-2-deoxy-2-fluoro-galactopyranosyl)-α-D-mannopyranoside (8)



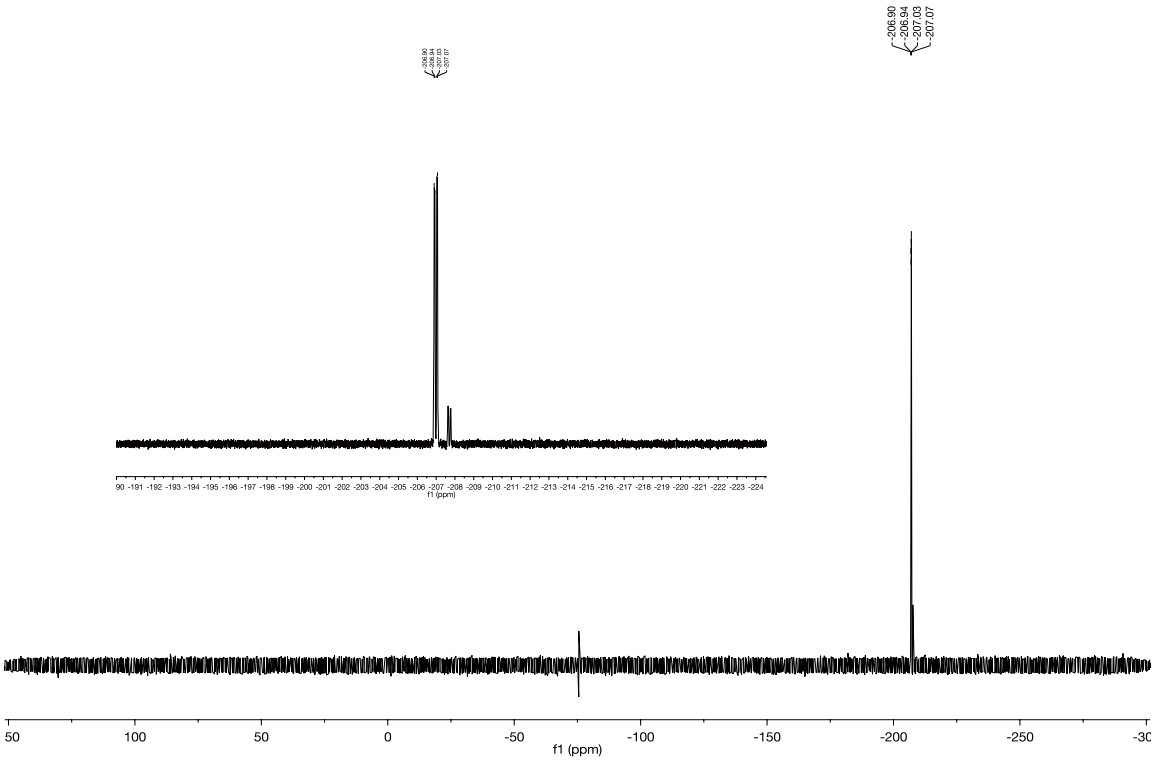
^{1}H NMR (800 MHz, D_2O)



^{13}C NMR (201 MHz, D_2O/CD_3OD)

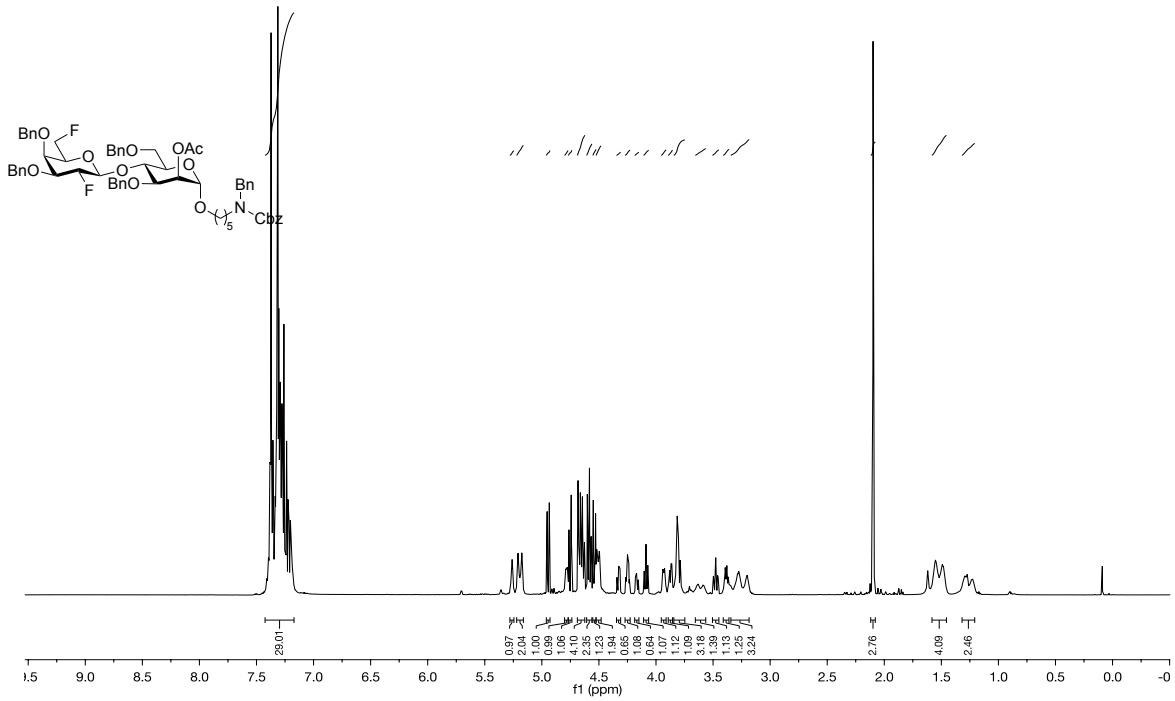


HSQC (800 MHz/201 MHz, D_2O)

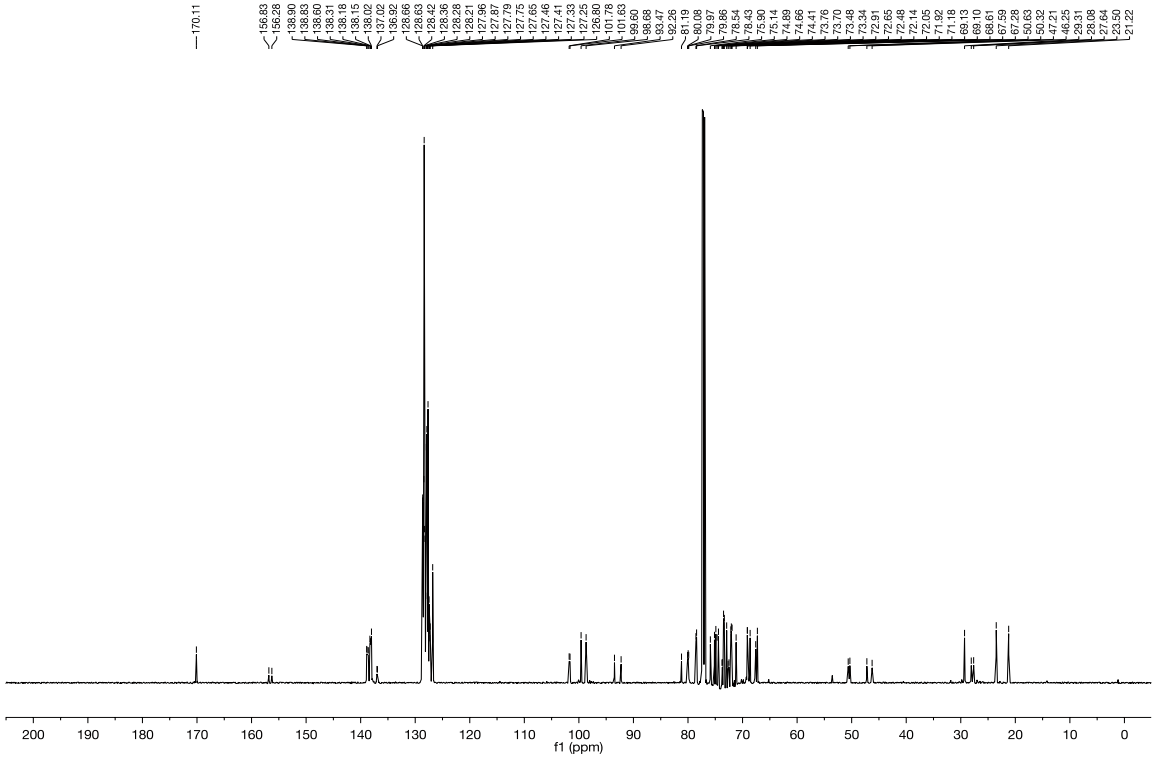


^{19}F NMR (376 MHz, D_2O)

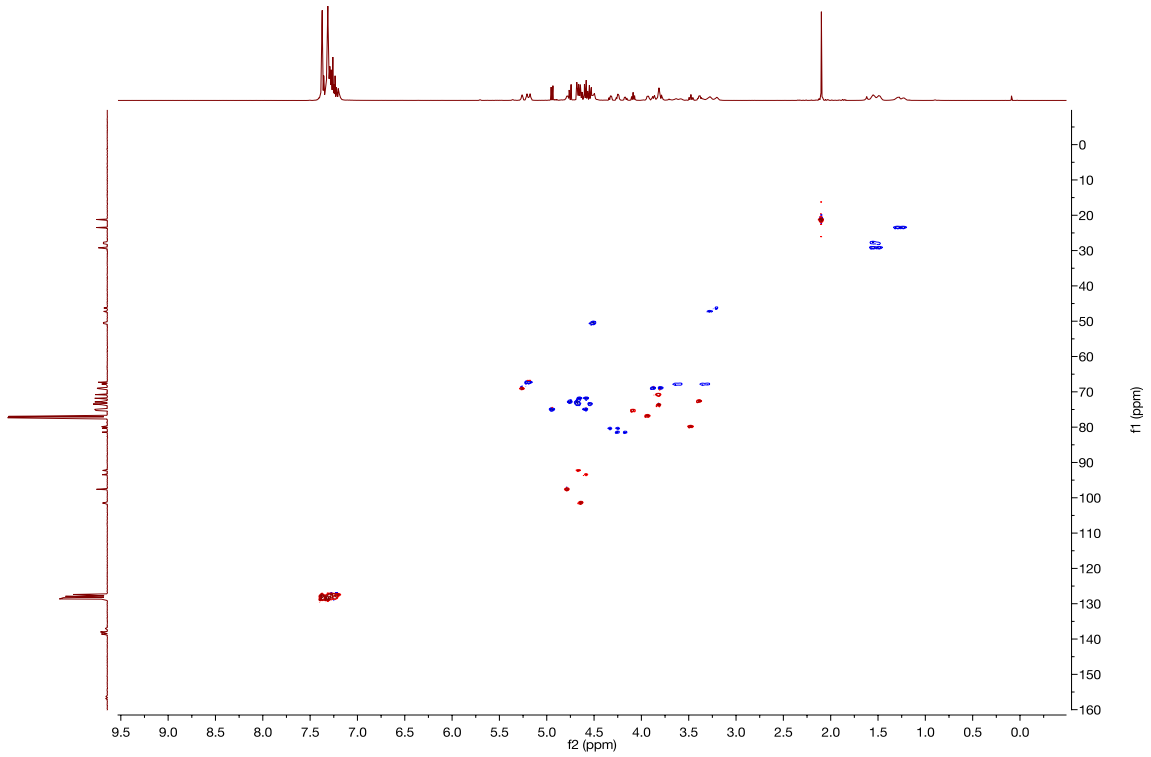
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4-di-_O_-benzyl-β-D-2,6-dideoxy-2,6-difluoro-galactopyranosyl-(1→4)-2-_O_-acetyl-3,6-di-_O_-benzyl-α-D-mannopyranoside (89)



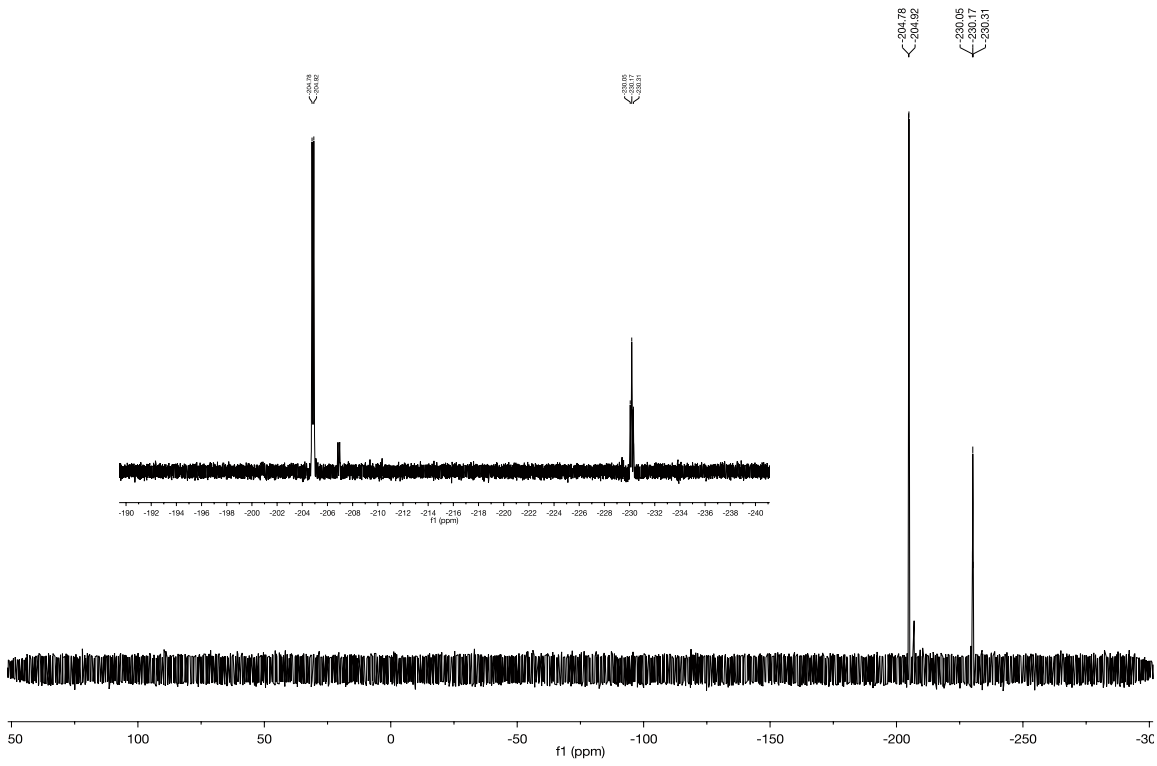
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

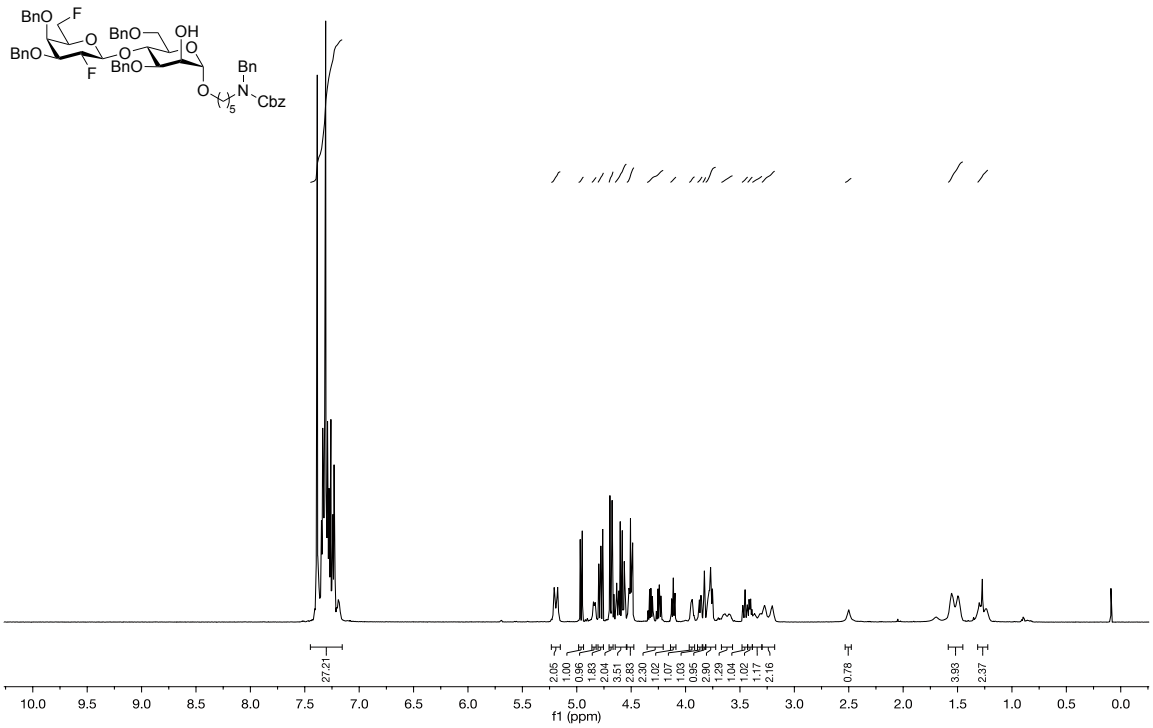


HSQC (599 MHz/151 MHz, $CDCl_3$)

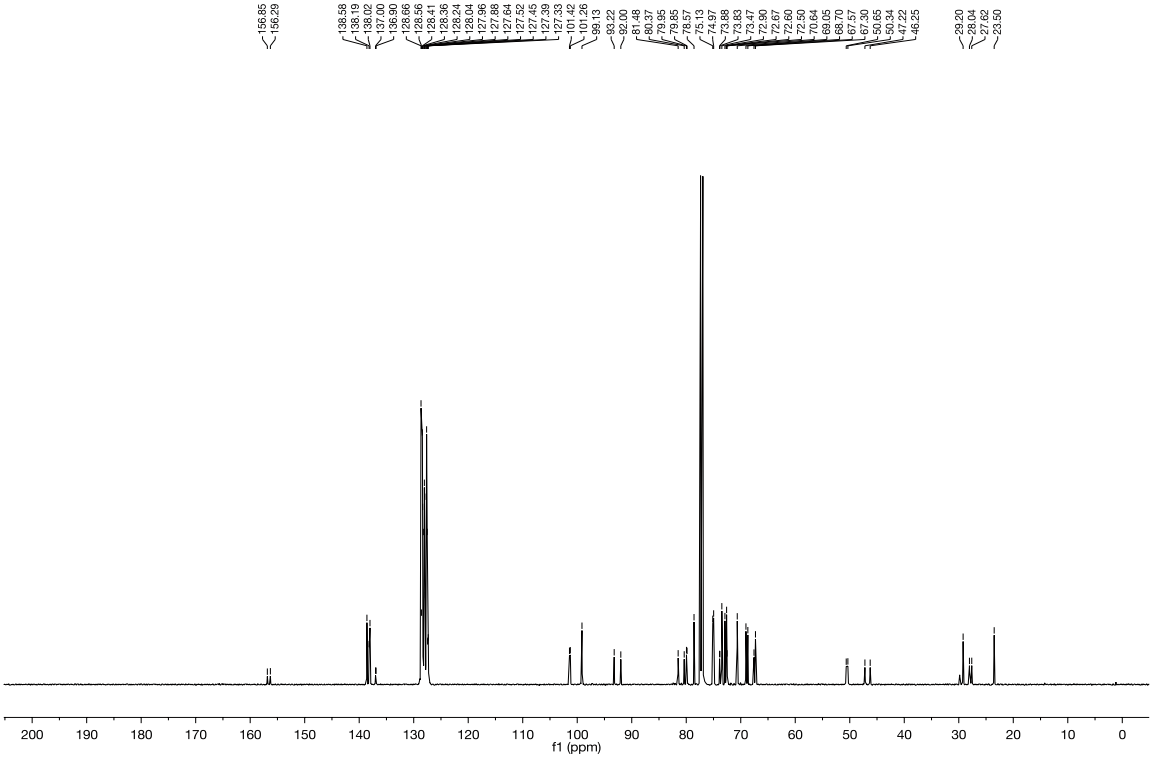


^{19}F NMR (376 MHz, $CDCl_3$)

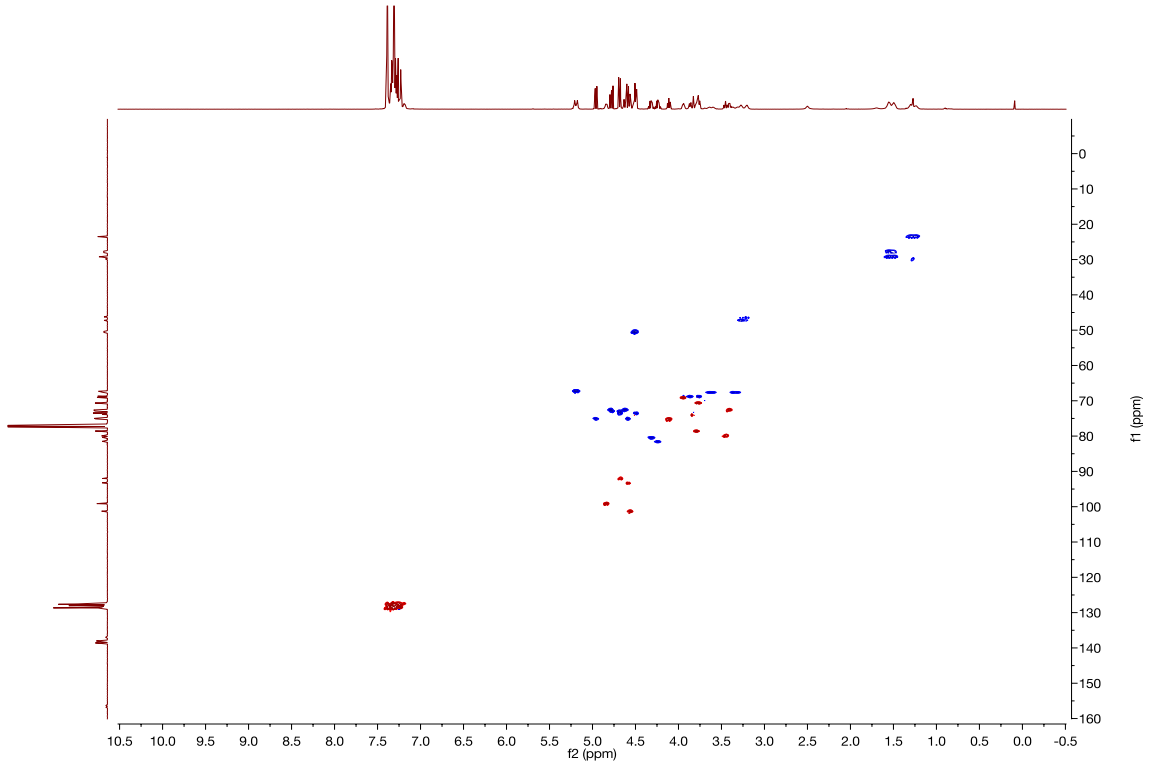
_N_-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4-di-_O_-benzyl-β-D-2,6-dideoxy-2,6-difluoro-galactopyranosyl-(1→4)-3,6-di-_O_-benzyl-α-D-mannopyranoside (93)



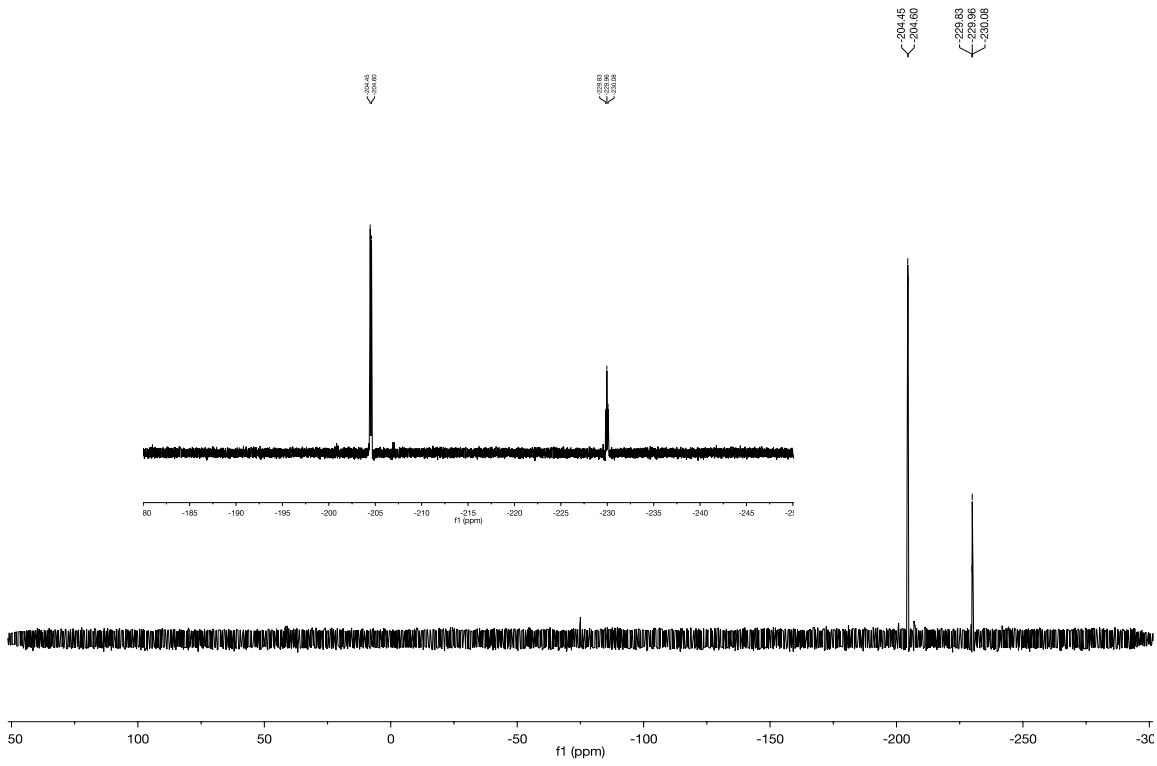
^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (101 MHz, $CDCl_3$)

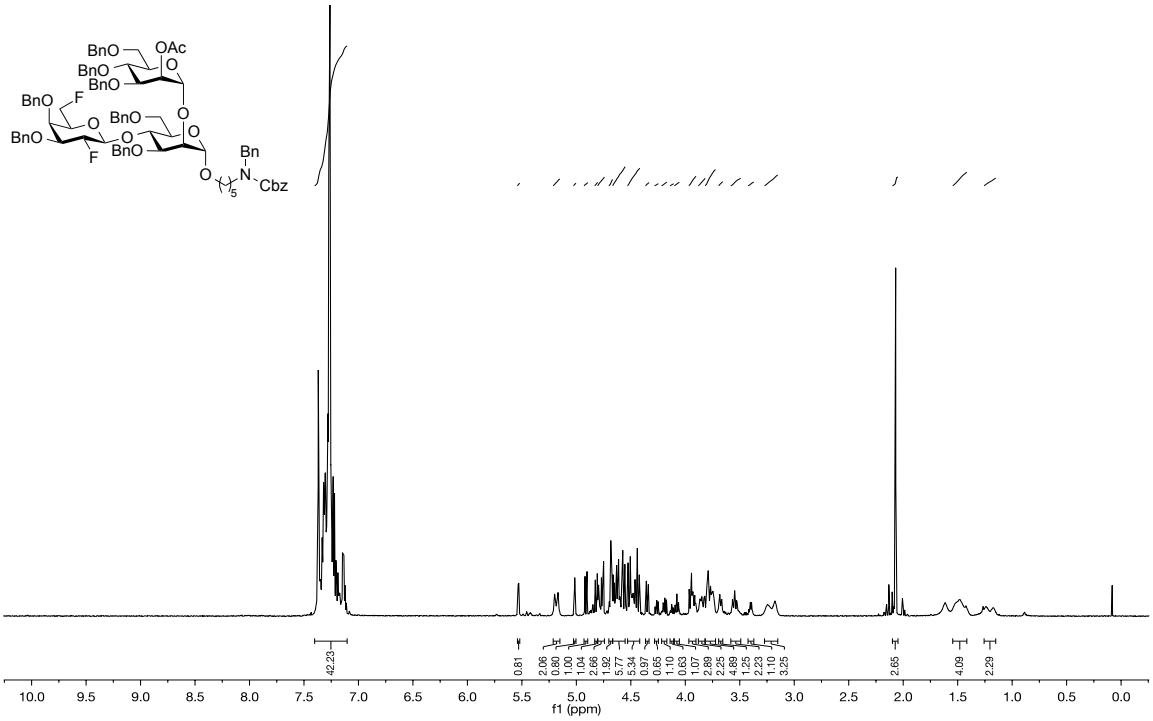


HSQC (400 MHz/101 MHz, $CDCl_3$)

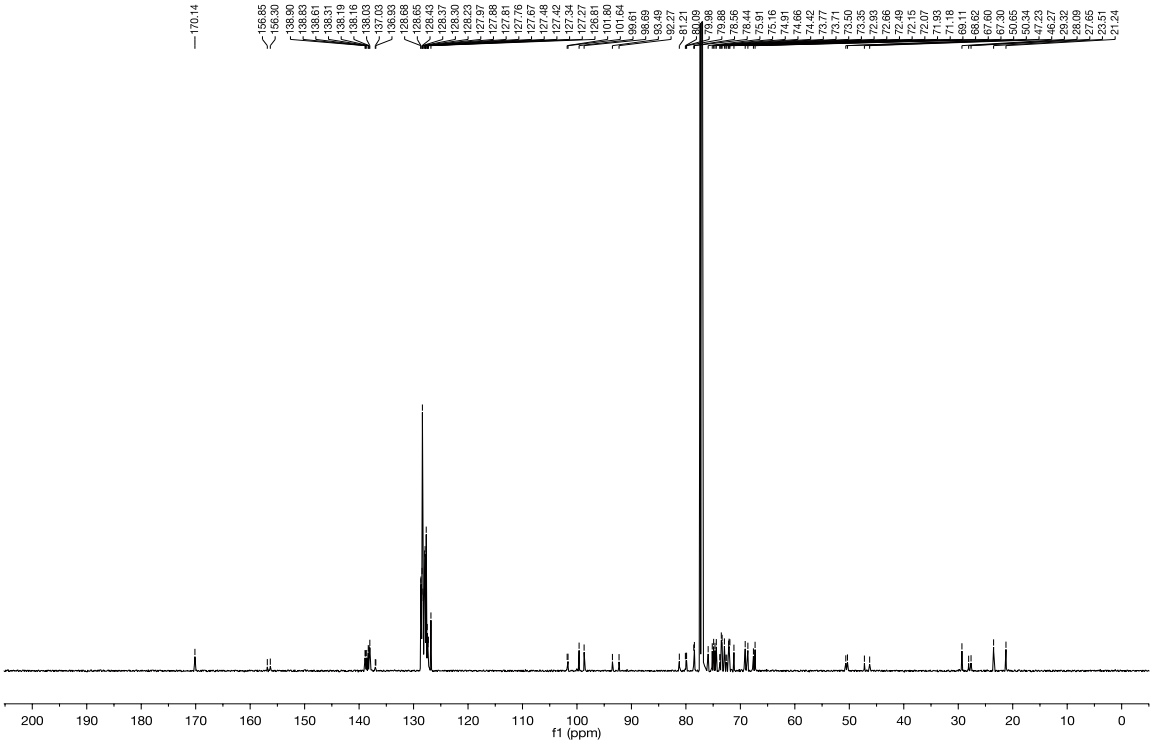


^{19}F NMR (376 MHz, $CDCl_3$)

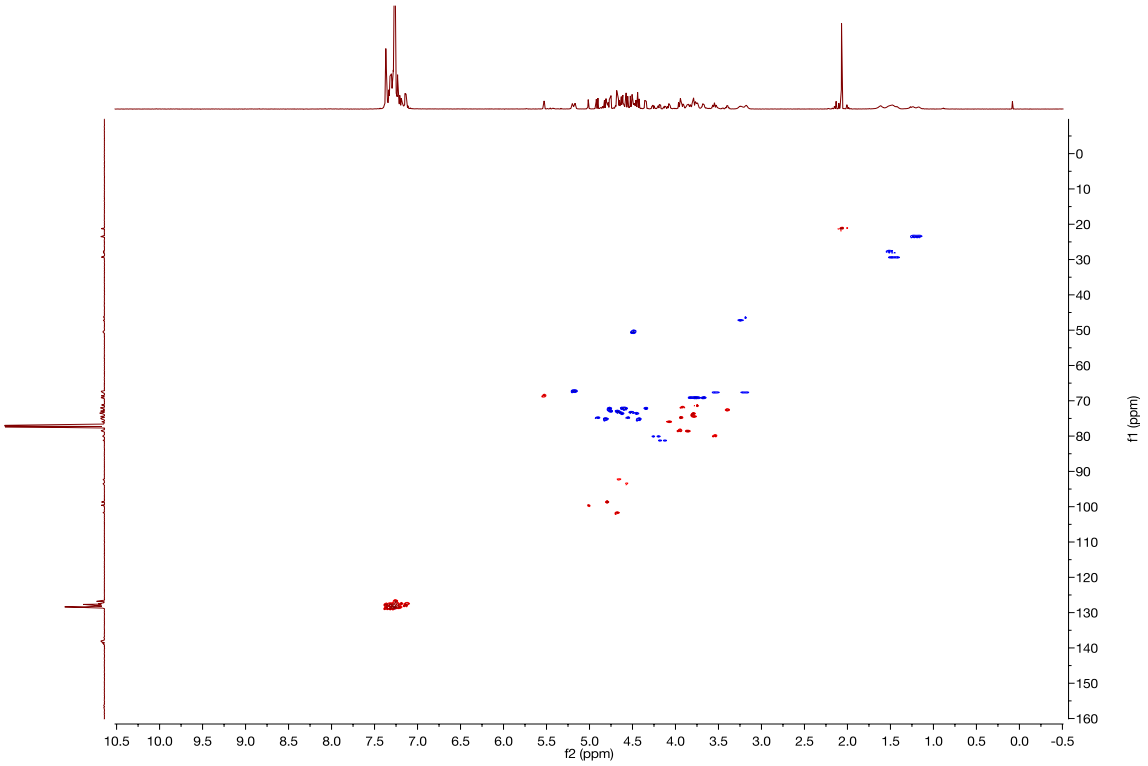
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4-di-*O*-benzyl-β-D-2,6-dideoxy-2,6-difluoro-galactopyranosyl)-α-D-mannopyranoside (99)**



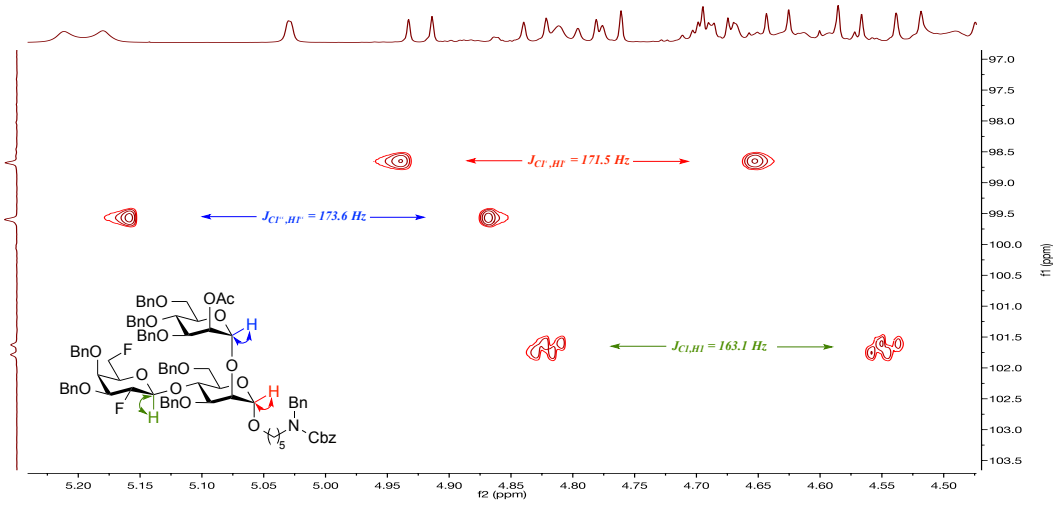
^{1}H NMR (599 MHz, $CDCl_3$)



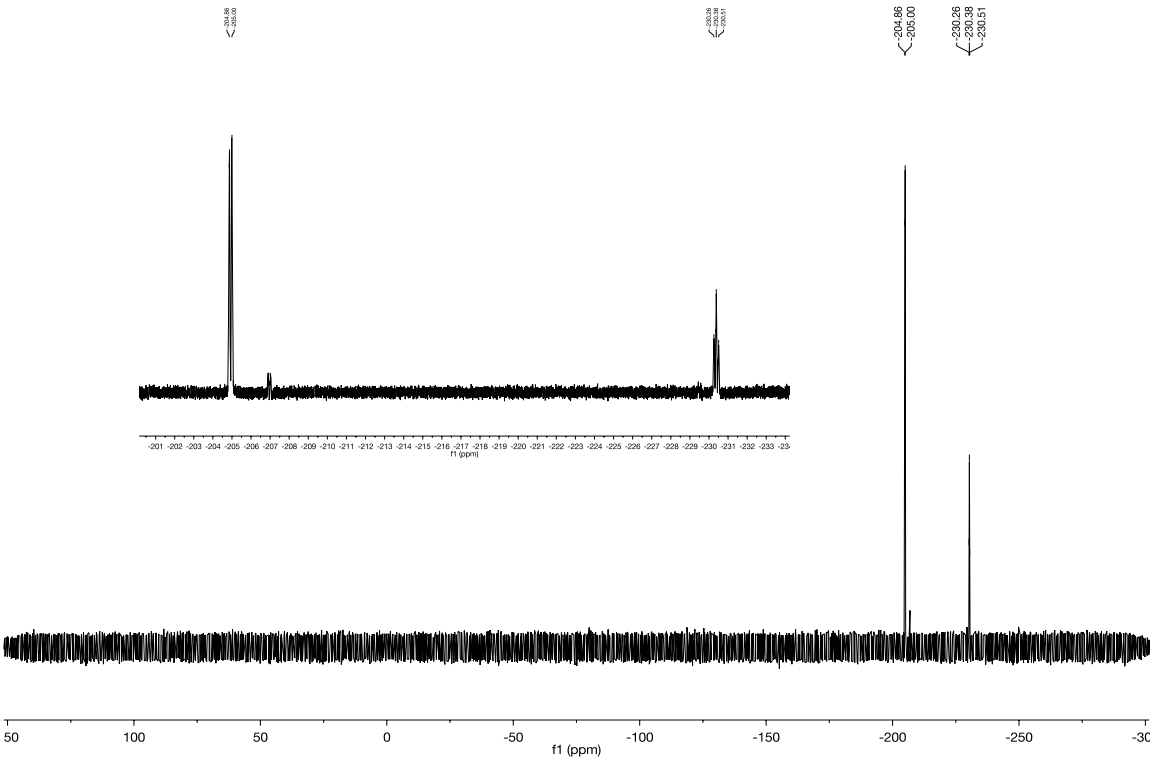
^{13}C NMR (151 MHz, $CDCl_3$)



HSQC (599 MHz/151 MHz, $CDCl_3$)

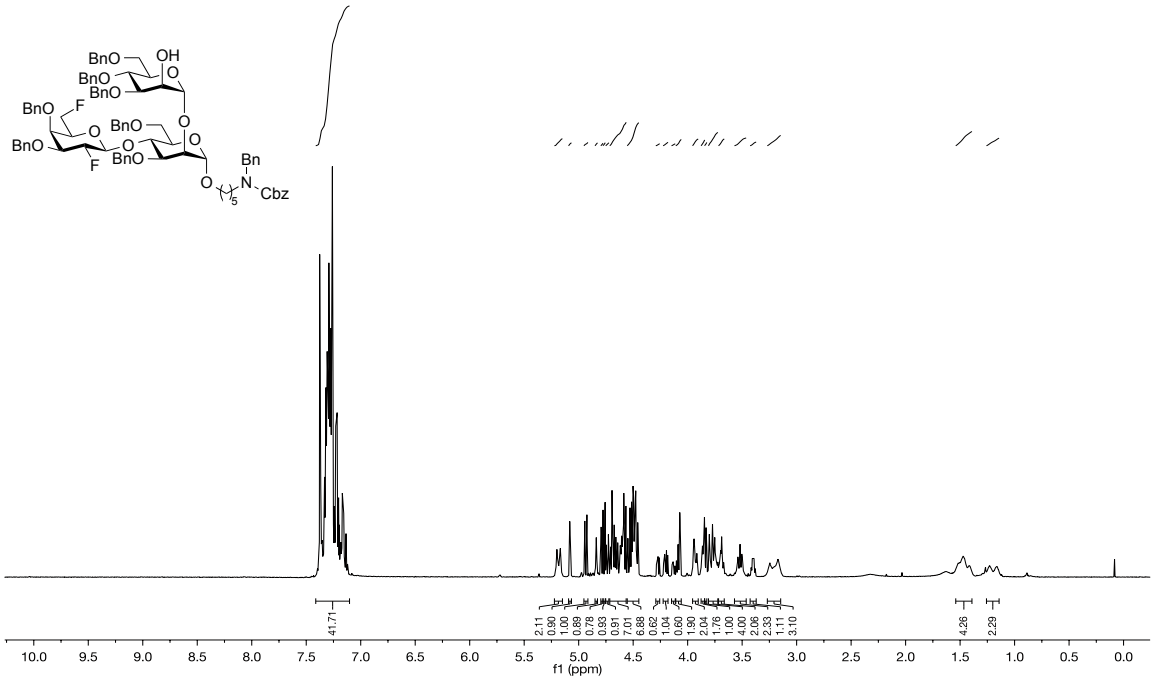


^{1}H-^{13}C coupled HSQC-NMR

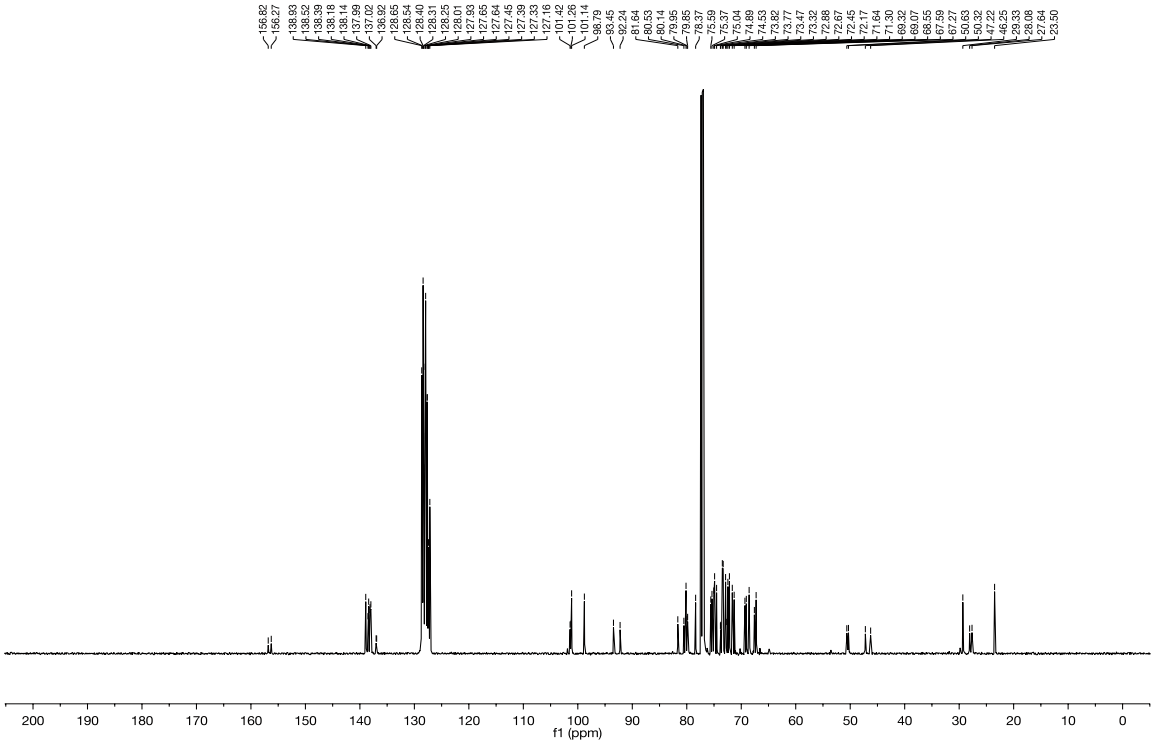


^{19}F NMR (376 MHz, $CDCl_3$)

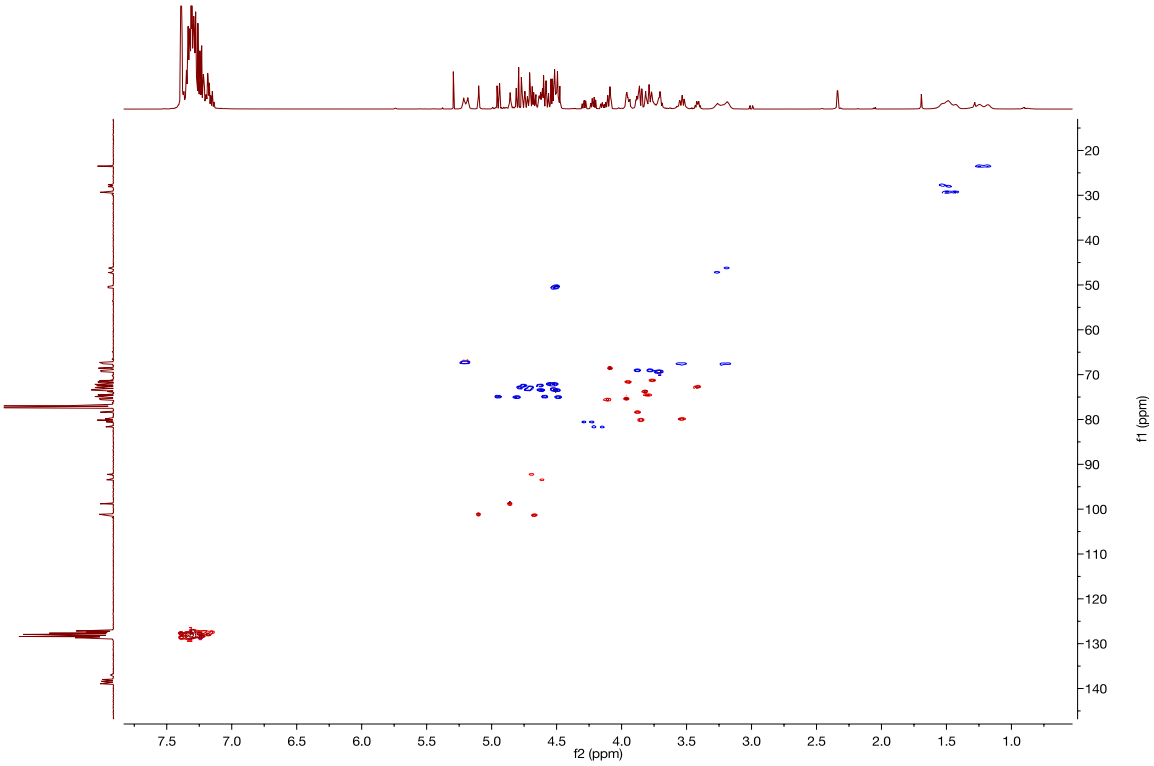
***N*-(Benzyl)benzyloxycarbonyl-5-aminopentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4-di-*O*-benzyl-β-D-2,6-dideoxy-2,6-difluoro-galactopyranosyl)-α-D-mannopyranoside (103)**



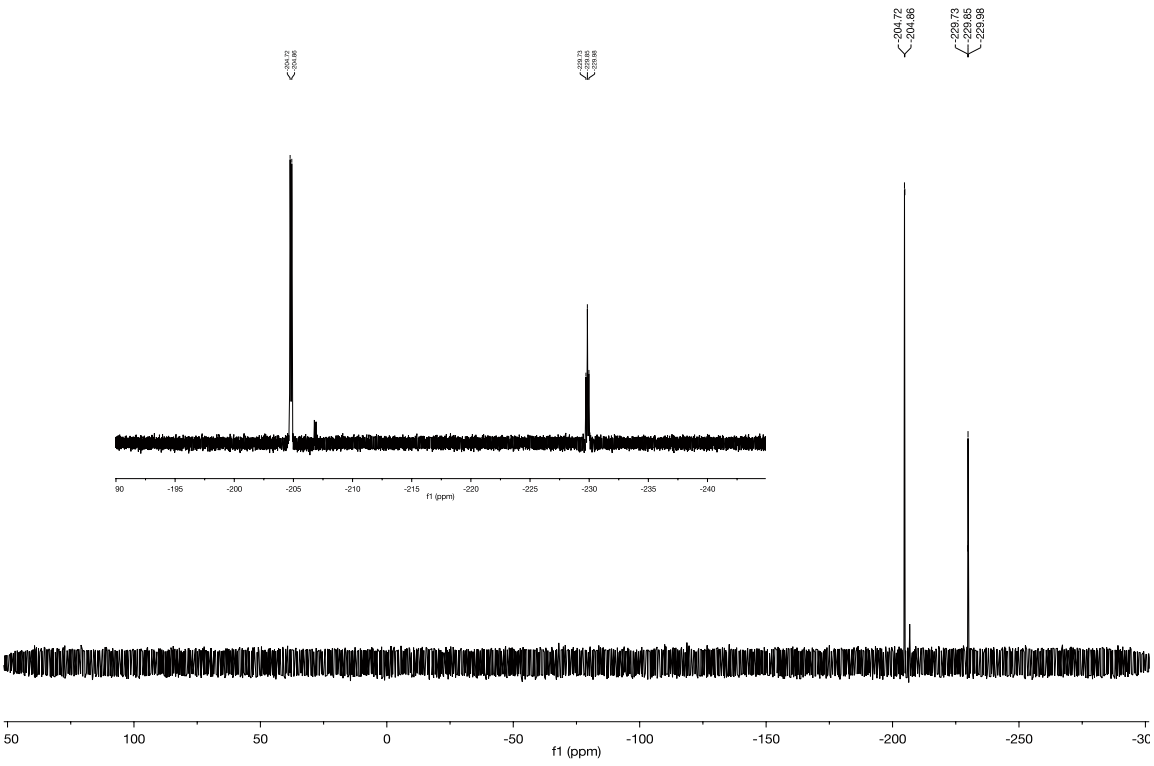
^{1}H NMR (599 MHz, $CDCl_3$)



^{13}C NMR (151 MHz, $CDCl_3$)

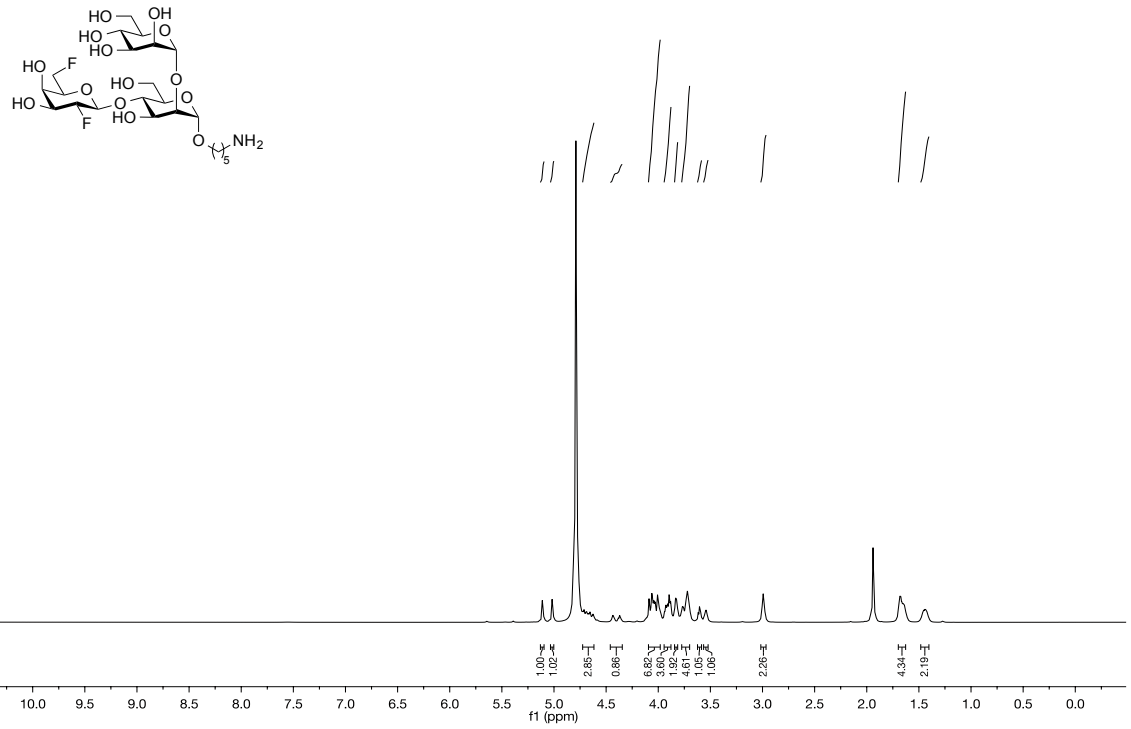


HSQC (599 MHz/151 MHz, $CDCl_3$)

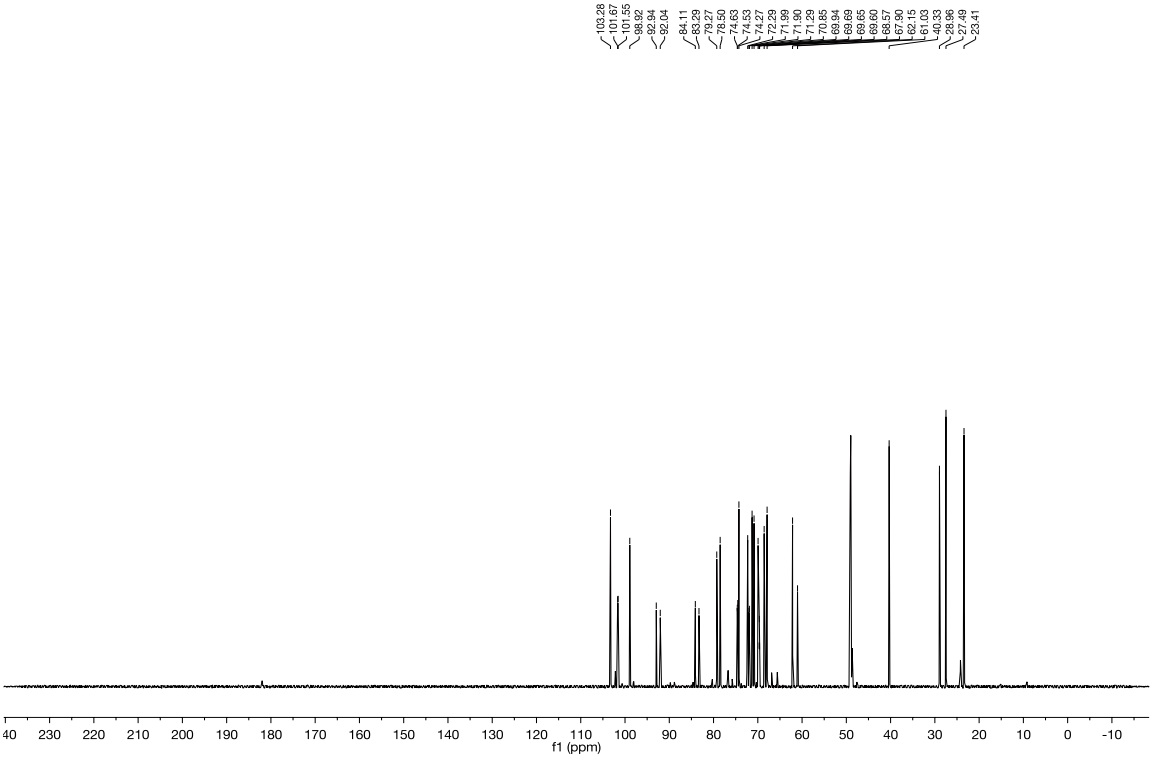


^{19}F NMR (376 MHz, $CDCl_3$)

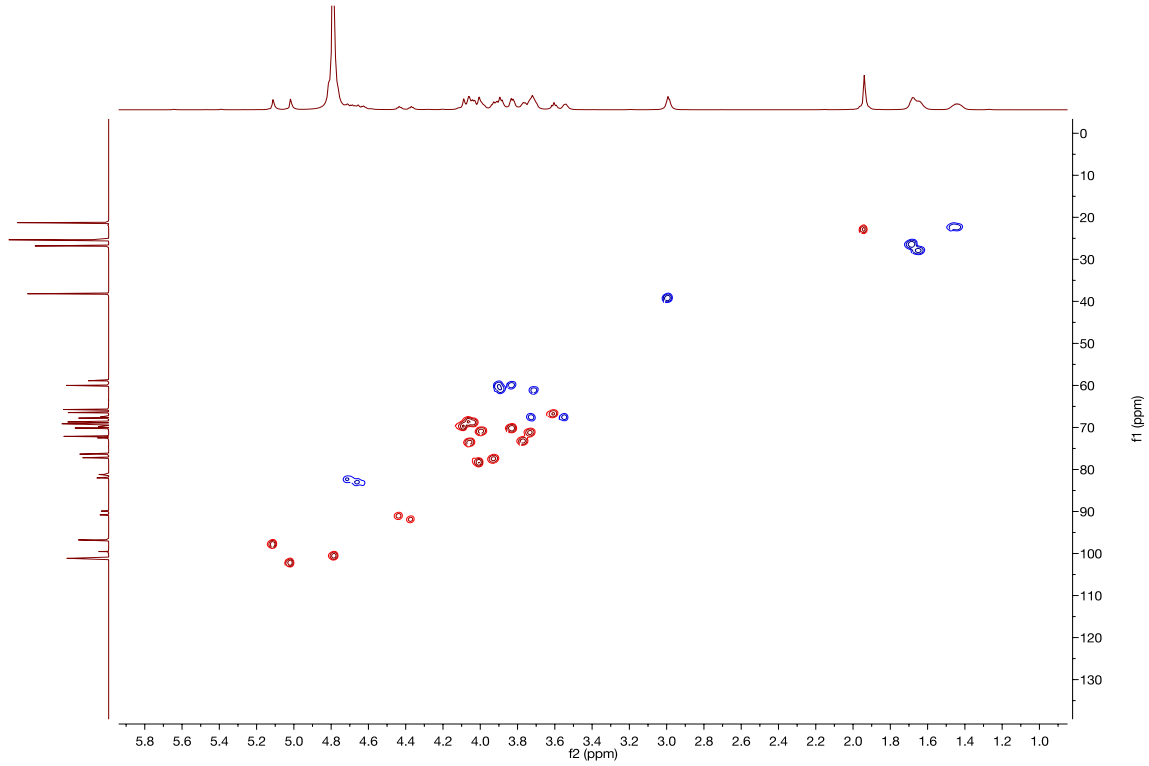
5-Aminopentyl α-D-mannopyranosyl-(1→2)-4-*O*-(β-D-2,6-dideoxy-2,6-difluoro-galactopyranosyl)-α-D-mannopyranoside (9)



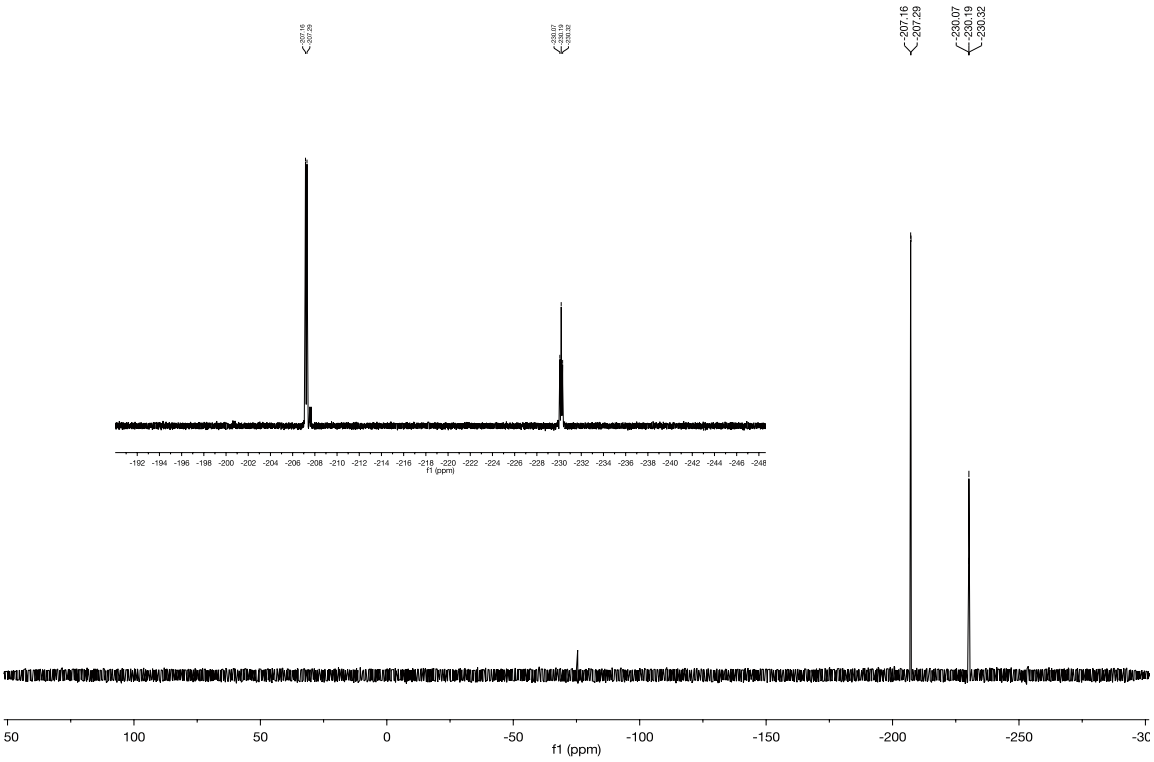
^{1}H NMR (800 MHz, D_2O)



^{13}C NMR (201 MHz, D_2O/CD_3OD)

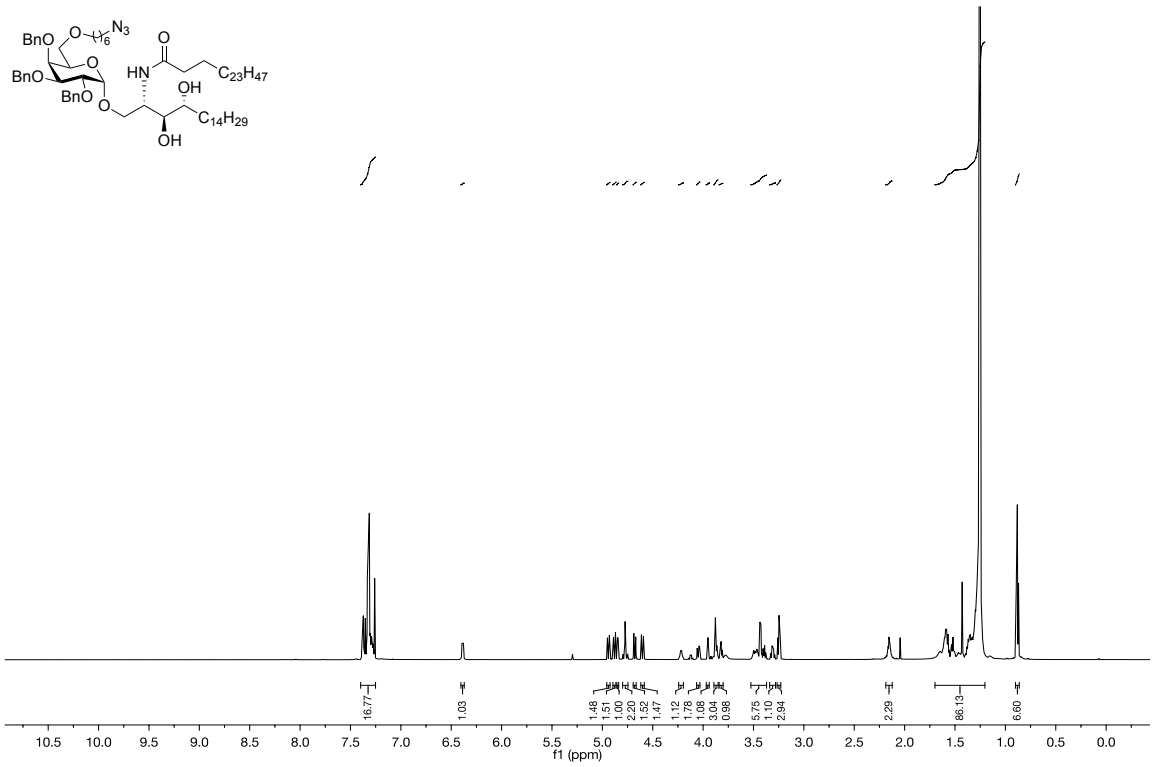


HSQC (800 MHz/201 MHz, D_2O)

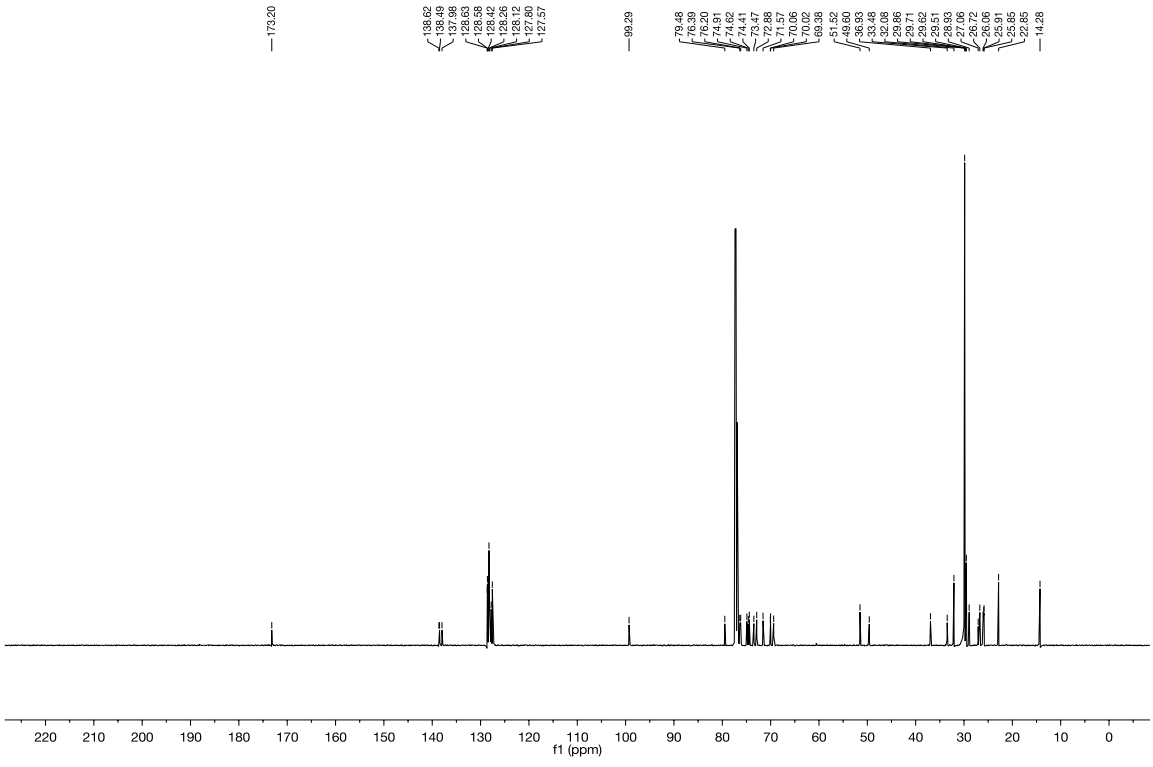


^{19}F NMR (376 MHz, D_2O)

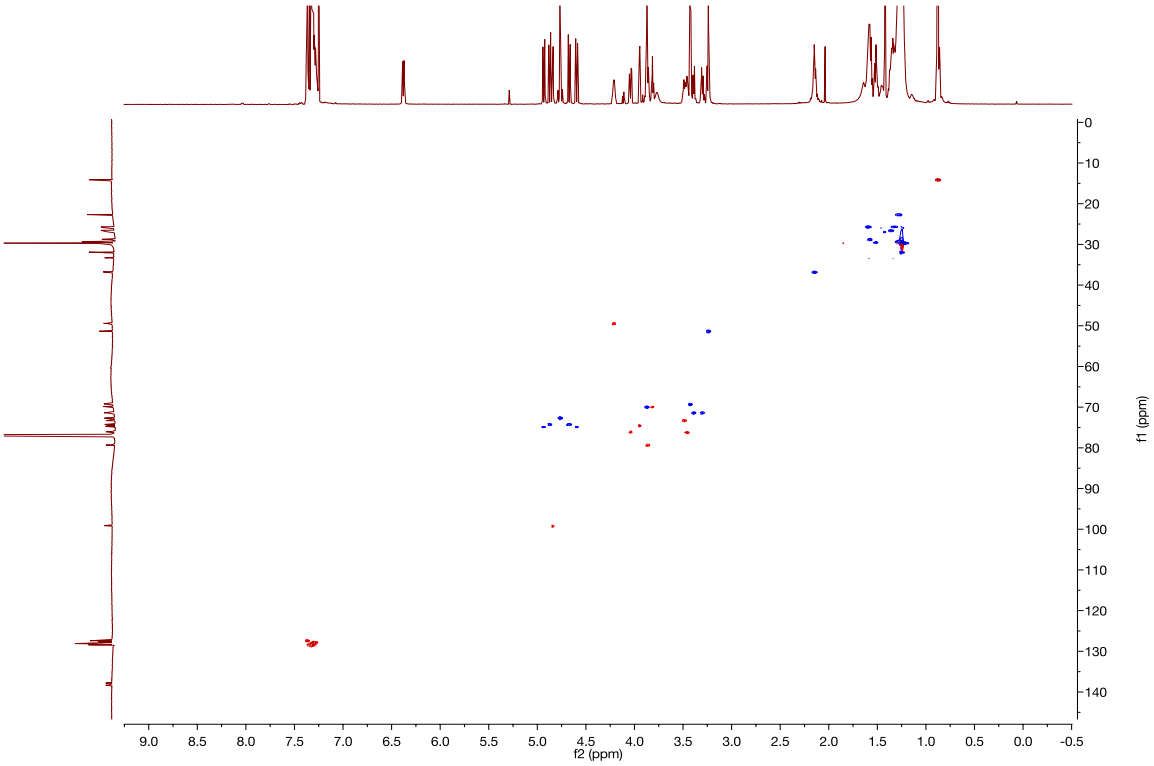
(*2S*,*3S*,*4R*)-1-(6-(6'-Azidohexyl)-2,3,4-tri-O-benzyl-α-D-galactopyranosyl)-2-hexacosanoylamino-octadecane-3,4-diol (33)



^{1}H NMR (599 MHz, $CDCl_3$)

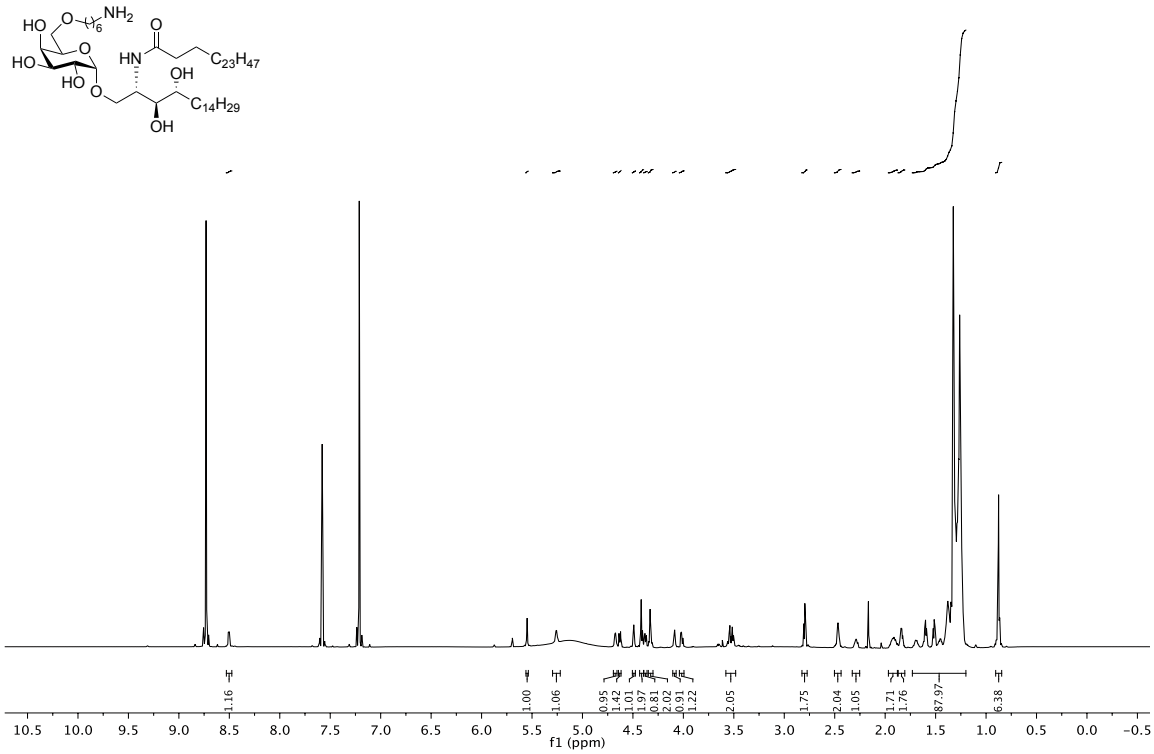


^{13}C NMR (151 MHz, $CDCl_3$)

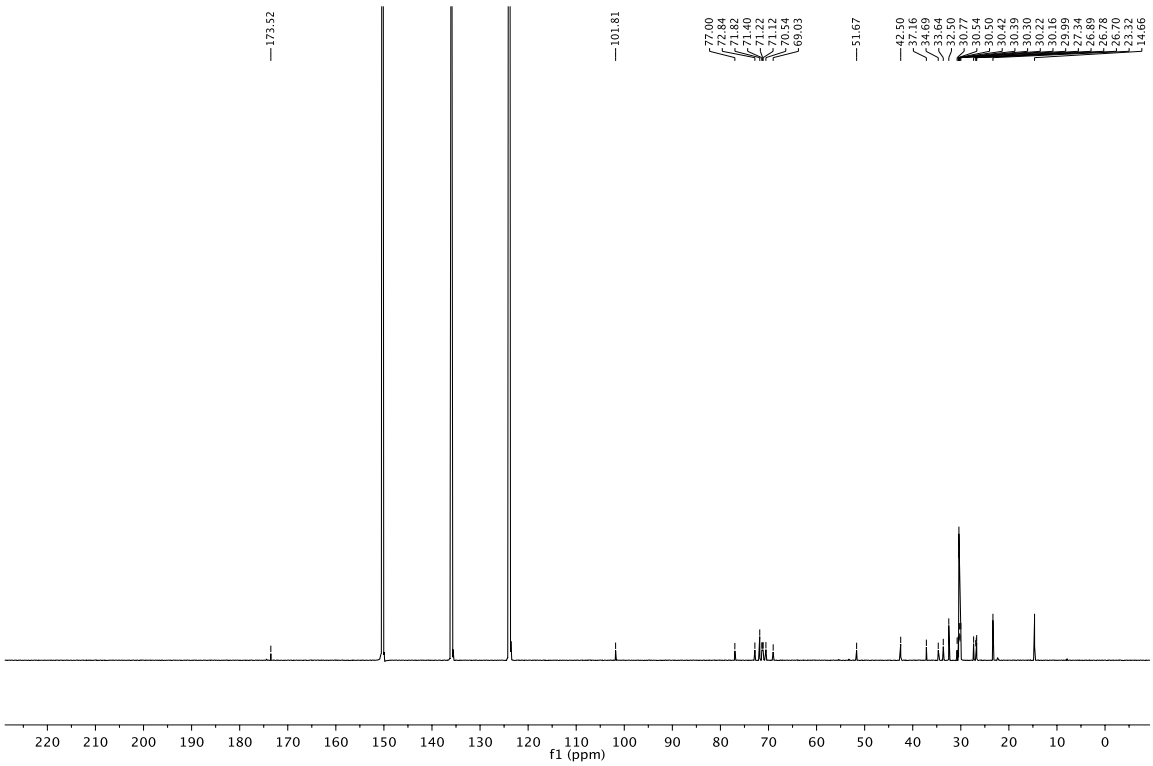


HSQC (599 MHz/151 MHz, $CDCl_3$)

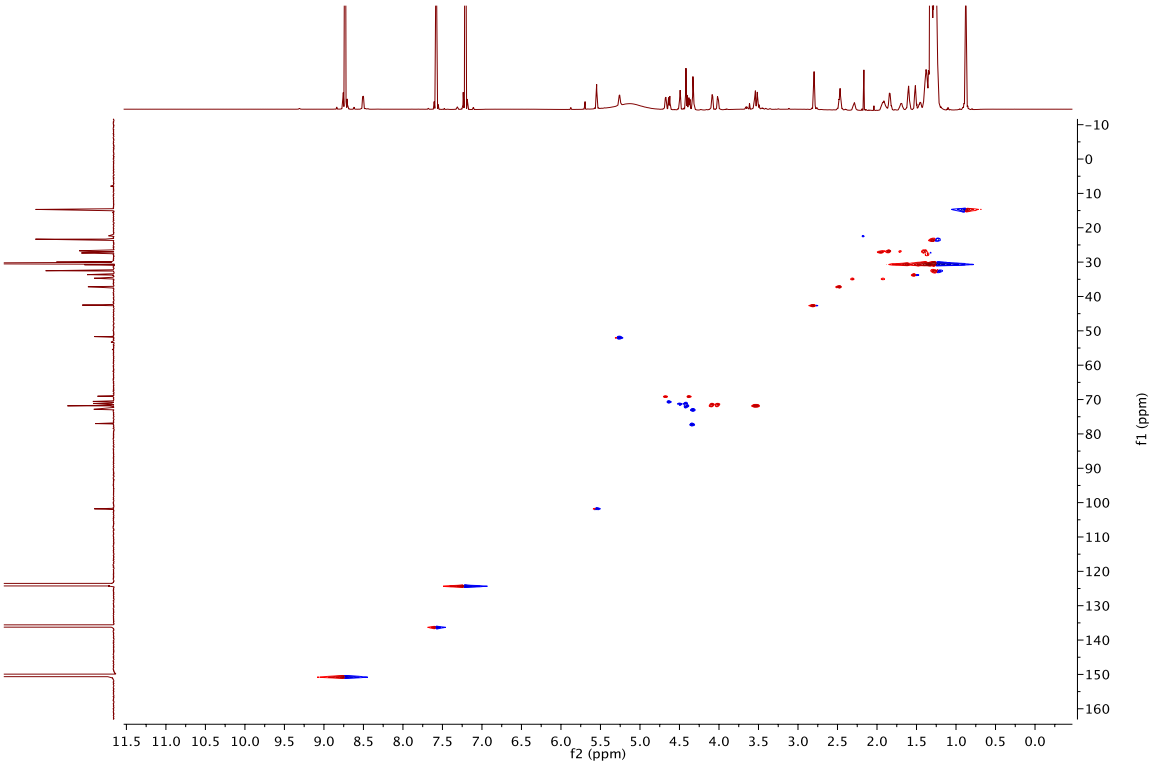
(*2S*,*3S*,*4R*)-1-(6-(6'-Aminohexyl)-α-D-galactopyranosyl)-2-hexacosanoylamino-octadecane-3,4-diol (29)



^{1}H NMR (800 MHz, C_5D_5N)

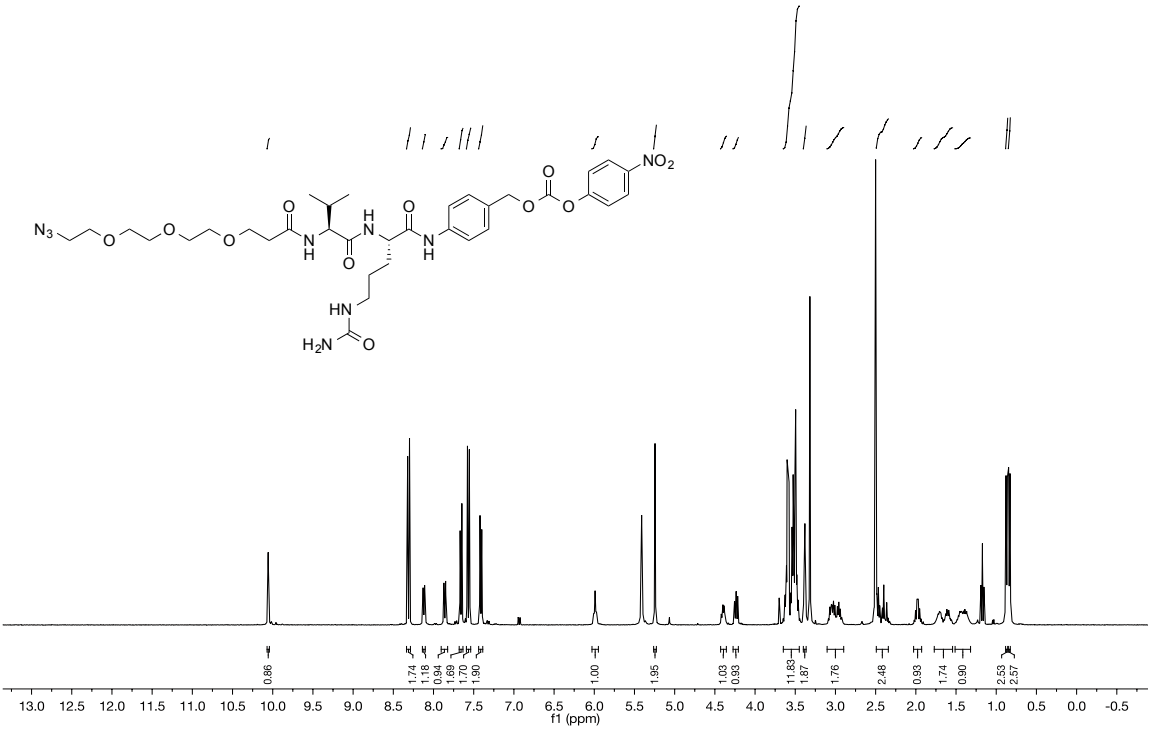


^{13}C NMR (201 MHz, C_5D_5N)

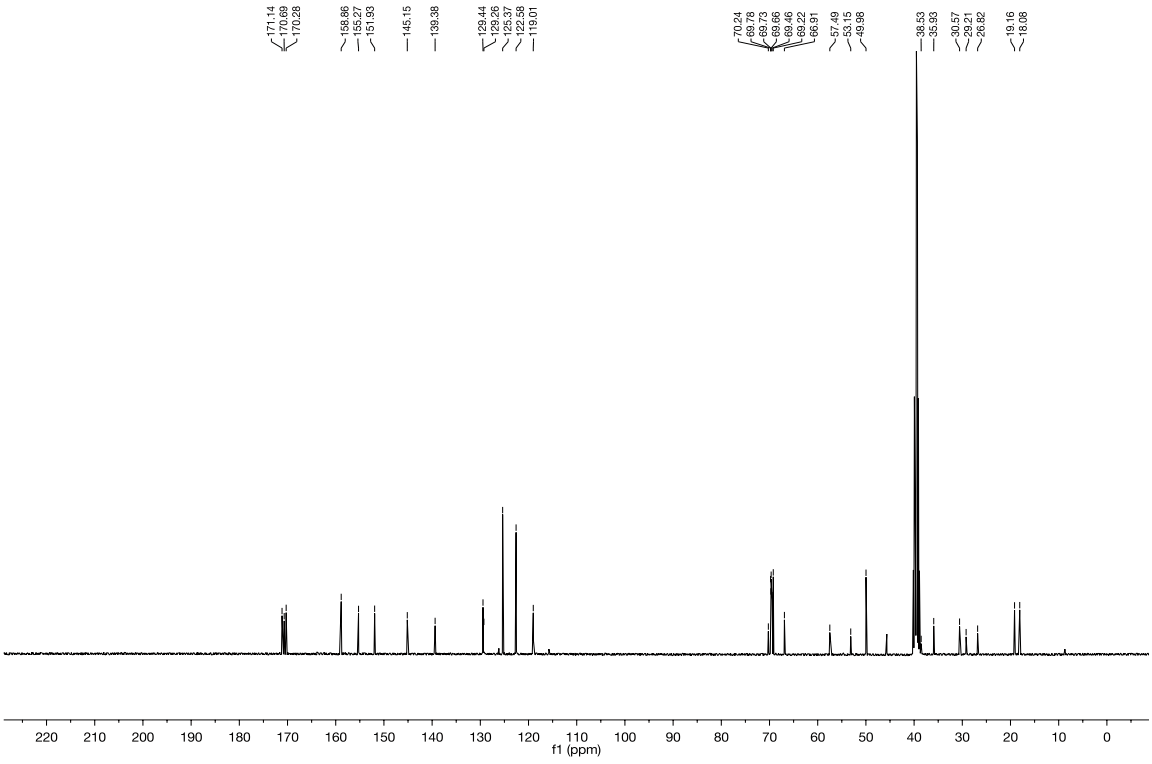


HSQC (800 MHz/201 MHz, C_5D_5N)

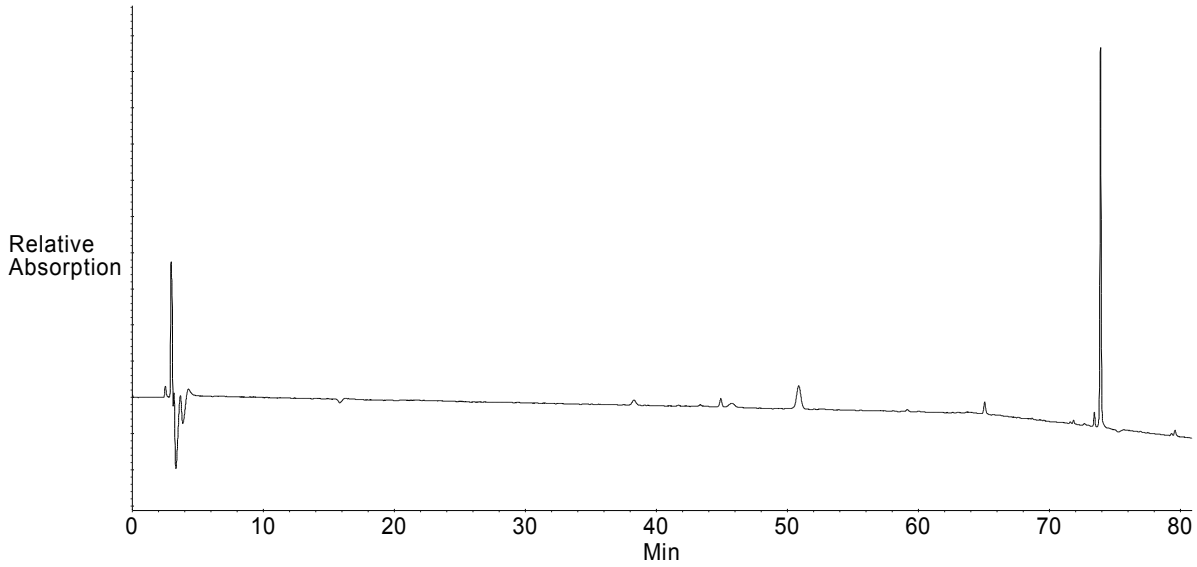
***N*-(12-Azido-4,7,10-trioxadodecanoyl)-Val-Cit-4-aminobenzyl 4-nitrophenyl carbonate (28)**



^{1}H NMR (400 MHz, $(CD_3)_2SO$)

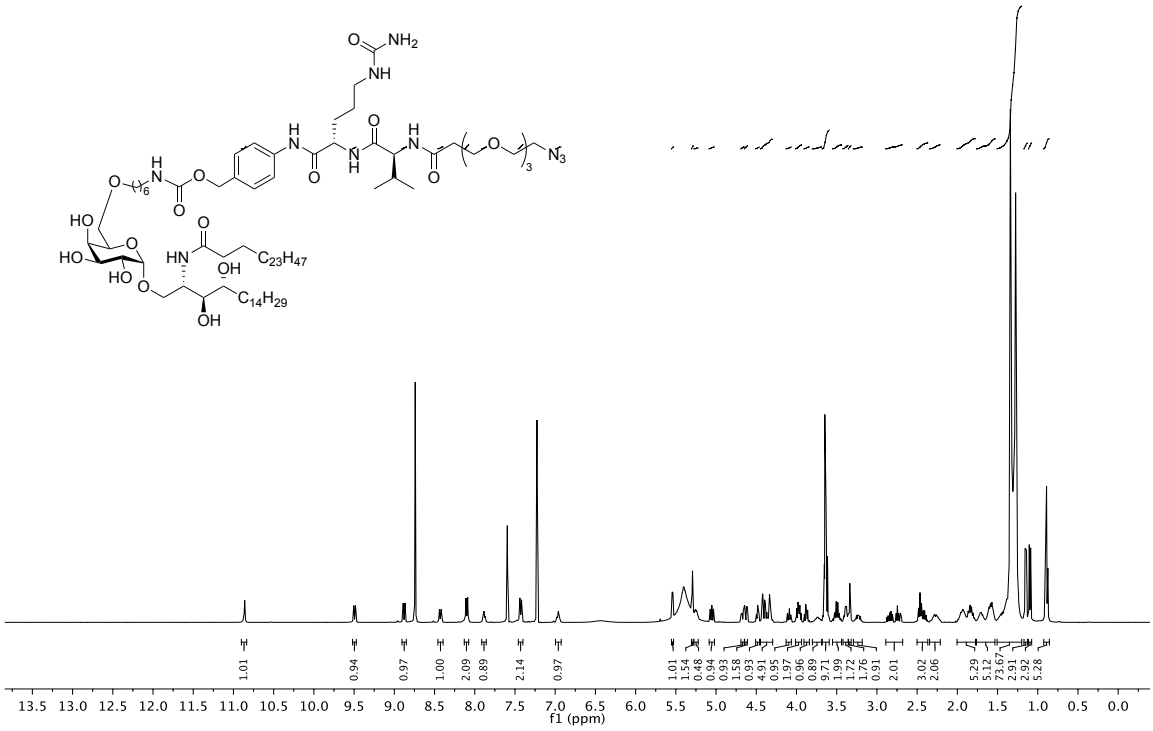


^{13}C NMR (101 MHz, $(CD_3)_2SO$)

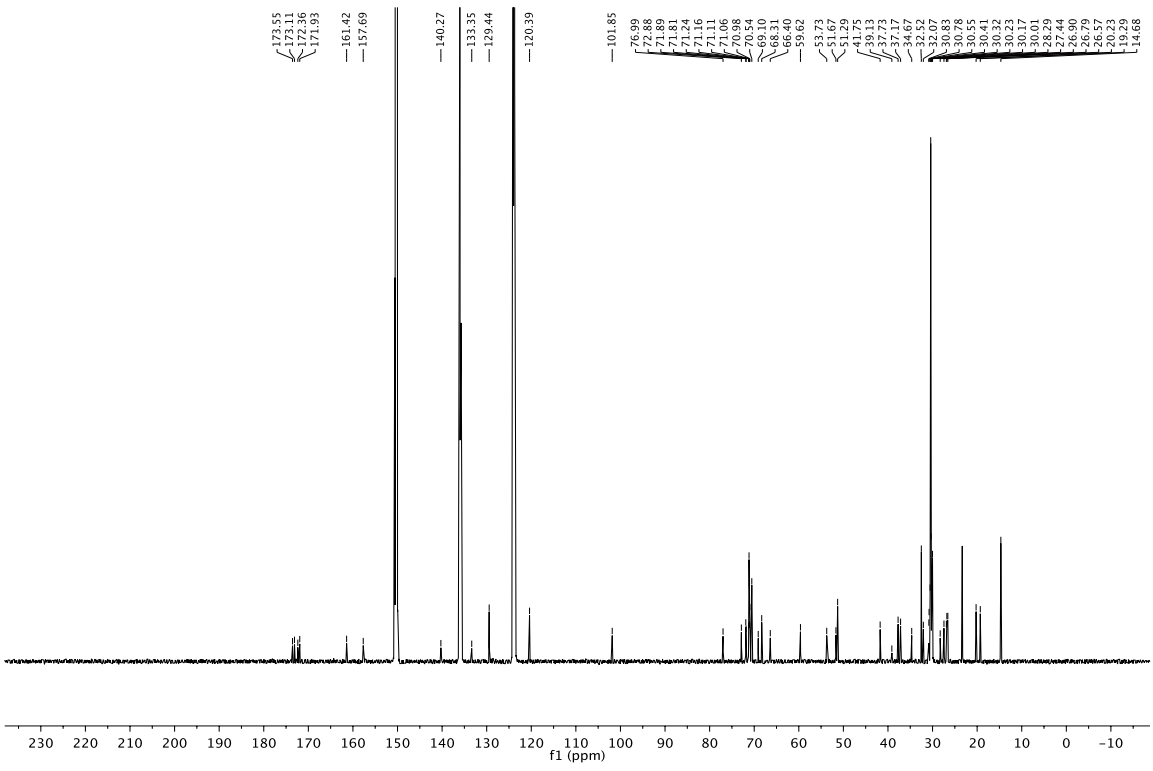


RP-HPLC: t_R = 73.9 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(30:70) 60 min, →(80:20) 90 min.

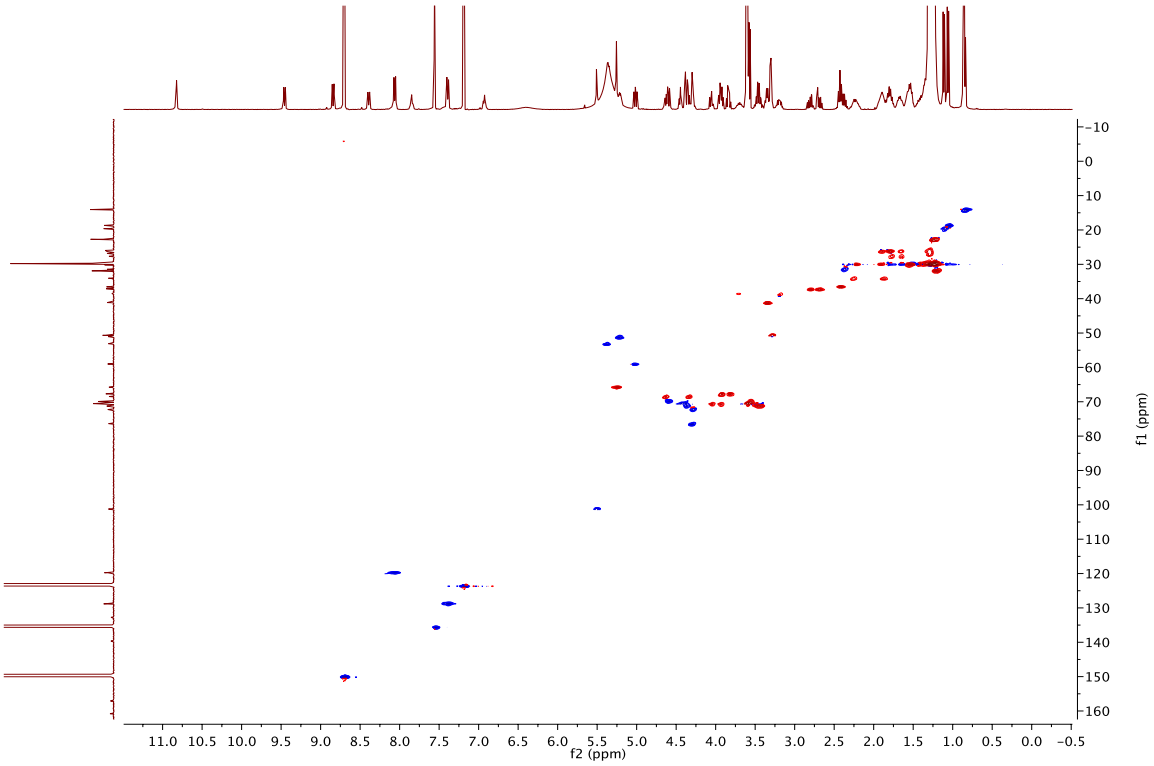
(*2S*,*3S*,*4R*)-1-(6-(6‘-(*N*-(12-Azido-4,7,10-trioxadodecanoyl)-Val-Cit-4-aminobenzyloxycarbonylamino)-hexyl)-α-D-galactopyranosyl)-2-hexacosanoylamino-octadecane-3,4-diol (11)



^{1}H NMR (400 MHz, C_5D_5N)

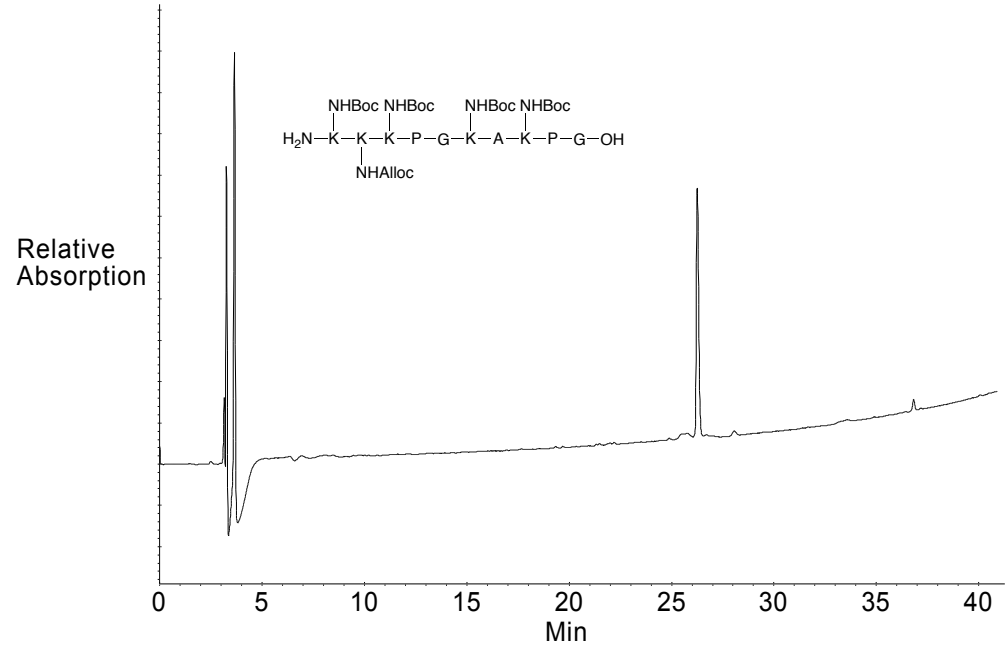


^{13}C NMR (201 MHz, C_5D_5N)



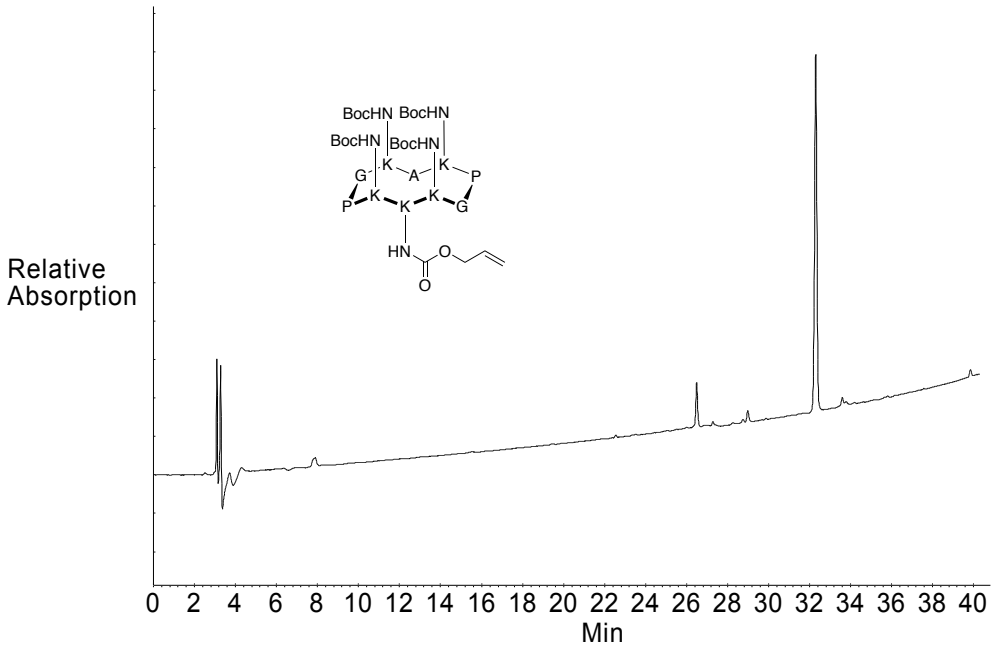
HSQC (400 MHz/201 MHz, C_5D_5N)

Lys(Boc)-Lys(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly (40)



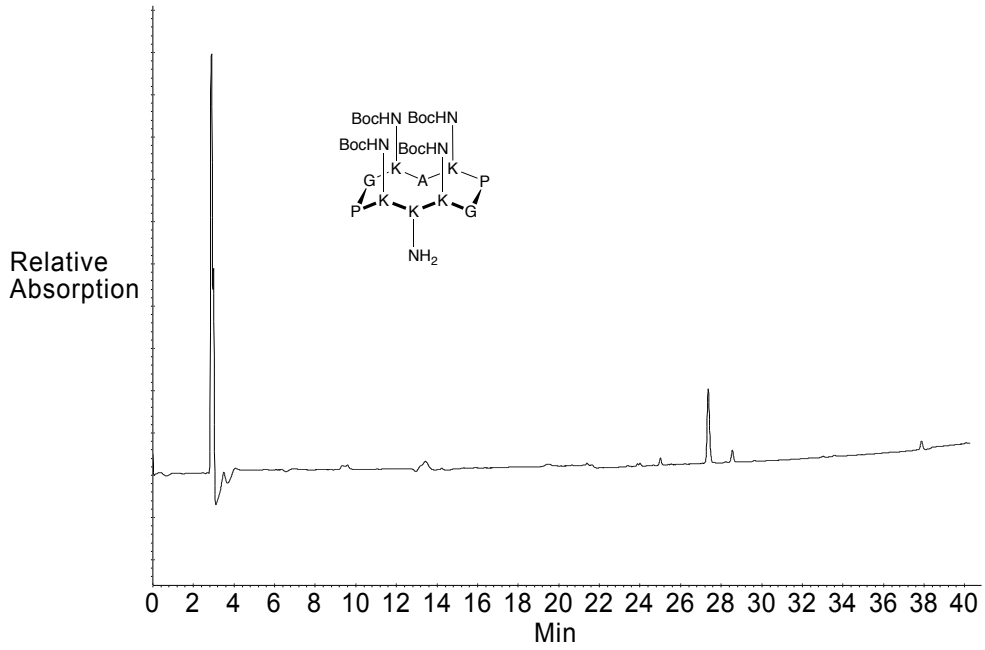
RP-HPLC: t_R = 26.3 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min

c[Lys(Boc)-Lys(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (41)



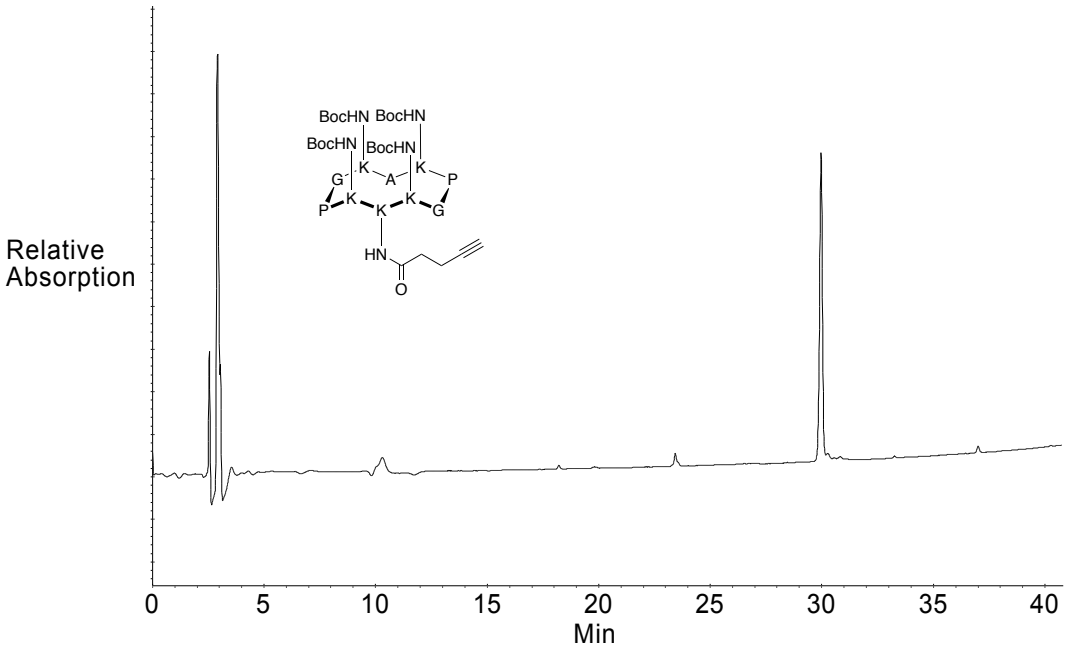
RP-HPLC: t_R = 32.3 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min

c[Lys(Boc)-Lys-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (126)



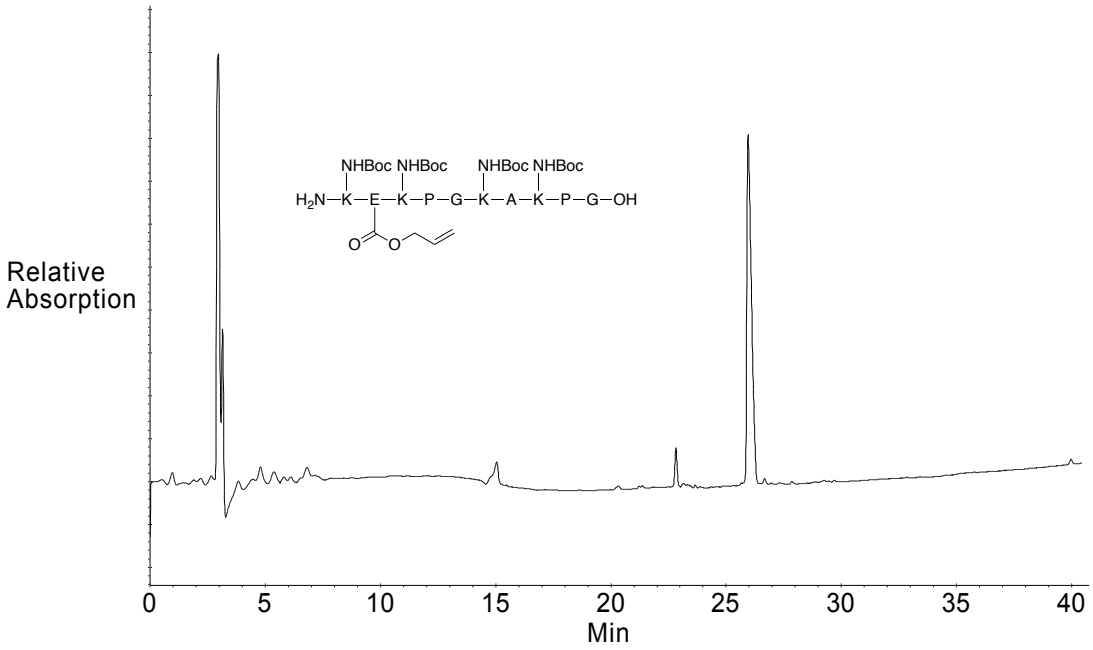
RP-HPLC: t_R = 27.4 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min

c[Lys(Boc)-Lys(4-Pentynoyl)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (127)



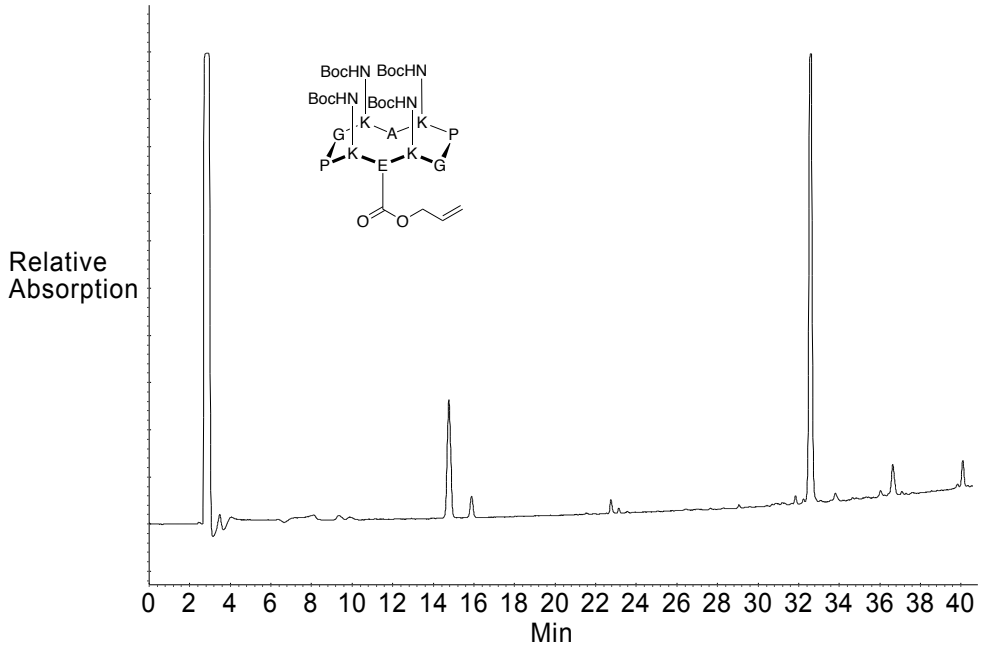
RP-HPLC: t_R = 30.0 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min.

Lys(Boc)-Glu(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly (128)



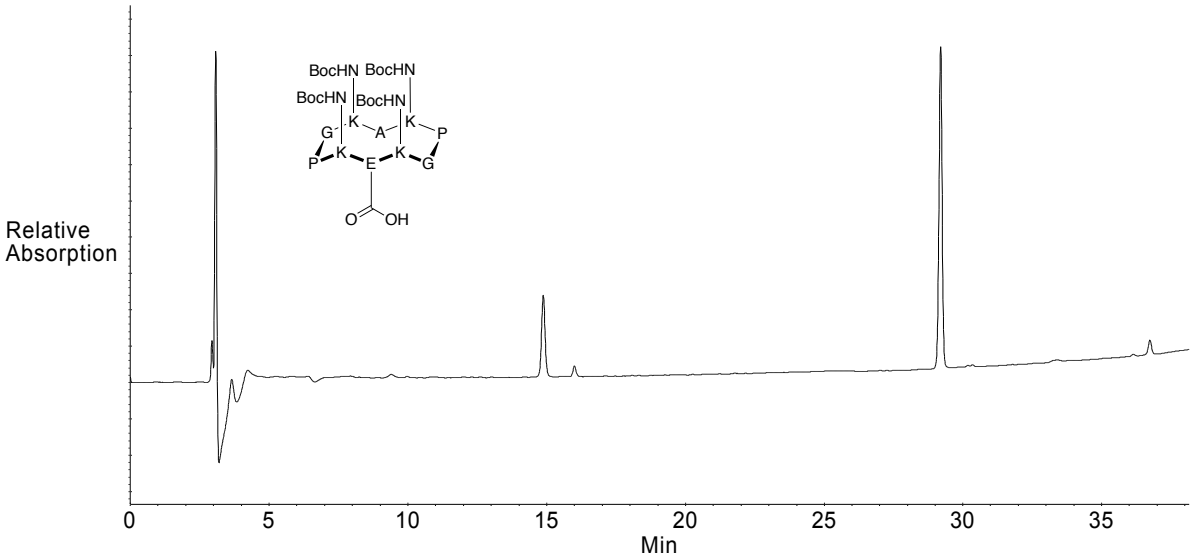
RP-HPLC: t_R = 26.0 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min

c[Lys(Boc)-Glu(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (130)



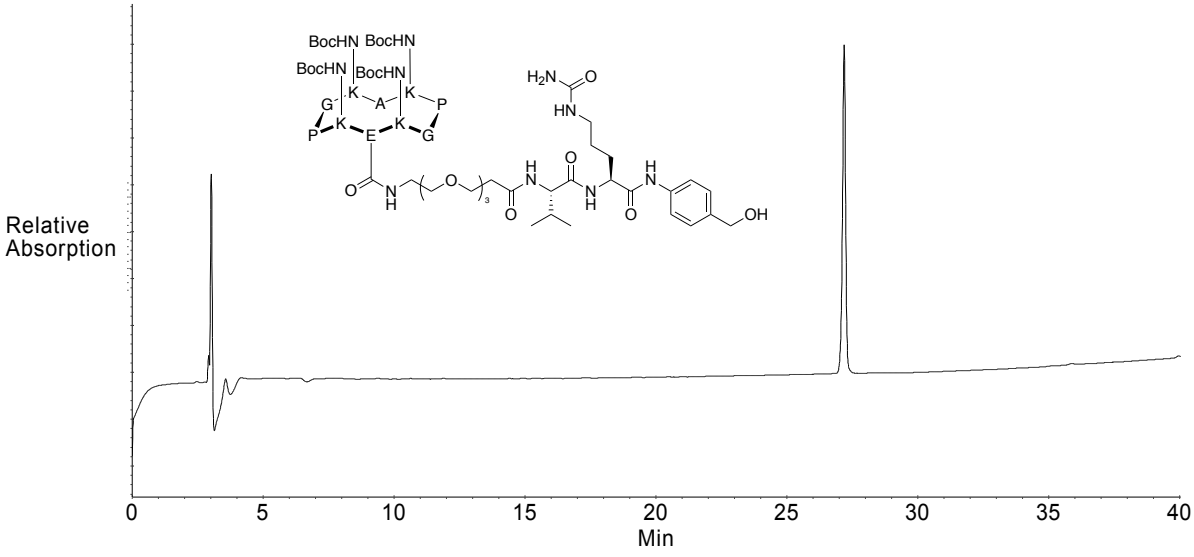
RP-HPLC: t_R = 32.6 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min

c[Lys(Boc)-Glu-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (131)



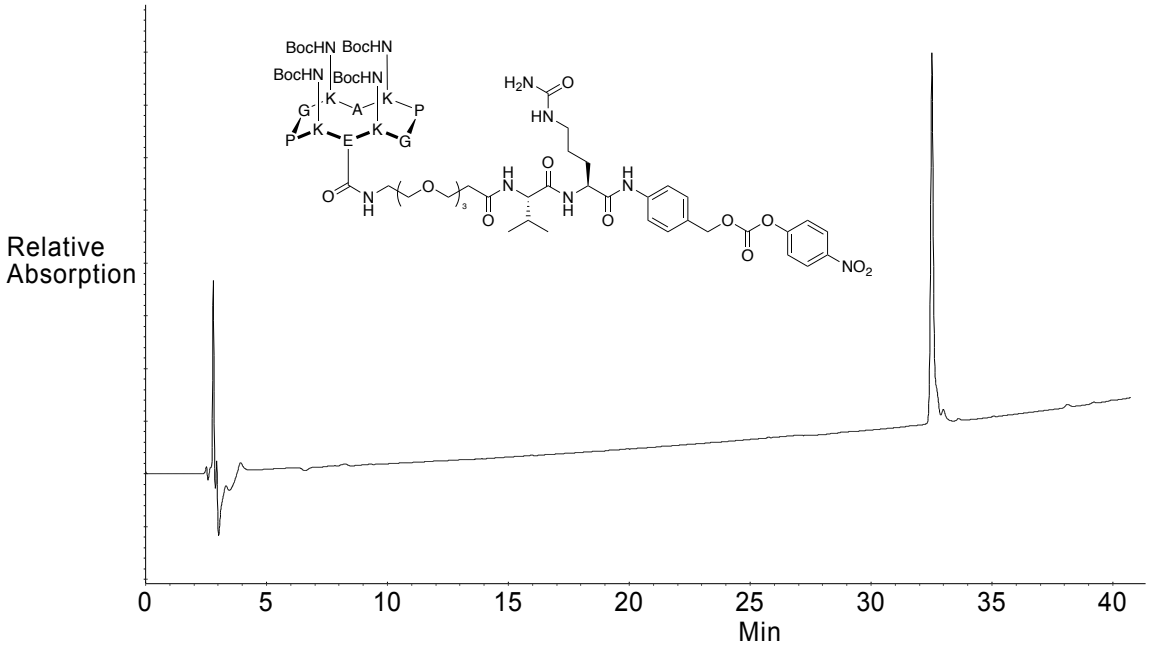
RP-HPLC: t_R = 29.2 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min

c[Lys(Boc)-Glu(PEG-Val-Cit-4-aminobenzyl alcohol)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (134)



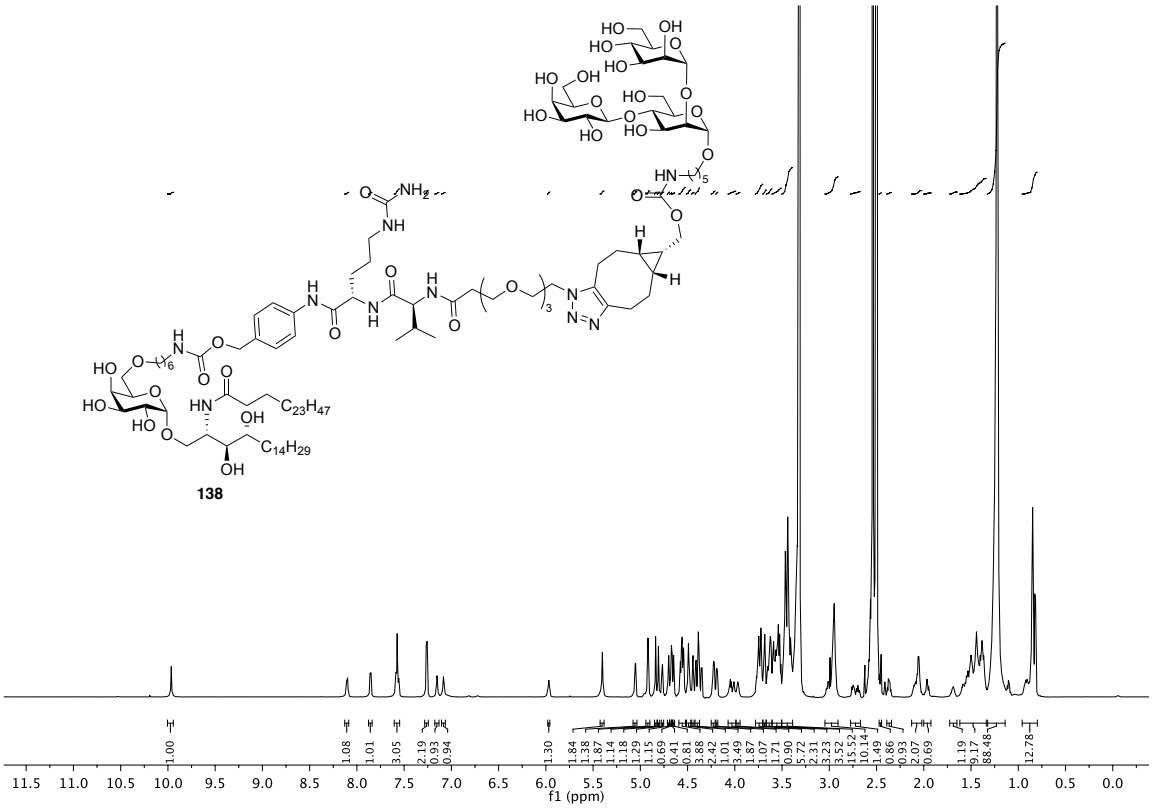
RP-HPLC: t_R = 27.2 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min

c[Lys(Boc)-Glu(PEG-Val-Cit-4-aminobenzyl 4-nitrophenyl carbonate)-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly] (129)

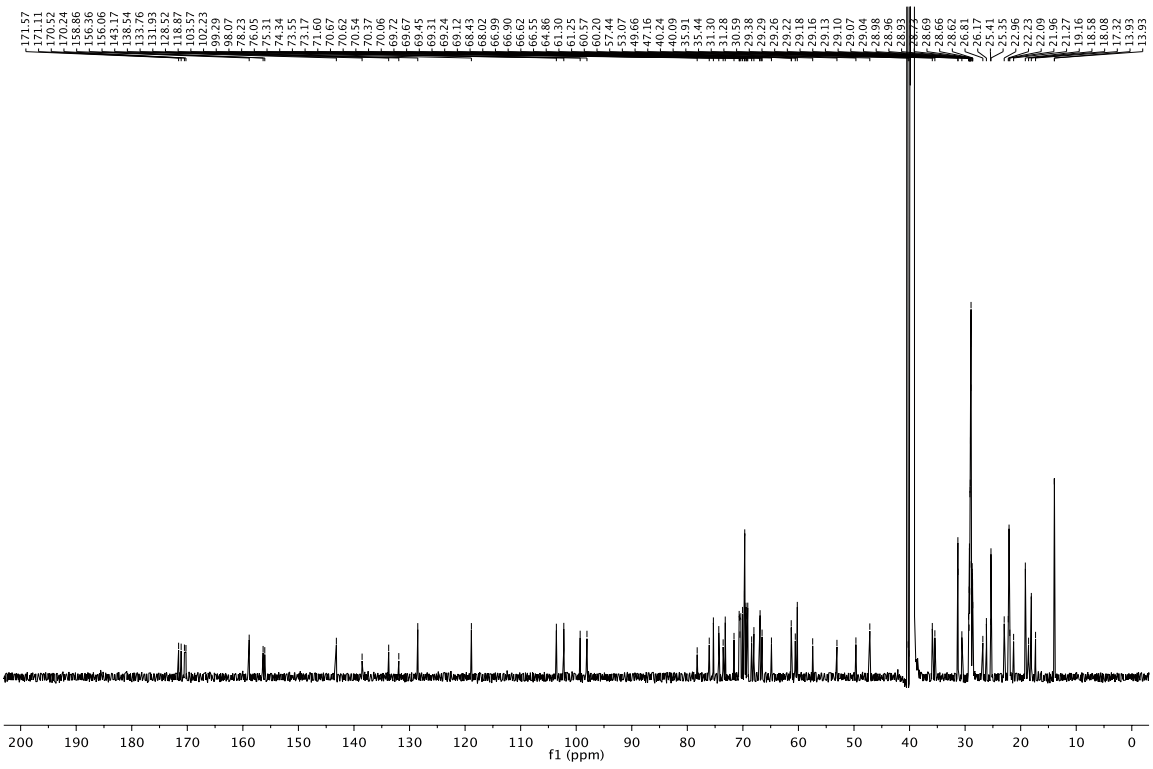


RP-HPLC: t_R = 32.5 min, Phenomenex Aeris C18, λ = 214 nm, Grad.: MeCN:H_2O + 0.1% TFA (5:95) →(20:80) 40 min

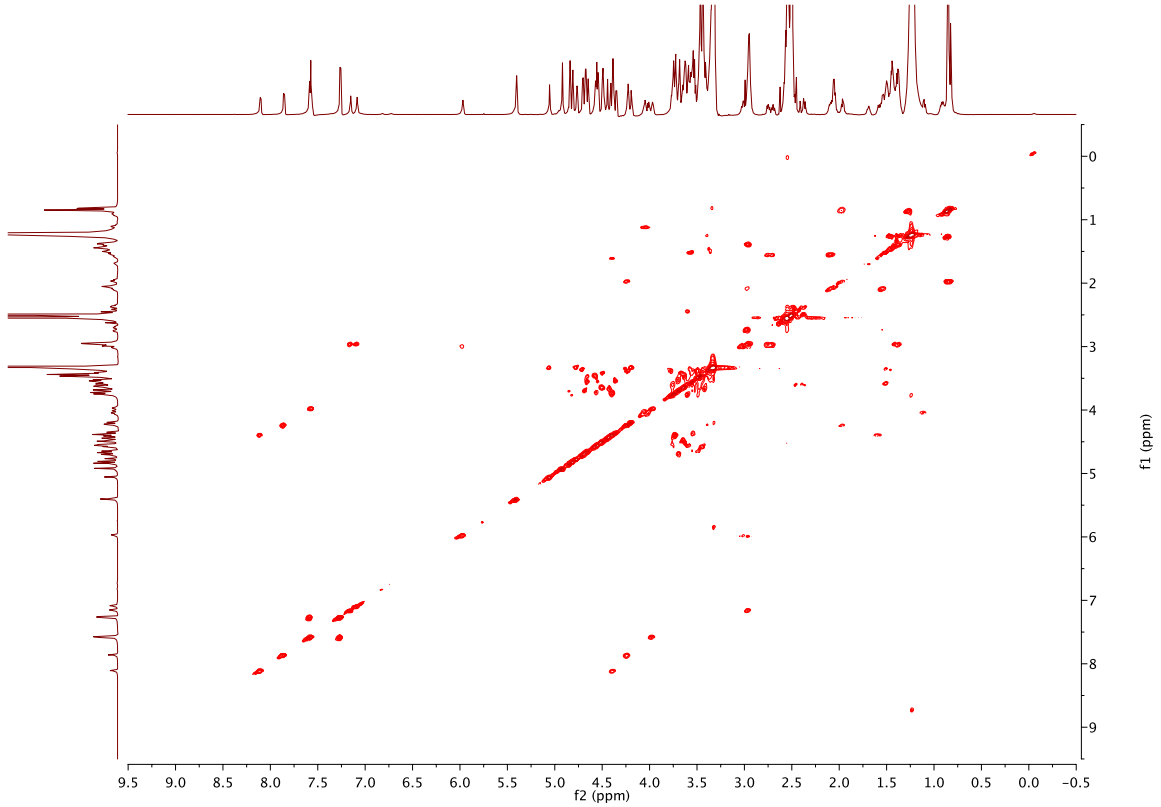
SPAAC conjugate (138)



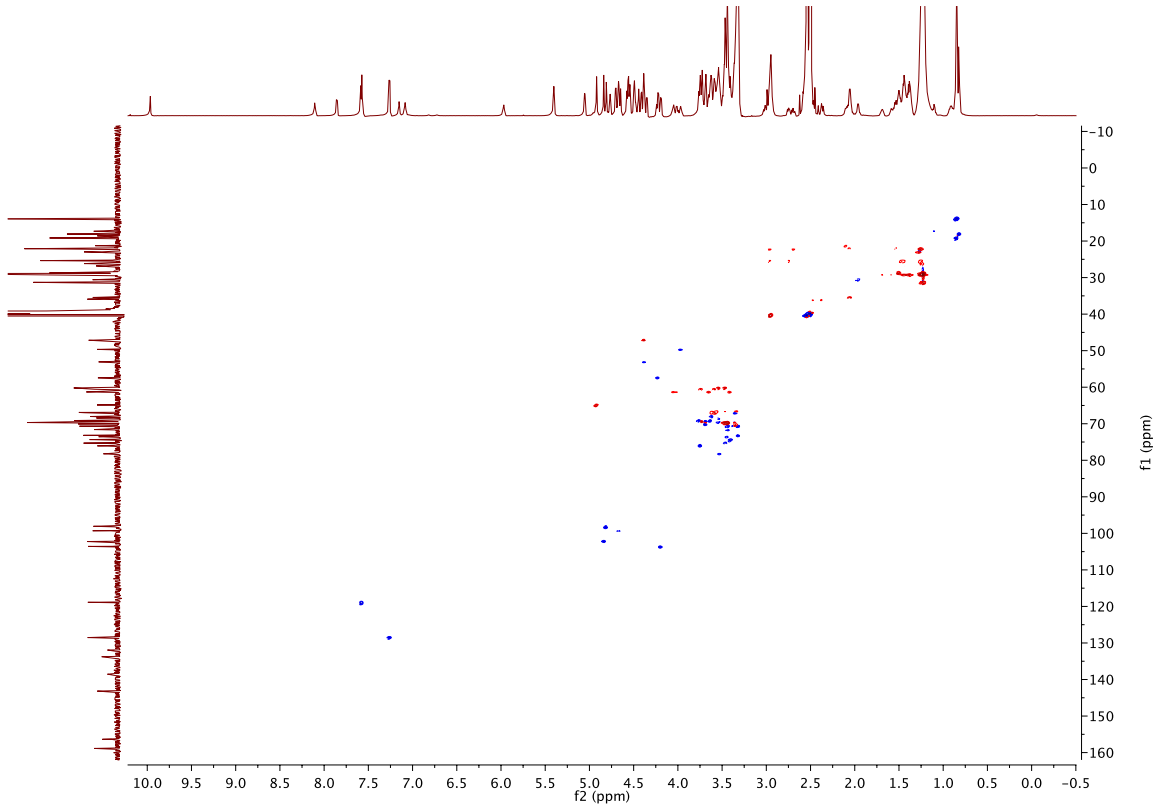
^{1}H NMR (800 MHz, $(CD_3)_2SO$)



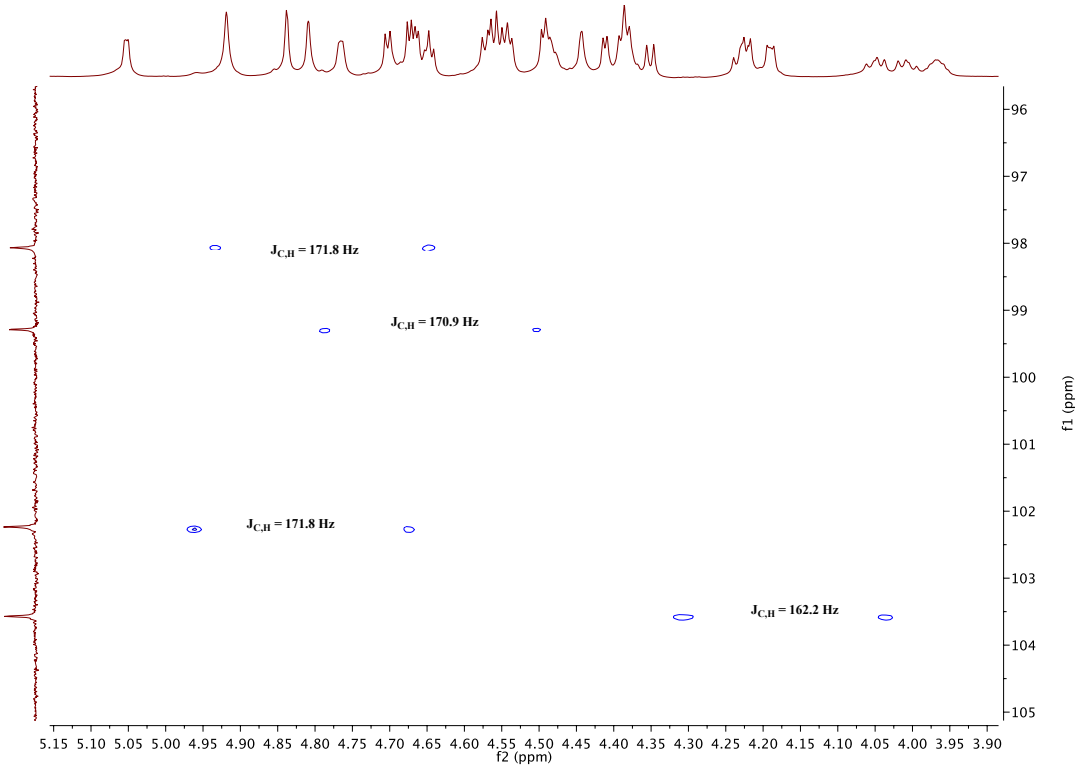
^{13}C NMR (201 MHz, $(CD_3)_2SO$)



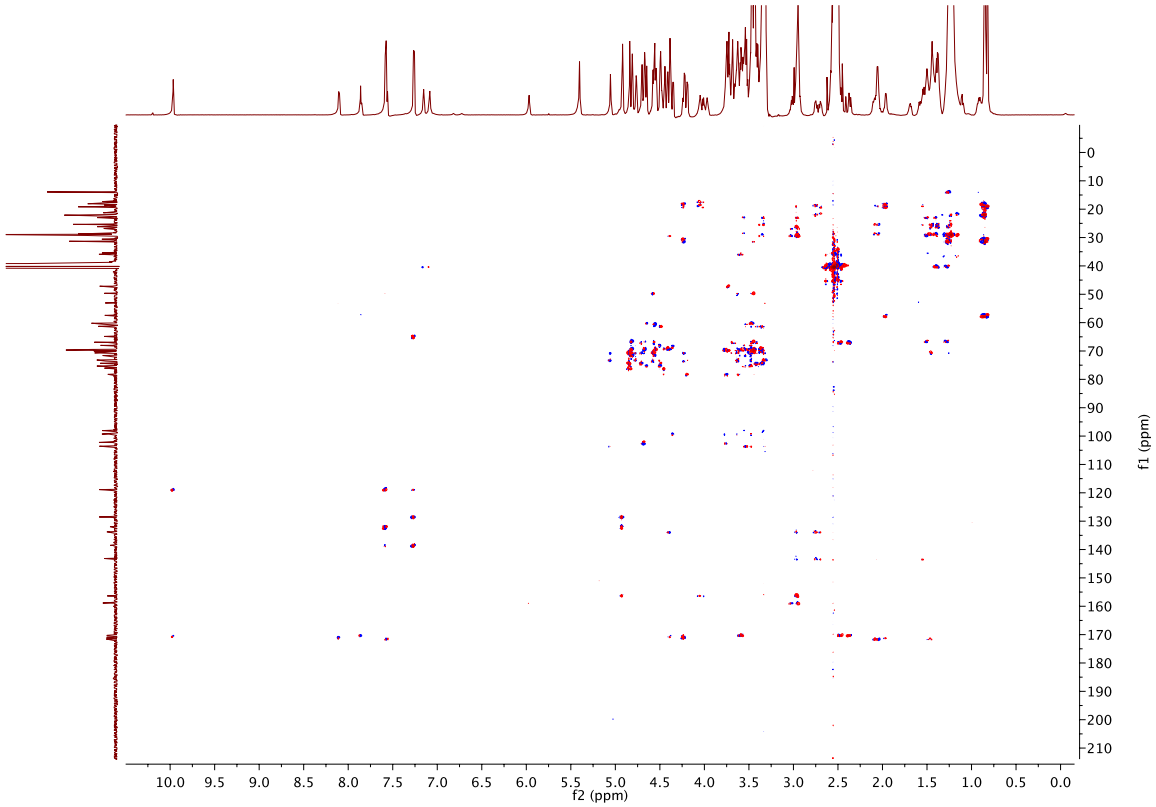
COSY (800 MHz/201 MHz, $(CD_3)_2SO$)



HSQC (800 MHz/201 MHz, $(CD_3)_2SO$)



^{1}H-^{13}C coupled HSQC (600 MHz/151 MHz, $(CD_3)_2SO$)



HMBC (800 MHz/201 MHz, $(CD_3)_2SO$)