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Synthesis of fluorinated *S. pneumoniae* serotype 8 glycotope mimetics for the assembly of synthetic vaccine candidates

and

A set of rhamnosylation-specific antibodies enables the detection of novel protein glycosylation in bacteria

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aus

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Erklärung

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Eidesstattliche Versicherung

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-Für meine Familie-

[…]

Ja mach nur einen Plan!

Sei nur ein großes Licht!

Und mach dann noch´nen zweiten Plan

Gehn tun sie beide nicht.

[…]

Aus „Ballade von der Unzulänglichkeit Menschlichen Planens“

Bertold Brecht

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Publications and Communications

Parts of this work have been published in a peer-reviewed journal:

1. Daniel Gast‡, Franziska Koller‡, Ralph Krafczyk, Lukas Bauer, Swetlana Wunder, Jürgen Lassak* and Anja Hoffmann-Röder*, A set of rhamnosylation-specific antibodies enables detection of novel protein glycosylations in bacteria, *Organic & Biomolecular Chemistry* **2020**, *18*, 6823 -6828.

   ‡ Authors contributed equally to this work. * Corresponding Authors

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3. Synthetic glycopeptides for investigation of protein rhamnosylation in bacteria, Poster, *International Conference on Molecular System Engineering* **2017**, Basel, Swiss.
4. Chemical synthesis of *N*-rhamnosylated glycopeptides promote the discovery of novel glycosylations, Poster, *International Carbohydrate Symposium* **2018**, Lisbon, Portugal.
5. Chemical synthesis of *N*-rhamnosylated glycopeptides promote the discovery of novel glycosylation, Poster, *Synthetic Biology III Conference* **2018**, Landshut, Germany
6. Chemical synthesis of *O*- and *N*-rhamnosylated glycopeptide haptens for antibody generation, Poster, *European Carbohydrate Symposium* **2019**, Leiden, Netherlands.

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List of Schemas:

Abbreviation

[α]	Optical rotation
4-DMAP	4-Dimethylaminopyridine
Å	Ångström
Ac_2O	Acetic anhydride
AcOH	Acetic acid
All	Allyl
APC	Antigen presenting cell
aq.	Aqueous
Arg	Arginine
Asn	Asparagine
Asn^{Rha}	*N*-rhamnosyl asparagine
Asp	Aspartic acid
BADA	Benzaldehyde dimethyl acetal
BCN	Bicyclo[6.1.0]nonyne
Bn	Benzyl
BSA	Bovine serum albumin
Bz	Benzoyl
^{c}Hex	Cyclohexane
Conc.	Concentrated
COSY	^{1}H correlation spectroscopy
CPS	Capsular polysaccharides
CSA	Camphorsulfonic acid
Cys	Cysteine
DAST	Diethylaminosulfur trifluoride
DATDH	2,4-diacetamido-2,4,6-trideoxyhexose
DBTO	Dibutyltin oxide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIC	*N,N´*-Diisoproylcarbodiimide
diNAcBac	*N,N´*-Diacetylbacillosamine
DMAPA	Dimethylaminopropylamine
DMF	*N,N´*-Dimethylformamide
DMSO	Dimethyl sulfoxide
dTDP	Deoxythymidine diphosphate
E. coli	*Escherichia coli*
EF-P	Elongation Factor P
ELISA	Enzyme-linked immunosorbent assay
eq.	equivalents
Et_2O	Diethyl ether

EtOAc	Ethyl acetate
EtOH	Ethanol
Fmoc	Fluorenylmethyloxycarbonyl
Gal	Galactose
GalCer	Galactosylceramide
GalNAc	*N*-Acetylgalactosamine
GATDH	Glyceramido acetamido trideoxyhexose
gg	*Gauche, gauche*
Glc	Glucose
GlcNAc	*N*-acetylglucosamine
Gly	Glycine
gt	*Gauche, trans*
H_{ax}	Axial proton
H_{eq}	Equatorial proton
His	Histidine
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond connectivity
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear single quantum coherence
Ig	Immunoglobulin
Ile	Isoleucine
iNKT cell	Invariant natural killer T cell
IPD	Invasive pneumococcal disease
J	Coupling constant
KLH	Keyhole limpet hemocyanin
Leg	Legionaminic acid
Le^Y	Lewis Y
MAG	Multiple antigen glycopeptide
MCF 7	Michigan cancer foundation-7
MeCN	Acetonitrile
MeOH	Methanol
MHC	Major histocompatibility complex
MUC	Mucin
NBS	*N*-Bromosuccinimide
NFM	*N*-Formylmorpholine
NHS	*N*-Hydroxysuccinimide
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance
NP	Naked Peptide
OD	Optical density
Oxyma	Ethyl cyanohydroxyiminoacetate

P. pu	*Pseudomonas putida*
PADRE	non-natural Pan-DR T cell epitope
PBS-buffer	Phosphate-buffered saline buffer
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
Pse	Pseudaminic acid
P-site	Peptidyl site
PTFA	*N*-(phenyl) trifluoroacetimidate
PTM	Post-translational modification
p-TsOH	*para*-Toluenesulfonic acid
Pup	Prokaryotic ubiquitin-like protein
PyBOP	Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate
RAFT	Regioselective adressable functionalized template
Rha	L-Rhamnose
RP	Reversed-Phase
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
sat.	Saturated
SDS-Page	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
Sec	General secretory pathway
Ser	Serine
Ser^{Rha}	*O*-rhamnosyl serine
S-layer	Surface layer
SNC	Succinyl norcocaine
SP	*Streptococcus pneumoniae*
SPAAC	Strain-promoted azide alkyne cycloaddition
SPPS	Solid-phase peptide synthesis
SPR	Surface plasmon resonance
ST	Serotype
ST 8	*Streptococcus pneumoniae* serotype 8
STD	Saturation-transfer difference
STn	Sialyl Tn antigen
TACA	Tumour associated carbohydrate antigen
TBAB	Tetrabutylammonium bromide
TBS	*t*-Butyldimethylsilyl
t-BuOH	*t*-Butanol
TCA	Trichloroacetimidate
T_D	Thymus dependent
TEG	Triethylene glycol

TF	Thomsen-Friedenreich antigen
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
tg	*Trans, gauche*
T_H	T helper
THF	Tetrahydrofuran
Thr	Threonine
Thr^{Rha}	*O*-rhamnosyl threonine
T_I	Thymus independent
TLC	Thin-layer chromatography
TLR	Toll-like receptor
TMSOTf	Trimethylsilyl trifluoromethansulfonate
Tol	4-methyl phenyl residue
tRNA	Transfer-RNA
TT	Tetanus toxoid
Und-P	Undecaprenyl phosphate

Summary

Part I: Synthesis of fluorinated *S. pneumoniae* serotype 8 glycotope mimetics for the assembly of synthetic vaccine candidates

Unique structural properties and dense distribution on cell surfaces (pathogens or tumour cells) mark polysaccharides as attractive target structures for the development of modern subunit vaccines. Recently, synthetic and structurally well-defined glycan epitopes gained considerable attention for addressing the limitations of current commercial glycoconjugate vaccines. However, sufficient intrinsic immunogenicity and *in vivo* stability of those synthetic carbohydrate epitopes are key requirements for their application in synthetic or semisynthetic vaccine formulations. In these regards, chemical derivatisation (e.g. fluorination) of given epitopes emerged as a promising approach to enhance their *in vivo* stability and/or immunogenicity.

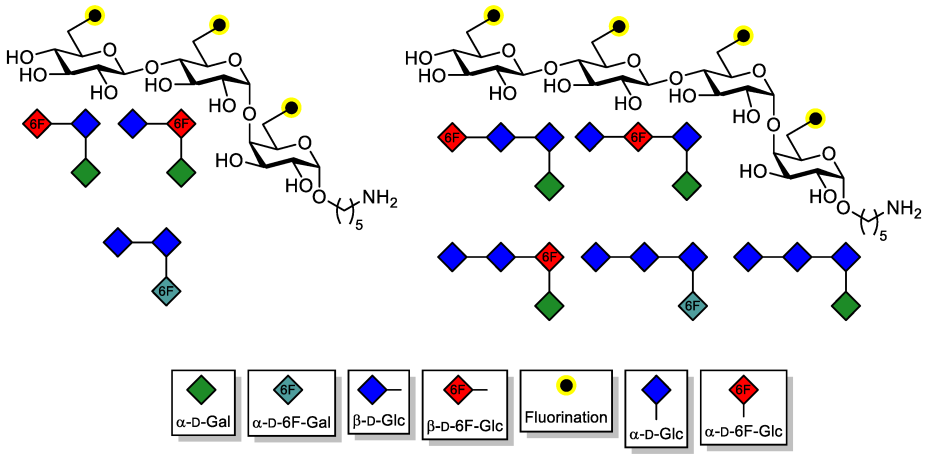

Figure 1: Synthesized glycan epitope mimetics derived from *Streptococcus pneumoniae* serotype 8.

Within the first project of this thesis, synthetic approaches towards C-6-fluorinated epitope mimetics of *S. pneumoniae* serotype 8 (ST 8; see Figure 1) were developed. Further, these synthetic approaches were used for the preparation of a known (non-fluorinated) tetrasaccharide epitope of ST 8. This selection of glycotope mimetics in turn should provide a useful tool to study the influence of epitope fluorination onto antibody recognition and immunological potency these antigenic determinants.

In a continuative project, three of these synthetic glycans were used to assemble first synthetic two-component vaccine candidates, comprising a α-GalCer derivative as a T helper-like epitope (see Figure 2). In addition, these vaccines candidates were equipped with a self-immolative dipeptide linker to provide sufficient serum stability and effective epitope liberation within B cells.

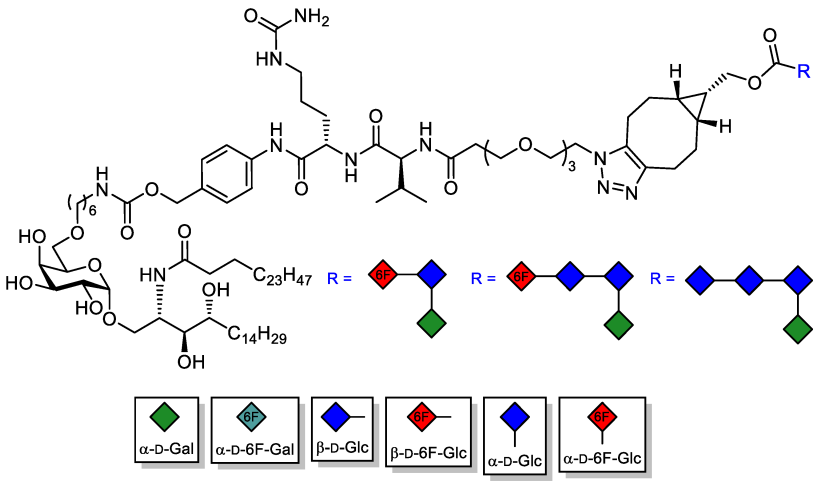

Figure 2: First synthetic vaccine candidates assembled in a continuative project.

Part II: A set of rhamnosylation-specific antibodies enables the detection of novel protein glycosylation in bacteria

The discovery of arginine mono-rhamnosylation (Arg^{Rha}) in various pathogens, attracted the idea of protein mono-rhamnosylation being a more common post-translational modification in bacteria. Assuming that a putative rhamnoproteome might also include linkages beyond that of known Arg^{Rha} we aimed at expanding the limited toolbox of polyclonal *anti*-Arg^{Rha} antibodies by a set of antibodies covering the most prominent sites of bacterial *O*- and *N*-glycosylation (namely Asn^{Rha}, Ser^{Rha} and Thr^{Rha}).

Accordingly, synthetic approaches towards stereochemically well-defined rhamnosyl amino acid building blocks and their incorporation into glycopeptide haptens were development (project overview see Figure 3). Generation of polyclonal sera using these synthetic rhamnopeptide haptens were conducted commercially and furnished the intended set of specific *anti*-Asn^{Rha}, *anti*-Ser^{Rha} and *anti*-Thr^{Rha} antibodies. Subsequent immunoblotting experiments indeed disclosed the existence of rhamnose-modified proteins in various bacterial species and provided a promising starting point for further investigations on the rhamnoproteome of bacteria.

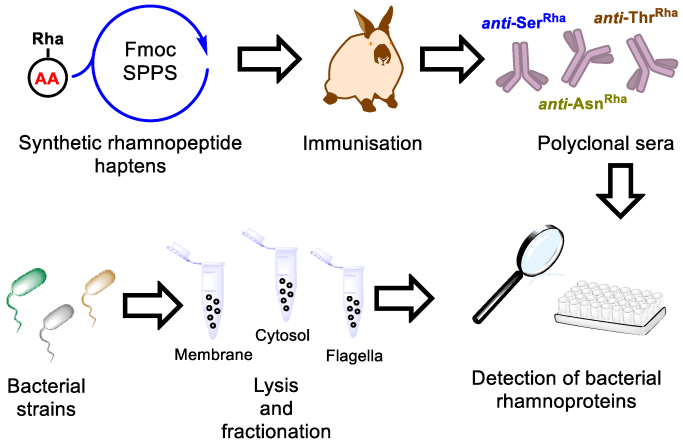

Figure 3: Generation of *anti*-Ser^{Rha}, *anti*-Thr^{Rha} and *anti*-Asn^{Rha} antibodies and detection of rhamnoproteins in various bacterial species.

General Introduction

Carbohydrates, proteins, lipids and nucleic acids are the major constituents of cells. Amongst these building blocks of life, carbohydrates are unique in their capability to encode biochemical information in a high-density fashion.[1] Anomeric configuration, regiochemistry and branching are some of the structural elements that establish the unsurpassed chemical variability of carbohydrates. Hence, it is not surprising that nature employs these entities in numerous biological processes (e.g. immune responds, cell proliferation) and that aberrant glycosylation patterns may have severe effects on cell functionality.[2, 3]

Regardless of their importance in biological processes, carbohydrates are the least well understood class of biomolecules in terms of their physiological function beyond that of nutrition.[4] This in parts is attributed to their non-template driven biosynthesis, which renders established approaches from protein science (e.g. genomic analysis or genetically encoded tagging) inapplicable.[3, 5] Furthermore, the natural appearance of heterogenous glycoforms, substoichiometry and reversibility are additional obstacles that complicate isolation from natural sources and elucidation of biological functions.[4, 6]

Consequently, chemical synthesis has long been an indispensable ally of glycobiology, providing specialized tools to study the glycome of living organisms. Chemically modified monosaccharide probes (e.g. for metabolic labelling approaches) or glycan specific antibodies are just two examples which facilitated investigations of protein glycosylations in cells.[5, 7-11]

Pharmaceuticals such as *Zanamivir* or the Cuban *Haemophilus influenzae* type b vaccine illustrate the potential of synthetic glycans (or derivatives thereof) beyond fundamental research applications.[4, 12-14] Here, chemical synthesis offers defined glycan structures with little to no batch-to-batch variability. Moreover, chemically modified mimetics that resemble the function of native entities can provide improved metabolic stability or pharmacological properties.[2, 15, 16]

The potential of chemical glycobiology in fundamental research and medicinal chemistry, in turn fosters the development of synthetic approaches towards sophisticated glycan targets. In particular, stereocontrolled assembly of 1,2-*cis* glycosidic linkages (α-glucose/galactose or β-rhamnose/mannose) has always been a significant challenge for carbohydrate chemists.[17, 18] In contrast to 1,2-*trans* selective glycosylations in which stereoselectivity can conveniently be established by means of participating ester protecting groups, 1,2-*cis* selectivity is generally obtained by a more complex interplay of a variety of factors. For example, acceptor nucleophilicity, protecting group pattern, solvents or electron withdrawing substituents have severe impact on the stereochemical outcome of these glycosylation reactions.[17, 19-22]

The inherent challenges associated with carbohydrate synthesis was already concisely summarized by Professor *Hans Paulsen* back in 1982: "*Although we have now learned to synthesize oligosaccharides, it should be emphasized that each oligosaccharide synthesis remains an independent problem, whose resolution requires considerable systematic research and a good deal of know-how. There are no universal reaction conditions for oligosaccharide synthesis*."[23] Of course, since then tremendous progress has been made in terms of understanding glycosylation reactions and improving chemical methodologies.[17, 24-27] Nevertheless, assembly of oligosaccharides generally still demands for a combination of various glycosyl donor types and careful optimization of glycosylation conditions.

The following thesis is divided into two parts, each presenting a synthetic project dedicated to a specific area of glycoscience. In the first part, a synthetic approach towards a small library of fluorinated glycotope mimetics of *Streptococcus pneumoniae* Serotype 8 (ST 8) is presented. In a long-term perspective these mimetics could help to gain a deeper understanding of the influence of fluorine modifications onto the immunological properties of pathogen-derived antigens and may foster the development of improved glycoconjugate vaccines.

The project presented in the second part aims towards the generation of novel glycosylation-specific antibodies from synthetic glycopeptide haptens. These novel antibodies directed against yet unappreciated protein rhamnosylation sites, are specific biochemical tools to unveil novel (functional) protein glycosylations in bacteria.

Part I:

Synthesis of fluorinated *S. pneumoniae* serotype 8 glycotope mimetics for the assembly of synthetic vaccine candidates

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Introduction

I.1 A brief history of vaccines: From early attempts to modern design principles

The world´s first vaccination was performed by *Edward Jenner* in 1796. *Jenner*, who inoculated a child with pus from a cowpox lesion observed "*that the cow-pox protects the human constitution from the infection of smallpox*".[28-30] Thus, the foundation of vaccination was laid at a time when the connection between bacteria and diseases has not been discovered yet. Interestingly, the first anti-vaccination movements emerged soon after in Europe and the United States.[28, 31] Until 1885, when *Louis Pasteur* developed his rabies vaccine, the term vaccine only referred to cowpox inoculation for smallpox.[28, 32] Extending the concept of vaccination to a more general principle, *Louis Pasteur* largely contributed to today´s definition of vaccines.

Figure 4: Some (but not all) of the pioneers in the history of vaccine development: *Edward Jenner* (upper left) conducted the first successful vaccination against smallpox. [33] *Louis Pasteur* (lower left, in the back) extended the concept of vaccination by introducing his rabies vaccine.[34] Sir *Almroth Wright* (upper middle) conducted the first pneumococcal vaccine study amongst South African gold miners.[35] *Alphonse Raymond Dochez* (lower middle)[36], *Oswald Avery* (lower right)[37] and *Michael Heidelberger* (upper right)[38] largely contributed to the elucidation of the immunological active soluble substance of pneumococcus.

The first pneumococcal vaccine study was conducted in 1911, when pneumonia infection rate threatened the existence of the mining industry in South Africa.[39, 40] Sir *Almroth Wright* immunized gold miners with a whole-cell pneumococcal vaccine, claiming a *"...reduction in the incidence-rate and death rate of pneumonia in the inoculated... "*.[39, 40] However, the raising awareness about heterogeneity and immunogenicity of pneumococcal strains suggested later that the number of pneumococci included in *Wright´s* vaccine was insufficient to stimulate effective antibody response.[40, 41] Hence, *Wright´s* findings remain at least questionable.[42]

A landmark in the history of pneumococcal vaccines was the description of the immunologically active soluble substance of pneumococcus in 1917. Despite falsely attributing the active substance to be "*...of protein nature or associated with protein*", *Dochez* and *Avery* laid the foundation for a later study which identified polysaccharides as building blocks of the pneumococcal capsule.[43-46] By 1929, the immunogenicity of pneumococcal-derived capsular polysaccharides of Serotype 2 and 3 were confirmed by *Schiemann* and *Casper*.[47, 48] However, a field trial

of a vaccine containing these capsular polysaccharides again furnished only inconclusive results, mainly due to the lack of complete bacteriological data and follow-up studies.[40, 49] This however changed in the course of the 1940´s when field studies under controlled conditions provided strong evidence on the efficiency of bi- to quadrivalent polysaccharide vaccines and enabled commercialization of a first hexavalent vaccine.[50-52] Unfortunately, the advent of penicillin (and other antibiotics) rendered the formerly feared pathogen unserious and led to withdrawal of pneumococcal vaccines from the market.[40, 52, 53]

This changed in 1964 with *Austrian* and *Gold* reporting on a case study of approximately 2000 patients from which nearly 25 % died from pneumococcal infections despite the deployment of antimicrobial drugs.[54] As a result, polysaccharide vaccines experienced a renaissance and by 1977 a 14-valent polysaccharide vaccine was introduced to the market by *Merck*. Six years later, this vaccine was replaced by the 23-valent *Pneumovax 23*®, containing at least 85 % of *S. pneumoniae* serotypes that are responsible for invasive infections in adults and children.[52] However, the vaccines´ limited efficacy especially amongst high risk patients (elderly, children and patients with underlying illnesses) remained as a major driving force for further development of vaccines.[52, 55-69]

Second generation vaccines: glycoconjugate vaccines

In addition to the limited efficacy in the high-risk groups, short effectiveness is a common obstacle of purely polysaccharide-based vaccines. This in large parts is attributed to polysaccharide antigens being T lymphocyte independent class 2 antigens (T_I type 2) possessing no intrinsic B lymphocyte stimulation activity.[70] Such T_I type 2 antigens activate antigen-specific mature B cells *via* cross-linking of their surface B cell receptors (see Figure 5).[71, 72] As a result, immune responses against these antigens are short-living, IgM mediated and lack the formation of immunological memory.[73] In contrast, proteinic or peptidic antigens are T lymphocyte dependent antigens (T_D), that induce formation of high affinity IgG antibodies. Beyond that, proliferation of resting B cells into memory cells establishes immunological memory.[14, 70]

In 1931 *Avery* and *Goebel* first described conjugation of capsular polysaccharides to a carrier protein to enhance their immunogenicity (*"Carrier Effect"*).[74] Since this early finding, further research demonstrated that T_I antigens, such as polysaccharides, can be converted into T_D immunogens upon conjugation onto a suitable proteinogenic carrier. Thus, protein conjugation provides the necessary T cell support which is crucial for IgM to IgG class switching and formation of an immunological memory targeted against the carbohydrate antigen.[14, 75] In this regard, the work of *Avery* and *Goebel* laid the foundation for the development of modern glycoconjugate vaccines that provide enhanced efficacy in the high-risk groups.[76, 77] Currently, two pneumococcal glycoconjugate vaccines are commercially available. The first *Prevnar 13*® was approved in 2009 in the European Union containing thirteen serotypes of pneumococcus conjugated to a non-toxic mutant of *diphteria toxin* (CRM_{197}) as carrier protein. The second, *Synflorix*®, is produced by *GlaxoSmithKline* and contains ten serotypes conjugated to different carrier proteins.[78]

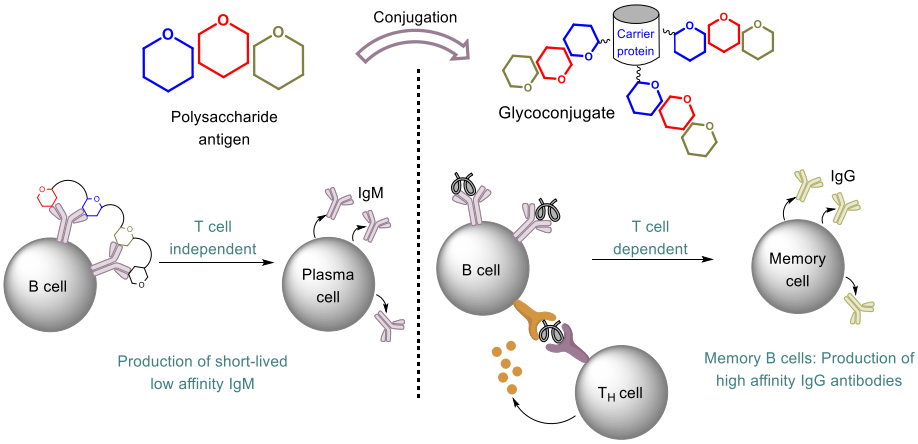


Figure 5: Simplified depiction of the *Carrier Effe*ct, first described in 1931 by *Avery* and *Goebel* (adapted and modified from reference[79]). Since then, a large body of research provided evidence that T_I type 2 polysaccharide antigens can be converted into T_D immunogens *via* conjugation onto a suitable carrier protein. These findings laid the foundation for the development of a variety of commercially available conjugate vaccines.

Their ability to prevent infectious diseases in high-risk groups render glycoconjugate vaccines one of the most successful inventions in the history of biomedical science.[14] Despite their success, modern glycoconjugate vaccines still suffer from several limitations that need to be addressed.

Commercially available glycoconjugate vaccines are generally heterogenous mixtures containing a variety of immunological active substances, such as isolated bacterial capsular polysaccharides, protein carriers and additional adjuvants to enhance *in vivo* immunogenicity. Manufacturing thereby relies on the isolation of capsular polysaccharides (CPS) from natural sources and their conjugation to suitable carrier proteins.[80] Thus, formation of artificial non-protective epitopes, insufficient antigen stability and the presence of toxic contaminants are just some aspects that might hamper efficacy of commercially available glycoconjugate vaccines.[14, 81-90] In addition, intrinsic heterogeneity of natural isolates and unspecific conjugation methods counteracts the establishment of sound structure-activity relationships and increases the risk of batch-to-batch variability.[84-89, 91]

I.1.2 A promising liaison: Chemical synthesis meets vaccinology

As described earlier, classic glycoconjugate vaccines are heterogenous mixtures including diverse immunological active components derived from natural sources and their distinct molecular mode of action and structure-activity relationships often remained ill-defined.[80] Increasing knowledge about the underlying immunological processes accountable for effective prevention of diseases, prompted the identification and synthesis of immunological active subunits with a defined task and precise molecular composition. Eventually this approach may enable modular vaccine constructs consisting solely of essential and optimized elements for evoking effective immunization amongst all population. Thus far, a large variety of vaccine constructs have been developed ranging from semi-synthetic constructs including only a single synthetic module (mostly carbohydrate epitopes) to fully synthetic multi-component vaccine constructs (for a graphical summary on the “evolution” of modern subunit vaccines see Figure 6). To this end, synthetic carbohydrate epitopes appear to be the most extensively studied module due to their crucial role in promoting vaccine specificity. However, synthetic T cell epitopes, adjuvants and multivalent non-immunogenic scaffolds are further modules developed to enhance immunogenicity of synthetic vaccines.

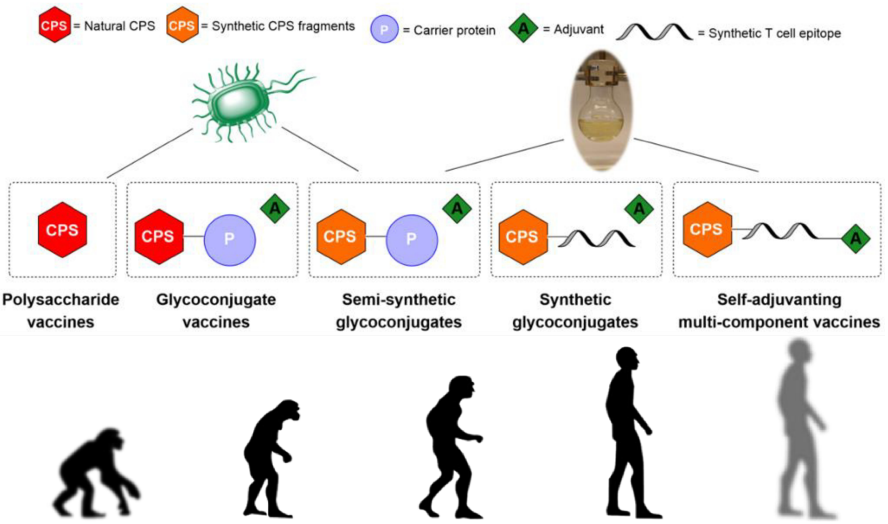


Figure 6: The evolution of modern subunit vaccines (evolution picture adapted from[92]). Limited efficacy of early polysaccharide vaccines especially in high-risk groups was a major driving force for the development of glycoconjugate vaccines. Progress in immunobiology and chemical synthesis further pathed the way for modern vaccine candidates consisting of precisely assembled synthetic immunological modules.

In the following sections, individual parts of modern synthetic vaccine designs will be discussed in more detail. It is worth mentioning that most research in this area has been directed towards the development of anti-tumour vaccines. Nevertheless, findings from this vivid field of vaccine research also stimulates development of anti-bacterial vaccine constructs and will hence be included herein to provide a more complete picture.

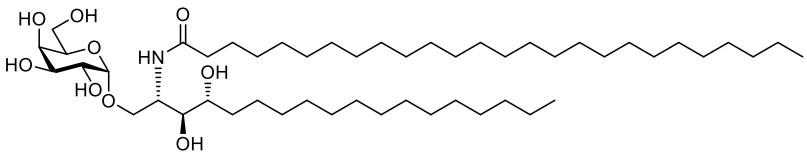
T cell epitopes

Since the discovery of the *Carrier Effec*t by *Avery* and *Goebel* it became obvious that bacterial carbohydrate antigens require additional T cell help to elicit durable and strong immune responses. Classic glycoconjugate vaccines utilize immunogenic carrier proteins that facilitate uptake and presentation by antigen presenting cells (APC) and activation of $CD4^+$ T helper cells.[14] Prominent examples of commonly used immunogenic carriers are nontoxic cross-reactive material of the *diphtheria toxin* (CRM_{197}), *tetanus toxoid* (TT) or the *keyhole limpet hemocyanin* (KLH). However, immunogenic carrier proteins are frequently fraught by limitations. For instance, unspecific conjugation methods provide mixtures of glycoforms (e.g. carbohydrate to protein ratio and distribution) which may vary in pharmacokinetics and immunological properties.[91] In addition, a pre-existing immunity against the respective carrier protein may render the vaccine ineffective due to carrier-induced immune suppression.[93]

In regard to synthetic vaccine constructs, use of rather small synthetic T cell epitopes possesses several advantages compared to the classical approach using intact immunogenic proteins. These advantages include controlled manufacturing and characterisation, a defined carbohydrate to peptide ratio and eventually the promise of improved vaccine effectiveness.[94-96] However, the use of a defined T cell epitope is hampered by polymorphism of the major histocompatibility complex (MHC) resulting in diminished T cell help.[95, 97, 98] Consequently, promiscuous peptidic T cell helper epitopes (*e.g.* the non-natural Pan-DR T cell epitope; PADRE) have emerged as a strategy to address this challenge.[95, 97, 99-106] On the other hand though, α-Galactosyl ceramide (KRN 7000) recently provided an elegant approach to circumvent the use of proteinogenic carrier proteins or synthetic peptide-based T cell epitopes and the limitations thereof.

α-Galactosyl ceramide (KRN 7000) as T_H-like epitope

α-Galactosyl ceramide (α-GalCer) has gained reasonable attention in the last decades due to its diverse biological activity and potential in various applications from cancer therapy to asthma prevention.[107-111] α-GalCer, also known as KRN 7000, is a synthetic glycolipid identified from structure-activity studies of marine sponge extracts (see Scheme 1).[112-115]



Scheme 1: Structure of CD1d agonist α-Galactosylceramide (α-GalCer, KRN 7000).

There are several characteristics that render α-GalCer derivatives particularly interesting entities for the design of synthetic vaccines. Upon presentation of α-GalCer within the context of the non-polymorphic MHC-like Cd1d protein, it is a potent activator of invariant natural killer T cells (*i*NKT).[116, 117] These particular immune cells respond rapidly by secreting substantial amounts of cytokines (T_H1 and T_H2 type), hence triggering a cascade of immune cells including dendritic cells, B cells and conventional T cells.[118-123] Furthermore, *i*NKT cells can adopt a T_H-like function, thereby providing cognate help to B cells.[117, 124, 125] This in turn facilitates antibody production, class-switching and formation of immunological memory (see Figure 7), without the need of additional peptide epitopes for MHC II mediated T cell help. The mechanism of *i*NKT mediating B cell help is in large parts similar to the interaction MHC II with B cells.[117] However, CD1d targeting possesses several advantages compared to MHC molecules. A first advantage is the number of *i*NKT cells that exceed the number of naïve peptide-specific T cells.[126] Secondly, CD1d possesses little to no polymorphism (in stark contrast to MHC molecules) thus providing a more predictable immune response amongst all vaccinated individuals.[117, 127-130]

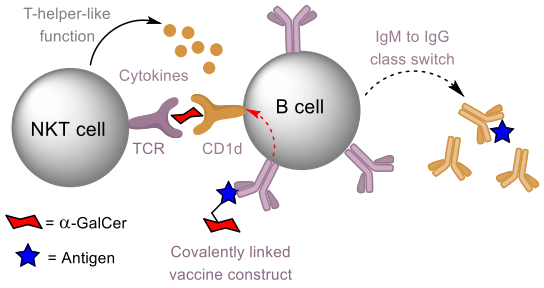
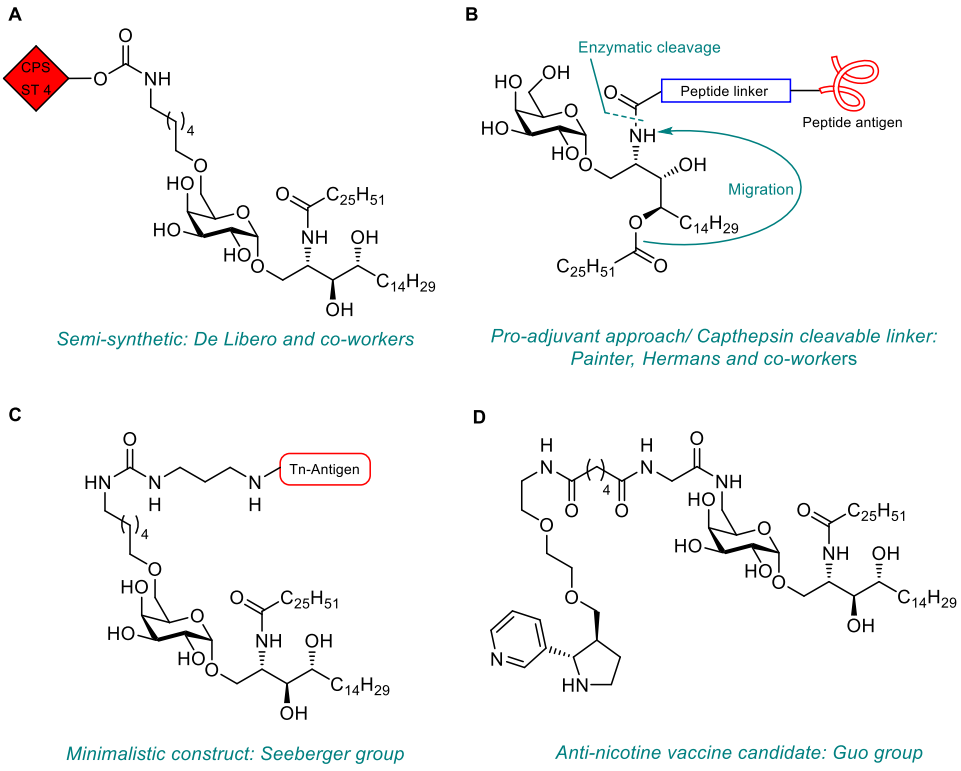


Figure 7: Schematic presentation of cognate NKT cell help for B cells (adapted from reference[117]). The α-GalCer ligand is presented by the MHC I-like molecule CD1d on the surface of B cells. Formation of an immunological synapse between the CD1d:glycolipid complex and the TCR of the *i*NKT cell stimulates cytokine release, resulting in antibody production and class switching.

Cognate *i*NKT cell mediated B cell help however demands for covalent attachment (or co-administration within the same microparticle) of KRN 7000 and the respective antigen.[117] A first example of a covalently linked, semisynthetic carbohydrate-lipid vaccine against *Streptococcus pneumoniae* serotype 4 (*S. pneumoniae* ST 4) was reported in 2014 by the group of *De Libero*.[126] *De Libero´*s vaccine showed all hallmarks of effective vaccination such as polysaccharide-specific IgG and IgM antibody generation, affinity maturation and formation of memory B cells. A lysosomal cleavable linker facilitated internalization and intracellular release of α-GalCer (see Scheme 2A). Contemporaneously, *Anderson et al.* reported on a peptide conjugate using an inactive regioisomer of α-GalCer, which becomes activated upon esterase processing and spontaneous $O \rightarrow N$ acyl migration.[131, 132] This

approach was latter developed further by *Hermans* and *Painter* utilizing a cathepsin sensitive di-peptide linker to provide sufficient serum stability and subsequent release of the active compound within antigen presenting cells (APCs; see Scheme 2B).[133] In addition, *Hermans*, *Painter* and others, demonstrated the potential of click-chemistry (copper-mediated or strain-promoted) to facilitate assembly of conjugate vaccine constructs, despite the use of triazoles has been discussed controversially.[117, 133-135]



Scheme 2: Selected examples of glycolipid conjugates comprising αGalCer derivatives as Cd1d agonist. **A**) First example of a semisynthetic covalent conjugate vaccine by *De Libero* and co-workers.[126] **B**) Pro-adjuvant approach of *Painter* and *Hermans* employing a Cathepsin B cleavable linker.[133] **C**) A minimalistic anti-tumour vaccine from the *Seeberger* group employing the small and weakly immunogenic Tn-antigen.[136] **D**) Anti-nicotine vaccine candidate from the *Guo* group.[137]

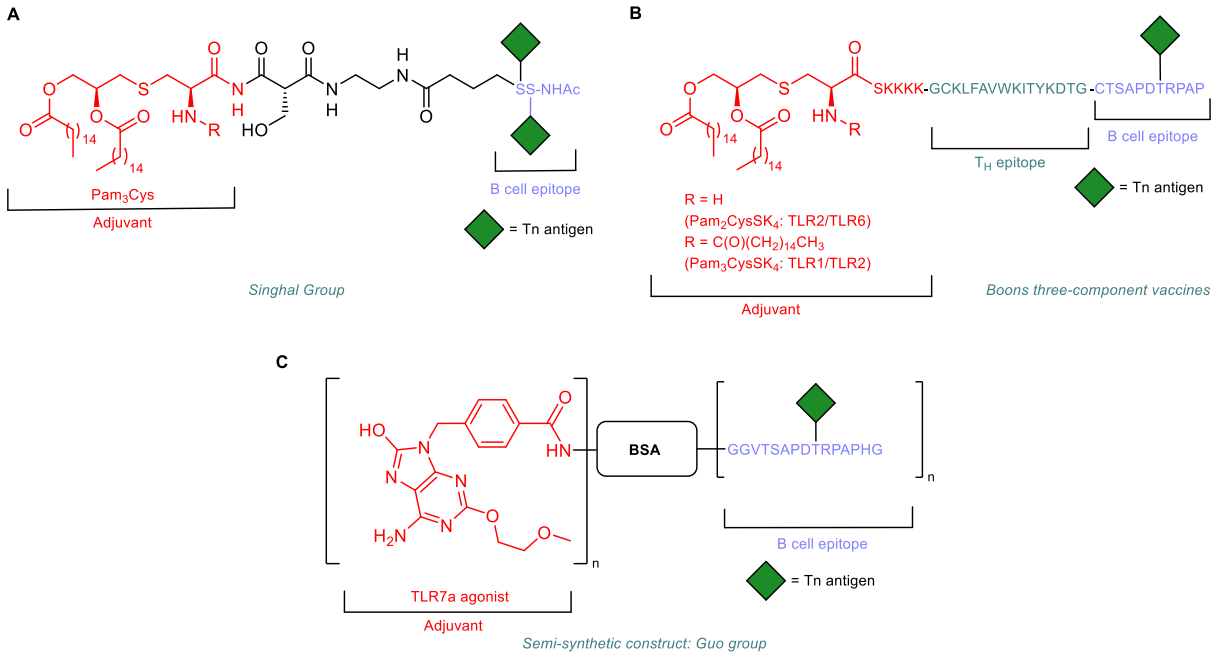
A simple but effective minimalistic carbohydrate-α-GalCer vaccine construct was introduced by *Seeberger et al.* which elicited a robust anti-glycan immune response against the small and weakly immunogenic tumour-associated Tn antigen in a liposomal and adjuvant free administration (see Scheme 2C).[136] Recently, the *Seeberger* group further demonstrated that C-6-functionalisation of α-GalCer derivatives does not significantly influence their structural properties.[138] To this end, C-6 functionalised α-GalCer derivatives have been utilized in a variety of vaccine candidates for anti-cancer immunotherapy, *S. pneumoniae* ST4, or immunopharmacotherapy (see Scheme 2D).[117, 126, 135-137, 139] These promising results illustrate the potential of α-GalCer for future vaccine designs.

Adjuvants

Subunit vaccines generally demand for additional immune potentiators (adjuvants) to evoke protective and long-lasting immunisation.[140] Classically the term adjuvant refers to "*…components added to vaccine formulations that enhance the immunogenicity of antigens in vivo*."[141] More recently, a more precise definition based on the respective molecular mode of action was proposed. Thus, adjuvants need to be divided into immune potentiators and delivery systems.[140, 141] In this regard, immune potentiators directly target innate immune system activation (e.g. toll-like receptor (TLR) ligands or cytokines), while delivery systems augment immune response by APC targeting, concentration/colocalization or depot effects (e.g. mineral salts or liposomes).

Until recently, only a handful of adjuvants have been approved for human administration.[140, 141] Mostly obtained from empirically observations, classic adjuvants are frequently hampered by side-effects or insufficient T cell responses.[14, 141] Hence, identification of immune potentiators with well-defined chemical structures and improved safety profiles are a worthwhile goal of vaccine development.[14, 142]

In this regard, TLR ligands gained reasonable attention as molecular adjuvants due to their ability to activate dendritic cells.[14, 143] Prominent examples are palmitoylated peptide derivatives such as $Pam_2Cys(SK_4)$ and $Pam_3Cys(SK_4)$, which have been employed as build-in adjuvants in a variety of synthetic anti-tumour vaccine constructs (selected examples see Scheme 3A and B).[78, 106, 144-146] A recent example that uses a build-in adjuvant in a semi-synthetic construct was reported by *Guo* and co-workers.[147] Here a small molecule TLR7a agonist and the MUC1 antigen where covalently linked to bovine serum albumin (BSA) to provide a three-in-one protein conjugate vaccine (Scheme 3C).



Scheme 3: Selected examples of (semi-) synthetic vaccines using TLR agonists as build-in adjuvants. **A**) and **B**): Synthetic multi-component anti-tumour vaccine candidates comprising TLR ligands as build-in-adjuvants. **C**) A recent example of a semi-synthetic three-in-one protein conjugate with a small-molecular TLR7a agonist.

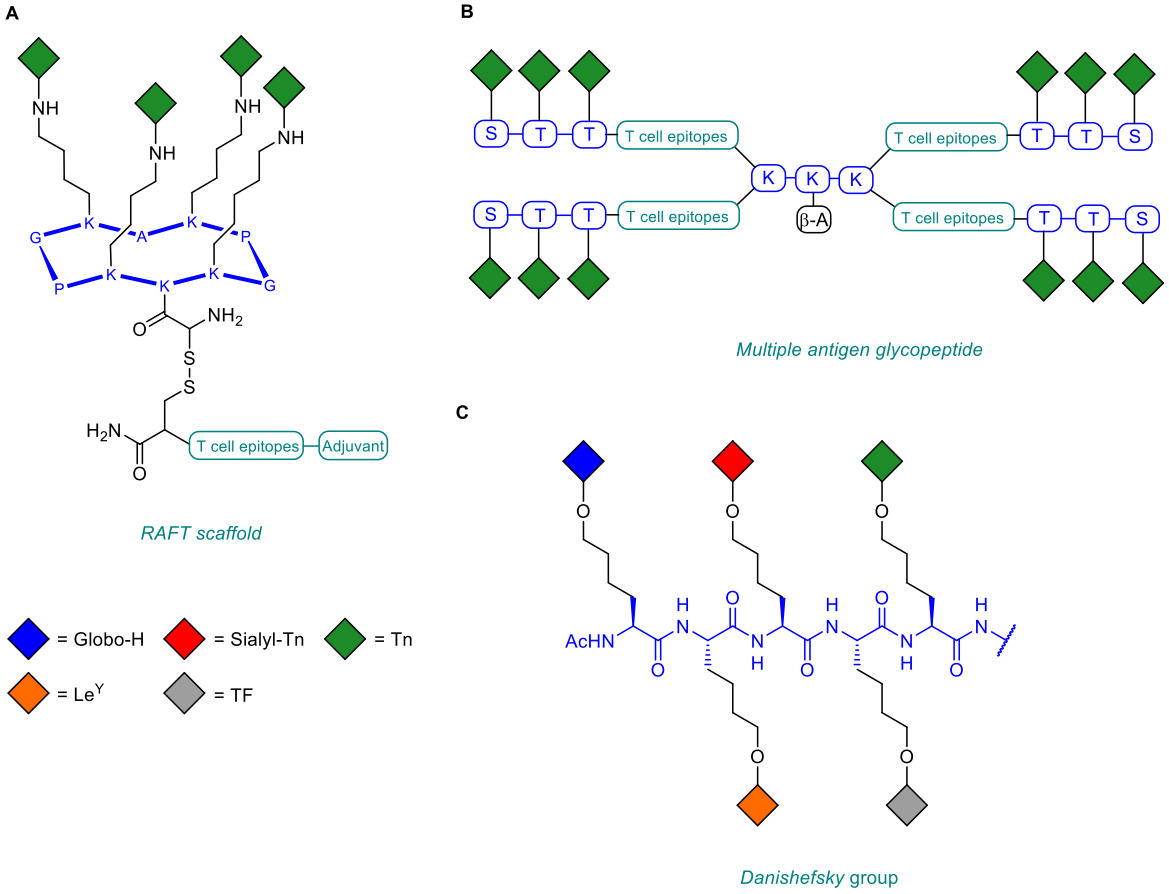
In addition, the previously described KRN 7000 glycolipid gained considerable attention as molecular adjuvant in modern vaccine formulations.[117, 142] Here its ability to effectively activate *i*NKT cells, which serve as bridging point between innate and adaptive immune system, enabled strong antigen-specific immune responses while surpassing the need for further external adjuvants.[117, 126, 135, 136, 142, 148] Hence, KRN 7000 derivatives hold great potential for the development of structurally simplified but yet effective fully- or semisynthetic vaccines candidates.

Multivalency

Protein-carbohydrate binding of single carbohydrate ligands is typically characterized by low affinity and dissociation constants in the mM range.[149] Nature addresses this limitation by presentation of carbohydrate ligands in a multimeric fashion (*"cluster glycoside effect"*), creating high-avidity interactions and dissociation constants up to a nM range.[149, 150] In regard to glycoconjugate vaccines, high valency of carbohydrate epitopes was found to

promote B cell receptor binding and clustering, both being of importance for antigen internalization and T cell signaling.[151]

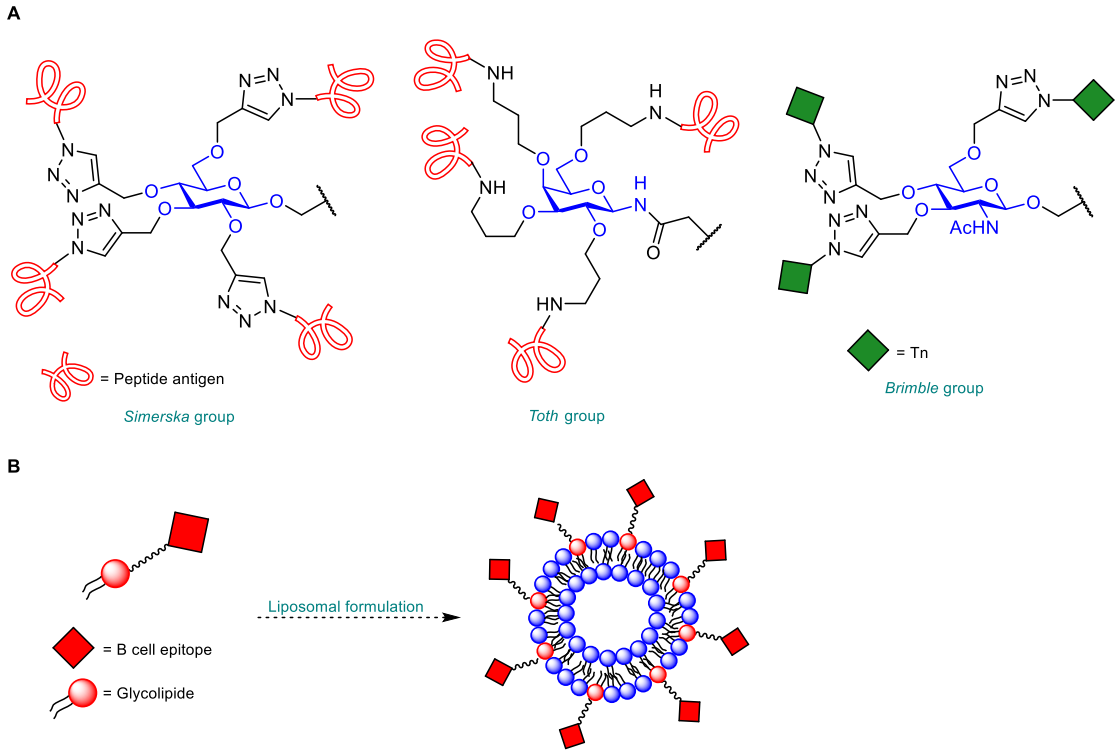
Various synthetic scaffolds for clustered antigen presentation have been developed in the last decades. For instance, *Mutter* and *Dumy* pioneered the *regioselectively addressable functionalized template* (RAFT) peptide for multivalent antigen presentation.[152-155] This non-immunogenic cyclic decapeptide elegantly enables modular presentation of immunogenic components and has been utilized in various synthetic vaccine constructs (for a selected example, see Scheme 4A).[106, 156-165] Another prominent example in this regard is the dendrimeric *multiple antigen glycopeptide* (MAG) scaffold (see Scheme 4B).[166-169] A milestone, in terms of synthetic efforts, was arguably contributed by *Danishefsky* who assembled five (up to six) different tumour-associated antigens (Globo H, Sialyl-Tn, Tn, Le^Y, TF) in a single synthetic construct (see Scheme 4C).[170]



Scheme 4: Examples of peptide-derived chassis for multivalent epitope presentation. **A**) Regioselectively addressable functionalized template (RAFT) pioneered by *Mutter* and *Dumy*.[106] This non-immunogenic cyclic peptide allows a regioselective assembly of multivalent vaccine candidates. **B**) The multiple antigen glycopeptide (MAG) for multivalent antigen presentation in a dendritic fashion.[167] **C**) Synthetic milestone by the *Danishefsky* group: A fully synthetic antitumor vaccine comprising multiple different carbohydrate antigens on a single peptide scaffold.[170]

In addition to these peptide derived scaffolds, carbohydrate derivatives emerged as promising chassis to create multivalency artificially. It was proposed that spatial antigen arrangement can be influenced by the stereochemistry of the respective carbohydrate anchoring point, thus allowing for optimized epitope presentation.[171] To this end, glucose- and galactose based chassis have been utilized to create epitope clustering and underlining their potential for future vaccine design (selected examples see Scheme 5A).[171-176]

A rather convenient method to create multivalence is liposomal formulation, which was used for instance by *Savage* and co-workers to create a multivalent vaccine candidate against *S. pneumoniae* ST 14.[177] It´s worth mentioning, that liposomal-formulation is usually employed for α-GalCer conjugate vaccines, suggesting a similar antigen presentation (see Scheme 5B).[117, 136]



Scheme 5: **A**) Examples for glucose and galactose derived scaffolds for spatial antigen-arrangement in a multivalent manner.[172, 174, 175] **B**) Simplified illustration of multivalency provided by liposomal formulation.[117, 136, 177]

Synthetic polysaccharides in vaccine development

Structural uniqueness and dense expression on the surface of cells (malign tumour cells or pathogens) render polysaccharides particularly attractive target structures in vaccine development.[81] Furthermore, carbohydrate-specific antibodies are generally held accountable for mediating immune protection against encapsuled pathogens.[14] Thus, the ability of eliciting carbohydrate-specific antibodies is essential for the effectiveness of anti-bacterial vaccines.

As discussed earlier, isolation and conjugation of naturally derived glycan antigens is generally associated with severe limitations regarding sample heterogeneity, stability and quality control.[81] In principle, these limitations can be elegantly addressed by chemical synthesis to provide considerable amounts of well-defined and thoroughly characterized glycan epitopes with little to no batch-to-batch variability. In addition, linker molecules installed at the reducing end of these epitopes enable a controlled conjugation to a carrier of choice under preservation of structural integrity.[14] Hence, synthetic carbohydrate antigens hold the promise of advancing carbohydrate vaccines, which has been impressively illustrated by the Cuban *Haemophilus influenzae* type b vaccine.[12-14]

Nevertheless, synthetic glycotopes come with their own unique challenges. For instance, polysaccharides isolated from natural sources are structurally divers and thus have a high probability of harbouring a suitable epitope

ensuring protective immunization. In contrast, homogeneous and comparatively small synthetic glycan epitopes do not necessarily comprise the respective protective epitope.[81] Thus, a more profound knowledge about protective substructures and their respective immunogenicity is of fundamental importance. Accordingly, identification of minimal protective epitopes is particularly relevant to reduce the synthetic effort (and costs) involved.[81]

Carbohydrate epitopes can be generally divided into two distinct classes: conformational and sequential epitopes. Conformational epitopes, require a distinct spatial arrangement to induce protective immune response (e.g. helical *Group B Streptococcus* type III CPS).[81, 178, 179] Here, large fragments are necessary to induce efficient immune responses.[180, 181] Given the challenges associated with glycan synthesis of increasing length, conformational epitopes are hard to address by synthetic approaches. In contrast, sequential epitopes are recognized by their primary structure and protective immunization can be achieved by relatively small epitope fragments.[182-188] Consequently, substantial efforts have been dedicated to unveil minimal protective glycotopes of bacterial pathogens comprising those sequential epitopes.[14, 81] Classically, this task has been approached iteratively.[182, 188-190] However, glycan microarrays, surface plasmon resonance (SPR) and saturation-transfer difference (STD)-NMR recently fostered a more efficient elucidation of minimal protective epitopes and crucial recognition elements.[81, 187, 191-195]

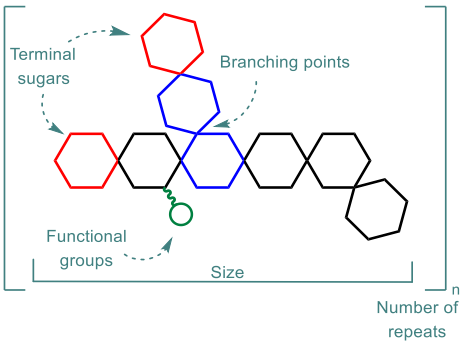


Figure 8: Immunogenic determinants of synthetic carbohydrate antigens: epitope size, terminal sugar (e.g. rare sugars), branching points, functional groups, number of repeating units (according to *Anish et al.*[81]).

Thus far, several factors have been identified or hypothesized to influence the immunogenicity/effectiveness of carbohydrate antigens (see Figure 8). Thus, anomeric configurations, glycan sequence as well as branching points and substituents may have crucial influence on the immunogenicity of antigens.[81, 196, 197] In addition, it is assumed that terminal glycans may have a larger influence on immune recognition than internal structures.[198-201] For instance, rare bacterial sugars located at an exposed side of the antigen might render it non-self and provide enhanced immunogenicity.[199] Indeed, inclusion of exposed rare sugars into epitopes has been occasionally reported to be vital for immune recognition. [202-204]

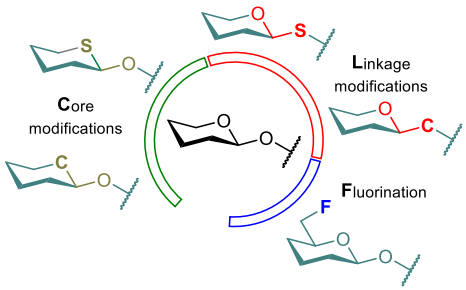
In contrast, carbohydrates that mimic a self-character (e.g. sialic acid) may diminish their immunogenicity.[73] For instance, *Kasper* and co-workers observed enhanced IgM to IgG class-switching upon utilizing desialylated analogues of *Group B Streptococcus* type V CPS in a glycoconjugate vaccine.[205] In this regard, the authors suggested that "*Targeted chemical engineering of a carbohydrate to create molecule less like host self may be a rational approach for improving other glycoconjugates.*"[205] Of course, enhanced immunogenicity does not necessarily provide improved protection (e.g. non-protective epitopes) and thus the influence of modifications onto immunological properties need to be carefully examined.[199] Nevertheless, chemical modification of given

antigenic structures has recently emerged as an exciting field in vaccine development and will be presented in further detail in the next section.

Chemical modified carbohydrate mimetics

Rapid degradation of natural saccharides by digestive, plasma and cellular glycosidases has ever been a challenge associated with the use of carbohydrate derived therapeutics.[15] In view of using minimal protective glycotopes in synthetic vaccines, *in vivo* degradation also constitutes a point of concern, as it may lead to a loss of crucial recognition elements and may hamper antibody specificity and effectiveness of the immune response.[206]

Chemically-modified mimetics of known antigenic structures have thus come to the fore as fascinating entities to improve antigen stability and preserve crucial recognition elements. In this regard, glycomimetics comprising non-natural glycosidic linkages such as *C*-glycosides[207-212], *S*-glycosides[213-216] as well as core modifications including carbasugars[217-225] and thiasugars[226] have been reported amongst others[160, 227-229] to enhance *in vivo* stability (see Scheme 6). However, the unique structural properties of *O*-glycosidic linkages, render imitation of native antigens in terms of size and conformation not an easy task. For instance, substitution of oxygen by sulphur causes an increase in bond length and weakens stereoelectronic effects. This in turn can result in a higher conformational flexibility around the glycosidic bond and formation of non-natural conformers.[213, 230, 231] Consequently, biological activity of such analogues is hard to predict and may be either diminished or in some cases enhanced due to a more distinct non-self character.[213]

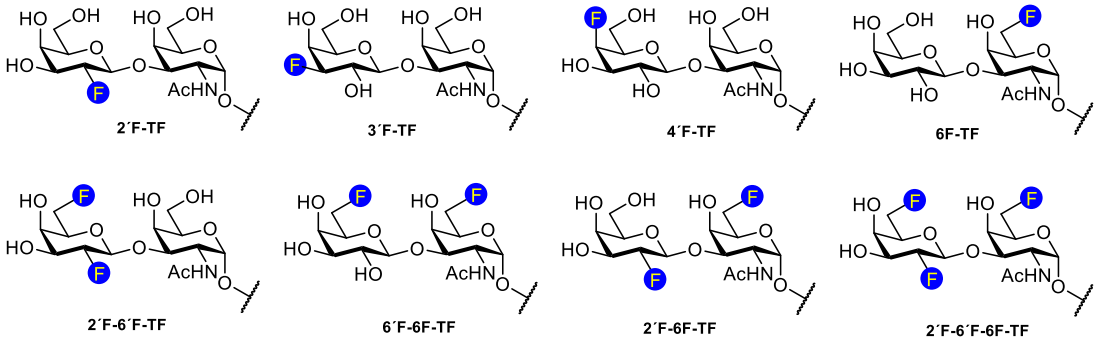


Scheme 6: Selected examples of carbohydrate mimetics. Common classes of glycomimetics include alterations of the core structure (depicted in green), linkage modifications (red) or substitution of defined hydroxy groups by fluorine atoms (blue).

Another point of consideration is the generally poor quality of antibody response directed against carbohydrate antigens.[206] Enhancing the intrinsic immunogenicity of carbohydrate antigens using chemical derivatization has gained reasonable attention for the development of anti-tumour vaccines.[232] Here, immune tolerance against self-antigens marks tumour-associated carbohydrate antigens (TACA) poor immunogens. [206, 232, 233]

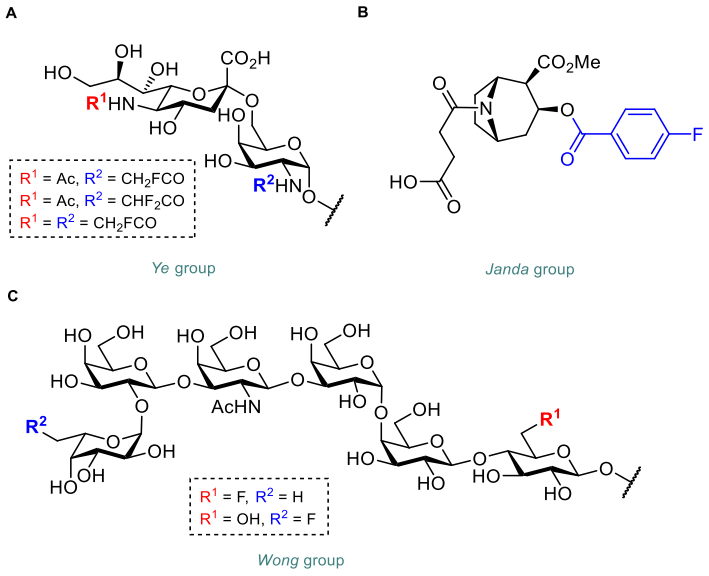
From a conceptional point of view, fluorination of such antigenic structures is particularly interesting due to the unique properties of fluorine. For instance similar *van der Waals* radii (F: 1.47 Å, O: 1.52 Å) make fluorine a known bioisoster for hydroxy groups.[234-236] In addition, the destabilizing effect of fluorine onto the oxocarbenium ion transition state has been utilized earlier by *Withers* and co-workers in the development of mechanism-based glycosidase inhibitors.[237-240] Besides that, a more recent work of *Hoffmann-Röder* and co-workers provided evidence for enhanced *in vitro* stability of a C-4´-fluorinated Thomsen-Friedenreich antigen analogue (4´F-TF, see Scheme 7) against β-galactosidase degradation.[241] Immunization of mice with the respective 4´F-TF-MUC-TTox conjugate, elicited IgG antibodies, cross-reactive with MUC1 epitopes on MCF7 human tumour cells. Comparable

results were found for a 6´F-6F-TF-MUC-TTox conjugate.[241, 242] Interestingly, sera from immunization with 6´F-6F-TF-MUC-TTox conjugate or its non-fluorinated TF[6]-MUC1-TTox counterpart showed very little differences in the recognition of glycopeptide-BSA conjugates comprising fluorinated TF-antigen analogous (Scheme 7: 3´F-TF, 2´F-6F-TF, 2´F-6´F-6F-TF, 2´F-6´F-TF, 6F-TF, 2´F-TF).[242-244]



Scheme 7: Fluorinated analogous of the Thomson-Friedenreich antigen (TF) reported by the *Hoffmann-Röder* Group.[241-244]

Given the absence of covalently bound fluorine from most organisms, it has long been discussed that fluorination might increase the non-self-character of a given antigen and can thus be utilized to enhance its immunogenicity.[206] This hypothesis was supported by the findings of the *Yu* group, observing significantly increased *anti*-STn IgG titres as well as an improved IgG to IgM ratio in mice when using a fluorine-modified STn antigen analogue (see Scheme 8A).[245] A further example underlining the potential of fluorination to enhance the immunogenicity of small molecular haptens has been reported by *Janda* and co-workers.[246] Here a succinyl norcocaine (SNC) derivative comprising a mono-fluorinated benzoyl substituent was found to elicit higher anti-cocaine antibody concentrations (compared to parent SNC), while retaining a potent cocaine affinity (Scheme 8B).



Scheme 8: Examples of fluorinated haptens: **A**) Fluorinated STn antigen analogues from the *Ye* and co-workers.[245] **B**) Fluorine containing small molecule hapten for the development of an *anti*-cocaine vaccine by *Janda* and co-workers.[246] **C**) Fluorinated Globo H analogues of *Wong* and co-workers.[247]

Despite these encouraging results, correlations (if there are any) between antigen fluorination and immunogenicity are far from being fully understood. For instance, *Wong* and co-workers observed a clear dependence between immunogenicity modulation and the position of the fluorine substituent in Globo H mimetics (see Scheme 8C).[247]

Thus, fluorination of the C-6-Position of glucose at the reducing end resulted in antibody titres comparable to the native Globo H antigen, while titre induced from an analogue comprising a more exposed C-6-fluorinated fucose moiety at the non-reducing end showed lower reactivity against Globo H.

Bacterial pathogens use molecular mimicry to induce immune tolerance by expressing carbohydrate structures similar to host-self glycans.[14] Here fluorination may provide a powerful tool to emphasize the "foreign" character of such antigens and support effective immune response.[14] However, this potential benefit of fluorination with regard to poorly immunogenic pathogenic epitopes, has barely been investigated.[162, 248-252] Thus, the development of effective synthetic approaches towards well-defined and selectively fluorinated antigen mimetics is a fundamental prerequisite to further explore the influence of this modification onto immunological properties of antigens (tumour-associated or pathogen-derived).

I.1.3 Streptococcus pneumoniae and serotype 8

Streptococcus pneumoniae (SP) is a lancet-shaped, gram-positive and facultative anaerobic bacterium which mostly occurs as diplococci but may also appear single or in short chains.[199, 253] Inhabiting in the upper respiratory tract as part of the normal flora, asymptotic carriage of this pathogen is especially high in children (20 – 50 % of healthy children) and plays a fundamental role in pneumococcal spread (see Figure 9A).[253-255] Transmission of infection occurs *via* respiratory droplets and epidemic spreads may occur in closed populations such as day care facilities.[256-259] Invasive pneumococcal diseases (IPDs) such as pneumonia, sepsis and meningitis are still a threat for human live, especially in the high-risk groups (immunocompromised patients, the elderly and children). For instance, during influenza pandemic SP infections has been associated to lethal courses and IPD are still a major cause for mortality and morbidity in HIV patients.[199, 260, 261] Just recently, infections with SP have also been discussed in connection with COVID-19 due to indistinguishability of radiological pattern that might lead to misdiagnosis and delayed therapy.[262, 263] The risk for IPD appears especially high in regions with poor health standards and for children under the age of five. Incidence rate amongst children (< 5 years) has been estimated to be 14.5 million annual cases, with more than 800.000 deaths per year.[199, 264]

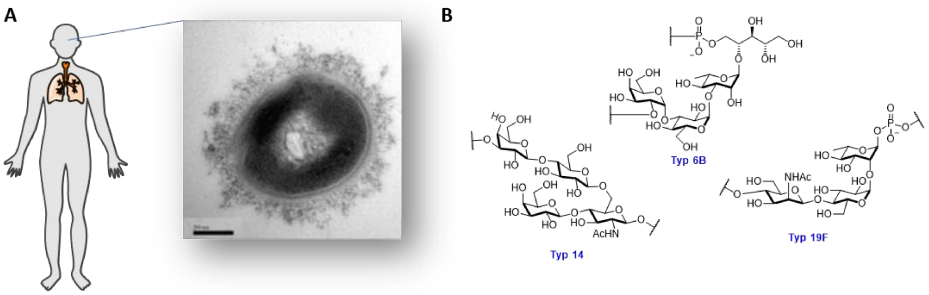


Figure 9: **A**) EM picture of encapsulated TIGR4 strain (ST4) from *Hyams et al.*[265] **B**) Selected examples of SP repeating units, illustrating their chemical diversity.

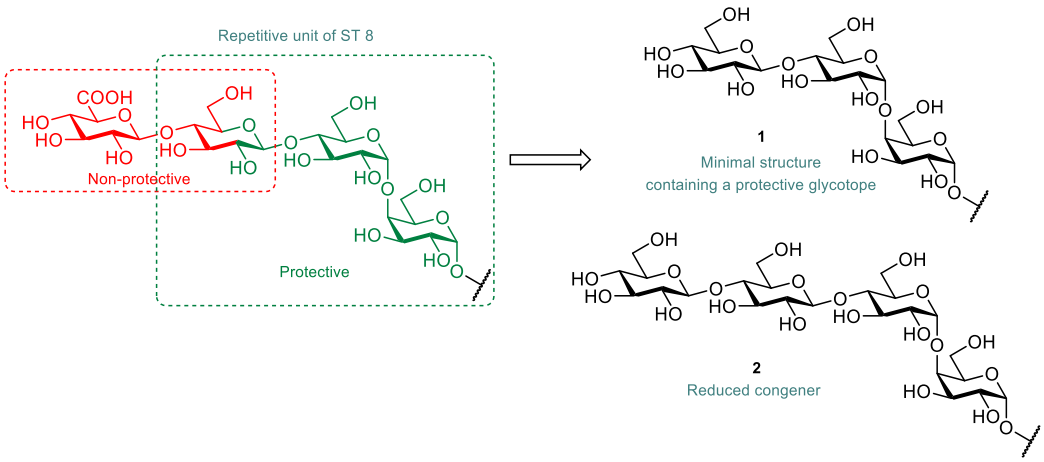
The bacterial cell membrane of SP is covered by several layers of peptidoglycans, with the outmost layer being formed by the capsule.[266] This capsule consists of a high-molecular-weight polymer built from oligosaccharide repeating units (CPS, 2-8 monosaccharides per repeat).[267] The chemical composition of these repeats is highly diverse and allows for a categorization of SP into more than 90 different serotypes.[268] Antigenically closely related

types are thereby summarized within a certain group and marked by alphabetic characters (*e.g.* serotype 9 consists of 9A, 9L, 9N and 9V; examples see Figure 9B). However, cross-reactivity between these subtypes varies between groups.[269] The capsule of SP has long been recognized as indispensable for virulence, although further factors might be needed for full virulence.[267, 270, 271] Here, capsule structure and to a lesser extend its quantity are determinants for SP virulence and removal of the capsule has been reported to abolish virulance.[266, 270-272] Besides being involved in resisting phagocytosis, the capsule contributes to antibiotic tolerance by withstanding antibiotic induced autolysis.[266, 273] Interestingly, the latter effect appeared to be serotype-dependent.

It is assumed that most, if not all of the 90 SP serotypes induce serious disease in human.[269] However, significant differences in their potential of causing infections has been reported, which suggests that only a limited number of serotypes accounts for most cases.[267, 269] Serotype 8 (ST 8) is a highly invasive pneumococcal serotype and IPDs caused by this serotype occur mainly amongst adults.[269, 274, 275] Broad resistance against common antibiotics and invasive outbreaks in high-risk groups has been often reported in connection with this particular serotype.[276-279]

A first discovery of ST 8 dates back to 1928, when *Neil* and co-workers reported on a virulent *"...strain of Pneumococcus which is related but not identical with typical Type III strains."*[280] Classification as distinct serotype followed one year later.[281] The chemical structure of the ST 8 CPS was first elucidated by *Perry* and co-workers, who reported that *"...S VIII polysaccharide is essentially a linear chain, high molecular weight polymer with the repeating unit -O-β-D-glucopyranosyluronic acid-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-O-α-D-galactopyranosyl-(1→4)-".*[282] Recent gene cluster analyses further unpinned their findings.[268, 283]

Thus far, the polysaccharide vaccine *Pneumovax23* is the only commercially available vaccine including ST8. Regarding semi-synthetic approaches, *Schumann et al*. conducted a comprehensive study to uncover the minimal protective epitope of ST 8 using a combination of automated/conventional glycan synthesis and monoclonal antibody reverse engineering.[194]



Scheme 9: Repetitive unit of ST 8 CPS with protective (Green) and non-protective (red) glycotopes highlighted. Chemical structure of minimal structure containing a protective glycotope **1** and the reduced congener **2**.[194]

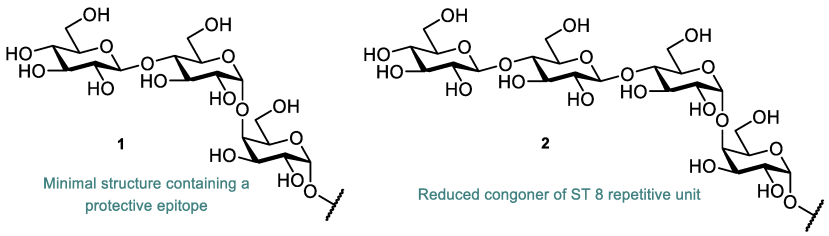
A first major finding of this study was that the terminal glucuronic acid of the repetitive unit of ST 8 is part of an immunodominant but non-protective glycotope that does not confer cross-reactivity against ST8 CPS (see Scheme 10). Furthermore, trisaccharide (**1**), lacking the terminal glucuronic acid, was unveiled as minimal structure containing a protective glycotope against ST 8. Unfortunately, including this trisaccharide **1** in a semisynthetic CRM_{197} glycoconjugate vaccine failed to induce ST8-directed immune responses in mice. Elongation of

trisaccharide **1** by a terminal glucose moiety furnished a "reduced" congener (**2**) that lacks the negative charge of the native ST8 repetitive unit (**2**). A semisynthetic glycoconjugate vaccine including this epitope (**2**) showed improved, but still poor immunogenicity in mice, while inducing reliable immune response in a rabbit model. Interestingly, a more recent study published by the same group employed the native repetitive unit comprising the terminal glucuronic acid, despite its role in a non-protective epitope.[284]

Although the reasons for these findings remain unclear, *Schumann* speculated that presentation on a carrier protein may not be ideally suited for inducing antibodies against internal epitopes of ST 8.[199] Furthermore, weak glycan immunogenicity seems to be a main challenge *en route* to synthetic ST 8 vaccine candidates.[194] Consequently, further optimizations of this existing system may include glycotope presentation (e.g. fully synthetic vaccine constructs) and/or derivatization of the glycan epitopes (**1** and **2**) to enhance targeted immune responses.

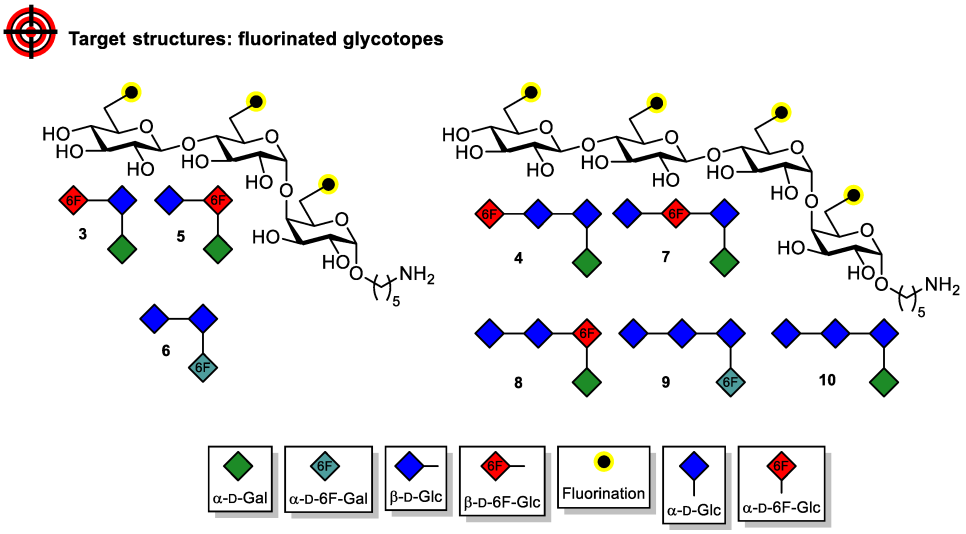
I.2 Objective part I

S. pneumoniae is a lancet-shaped bacterial pathogen that frequently causes severe invasive diseases in humans.[253-255] Based on the chemical composition of its capsule, *S. pneumoniae* can be categorized into more than 90 serotypes.[268] ST 8 is a highly virulent serotype that has been frequently reported in association with IPD in adult patients and broad resistance against common antibiotics.[276-279] Its absence from commercial pneumococcal glycoconjugate vaccines (e.g. *Prevnar 13*) further underlines the need to develop effective preventing therapies against this particular pathogen. In 2017, *Schumann et al.* conducted a comprehensive study to unveil the minimal protective epitope of ST 8 (see Scheme 10).[194] Thereby trisaccharide **1** was identified as a minimal glycan structure harbouring a protective glycotope against ST8, but poor immunogenicity hampered its use in semisynthetic vaccine formulations. In contrast, a semisynthetic glycoconjugate vaccine including a reduced congener of the ST 8 repetitive unit (**2**) showed enhanced immunogenicity in a rabbit model.



Scheme 10: Synthetic glycotopes reported by *Schumann et al.*[194]

In the course of this project, we aimed towards the synthesis of fluorinated glycotope mimetics corresponding to the known glycotopes **1** and **2**. The assumption that the C-6-position of the terminal glucose moiety should be amongst the most exposed positions of these glycotopes, lead us to embark on the synthesis of derivatives **3** and **4** comprising a C-6-fluorinated glucose moiety at the non-reducing end (see Scheme 11). Considering that positioning of fluorine might affect immune recognition[247], we further extended our targets to a complete set of hitherto unknown C-6-fluorinated tri- and tetrasaccharide mimetics (**5** – **9**).

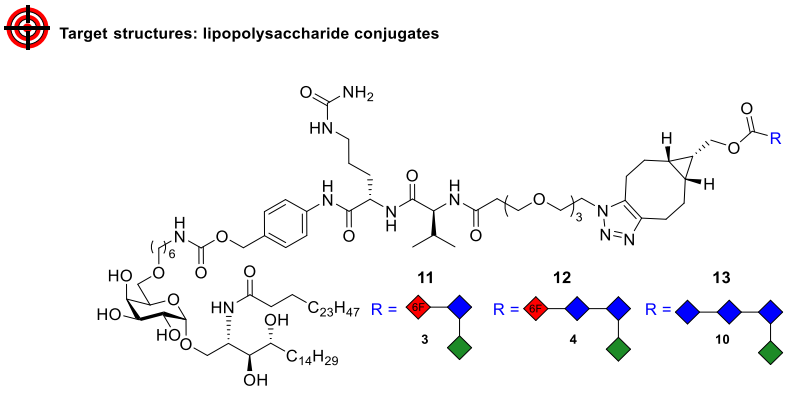


Scheme 11: Targeted library of C-6-fluorinated tri- and tetrasaccharide glycotopes.

In addition, non-fluorinated tetrasaccharide congener **10** was included, serving as a reference in future biological evaluations. In accordance with the previously reported *Seeberger* synthesis, we opted for a convergent [2+1] or

[2+2] synthetic strategy, which should facilitate the assembly of both chemically more challenging *1,2-cis* glycosidic linkages at a rather early stage of the synthesis.[194] A more detailed description of the underlying synthetic consideration is provided in the chapter "*I.3.1 Previous work and synthetic strategy*".

In a continuative collaboration with *Sebastian Neidig* from the *Hoffmann-Röder* group, a selection of these synthetic glycotopes should subsequently be used to assemble first synthetic liposaccharide conjugate vaccine candidates against ST 8 (**11** - **13**). Utilizing a synthetic KRN 7000 precursor, previously described by *Dr. Andreas Baumann*, we planned the assembly of three vaccine candidates *via* copper free strain-promoted alkyne-azide cycloaddition (SPAAC).[285, 286] Here we targeted towards vaccine candidates comprising either a non-fluorinated tetrasaccharide congener **10** or the terminal fluorinated analogues **3** and **4** (see Scheme 12).

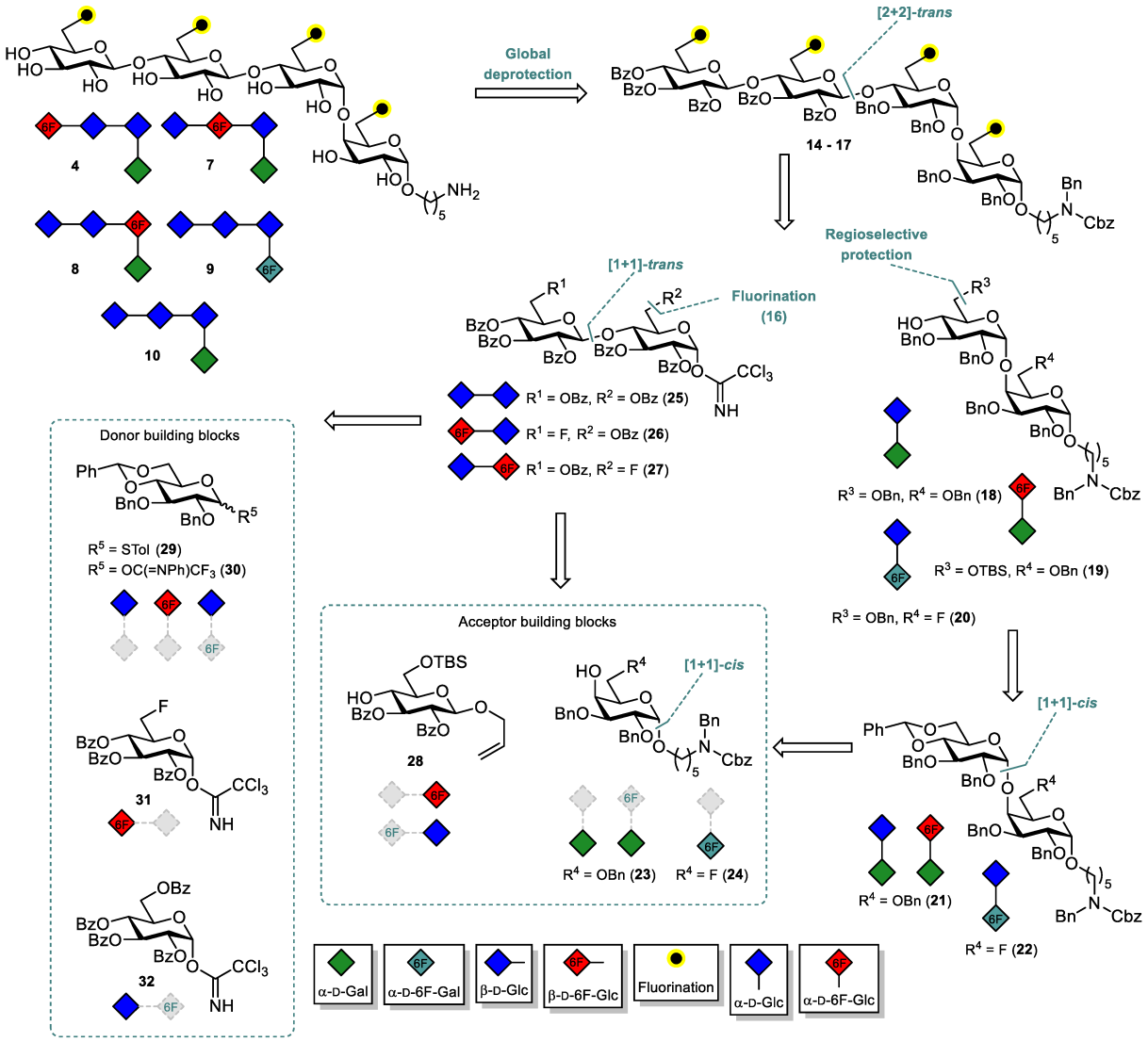


Scheme 12: Targeted conjugate vaccine candidates.

I.3 Results and Discussion

I.3.1 Previous work and synthetic strategy

First chemical syntheses of di- and trisaccharide fragments of the ST 8 CPS have been reported by *Vliegenthart* and co-workers, using a [1+1] and [2+1] synthetic strategy.[287] In *Vliegenthart´s* approach, the synthetically challenging α-D-Glc-(1 → 4)-α-D-Gal glycosidic linkage was formed during the last glycosylation step. More recently, *Seeberger* and co-workers reported the syntheses of several synthetic fragments of the ST 8 CPS.[194] Assuming that, the assembly of the α-D-Glc-(1 → 4)-α-D-Gal fragment represents one of the key challenges *en route* to a selection of fluorinated ST 8 congeners (**4**, **7** - **9**), we opted for a [2+2] synthetic strategy in accordance to *Seeberger´s* approach (see Scheme 13). Here both α-glycosidic linkages can be assembled in a single precursor (**21** or **22** respectively), while neighbouring group participation should provide full control of the stereochemistry during the final assembly of the tetrasaccharide core structure.



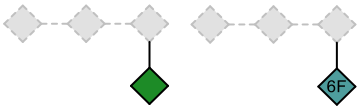
Scheme 13: Retrosynthetic considerations for the assembly of fluorinated ST8 glycotope mimetics. For ease of presentation, only the retrosynthetic analysis of tetrasaccharides (**4**, **7** - **10**) is presented. Respective fluorinated trisaccharide analogues can be accessed following a [2+1] glycosylation approach in accordance with these considerations.

Hence, fluorinated tetrasaccharides (**14** - **17**) may be accessed from the respective disaccharide acceptors (**18** -**20**) that comprise both α-glycosidic linkages of the α-D-Glc-(1→4)-α-D-Gal structural element. **18** - **20** in turn can be

easily traced back to disaccharide precursors (**21** and **22**) comprising a 4,6-*O*-benzyliden protecting group. This protecting group was chosen, as it should enable facile formation of a C-6-*O*-benzyl ether *via* regioselective benzylidene opening (**18** or **20**) or alternatively the introduction of an orthogonal protecting group for subsequent late-stage fluorination at the C-6´ position (**19**). Besides that, previous studies of *Crich* and others established an inherent 1,2-*cis* directing effect of the 4,6-*O*-benzyliden acetal protecting group in glycosylation reactions employing glycosyl donors from the mannose or glucose series (for further details see chapter *I.3.4 Synthesis of disaccharide acceptors **18** to **20***).[20, 288, 289] Hence, the 4,6-*O*-benzylidene acetal protecting group was assumed to not only facilitate regioselective protecting group manipulation but in addition support α-selectivity during assembly of precursor **21** and **22** from the respective galactosyl acceptors **23** and **24**.

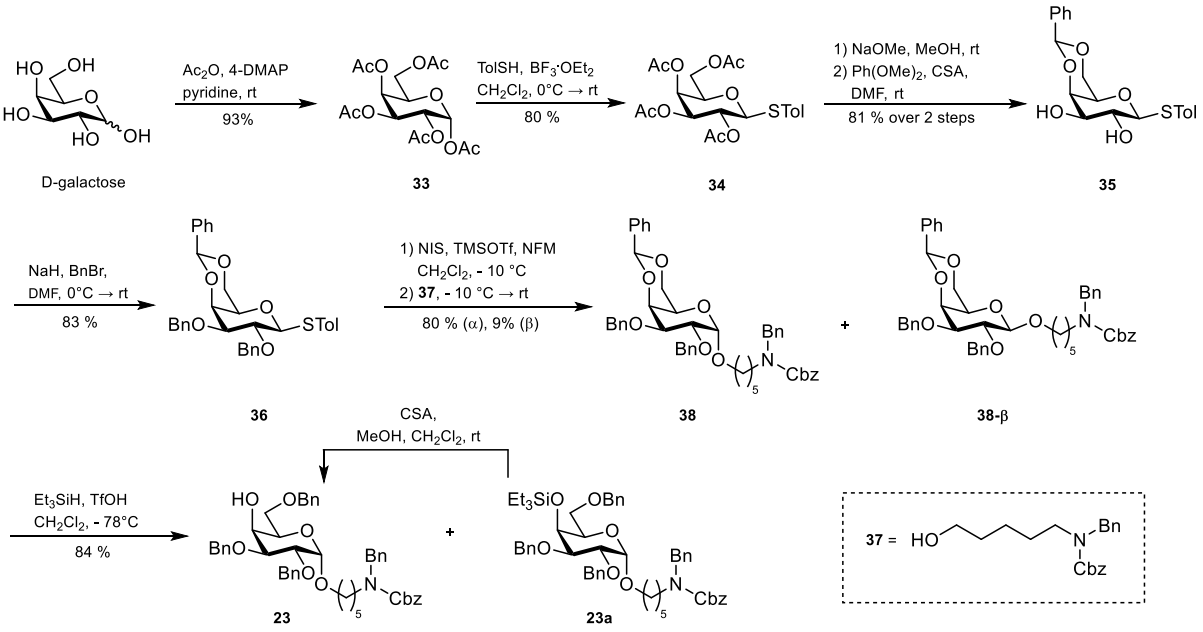
In addition, a series of cellobiose donors (**25** - **27**) can be identified as important building blocks. While the non-fluorinated cellobiose derivative **25** should be readily accessible from inexpensive and commercially available D-cellobiose, the respective fluorinated analogues (**26** and **27**) are obtainable by a neighbouring group-assisted [1+1]-*trans* glycosylation. Here an orthogonal cleavable protecting group (TBS-ether) on the monomeric glucose acceptor **28** should enable DAST-mediated fluorination after assembly of the glycosidic linkage (**27**). In summary, retrosynthetic considerations reveal a few glucose (**28** - **32**) and galactose derivatives (**23** and **24**) as key synthons to assemble our selection of C-6-fluorinated tetrasaccharides. In accordance with this considerations, C-6-fluorinated trisaccharide derivatives (**3** - **6**) should be accessible from the same building blocks, following a comparable [2+1] glycosylation strategy.

I.3.2 Synthesis of galactosyl acceptors 23 and 24



Synthesis of native galactosyl acceptor 23

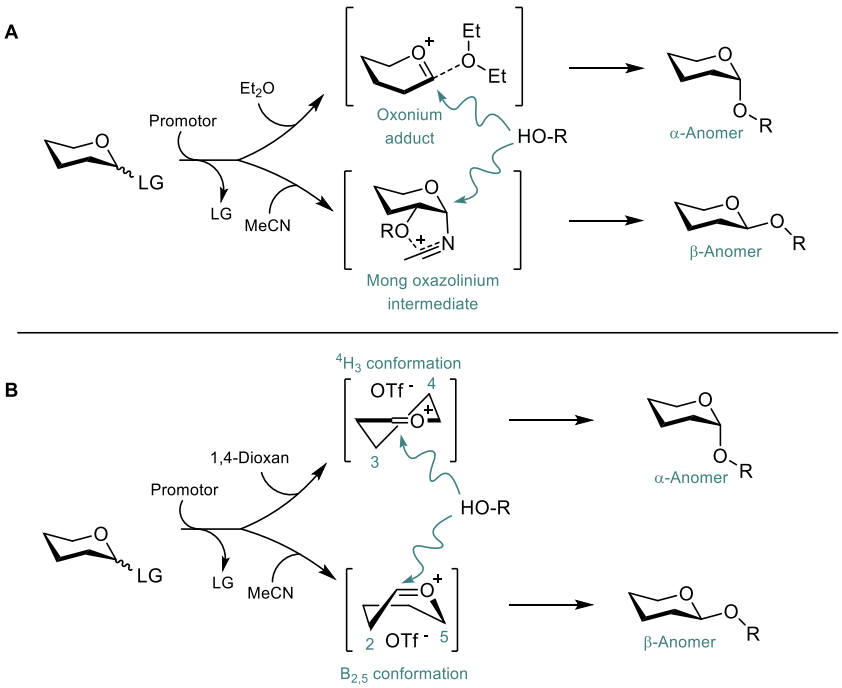
Starting from commercially available D-galactose, acceptor **23** was synthesized according to the synthetic route depicted in Scheme 14.



Scheme 14: Synthesis of galactosyl acceptor **23** over seven synthetic steps.

Thus, 4-DMAP-catalysed acetylation[290, 291] provided the penta-*O*-acetyl ester **33** in 93 % yield.[292] Thioglycoside **34** in turn was obtained from treatment of **33** with *p*-thiocresol and boron trifluoride etherate.[293] Saponification under *Zemplén* conditions[294] followed by benzylidene acetal-protection of C-4 and C-6 furnished diol **35** in 81 % yield.[295] *Williamson* etherification of **35** with benzyl bromide provided **36** after crystallization from EtOH.[295]

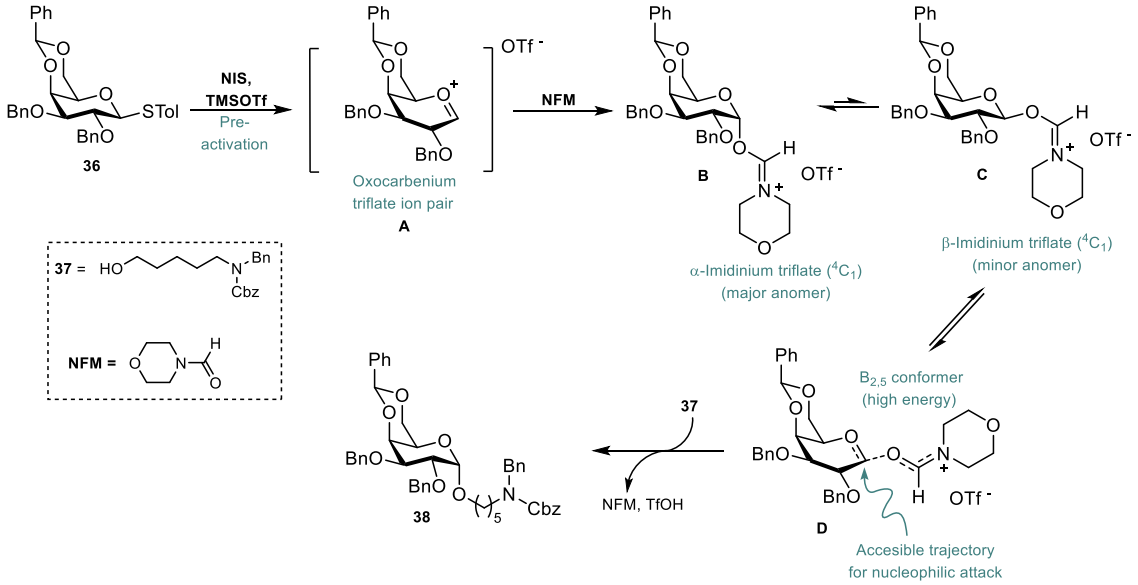
Stereoselective *1,2-cis* glycosylation of highly reactive alcohols (e.g. 5-amino pentyl alcohol derivatives) is particularly challenging due to erosion of selectivity[296] and hence represents the key step in the synthesis of **23**. Exploitation of the directing characteristics of co-solvents (e.g. Et_2O or MeCN) is a frequently used protocol to control the stereoselectivity of glycosylation reactions. In particular, ethereal solvents such as diethyl ether, THF or dioxane are known to facilitate the formation of α-glycosidic linkages, while nitrile solvents favour the formation of β-linked glycosides under kinetic conditions.[17, 297-299] These empirical observations, are generally explained by either solvent coordination or conformer and counterion distribution.[300] In this regard, the first hypothesis accounts for a spatial preference of solvent molecules upon coordination onto the intermediate oxocarbenium ion, leading to effective shielding of nucleophilic attacks from that side. In contrast, the conformer and counterion distribution hypothesis proposes conformational preferences of the oxocarbenium intermediate in different solvents as well as a solvent dependent side-preference of counter ion coordination (see Scheme 15 A and B).[300]



Scheme 15: Solvent effects in glycosylation reactions: **A**) Commonly used solvent coordination hypothesis; **B**) Alternative conformer and counterion distribution hypothesis.[17, 299, 300]

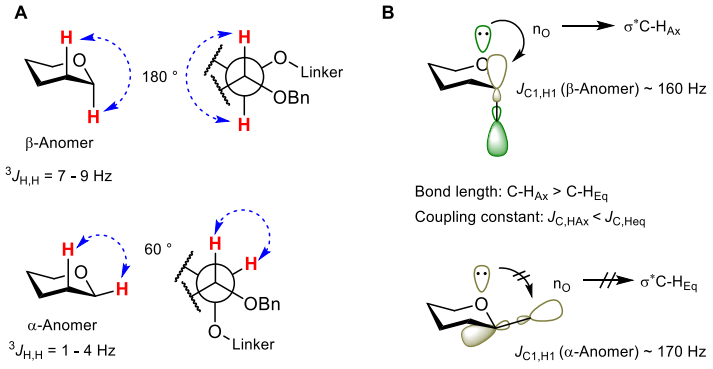
With these hypotheses in mind, thiodonor **36** was reacted in a first experiment with 5-amino pentyl linker **37**[301, 302] in a mixture of CH_2Cl_2/Et_2O using the NIS/TMSOTf promotor system. Unfortunately, the desired α-linked product **38** was isolated only as a minor product, while β-glycoside **38-β** was formed predominantly (84 % yield, α/β = 1:9). Since the highly reactive primary alcohol somehow outweighs the directing effect of Et_2O, a revised strategy based on exogenous nucleophiles to support 1,2-*cis* selectivity was applied. This concept was pioneered by *Lemieux* and *Schuerch* in the early 1970´s and a large variety of nucleophilic modulators have been reported thus far.[303, 304] In 2011, *Mong* and co-workers introduced formamide derived nucleophiles (e.g. DMF or *N*-formylmorpholine (NFM)) as universal modulators to facilitate *1,2-cis* glycosylations.[305, 306] Since then, this concept was successfully applied to different glycosyl donors and acceptors, enabling effective assembly of α-linked *O*-glycosides.[303, 306-308]

Hence, thioglycoside **36** was reacted with linker molecule **37**[301, 302] in the presence of *N*-formylmorpholine (NFM) and the targeted α-linked galactose derivative **38** was isolated in 80 % yield together with 9 % of the β-linked side product **38-β**.



Scheme 16: Schematic representation of a conceivable mechanism for the NFM-modulated glycosylation reaction used for the assembly of compound **38**.[306]

From a mechanistic point of view, it is assumed that the reaction commences with pre-activation of **36** using the NIS/TMSOTf promotor system to furnish an intermediate oxocarbenium triflate ion pair (**A**). This ion pair **A** reacts with NFM to form an equilibrium of α-galactosyl imidinium (**B**, major species) and β-galactosyl imidinium adducts (minor species, **C**). In the following, **C** may adopt a more reactive boat-conformation (**D**), which enables the formation of the desired α-linked product **38** *via* nucleophilic attack of the primary alcohol **37** (depicted in Scheme 16).[306] The anomeric connectivity of the obtained products **38** and **38-β** was assigned from $^{3}J_{H1,H2}$ coupling constants based on the *Karplus* relationship, which directly links the H1-H2 dihedral angle to the $^{3}J_{H1,H2}$ coupling constant (see Scheme 17 A). Accordingly, a coupling constant of $^{3}J_{H1,H2}$ = 7 – 9 Hz corresponds to a dihedral angel of approximately 180° as found for β-linked glycosides.[309-312] In contrast a coupling constant of $^{3}J_{H1,H2}$ = 1 – 4 Hz indicates a dihedral angle of 60° and corresponds to an α-glycosidic linkage. Consequently, the obtained coupling constant of $^{3}J_{H2,H1}$ = 3.6 Hz for glycosylation product **38** reveals the desired α-connectivity at the anomeric centre.



Scheme 17: Correlations between anomeric configuration and observed coupling constants in the ^{1}H NMR and ^{1}H,^{13}C-coupled HSQC NMR experiments. These correlations were used in due course to assign the anomeric connectivity of glycosylation products such as **38** and **38-β**. **A**) *Karplus* relationship between the H1-H2 dihedral angle and the coupling constant $^{3}J_{H1,H2}$. **B**) Schematic depiction of the *Perlin Effe*ct in carbohydrates and typical value-ranges for the $^{1}J_{C1,H1}$ coupling constants of different anomers.

This was further supported by the $^{1}J_{C1,H1}$-coupling constants derived from ^{1}H-^{13}C coupled HSQC experiments. Here the anomeric configuration can be assigned from the structural dependence of $^{1}J_{C,H}$ spin-spin coupling constants (*Perlin Effect).*[313] This correlation is generally attributed to C-H$_{ax}$ bonds comprising a larger bond length

than C-H_{eq}, due to a preferred electron delocalization among anti-periplanar oriented orbitals. Thus the $n_O \rightarrow \sigma^*_{C\text{-}Hax}$ interaction of a β-glycosidic linkage results in a larger bond length and a smaller $^1J_{C1,H1}$ coupling constant, while the absence of a suitable spatial orientation for $n_O \rightarrow \sigma^*_{C\text{-}Heq}$ in α-linked glycosides, gives rise to shorter C-H bonds and a larger $^1J_{C1,H1}$ coupling constant (depicted in Scheme 17 B).[314]

In the late 1960´s *Perlin* and *Casu* discovered a difference in the $^1J_{C1,\,H1}$ coupling constants of approximately 10 Hz between anomeric pairs of glycosides and since then extensive analysis of experimental data underpinned this effect.[315, 316] Determination of the anomeric configuration *via* the $^1J_{C,H}$ coupling constant is particularly useful in cases where the dihedral angle does not provide information about the anomeric configuration (e.g sugars from the L-mannose series) or where $^3J_{H1,H2}$ coupling constants cannot be accessed (e.g. due to signal overlapping).

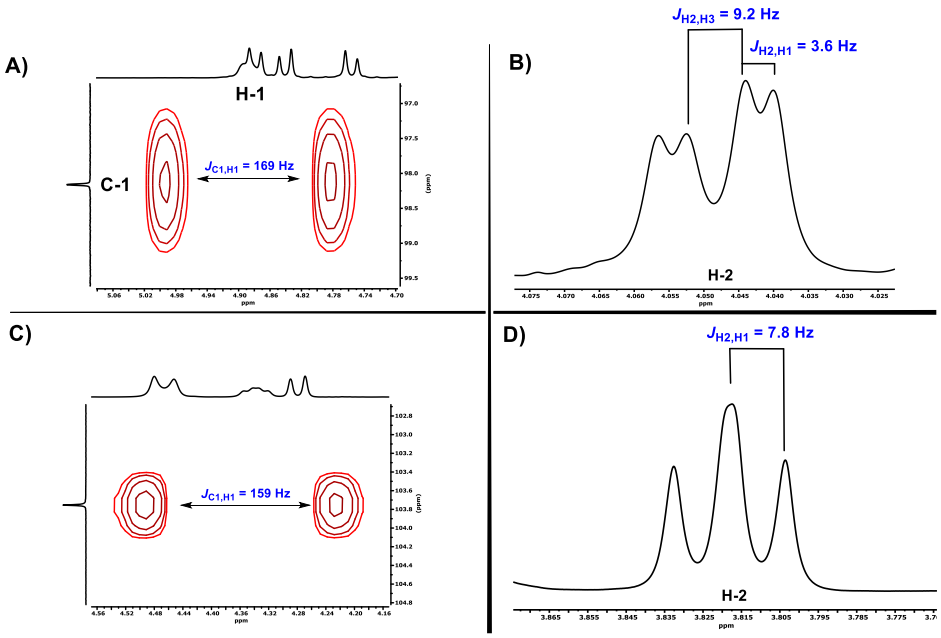


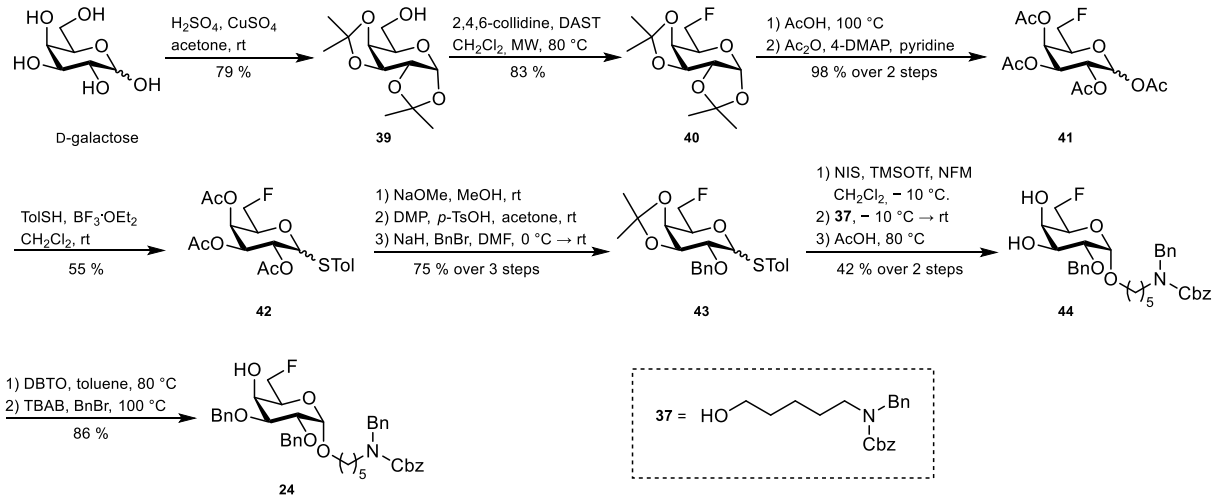
Figure 10: Excerption from NMR spectra of linker-modified products **38** and **38-β** to assign anomeric connectivity: **A**) ^{1}H-^{13}C-coupled HSQC NMR signal of the anomeric position of **38**. **B**) ^{1}H-NMR signal of H-2 (**38**) comprising a coupling constant of $^3J_{H2,H1}$ = 3.6 Hz in accordance with the *Karplus* relationship for 1,2-*cis* linked products. **C**) ^{1}H-^{13}C-coupled HSQC NMR signal of the anomeric position of **38-β**. **D**) ^{1}H-NMR signal of H-2 (**38-β**) comprising a coupling constant of $^3J_{H2,H1}$ = 7.8 Hz in accordance with the *Karplus* relationship for 1,2-*trans* linked glycosides.

In perfect accordance with the *Perlin Effect*, assignment of the $^1J_{C1,H1}$ coupling constant from a ^{1}H-^{13}C coupled 2D-HSQC NMR experiment furnished values of $^1J_{C1,H1}$ = 169 Hz for **38** and $^1J_{C1,H1}$ = 159 Hz for **38-β** ($^3J_{H2,H1}$ = 7.8 Hz) respectively (see Figure 10).

Having the α-glycosidic linkage successfully installed at the anomeric centre of **38**, subsequent regioselective benzylidene opening reaction finally provided acceptor **23**. In the last decades, numerous combinations of Lewis/Brönsted acids (e.g. $AlCl_3$, HCl, TFA) and hydride sources (e.g. $NaCNBH_3$[317], Et_3SiH[318], $LiAlH_4$[319, 320]) have been studied for this purpose, providing an extensive chemical repertoire for selective installation of primary and secondary benzyl ethers from cyclic acetals.[321] By following a synthetic protocol from *Wang* and co-workers, **38** was regioselectively converted into the C-6-*O*-benzyl ether **23** upon treatment with TfOH and triethyl silane in CH_2Cl_2.[322] However, in addition to the desired compound **23**, also significant amounts of a C-4-*O*-silyl ether by-product (**23a**) were obtained. Fortunately though, **23a** could be converted smoothly into **23** by treatment with CSA in MeOH/CH_2Cl_2 providing the desired product in a total yield of 84 %. Interestingly, formation of the C-4-*O*-silylether by-product **23a** was only observed upon up-scaling and neutralization with triethylamine.

Synthesis of fluorinated galactosyl acceptor 24

Starting from commercially available D-galactose, fluorinated galactosyl acceptor **24** was prepared over eleven synthetic steps as depicted in Scheme 18.



Scheme 18: Synthesis of fluorinated galactosyl acceptor **24** over eleven synthetic steps starting from D-galactose.

1,2:3,4-Di-*O*-isopropylidene acetal **39** was obtained from acid-mediated acetalization of D-galactose.[323] Microwave-assisted DAST fluorination furnished the respective 6-deoxy-6-fluoro galactose derivative **40** in 83 % yield.[285, 324, 325] Acidic hydrolysis of the isopropylidene acetals and subsequent acetylation gave the tetra-*O*-acetyl ester **41** in 98 % yield over two steps.[285, 324] Lewis-acid mediated thioglycosylation afforded the thioglycoside **42** in moderate yield (55 %) and as a mixture of anomers.[248] Transesterification under *Zemplén* conditions[294] yielded an intermediate tetrol, which was converted into the fully protected thioglycoside **43** upon regioselective acetalization and subsequent etherification under *Williamson* conditions (75 % yield over three steps).[326, 327] Incorporation of linker molecule **37**[301, 302] at the anomeric centre was achieved utilizing the previously described NFM-modulated glycosylation strategy[306] (see synthesis of compound **38** in previous chapter and Scheme 16). Thus, thioglycoside **43** was pre-activated with NIS and TMSOTf in the presence of NFM to form the intermediate galactosyl imidinium species. TLC monitoring indicated smooth conversion of the starting material to the imidinium intermediate after 1.5 h at – 10 °C. However, subsequent nucleophilic attack of **37** proceeded slowly and even after two days at ambient temperature, significant amounts of remaining imidinium intermediate were observed by TLC monitoring. Presumably, the reduced reactivity of the imidinium adduct is a result of the electron withdrawing character of the fluorine substituent at the C-6 position of **43**. Nevertheless, the NFM-modulated glycosylation protocol yielded the desired α-linked product albeit not in its pure form. Consequently, product containing fractions obtained from column chromatographic purification were pooled and subjected to acidic cleavage of the isopropylidene protecting group[324] to afford pure diol **44**. Assignment of the anomeric connectivity of **44** furnished a coupling constant of $^{3}J_{H2,H1}$ = 3.5 Hz together with a $^{1}J_{C1,H1}$ = 171 Hz coupling constant, both demonstrating the desired 1,2-*cis* configuration at the anomeric centre (see Figure 11).[316, 328]

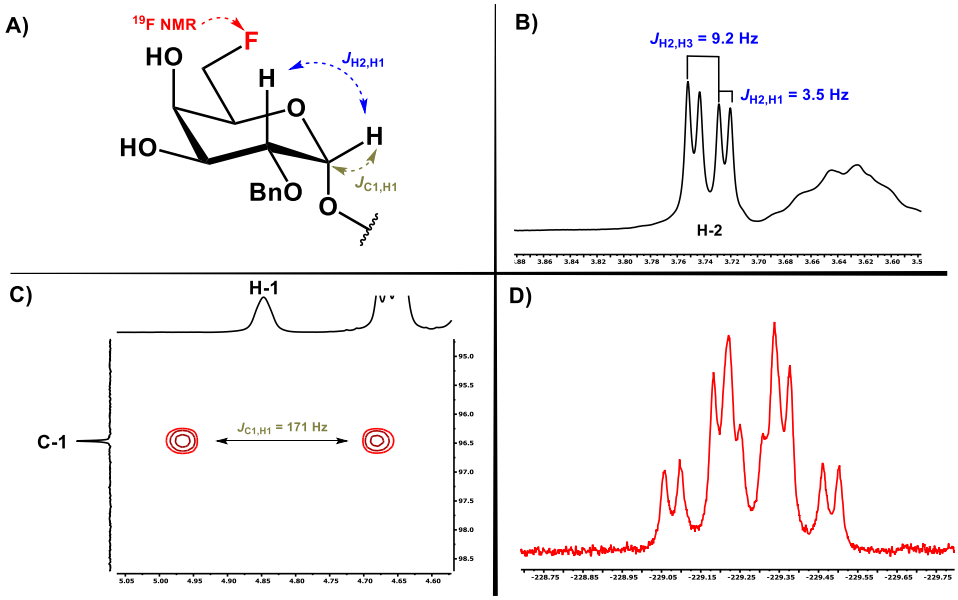
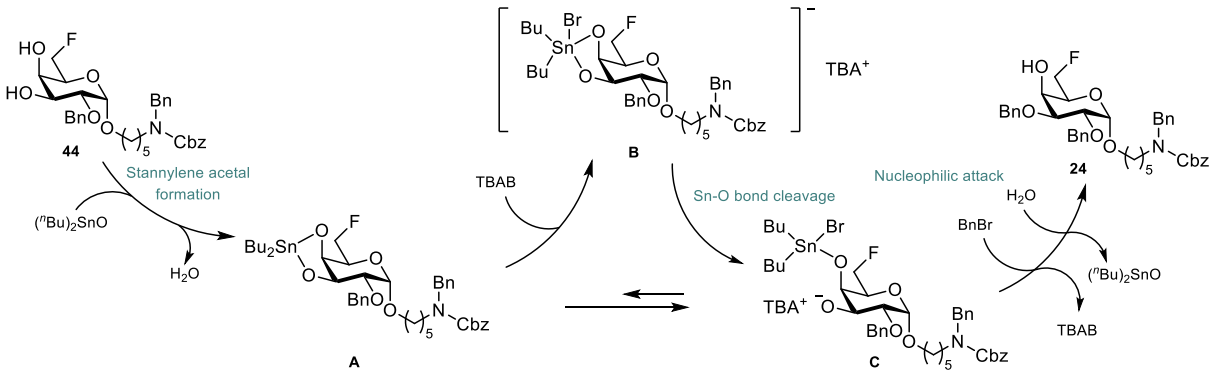


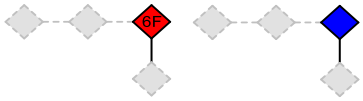
Figure 11: Depiction of relevant signals showing the correct regiochemistry of fluorinated compound **44**: **A**) Schematic representation of C-6-fluorinated galactose comprising the demanded 1,2-*cis* glycosidic linkage. **B**) Zoom into ^{1}H-NMR spectrum showing the signal of the H-2 proton and its $^{3}J_{H2,H1}/^{3}J_{H2,H3}$ coupling constants. **C**) Excerpt from its ^{1}H-^{13}C coupled HSQC spectrum showing the $^{1}J_{H1,C1}$ coupling constant of 171 Hz, typical for the desired *1,2-cis* glycosidic linkage. **D**) Excerpt from ^{19}F-NMR spectrum.

Finally, diol **44** was transformed into the C-6-fluorinated galactosyl acceptor **24** by regioselective etherification of the C-3 hydroxy group. Protocols for regioselective formation of alkyl ethers (besides reductive ring opening of benzylidene acetals[321]) usually demand for additional reagents (e.g. of tin[329, 330], boron[331], nickel (II)[332] and iron(III)[333, 334]) to foster selectivity. Thus diol **44** was treated with *n*-dibutyl tin oxide to form the intermediate stannylen acetal *in situ* (**A**, Scheme 19).[330] Subsequent addition of TBAB promotes selective Sn-O bond cleavage of the stannylene acetal ring *via* a pentacoordinated tin atom (**B**). The resulting anion **C** acts as a nucleophile to furnish the desired benzyl ether **24** (86 % yield). Even though an excess of benzyl bromide was utilized in the reaction, no etherification of the C-4 position was observed.[335]

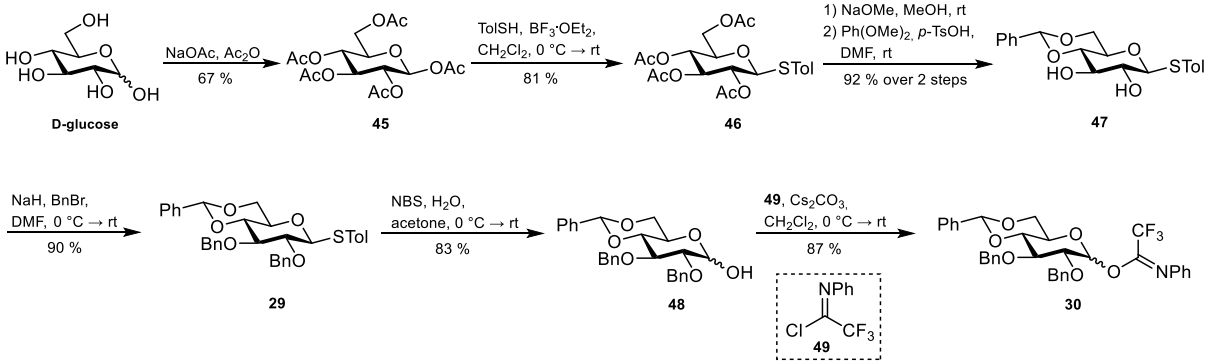


Scheme 19: Proposed mechanism for regioselective *O*-alkylation of the C-3 position of compound **44**.[335]

I.3.3 Synthesis of glucosyl donors 29 and 30



Starting from commercially available D-glucose, glucosyl donors **29** and **30** were synthesized in five or seven synthetic steps, respectively, as depicted in Scheme 20.

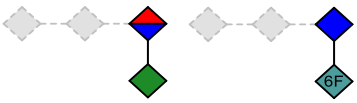


Scheme 20: Synthesis of glucosyl donor **29** and **30** from D-glucose.

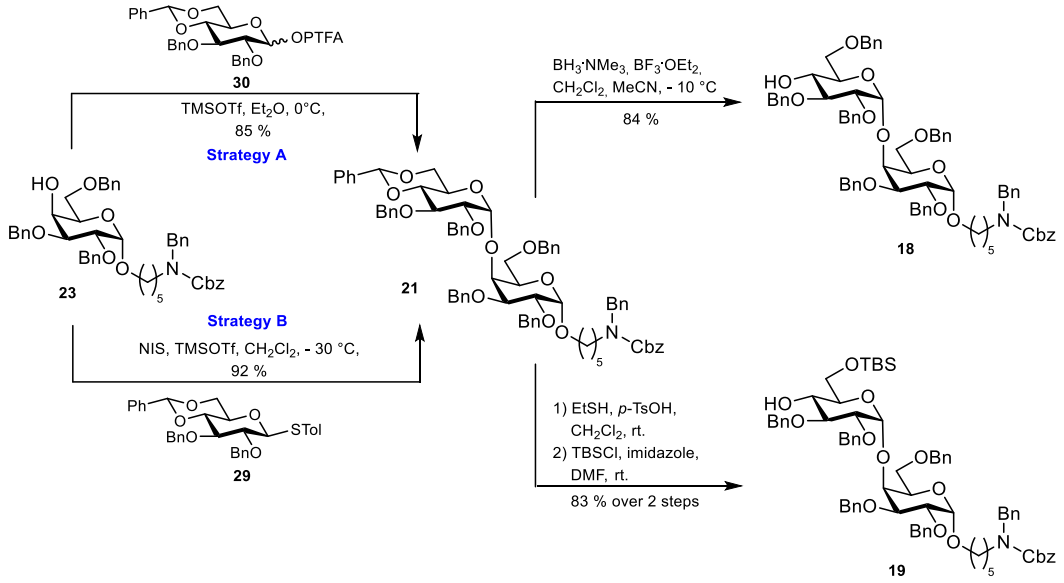
The syntheses commenced with peracetylation of D-glucose in acetic anhydride in the presence of sodium acetate to afford **45** after precipitation from water.[336] Thioglycoside **46** in turn was obtained from Lewis acid-mediated thioglycosylation of **45** in 81 % yield.[337] Transesterification under *Zemplén* conditions[294] followed by acetalization (**47**) and etherification gave the fully protected glucose derivative **29**, which was conveniently purified by crystallization from EtOH.[338, 339] It is worth mentioning that thioglycoside **29** was also employed as glucosyl donor in subsequent glycosylation studies (*vide infra*). Additionally, **29** was further derivatized to furnish *N*-phenyltrifluoroacetimidate (PTFA) donor **30**. Accordingly, NBS mediated anomeric deprotection[340] (**48**) and subsequent *O*-alkylation with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (**49**) and caesium carbonate effectively provided PTFA donor **30** in 87 % yield.[341, 342]

Since being first introduced almost 20 years ago by *Yu* and co-workers, PTFA donors found broad application in glycosylation reactions and many catalysts (e.g. TMSOTf [343], $BF_3{\cdot}OEt_2$[344], $Yb(OTf)_3$[345] and $TMSB(C_6F_5)_4$[346]) have been developed for their activation. In comparison to trichloroacetimidates (TCAs), this class of glycosyl donors generally shows a reduced reactivity and may be better suited for glycosylation reactions of rather unreactive acceptors.[347, 348] In particular, steric shielding of the nucleophilic imidate nitrogen by the phenyl substituent hampers formation of glycosyl amides arising from acid-mediated rearrangement. This side-reaction is often encountered in glycosylation reactions employing TCA donors in combination with weak nucleophiles.[25] Bearing in mind, that the C-4 position of galactose is the least reactive, PTFAs have been regarded particularly promising for the assembly of the α-D-Glc-(1→4)-α-D-Gal structural element.[349, 350]

I.3.4 Synthesis of disaccharide acceptors 18 to 20



With suitably protected glucoside donors **29** and **30** and galactosyl acceptors **23** and **24** in place, assembly of the corresponding disaccharide acceptors **18 - 20** commenced with a 1,2-*cis* selective [1+1] glycosylation step. In a first experiment, glycosylation of galactosyl acceptor **23** with PTFA glucosyl donor **30** was performed in Et_2O using TMSOTf as a promotor. This afforded product **21** in high yield (85 %) and with the desired 1,2-*cis* configuration (see Scheme 21, strategy A).

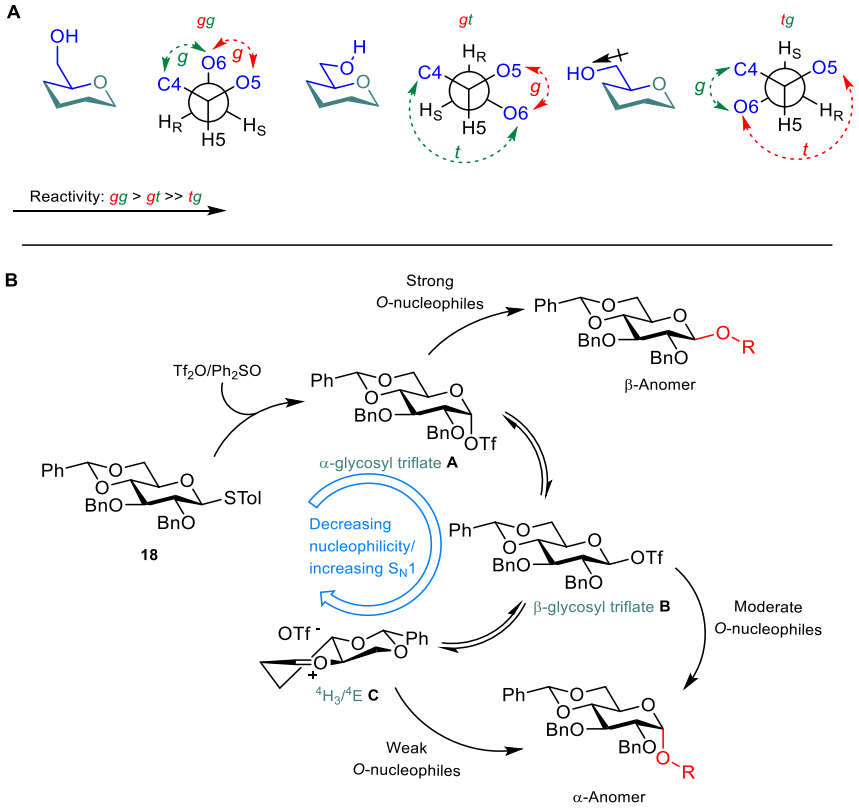


Scheme 21: Assembly of disaccharide acceptors **18** and **19**. In a first step, galactosyl acceptor **23** was 1,2-*cis* glycosylated using either PTFA donor **30** (strategy A) or alternatively utilizing thiodonor **29** (strategy B). Both approached furnished disaccharide intermediate **21** in very good yield and selectivity. In the following, **21** was either transformed into acceptor **18** (regioselective benzylidene acetal opening), or **19** (acidic benzylidene cleavage and regioselective introduction of primary TBS-ether).

The distinct stereoselectivity of this reaction is most likely explained by the inherent 1,2-*cis* directing effect of 4,6-*O*-benzylidene acetal protecting groups, which has been the subject of various studies. In brief, benzylidene acetals influence stereoselectivity by their disactivating properties giving raise to covalent intermediates during the glycosylation reaction.

In this regard, the *Fraser-Reid* group was the first to investigate the disarming properties of 4,6-*O*-benzyliden acetals, coining the term of *torsional disarmament* to explain the decreased reactivity of *trans*-fused bicyclic systems.[351, 352] Although the exact torsional interactions were not further specified, the lowered reactivity was attributed to a hampered transition from the chair-chair conformation of the 4,6-*O*-benzyliden acetal-protected donor to the chair-sofa state adapted by the respective oxocarbenium ion intermediate.[289, 351, 352] This concept was later challenged by *Bols* and co-workers stating that disarmament cannot be solely explained by torsional effects, but is mainly a result of electronic effects from the spatial arrangement of the hydroxylic substituents.[353, 354] Thus, the hydroxymethyl groups of flexible pyranosides may adopt three staggered conformations (*tg* (*trans, gauche*), *gt* (*gauche, trans*) and *gg* (*gauche, gauche*; see Scheme 22 A) from which the *tg* conformer is generally the least abundant.[355-357] By locking the glycoside (e.g. glucose) in the *tg* conformer, 4,6-*O*-benzylidene acetals give raise to a dipole moment with the negative terminus directing away from the anomeric centre thereby maximizing the

electron withdrawing effect on the oxocarbenium intermediate. However, a combination of both, torsional and electronic effects, seem accountable for the disarming character of 4,6-*O*-benzylidene acetals in the glucose series.[354]



Scheme 22: **A**) Different hydroxymethyl rotamers and their proposed relative reactivity. Electron-withdrawing character of the *tg* conformer as found in 4,6-*O*-benzylidene acetals is indicated by the black arrow.[354, 355] **B**) Glycosylation mechanism of benzylidene glucose as proposed by *Codée* and co-workers using Tf_2O/Ph_2SO as the promotor system.[20]

The influence of 4,6-*O*-benzylidene acetals on the stereochemical outcome of glycosylation reactions has been extensively studied for β-mannosylations.[20] However, in contrast to the distinct β-selectivity in the mannose system, a high degree of α-selectivity was observed by *Crich* in the benzylidene glucose series (*Glucose/Mannose Paradox*).[289] It is now widely accepted that this stereoselectivity originates from an equilibrium between the initially formed α-linked glucosyl triflate (**A**) and the respective β-linked triflate (**B**, see Scheme 22 B).[288] From this dynamic equilibrium the less stable and hence more reactive β-triflate (**B**) subsequently reacts with the respective nucleophile to selectively form the α-glycosidic linkage.[20, 358] Recently, *Codée* and co-workers highlighted the influence of acceptor nucleophilicity onto this mechanistic pathway and the observed stereoselectivity (see Scheme 22 B).[20] In brief, reactive nucleophiles (e.g. ethanol, C-6-OH) favour the formation of β-glycosidic linkages (*via* nucleophilic displacement of α-linked triflate **A**). In contrast, weak and moderate nucleophiles, which react with either the more reactive β-linked triflate **B** (moderate nucleophiles) or with a $^4H_3$-like oxocarbenium ion **C** (weak nucleophiles), furnish predominantly α-linked products.

Although mechanistic studies on the intermediates occurring during TMSOTf activation of PTFA donors are not available from the literature, it seems conceivable that the β-linked triflate **B** or the respective $^4H_3$-oxocarbenium like structure **C** are also intermediates during TMSOTf-mediated glycosylation of acceptor **23** with PTFA donor **30**. Hence, we concluded that disaccharide **21** may also be obtained from a respective glycosylation reaction using thioglycoside **29**, thus omitting the anomeric deprotection and *O*-alkylation step in the syntheses of PTFA donor

30 (see Scheme 20). Indeed, a second glycosylation experiment using acceptor **23** and thiodonor **29** in combination with the NIS/TMSOTf promotor system[359] (see Scheme 21, strategy B), afforded disaccharide **21** in even a slightly improved yield (92 %).

With a robust and high yielding access towards disaccharide precursor **21** in place, the latter was used in subsequent protecting group manipulations to afford either the 6-*O*-benzyl ether **18** or compound **19** equipped with an orthogonal protecting group for subsequent fluorination. Thus **18** was obtained from a regioselective benzylidene opening step using borane trimethylamine complex and boron trifluoride diethyl etherate (84 %).[360] The required regiochemistry of **18** was assigned from 2D-HMBC NMR spectrum, showing a distinct cross peak between C-6 (δ = 69.1 ppm) and the CH_2 group of the benzyl ether (δ = 4.38/4.20 ppm) (see Figure 12 B). In addition, anomeric configuration was reaffirmed at this stage based on the *Karplus* relationship ($^3J_{H1',H2'}$ = 3.5 Hz) and/or the $^1J_{C1,H1}$ coupling constant ($^1J_{C1,H1}$ = 169 Hz, see Figure 12 C and D respectively).

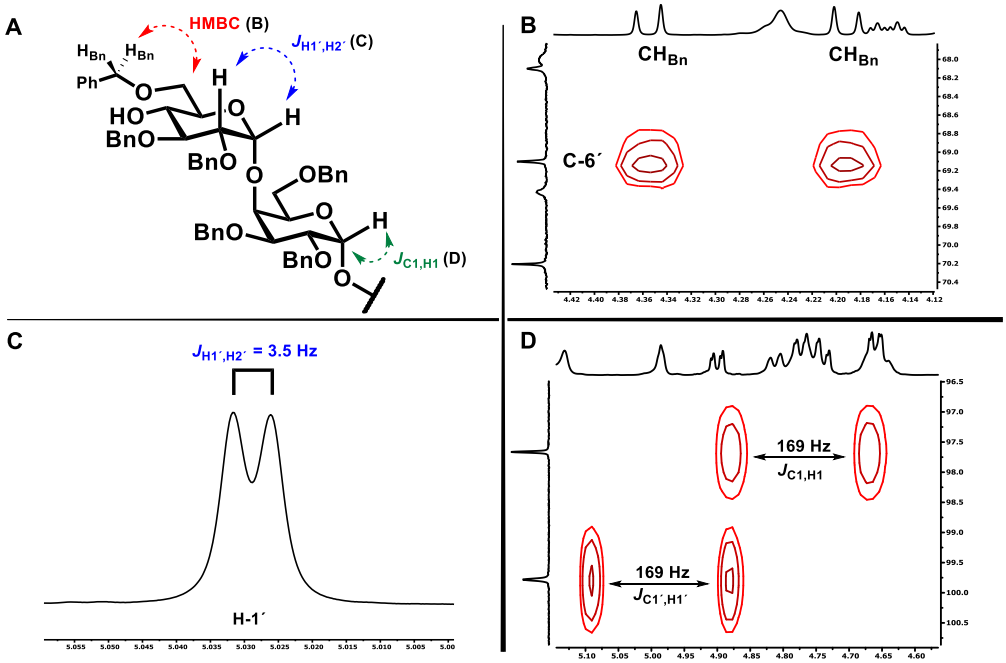
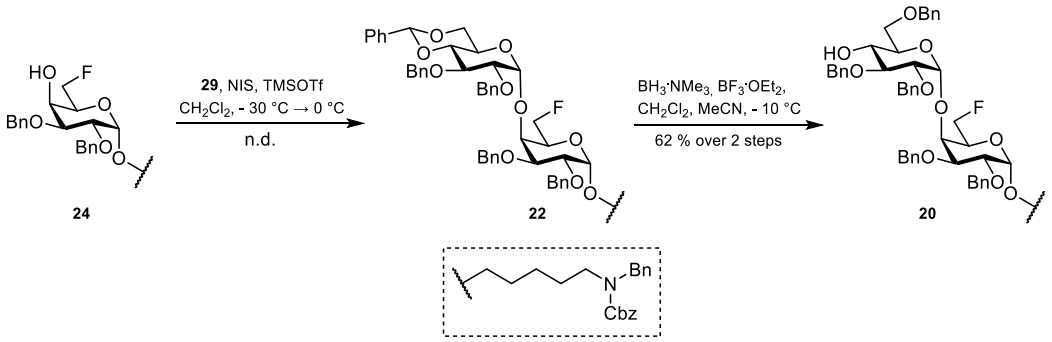


Figure 12: Presentation of NMR signals proofing the anomeric configuration and regiochemistry of disaccharide acceptor **18**. **A**) Presentation of disaccharide structure including NMR signals. **B**) Zoom into 2D-HMBC NMR spectrum of compound **18** showing the C-6´ → CH_{Bn} cross peak of 6-*O*-benzyl ether obtained from regioselective benzylidene opening. **C**) Anomeric proton of α-linked glucose and $^3J_{H1',H2'}$ coupling constant underpinning the desired α-glycosidic linkage. **D**) Zoom into ^{1}H-^{13}C-coupled HSQC NMR showing the $^1J_{C1,H1}$ and $^1J_{C1'H1'}$ coupling constant of the α-D-Glc-(1→4)-α-D-Gal structural element (Measured at 800 MHz NMR).

In addition, orthogonal protected acceptor **19** was provided over two synthetic steps from **21** (see Scheme 21). Here, cleavage of the 4,6-*O*-benzyliden protecting group under acidic conditions[194], followed by regioselective protection of the C-6´-position as a *O*-TBS ether[361] resulted in **19** in 76 % total yield over two steps.

Following the synthetic protocol for acceptor **18**, the corresponding fluorinated disaccharide acceptor **20** was assembled *via* NIS/TMSOTf-mediated glycosylation[359] of compound **24** with thioglycoside donor **29** (see Scheme 23). Although this proceeded smoothly, the product **22** could not be isolated in pure form. NMR analysis indicated the presence of approximately 10 % of an inseparable impurity most likely originating from decomposition of glycoside donor **29**. It is worth mentioning that at this point utilizing PTFA donor **30** did not improve the reaction outcome in terms of purity and yield. Thus, product **22** obtained from glycosylation with thioglycoside **29** was

used in the subsequent regioselective benzylidene opening[360] with borane trimethylamine complex in combination with boron trifluoride diethyl etherate to afford fluorinated acceptor **20** in 62 % yield over two steps.

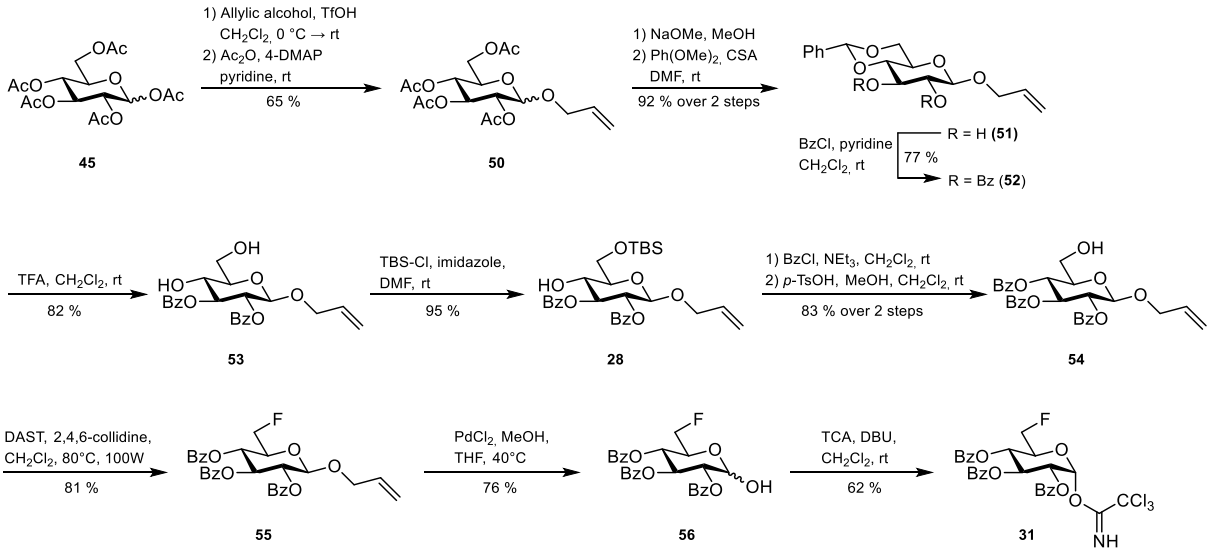


Scheme 23: Chemical synthesis of fluorinated disaccharide acceptor **20**.

In conclusion, by exploiting the inherent 1,2-*cis* selectivity of 4,6-*O*-benzyliden acetals, a high yielding synthesis of suitably protected disaccharide acceptor building blocks (**18** - **20**) was provided. These compounds in turn served as valuable precursors in the assembly of fluorinated tri- and tetrasaccharide congeners. In further course, the synthesis of monosaccharidic precursors (**28**, **31** and **32**) for the assembly of fluorinated cellobiose derivatives is described.

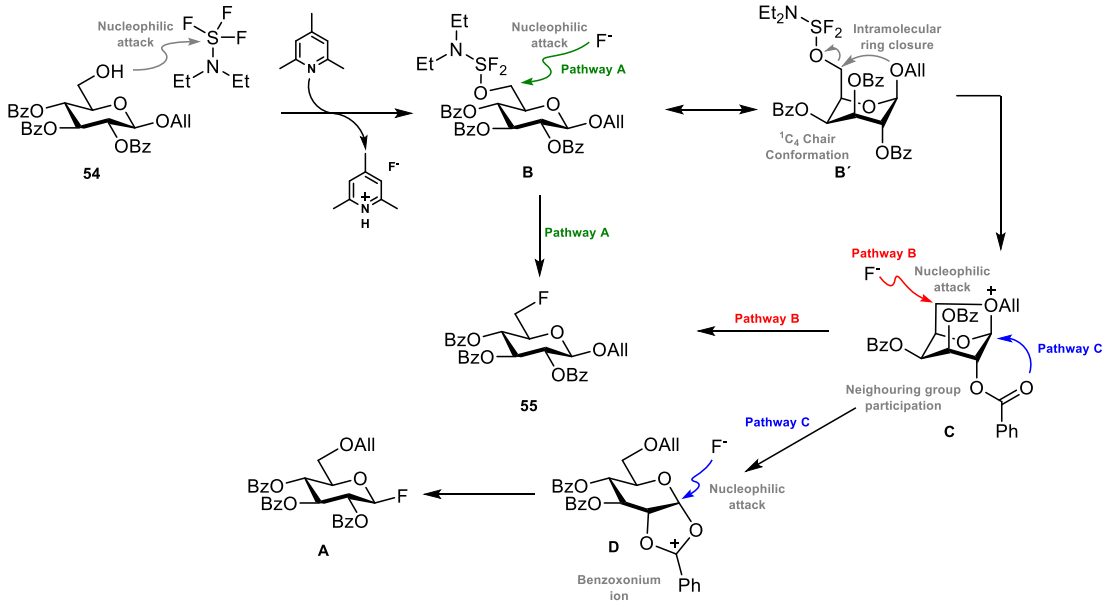
I.3.5 Synthesis of glucose building blocks 28, 31 and 32

Starting from previously described 1,2,3,4,6-penta-*O*-acetyl glucose **45**, fluorinated glucosyl donor **31** was synthesized in eleven synthetic steps as depicted in Scheme 24. It is worth mentioning that although a shorter synthetic route towards donor **31** have been developed in the course of this project (eight synthetic steps from **45**, see Master thesis of *Sebastian Neidig*[362]), the following synthesis also provides **28** as an orthogonal protected glucosyl acceptor for the synthesis of fluorinated cellobiose derivatives **26** and **27** (*vide infra*).



Scheme 24: Synthesis of glucose acceptor **28** and fluorinated glucose donor **31** starting from D-glucose derivative **45**.

Thus, allyl glycoside **50** was obtained by a TfOH-mediated glycosylation reaction of allylic alcohol according to a protocol from the *Demchenko* group.[363] Reacetylation of partially deacetylated side-products with acetic anhydride in the presence of 4-DMAP as nucleophilic catalyst finally afforded **50** (65 %) as a mixture of anomers which could be separated by standard column chromatography.[364] For subsequent reaction, the pure β-anomer (major product) was employed. Saponification under *Zemplén* conditions[294] led to an intermediate tetrol which was directly transformed into the corresponding 4,6-*O*-benzylidene acetal **51** upon treatment with benzaldehyde dimethyl acetal and CSA in DMF (92 % over two steps).[365] Esterification of the remaining hydroxy groups with benzoyl chloride afforded the fully protected glucose derivative **52** after crystallization from EtOH.[366] Acidolysis of the acetal protecting group gave diol **53**[366] (82 %) which was regioselectively protected as a C-6-*O*-TBS ether[361] (**28**) *via* treatment with TBS-Cl and imidazole (95 % yield). Esterification[322] of the C-4 hydroxy group followed by acidic removal of the 6-*O*-TBS protecting group[367] afforded compound **54** (83 % over two steps). Primary alcohol **54** was utilized in a subsequent fluorination reaction to introduce the fluorine substituent at the C-6 position of **55**. Thus far, DAST-mediated fluorination of primary alcohol **54** has only been described by *Lin et al.*, who reacted **54** with DAST at ambient temperature.[368] Under these conditions, they observed the formation of significant amounts of a side-product arising from an unexpected C-1 → C-6 migration of the anomeric protecting group (see Scheme 25), which resulted in an inseparable mixture of **55** and the corresponding migration product **A** (Product distribution: **55**: **A** = 1:1).



Scheme 25: Proposed mechanism of DAST-mediated C-1 → C-6 migration of the anomeric protecting group.[368]

Mechanistically, this rather uncommon C-1 → C-6 migration commences with a nucleophilic attack of the C-6-hydroxy group to form intermediate **B**. It was proposed that by adopting a $^1C_4$ chair conformation, intermediate **B´** facilitates an intramolecular nucleophilic attack to furnish oxygen bridged intermediate **C**. Neighbouring group participation of the *O*-2 benzoyl ester in turn gives rise to the stabilized cyclic benzoxonium ion **D** which finally provides the β-linked C-1 → C-6 migration product **A** upon nucleophilic attack of a fluorine ion at the anomeric carbon.

To avoid this undesired migration reaction and enable an effective synthesis of C-6-fluorinated glucose derivative **55**, a microwave-assisted fluorination protocol[324, 325] was applied. Indeed, this protocol furnished the desired

product **55** in 81 % yield. Regiochemistry of **55** was assigned by ^{19}F-NMR (δ_F = − 230.2 ppm) as well as HSQC-NMR ($^1J_{C6,F}$ = 176 Hz, δ_{C6} = 81.8 ppm) both proving the desired connectivity (see Figure 13).[368, 369]

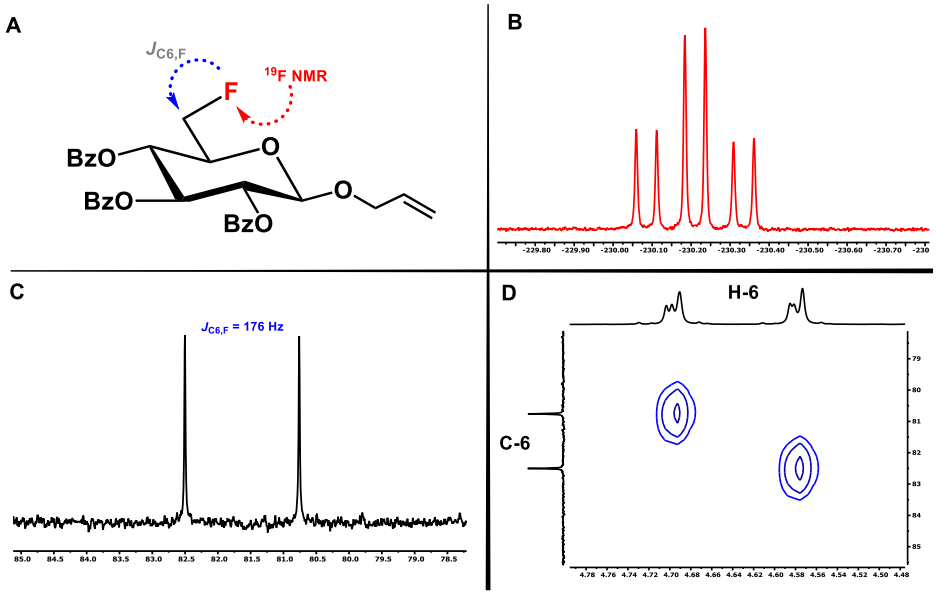
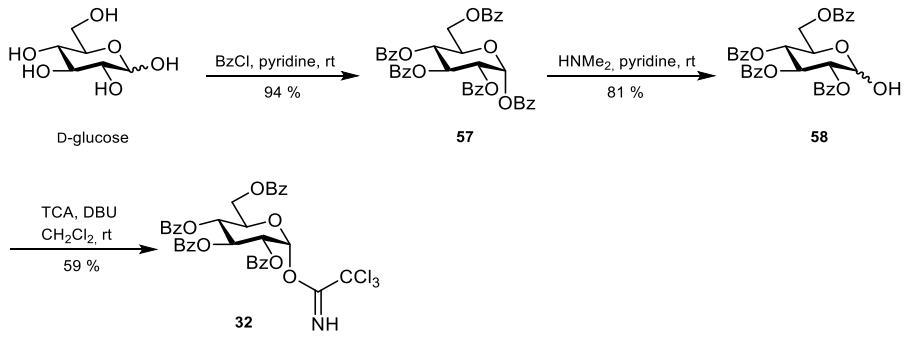


Figure 13: Schematic depiction of relevant signals proofing the correct regiochemistry of fluorinated compound **55**: **A**) C-6-fluorinated derivative **55** including the relevant signals for determination of C-6-F linkage. **B**) ^{19}F-NMR signal. **C**) ^{13}C signal of C-6 position and its $^1J_{C6,F}$ coupling constant of 176 Hz. **D**) HSQC NMR cross peak of C-6/H-6 signal supporting the correct regiochemistry of fluorine incorporation.

The anomeric position of product **55** was deprotected *via* $PdCl_2$-mediated deallylation[370] to give hemiacetal **56** in 76 % yield. Base-mediated *O*-alkylation[371] of **56** with trichloroacetonitrile and DBU finally gave the targeted glucosyl donor **31**.

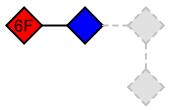
The corresponding non-fluorinated glucose donor **32** was synthesized in three synthetic steps from commercially available D-glucose as depicted in Scheme 26. Thus, benzoylation of D-glucose with benzoyl chloride in pyridine provided **57** after crystallization from EtOH.[372, 373] Regioselective anomeric deprotection was achieved by treatment of **57** with dimethylamine in pyridine (81 % of **58**).[374, 375] Subsequent *O*-alkylation of hemiacetal **58** with trichloroacetonitrile in DBU then afforded glucose donor **32**.[371]



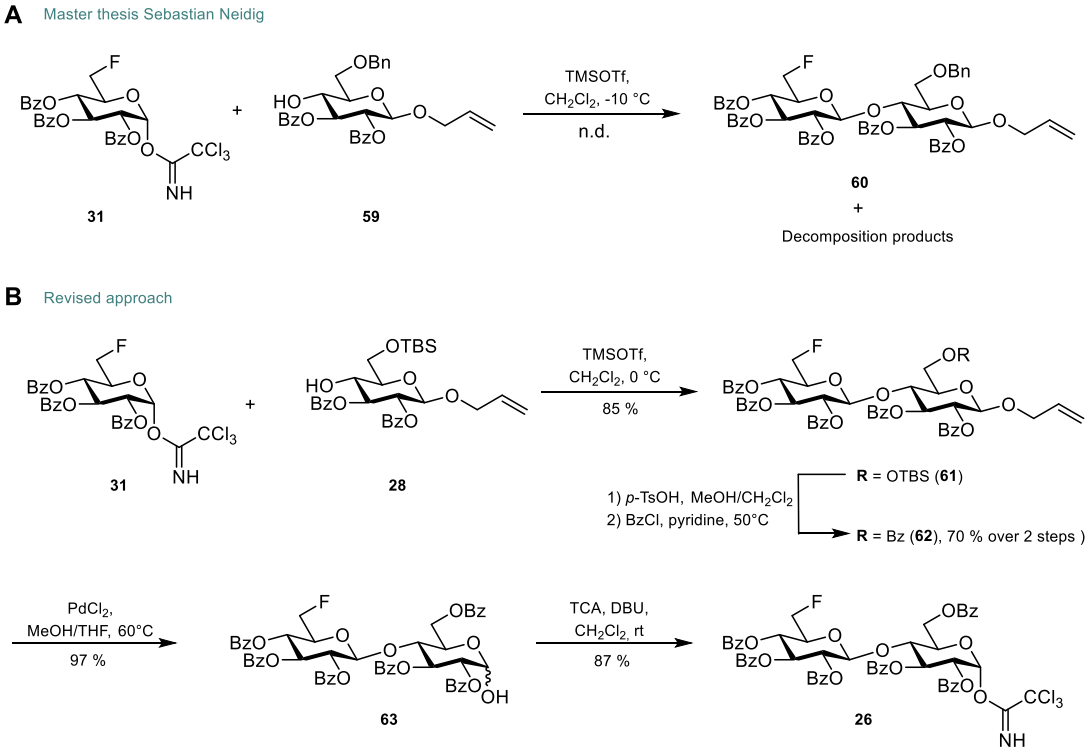
Scheme 26: Chemical synthesis of trichloroacetimidate donor **32** in three synthetic steps starting from D-glucose.

I.3.6 Synthesis of cellobiose donor building blocks 25 - 27

Synthesis of C-6´F-cellobiose donor 26



The synthesis of C-6´F-cellobiose donor **26** started with the assembly of the β-D-Glc-(1→4)-β-D-Glc glycosidic linkage under *Schmidt* conditions.[376, 377] Initial attempts to provide this structural element were conducted by *Sebastian Neidig* in the course of his master thesis and made use of glucosyl acceptor **59** comprising a 6-*O*-benzyl ether instead of the here employed 6-*O*-TBS protecting group.[362] This however resulted in inseparable mixtures of glycosylation product **60** and decomposition products from donor **31** (see Scheme 27 A). Consequently, a revised synthetic strategy is presented herein, employing an orthogonally cleavable TBS protecting group to facilitate purification (see Scheme 27 B).

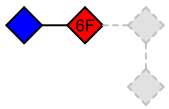


Scheme 27: Synthesis of C-6´-fluorinated cellobiose donor **26**. **A**) Initial synthetic route to assemble the demanded β-6F-D-Glc-(1→4)-β-D-Glc linkage conducted by *Sebastian Neidig*.[362] **B**) Revised synthesis of C-6´F-cellobiose trichloroacetimidate donor **26**.

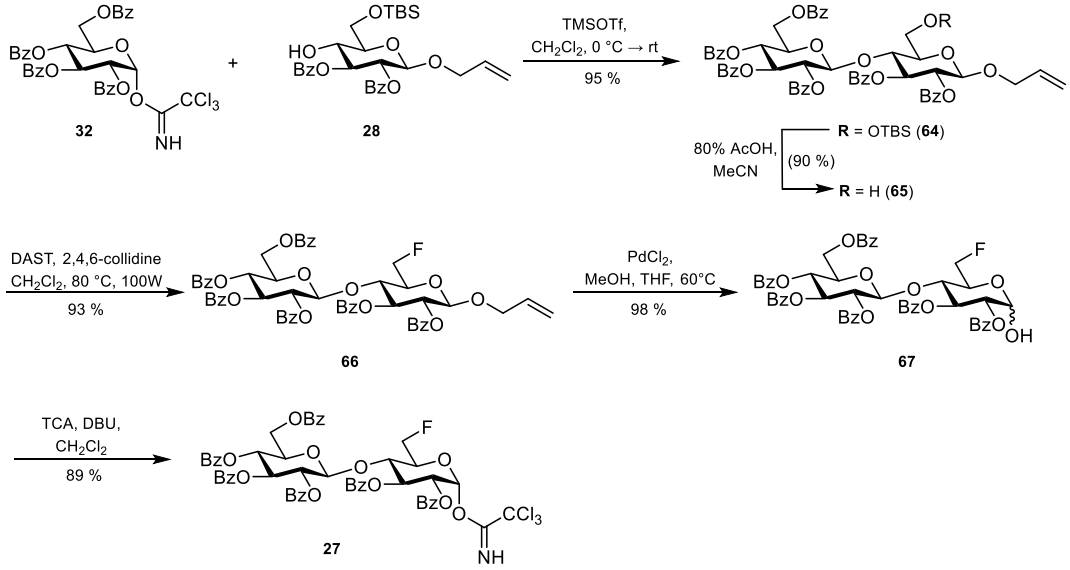
Thus, glycosylation of acceptor **28** under *Schmidt* conditions[376, 377] using TMSOTf as promotor yielded pure disaccharide **61** in 85 % yield. The β-anomeric connectivity was confirmed by ^{1}H-NMR experiments revealing a coupling constant $^{3}J_{H2',H1'}$ = 8.0 Hz in accordance to the *Karplus* relationship.[309-312]

Acidic cleavage of the silyl ether protecting group[367] and subsequent esterification[373, 378] afforded benzoyl ester **62** in 70 % yield over two steps. Ester **62** was selectively deprotected at the anomeric carbon *via* $PdCl_2$-mediated deallylation[370] to give lactol **63**. Base-mediated *O*-alkylation[379] finally furnished the desired fluorinated cellobiose donor **26** in 87 % yield.

Synthesis of C-6-F-cellobiose donor 27



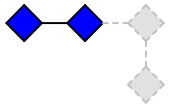
The synthesis of cellobiose donor **27** started again with the assembly of the β-(1→4)-*O*-glycosidic linkage under *Schmidt* conditions[376, 377] to afford **64** in 95 % yield (see Scheme 28). The anticipated β-glycosidic connectivity of **64** was confirmed by the $^{3}J_{H1',H2'}$ = 8.0 Hz coupling constant.[309-312]



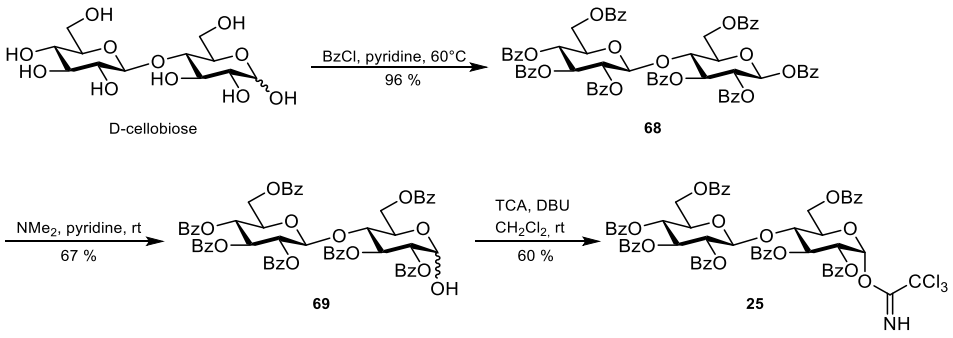
Scheme 28: Synthesis of C-6-fluorinated cellobiose donor **27**.

Acidic removal[380] of the silyl protecting group (**65**) followed by the previously described microwave-assisted DAST fluorination protocol[324, 325] furnished **66** in very good yield. The regiochemistry of the fluorination reaction was confirmed by 1D- and 2D-NMR experiments showing a $^{1}J_{F,C6}$ = 174 Hz coupling constant as well as a ^{19}F signal at δ = − 233 ppm, both in accordance with previously reported data of C-6-fluorinated glucose derivatives.[369] Anomeric deallylation[370] (**67**) and subsequent base-mediated *O*-alkylation[379] finally resulted in the desired trichloroacetimidate donor **27** comprising a fluorine substituent at the C-6-position.

Synthesis of native cellobiose donor 25

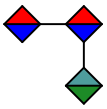


Starting from commercially available D-cellobiose, disaccharide donor **25** was obtained in three synthetic steps including aminolysis of the anomeric ester protecting group (see Scheme 29). In brief, esterification of D-cellobiose with benzoyl chloride afforded the fully protected derivative **68** upon heating to 60 °C.[381] Regioselective cleavage of the anomeric benzoyl ester[374, 375] afforded hemiacetal **69**, which was then converted into the trichloroacetimidate donor **25** in 60 % yield by base-mediated *O*-alkylation.[379]

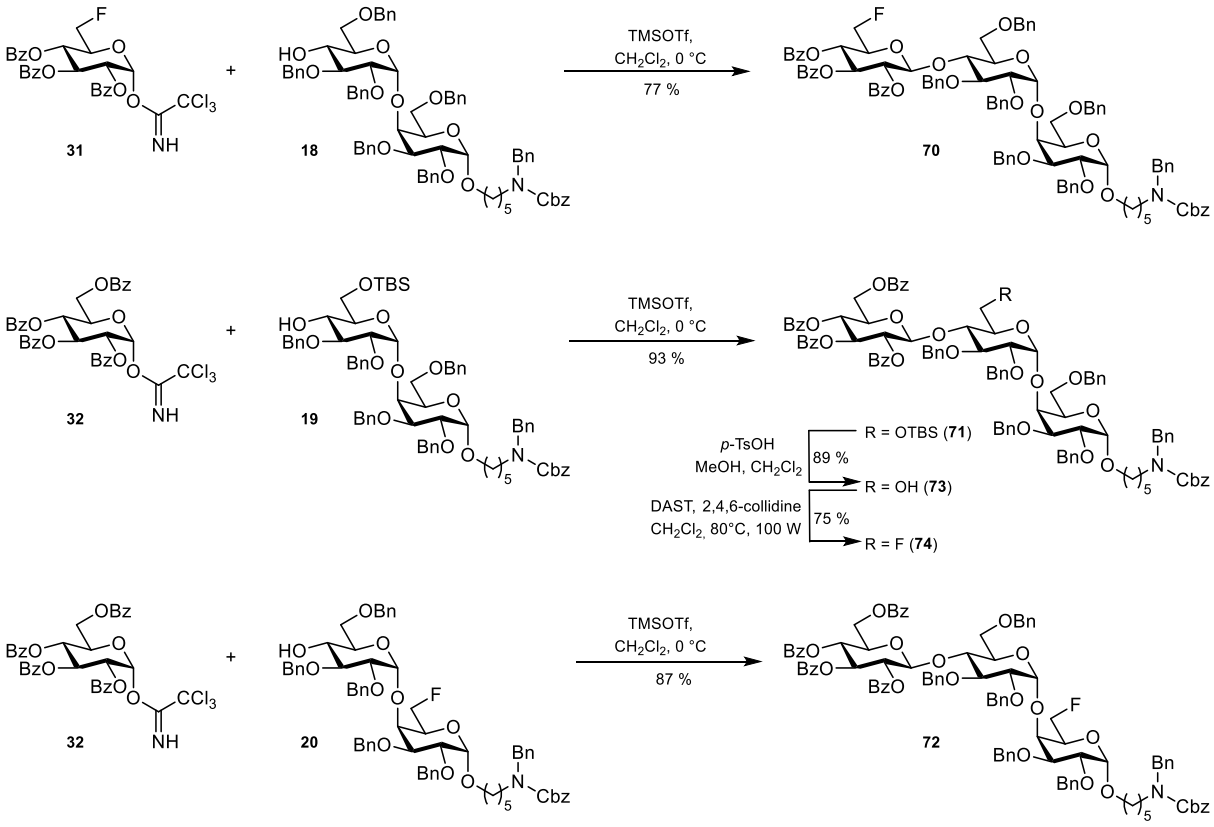


Scheme 29: Synthesis of cellobiose donor **25** from commercially available D-cellobiose.

I.3.7 Synthesis of fluorinated trisaccharides 3, 5 and 6



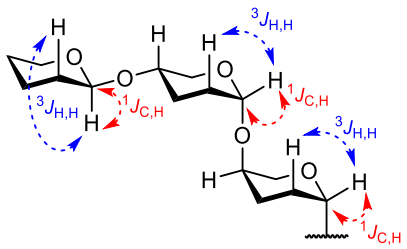
Trisaccharides **70** - **72** were synthesized from the respective disaccharide acceptors either comprising a 6-*O*-benzylether (**18** or **20**) or an orthogonal 6-*O*-TBS group (**19**). Glycosylations were conducted in dry CH_2Cl_2 under *Schmidt* conditions[376, 377] using TMSOTf as a promotor (see Scheme 30).



Scheme 30: Synthesis of fully protected trisaccharide structures **70**, **74** and **72**.

Glycosylation products **70** - **72** were obtained in high yields (77 % (**70**), 93 % (**71**) and 87 % (**72**)) and exclusively as the β-anomers due to neighbouring group participation of the *O*-Bz ester protecting group at the C-2 position. The resulting products **70**, **72** (and **74**) were carefully characterized by 1D (^{1}H, ^{13}C, ^{19}F) NMR and 2D NMR

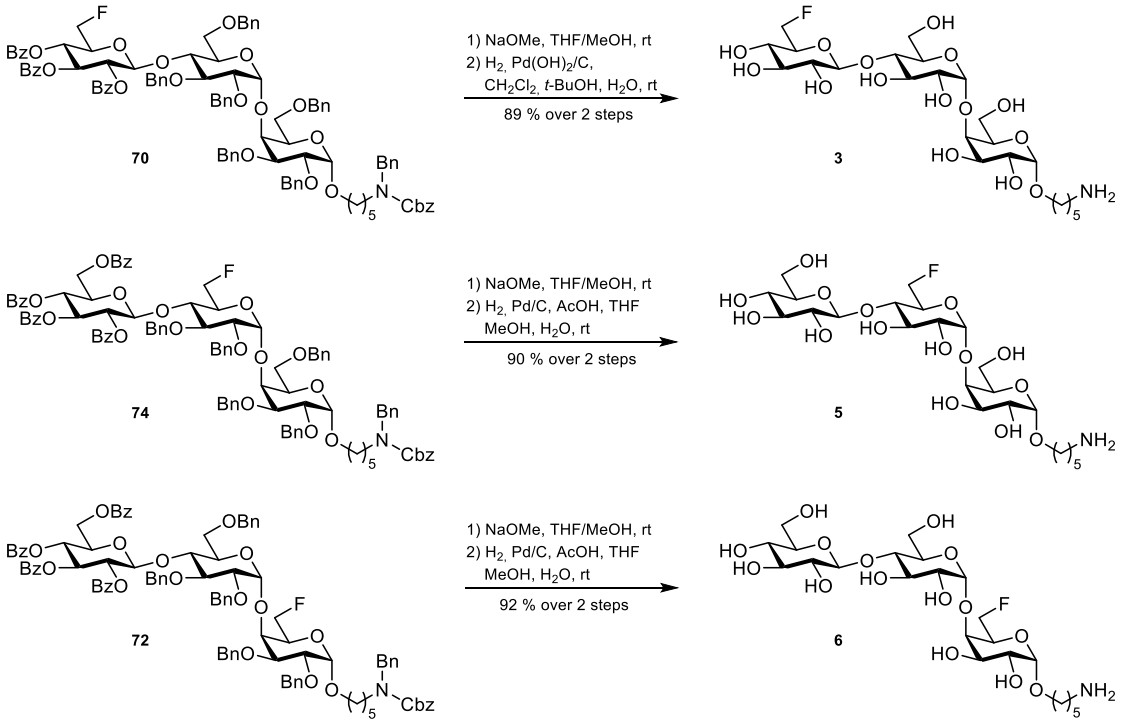
experiments (COSY, HSQC, HMBC, ^{1}H-^{13}C-coupled HSQC) to confirm the correct regio- and stereochemical connectivity of all glycosidic linkages (for selected data, see Table 1). Trisaccharide **74** comprising a fluorine substituent at the C-6´position of the bridging α-glucose moiety was provided by acidic removal of the TBS protecting group[367] (**73**) and subsequent microwave assisted[324, 325] late-stage fluorination. To the best of our knowledge, comparable examples of late-stage approaches for incorporation of fluorine into preassembled oligosaccharide structures are scarcely reported in the literature and appear to be limited to disaccharide motifs.[247, 249]



Coupling Constant	**6´´F-Tri (70)**	**6´F-Tri (74)**	**6F-Tri (72)**
$^{3}J_{H1,H2}$ [Hz]	bs (α-Gal)	bs (α-Gal)	m (α-Gal)
	3.5 Hz (α-Glc)	3.5 Hz (α-Glc)	3.5 Hz (α-Glc)
	m (β-Glc)	8.0 Hz (β-Glc)	8.1 Hz (β-Glc)
$^{1}J_{C1,H1}$ [Hz]	171 Hz (α-Gal)	172 Hz (α-Gal)	171 Hz (α-Gal)
	171 Hz (α-Glc)	171 Hz (α-Glc)	171 Hz (α-Glc)
	165 Hz (β-Glc)	164 Hz (β-Glc)	166 Hz (β-Glc)

Table 1: Coupling constants of fluorinated trisaccharides **70**, **72** and **74** proving the demanded anomeric connectivity.

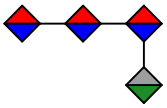
With all fluorinated trisaccharide derivatives (**70**, **72**, **74**) at hand, global deprotection was conducted in a two-step procedure in accordance to literature-known protocols (see Scheme 31).[194, 382] Thus, saponification of benzoyl esters with sodium methanolate in MeOH/THF and subsequent hydrogenolysis gave the targeted ensemble of fluorinated trisaccharides **3**, **5** and **6** in very good yields (89 % - 92 %) after RP-column chromatography.



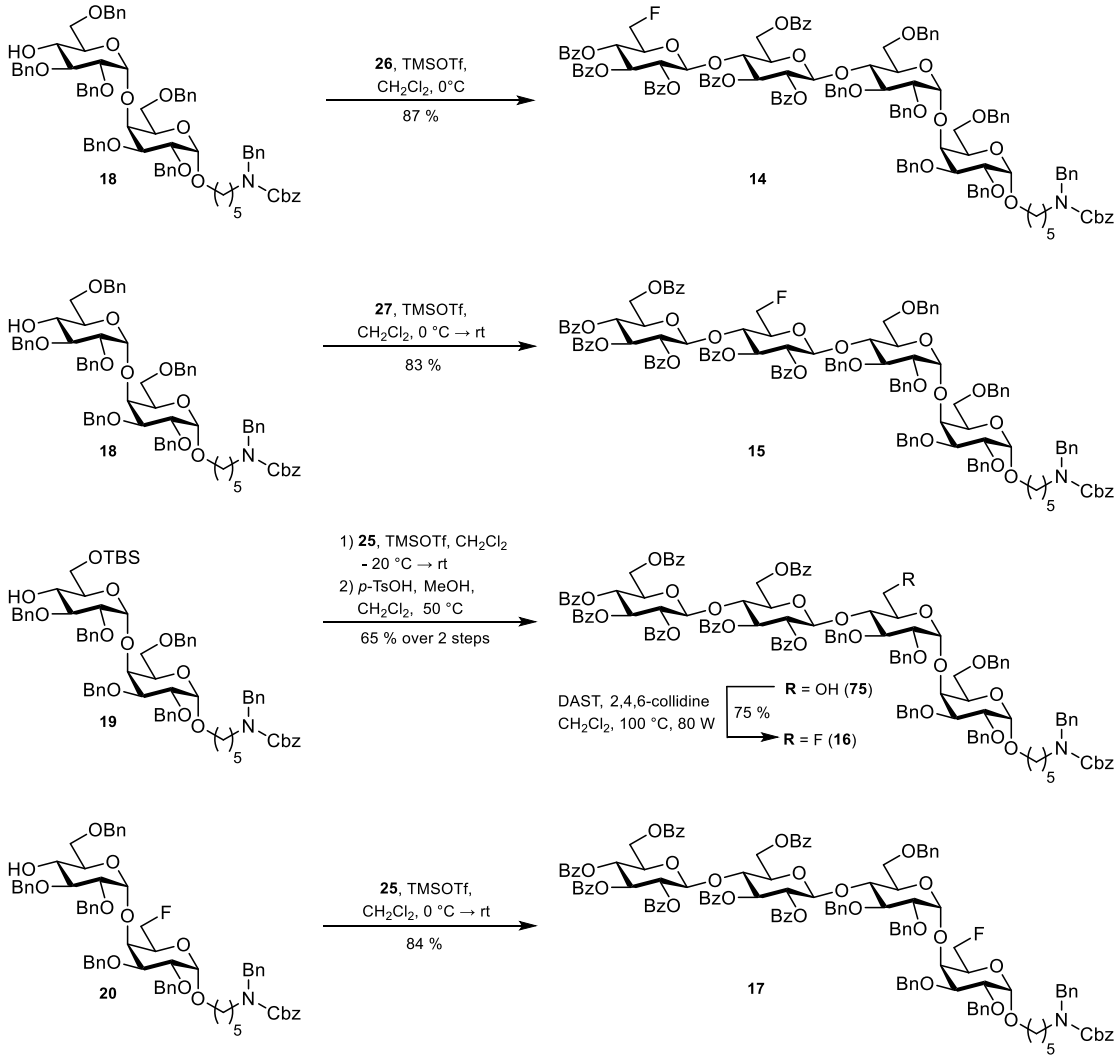
Scheme 31: Global deprotection of fluorinated trisaccharides **3**, **5** and **6**.

In summary, a synthetic approach towards an ensemble of C-6-fluorinated trisaccharides corresponding to the smallest glycan structure containing a protective epitope against ST 8 has been provided. This approach furnished sufficient amounts (22 – 34 mg) of the targeted compounds (**3**, **5** and **6**) for future preparation of fully synthetic vaccine candidates as for instance described in chapter *I.3.9 Synthesis of fully synthetic vaccine candidates against ST 8*) and/or subsequent antibody binding analysis using a glycan-specific monoclonal antibody.

I.3.8 Synthesis of tetrasaccharides 4 and 7 to 10



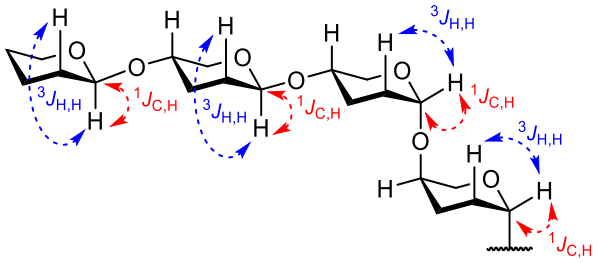
Tetrasaccharides **14**, **15**, **17** and **75** were assembled under *Schmidt* conditions[376, 377] using either fluorinated cellobiose donors (**26**, **27**) or the native cellobiose donor **25** in combination with the respective disaccharide acceptors **18** - **20** (see Scheme 32).



Scheme 32: Synthesis of fluorinated tetrasaccharides **14** - **17**.

All glycosylation reactions were conducted in dry CH_2Cl_2 using TMSOTf as promotor. Addition of 1.5 eq. of the respective donors (**25** - **27**) provided the fully protected tetrasaccharides **14**, **15**, **17** in high yields and purity after standard flash chromatography. On the other hand, tetrasaccharide precursor **75** was obtained in 65 % yield over two synthetic steps after cleavage of the C-6´-O-silyl ether under acidic conditions.[367] Subsequent DAST-mediated[324, 325] late-stage fluorination of tetrasaccharide **75** furnished the fluorinated derivative **16** in 75 % yield.

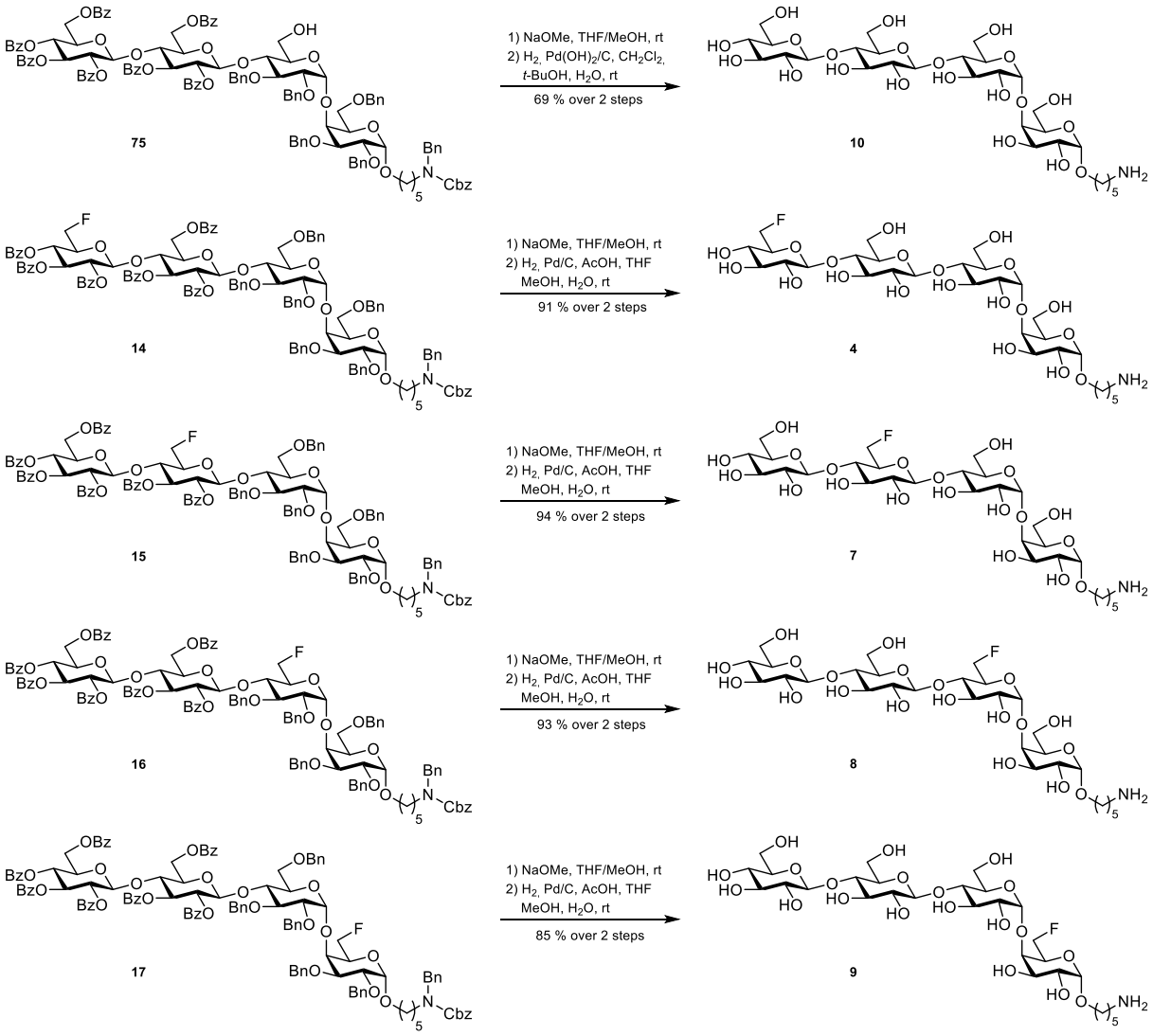
Fluorinated tetrasaccharides **14** - **17** were carefully characterized by 1D NMR (^1H, ^{13}C, ^{19}F) and 2D NMR experiments (COSY, HSQC, HMBC, ^1H-^{13}C-coupled HSQC) to confirm the correct regio- and stereochemical connectivity of all glycosidic linkages (for selected data please see Table 2).



	6´´´F-Tetra (14)	6´´F-Tetra (15)	6´F-Tetra (16)	6F-Tetra (17)
$^3J_{H1,H2}$ [Hz]	s (α-Gal)	m (α-Gal)	m (α-Gal)	m (α-Gal)
	3.7 Hz (α-Glc)	3.7 Hz (α-Glc)	3.6 Hz (α-Glc)	3.7 Hz (α-Glc)
	8.0 Hz (β-Glc)	7.8 Hz (β-Glc)	8.1 Hz (β-Glc)	m (β-Glc)
	7.9 Hz (β-Glc)	7.9 Hz (β-Glc)	m (β-Glc)	7.9 Hz (β-Glc)
$^1J_{C1,H1}$ [Hz]	169 Hz (α-Gal)	170 Hz (α-Gal)	170 Hz (α-Gal)	170 Hz (α-Gal)
	170 Hz (α-Glc)	169 Hz (α-Glc)	172 Hz (α-Glc)	168 Hz (α-Glc)
	165 Hz (β-Glc)	164 Hz (β-Glc)	162 Hz (β-Glc)	163 Hz (β-Glc)
	161 Hz (β-Glc)	162 Hz (β-Glc)	160 Hz (β-Glc)	163 Hz (β-Glc)

Table 2: Coupling constants of fluorinated tetrasaccharides **14 - 17** proving the demanded anomeric connectivity.

Global deprotection was conducted in a two-step procedure based on literature-known protocols.[194, 382] In a first approach, the benzoyl esters of compound **75** were cleaved by treatment with sodium methanolate in MeOH/THF, followed by hydrogenolysis in a mixture of CH_2Cl_2, *t*-BuOH and H_2O using $Pd(OH)_2$/C as described by *Schumann et al.*[194] This protocol furnished the non-fluorinated tetrasaccharide **10** in good yield of 69 %. However, an alternative debenzylation protocol employing hydrogenolysis of the fluorinated analogous in a mixture of THF, MeOH, H_2O and AcOH using Pd/C as catalyst was found supporior.[382] Under these conditions the fluorinated tetrasaccharides (**4**, **7** - **9**) were obtained in high yield (85 % - 94 %) after reversed-phase flash column chromatography and subsequent lyophilization (see Scheme 33).



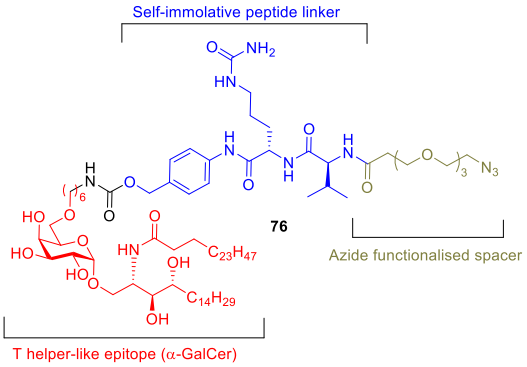
Scheme 33: Global deprotection of tetrasaccharide analogues **4, 7** - **10**.

In summary, various novel C-6-fluorinated tetrasaccharides (**4**, **7** - **9**) corresponding to a reduced congener of the ST 8 CPS repetitive unit were provided. In addition, the non-fluorinated tetrasaccharide **10** was prepared by the approach presented herein and in addition its previous synthesis reported by *Schumann et al*.[194] All target structures (**4**, **7** - **10**) were isolated in a 15 - 36 mg scale. This in turn should allow future conjugation reactions onto a suitable immunogenic carrier (e.g. a synthetic KRN7000 derivative as described chapter *I.3.9 Synthesis of fully synthetic vaccine candidates against ST 8*) for providing a selection of synthetic conjugate vaccine candidates against ST8.

I.3.9 Synthesis of fully synthetic vaccine candidates against ST 8

After successfully providing access to a library of synthetic glycotopes of ST 8, we targeted the assembly of first fully synthetic vaccine candidates. In collaboration with *Sebastian Neidig*, who kindly provided a suitably functionalized KRN 7000 derivative (**76**), we anticipated the assembly of two component vaccine candidates using copper-free strain promoted azide alkyne cycloaddition (SPAAC).[134, 286] This approach has recently been pursued in the group (*Dr. Andreas Baumann*) to provide a synthetic vaccine candidate against *Leishmania Donovani*.[285]

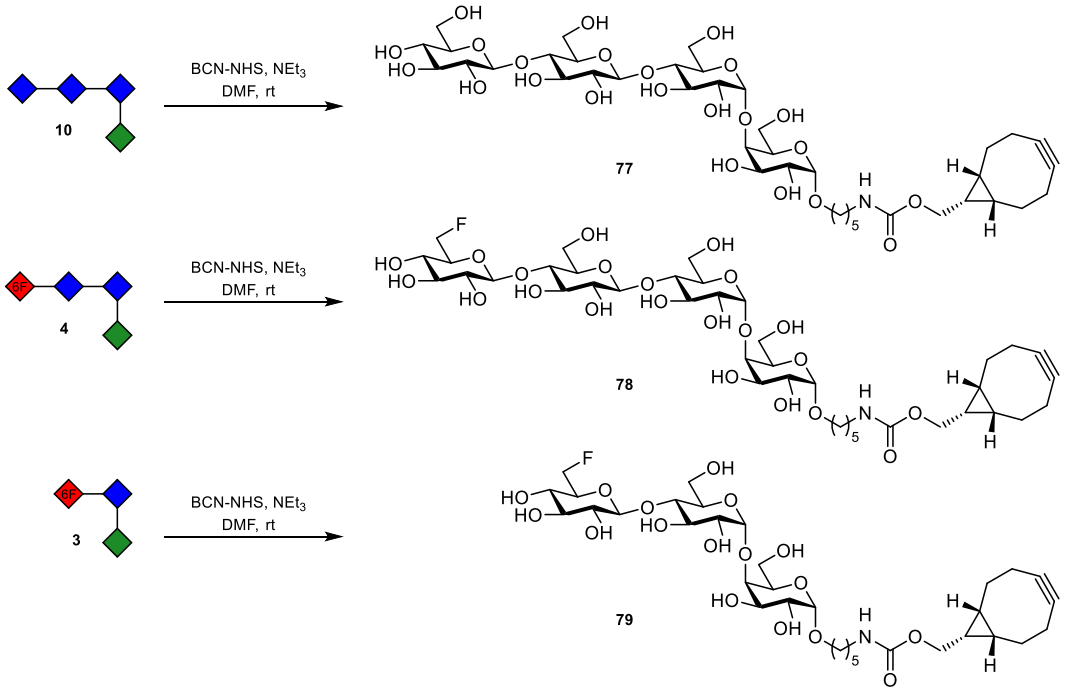
Scaffold **76** comprises a α-GalCer moiety (see Scheme 34, depicted in red) to serve as a T helper-like epitope.[117, 126] In addition, a self-immolative Valine-Citrulline dipeptide linker (see Scheme 34 depicted in blue) was introduced.[133] It is assumed that this Cathepsin B cleavable linker provides sufficient plasma stability to ensure co-delivery of both components (T helper-like epitope and B cell epitope) into the same APC.[134, 286, 383-385] Cleavage of this linker by lysosomal proteases in turn facilitates intracellular release and antigen presentation.[134] A triethylene glycol spacer attached to the *N*-terminus further provides the necessary azide functionality for SPAAC mediated conjugation (Scheme 34 depicted in green).



Scheme 34: T helper-like epitope **76** comprising a self-immolative peptide linker.[285]

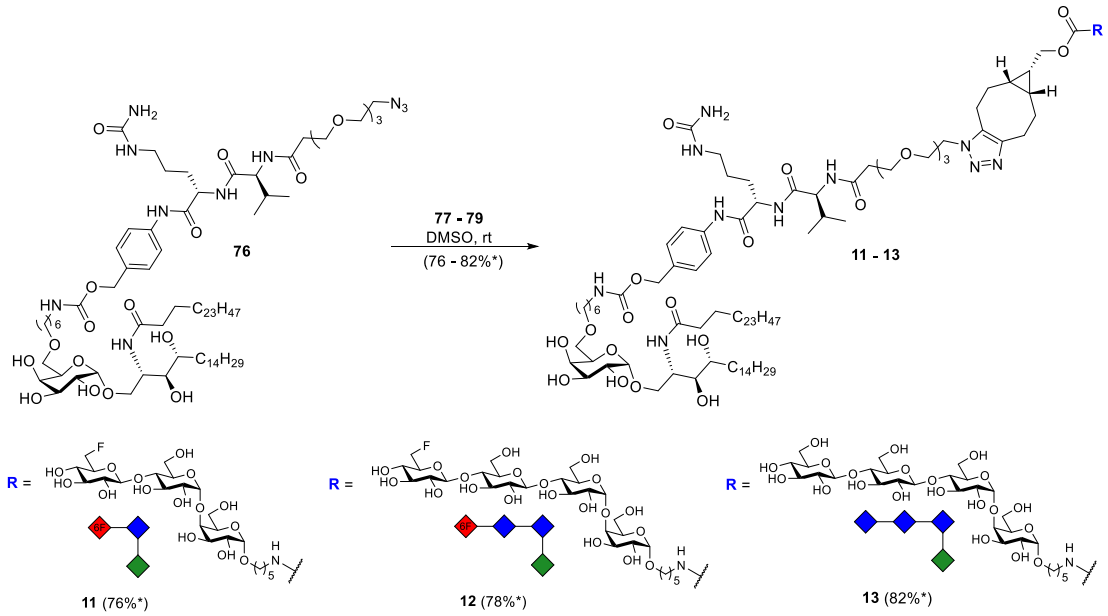
By opting for this SPAAC based approach, we anticipated a clean additive free assembly of the final vaccine construct which then should facilitate purification and provide contaminant-free conjugates for subsequent immunological testing.[134] It is worth noting that triazole linkages possess the risk of eliciting immune response targeted against this modification.[117] However, the use of click chemistry in recent glycolipid vaccine candidates underlines the potential of this approach.[134, 135, 286, 386]

For the assembly of our glycolipid conjugates, we chose a first selection of synthetic B cell epitopes synthesized in the course of this project. Hence, non-fluorinated tetrasaccharide congener (**10**) was included to serve as a reference in latter vaccination studies. Further, two fluorinated B cell epitopes **3** and **4** comprising a terminal C-6-fluorinated glucose moiety were selected. Including these fluorinated entities into our selection would provide the first representatives of fluorinated ST8 vaccine candidates. Consequently, synthetic B cell epitopes **3, 4, 10** were reacted with commercially available bicyclo[6.1.0]nonyne (BCN)-NHS ester in DMF in the presence of triethylamine as a base.[134, 285, 286] These conditions afforded the desired BCN-functionalized derivatives **77 - 79** after lyophilization and precipitation from Et_2O (see Scheme 35).



Scheme 35: Cycloalkyne functionalization of synthetic B cell epitopes **3**, **4** and **10**.

The obtained BCN carbamates **77 - 79** were utilized in the subsequent SPAAC reaction to assemble the fully synthetic vaccine candidates **11** - **13** (see Scheme 36). Accordingly, the BCN-equipped B cell epitopes **77** - **79** were dissolved in DMSO and added to a DMSO solution of the KRN derivative **76**.[134, 285, 286] After agitating these mixtures for four days at room temperature, the reaction was deemed complete and the crude products were lyophilized and thoroughly washed with water to furnish lipo-polysaccharide conjugates **11** - **13**.



Scheme 36: Assembly of fully synthetic lipo-polysaccharide vaccine candidates **11** - **13** (* preliminary yield after washing with water).

Characterization of these lipo-polysaccharide conjugates **11 - 13** by 1D- NMR experiments (^{1}H, ^{13}C, ^{19}F) and 2D-NMR (HSQC, HMBC, COSY) revealed characteristic signals for each vaccine component (e.g. anomeric signals belonging to the glycotope, anomeric signal of α-GalCer group, amide protons of the peptidic linker) providing

evidence for the desired identity of conjugates **11** - **13**. In addition, ^{19}F-NMR analysis of conjugates **11** and **12** revealed distinct fluorine signals at δ (DMSO-d_6) = – 232 ppm further supporting the presence of the fluorinated B cell epitopes in our vaccine candidates **11** and **12**.[369]

Unfortunately, all three spectra of the lipo-polysaccharide conjugates showed additional signals belonging to an aromatic small-molecular impurity. To effectively remove this impurity, glycoconjugate **11** comprising the fluorinated trisaccharide epitope was chosen as a test system for additional purification step. In a first approach, we opted for RP-HPLC purification using MeOH/H_2O/TFA for elution, as described by *Anderson et al*. for purification of a related lipopolysaccharide peptide vaccine.[286] Unfortunately, this protocol was found inapplicable due to poor sample solubility and extensive smearing. We therefore tested a precipitation strategy to remove the aromatic impurity from sample **11**. Dissolving **11** in MeOH (poorly soluble) and subsequently precipitating it from cold Et_2O effectively removed the aromatic impurity. This additional precipitation successfully provided the pure sample of vaccine candidate **11**, although the precipitation appeared to be incomplete resulting in significantly diminished yield. With regards to, the amphiphilic character and poor solubility of these compounds hampering available purification procedures, we are currently heading towards improving these protocols. Future strategies in this direction, could include for instance the use of less hydrophobic stationary phases in RP-HPLC purification (e.g. C-4 RP-HPLC columns[146]) to diminish sample smearing. This may eventually provide a more efficient purification protocol for vaccine candidates **12** and **13** and thus give access to a variety of pure synthetic vaccines candidates against *S. pneumoniae* ST 8.

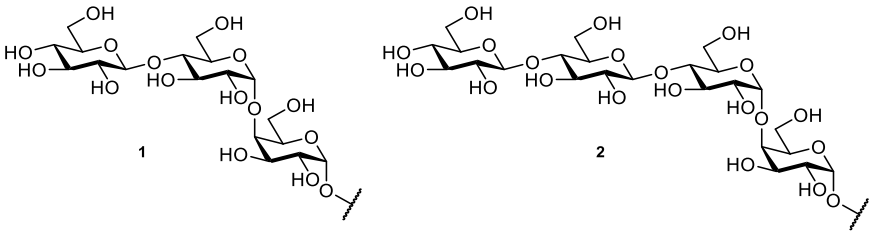
I.4 Conclusion and outlook

Severe diseases caused by bacterial pathogens are still a major threat to human health and the emerging spread of multiresistant germs emphasize the need for effective preventing therapies. In recent years, synthetic subunit vaccines came to the fore, holding the promise of effectively addressing the limitations of current glycoconjugate vaccine formulations.[14] In addition, synthetic approaches can offer new perspectives in terms of epitope derivatisation to improve immune potency and bioavailability.

Polysaccharides are attractive target structures in vaccine development and carbohydrate-specific antibodies are generally hold accountable for mediating immune protection against encapsuled bacteria. In the last decades, great efforts have been dedicated towards the identification of minimal protective glycan structures and the development of chemical approaches for their syntheses. However, sufficient *in vivo* stability and intrinsic immunogenicity are important requirements for application of such synthetic glycotopes in modern vaccine candidates. Here incorporation of fluorine is an attractive concept for stabilising crucial recognition elements and enhancing the immunogenicity of otherwise poor immunogens.[206, 245, 246]

Synthesis of fluorinated S. pneumoniae serotype 8 glycotope mimetics

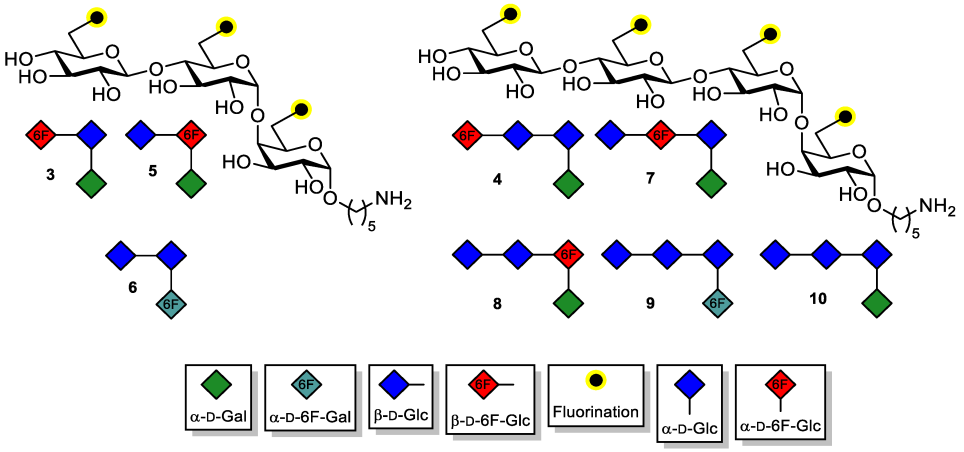
S. pneumoniae is a gram-positive bacterium which populates the upper respiratory tract of humans. Invasive diseases caused by this pathogen include *pneumonia*, *sepsis* and *meningitis*. The risk of acquiring such invasive pneumococcal diseases (IPD) is especially high in regions with poor health standards and for infants under the age of five. *S. pneumoniae* can be categorized in more than 90 serotypes and it is assumed that most, if not all serotypes induce serious disease in humans.[268, 269] Serotype 8 is a highly virulent serotype that has been reported in connection with IPD in adult patients and broad resistance against common antibiotics.[276-279] Its absence from commercially available conjugate vaccines could further facilitate the replacement of other serotypes by ST 8. In a recent study glycotopes **1** and **2** were identified as promising glycan structures for the development of synthetic vaccine candidates against ST 8 (see Scheme 37).[194] However weak glycotope immunogenicity seems to be a key challenge *en route* to synthetic vaccine candidates.



Scheme 37: Glycotopes of *S. pneumoniae* ST 8.

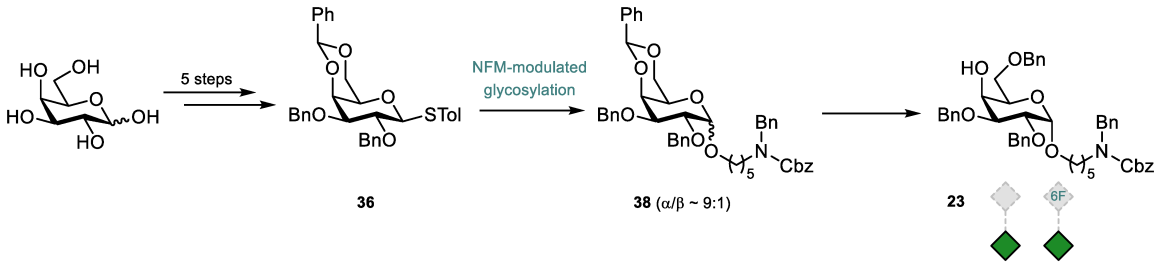
Within this project, synthetic approaches towards fluorinated tri- and tetrasaccharide mimetics of glycotopes **1** and **2** were developed. Hypothesizing that the C-6-position of the terminal glucose moiety should represent one of the most exposed positions in these glycotopes lead to the inclusion of the terminal fluorinated glycotopes in our envisioned selection (**3** and **4**, see Scheme 38). Given that positioning of the fluorine substituent has previously been reported to influence the immunological properties of such antigen mimetics, we further enlarged our selection of glycotope mimetics to provide an ensemble of C-6-fluorinated tri- and tetrasaccharide analogues (**3** -

9). This complete set of C-6-fluorinated glycotopes may provide a valuable tool to investigate the influence of fluorine incorporation onto antibody recognition and immunological potency of these glycotopes.



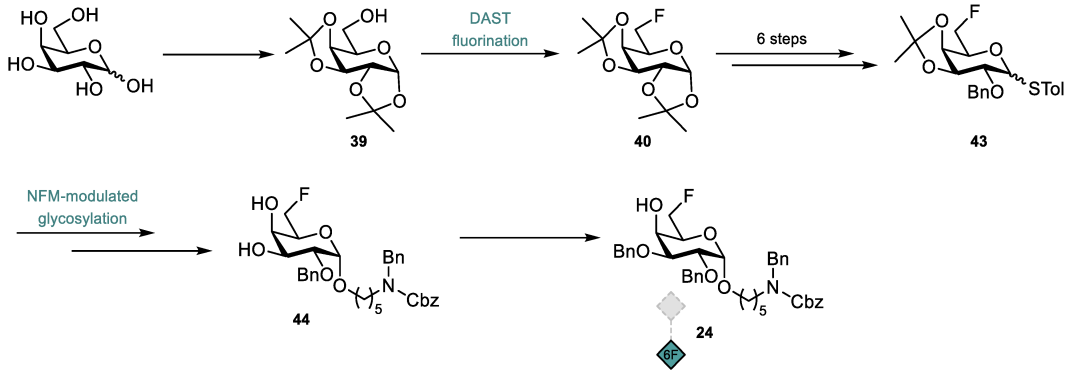
Scheme 38: Glycotope mimetics synthesized within the frame of this project.

Thus, target structures were accessed from the respective monomeric building blocks *via* a convergent strategy. Here effective fluorine incorporation and the assembly of both synthetically challenging 1,2-*cis* glycosidic linkages was of particular interest for the success of this project.



Scheme 39: Synthesis of galactosyl acceptor building block **23** in seven synthetic steps from D-galactose.

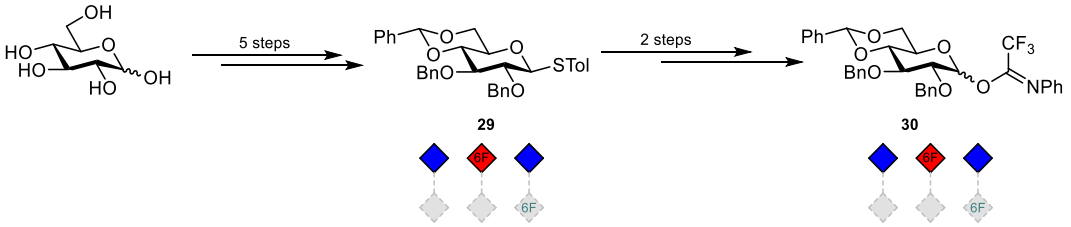
Hence, galactosyl acceptor **23** was synthesized over seven synthetic steps from D-galactose (see Scheme 39). Incorporation of the 1,2-*cis* configurated linker moiety constituted a key step *en route* to **23**, which was successfully addressed by a NFM-modulated glycosylation protocol.



Scheme 40: Synthesis of fluorinated galactosyl acceptor **24** in eleven synthetic steps from D-galactose.

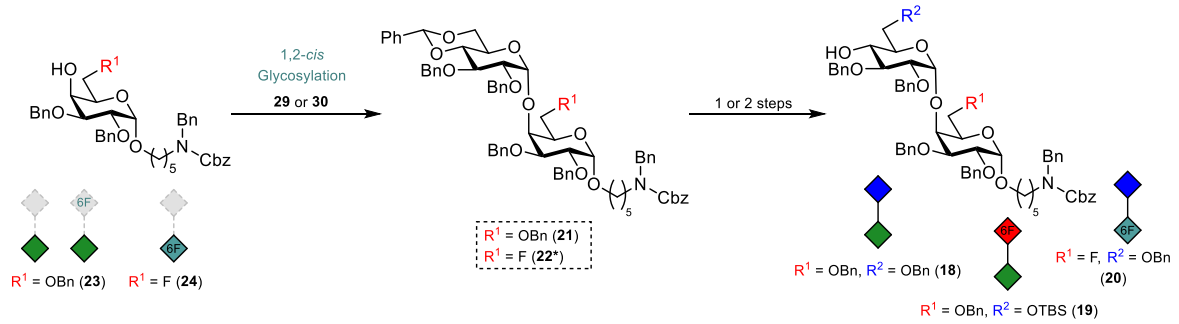
This NFM-modulated glycosylation approach was further employed in the synthesis of fluorinated galactosyl acceptor **24**, which was provided over eleven synthetic steps including DAST-mediated fluorination of the C-6-

position, NFM-modulated linker glycosylation and subsequent regioselective etherification of the C-3-position (see Scheme 40). Here, the impaired reactivity of thioglycoside **43** during linker glycosylation appeared as a bottleneck of this synthetic route.



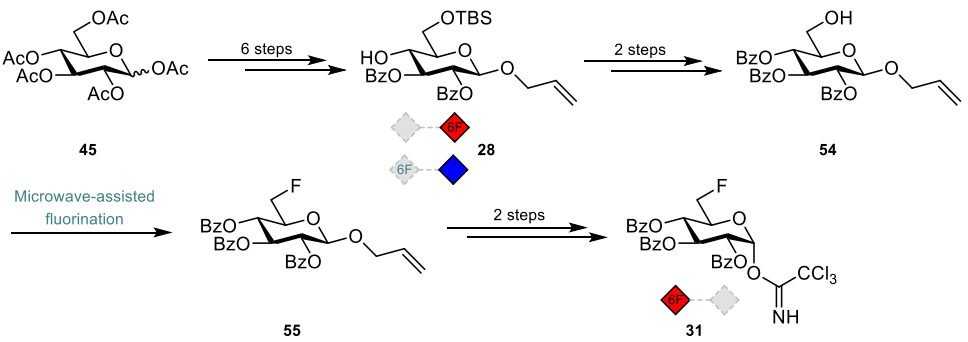
Scheme 41: Synthesis of glucose donor building blocks **29** and **30** from D-glucose in five or seven synthetic steps respectively.

Glucosyl donor building blocks **29** and **30**, both comprising a 4,6-*O*-benzyliden protecting group to support 1,2-*cis* selectivity and to facilitate subsequent regioselective protection of the C-6´-position were obtained over five and seven synthetic steps according to literature-known protocols (see Scheme 41). Subsequently, these building blocks were employed for the assembly of disaccharide acceptor (**18** - **20**) *via* 1,2-*cis* selective [1+1]-glycosylation of galactosyl acceptors **23** and **24** (see Scheme 42).



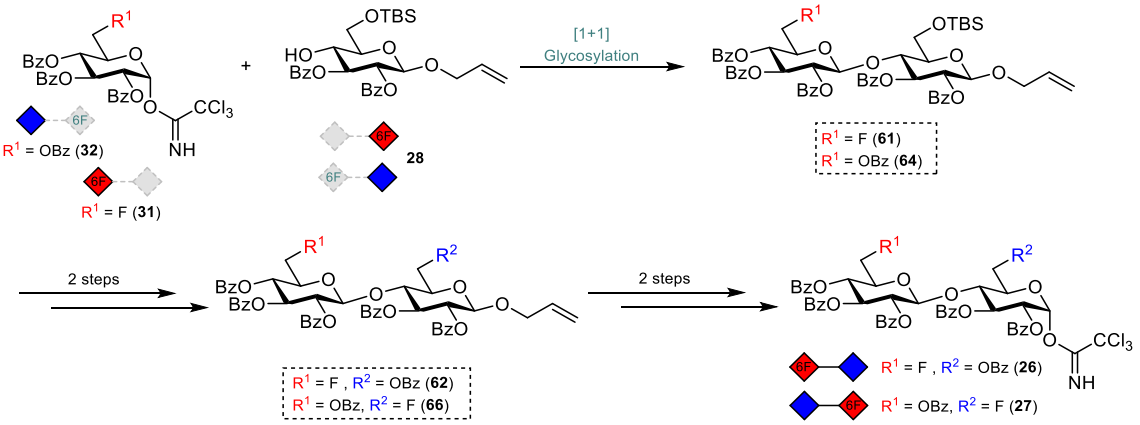
Scheme 42: Assembly of disaccharide acceptor building blocks **18** - **20** (* not isolated in pure form).

Here, glycosylation of acceptor **23** with thioglycoside donor **29** or PTFA donor **30** selectively furnished the desired α-linked product in high yield. However, in terms of the overall number of synthetic steps, thioglycoside **29** was identified as superior choice. Reductive benzylidene opening, or acidic hydrolysis and subsequent regioselective protection of the C-6´-position provided acceptor building blocks **18** and **19**, respectively. Accordingly, fluorinated acceptor **20** was synthesized *via* glycosylation of **24** with **29** and subsequent regioselective benzylidene opening. For the assembly of fluorinated cellobiose donors, monomeric building blocks **28** and **31** for were synthesized from previously described glucose derivative **45** in six and eleven steps, respectively (see Scheme 43).



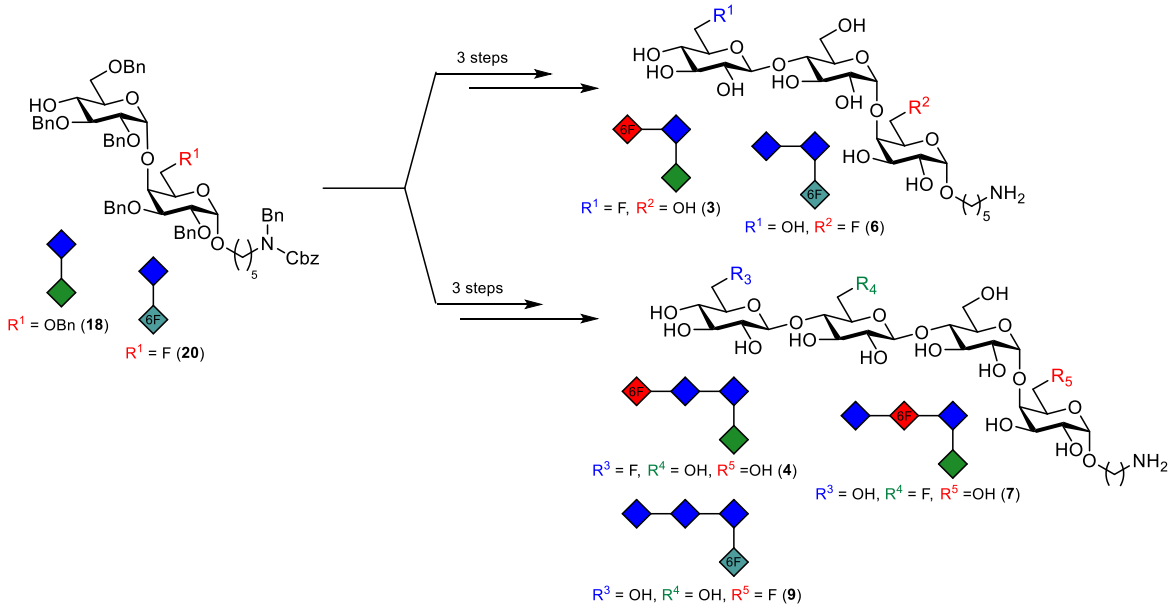
Scheme 43: Synthesis of glucose acceptor **28** and fluorinated glucose donor **31**.

Towards **31**, DAST-mediated fluorination of primary alcohol **54** was of particular interest. Compared to the existing protocol[368] from *Lin et al.* which suffers from extensive side-product formation, the here described microwave assisted fluorination approach provided a high yielding access to glucose derivative **55**. The non-fluorinated glucosyl donor **32** was provided in three synthetic steps *via* benzoylation and selective deprotection of the anomeric centre.



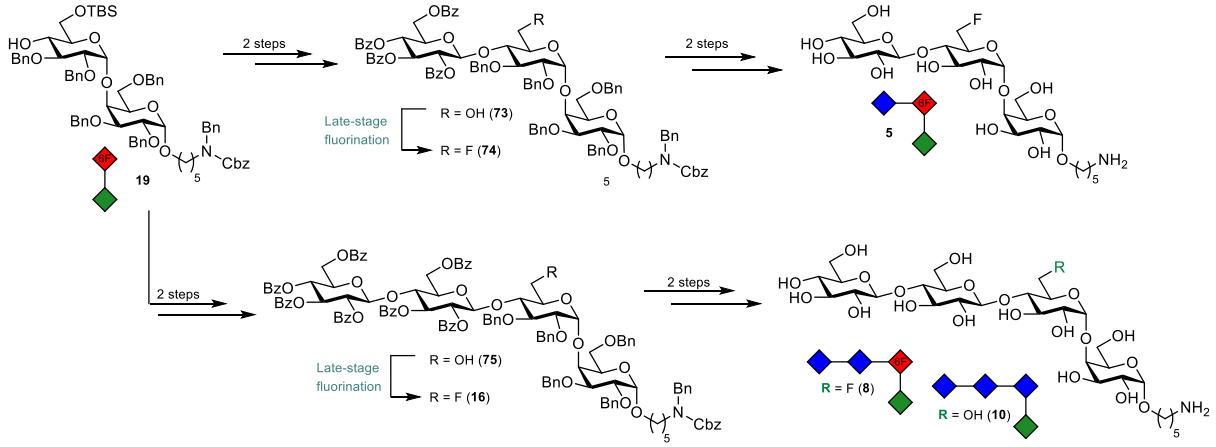
Scheme 44: Assembly of fluorinated cellobiose building blocks **26** and **27** over five synthetic steps from glucosyl donors **31** or **32** and acceptor **28**.

Fluorinated cellobiose building blocks **26** and **27** were assembled from the previously described monosaccharide building blocks **31** or **32** and glucosyl acceptor **28** in five synthetic steps, starting with a *trans*-selective [1+1] glycosylation reaction (**61**, **64**; see Scheme 44). In particular cellobiose derivative **66**, which comprises a fluorine substituent on the C-6-position, was obtained *via* the already mentioned *trans*-selective [1+1] glycosylation (**64**), followed by C-6 deprotection and subsequent microwave assisted disaccharide-fluorination. In addition, native cellobiose donor **25** was synthesized from D-cellobiose in a three-step protocol according to the synthesis of glucose donor **32**.



Scheme 45: Synthesis of fluorinated glycotopes **3**, **4**, **6**, **7**, **9**.

Fluorinated tri- and tetrasaccharides **3**, **4**, **6**, **7** and **9** were obtained from [2+1]- and [2+2]-*trans* glycosylation reactions of disaccharide acceptors **18** or **20** and the respective mono- (**31**, **32**) or disaccharide donors (**25** - **27**) followed by global deprotection (see Scheme 45). In contrast, derivatives **5** and **8** comprising a fluorine substituent at the bridging α-glucose moiety were obtained over five synthetic steps including a [2+1]- and [2+2]-*trans* glycosylation, a DAST-mediated late-stage fluorination and a global deprotection step (see Scheme 46). In addition, non-fluorinated tetrasaccharide **10** was obtained after global deprotection of intermediate **75**.

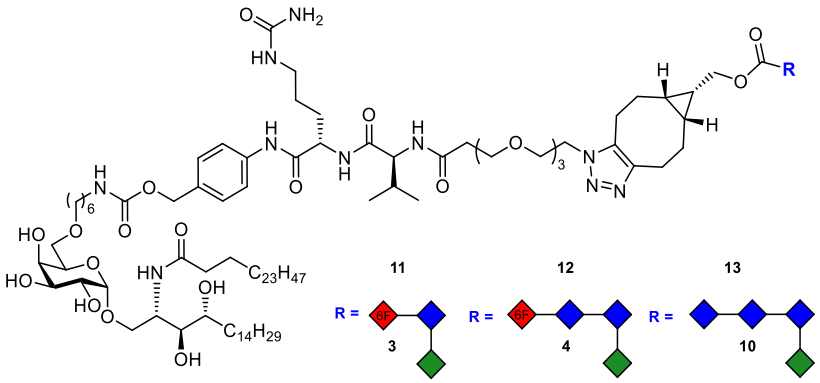


Scheme 46: Synthesis of compounds **5**, **8** and **10** in four synthetic steps from disaccharide precursor **19**.

In summary, a selection of seven fluorinated glycotope mimetics related to the repetitive unit of *S. pneumoniae* serotype 8 were successfully synthesized in the course of this project. Together with non-fluorinated glycotope **10**, all of these entities are now available in sufficient amounts (15 – 36 mg) for further assembly of full synthetic vaccine candidates (*vide infra*). In addition, these compounds should represent useful tools for evaluating the influence of fluorination onto binding of a glycan-specific monoclonal antibody[194] (e.g. *via* SPR or glycan microarray) and thus foster the identification of the most promising fluorinated glycotope mimetics for the design of efficient vaccine candidates.[285]

Assembly of fully synthetic vaccine candidates

In a continuative collaboration with *Sebastian Neidig*, three candidates from these synthetic glycotopes were used to assemble first synthetic vaccine conjugates. Thereby, an α-GalCer derivative comprising a self-immolative dipeptide linker equipped with an azide functionality were used in the assembly of full synthetic conjugates *via* SPAAC.



Scheme 47: Fully synthetic vaccine candidates **11** - **13**.

Accordingly, three vaccine candidates either comprising the terminal fluorinated glycotopes (**11**, **12)** or the non-fluorinated congener (**13**, see Scheme 47) were obtained. However, all conjugates showed a small molecular contaminant after precipitation from water, which could be successfully removed from conjugate **11** by an additional precipitation step. Since this approach was unfortunately hampered by incomplete precipitation, improved protocols are currently developed for purification of the remaining conjugate vaccine candidates **12** and **13**.

I.5 Experimental data

Synthesis of fluorinated *S. pneumoniae* Serotype 8 glycotopes and assembly of synthetic vaccine candidates

Methods and reagents:

If not otherwise noted, all reactions were magnetically stirred and conducted in oven-dried glassware. Moisture sensitive reactions were performed in an argon atmosphere using standard Schlenk techniques. Further, solvents for moisture sensitive reactions (Diethyl ether, Dichloromethane, Acetonitrile, Tetrahydrofuran) were dried according to standard procedures and distilled prior to use or purchased from *Acros Organics* as "extra dry" reagents stored over molecular sieve and under inert gas atmosphere (*N,N*-Dimethylformamide (DMF)). Commercially available reagents were purchased from *Sigma-Aldrich* or *TCI-Chemicals* and were used without further purification. Analytical thin-layer chromatography (TLC) was used for monitoring of reactions. TLC was performed on pre-coated silica gel 60 F254 aluminium plates (*Merck KGaA*, Darmstadt) and visualized by exposure to ultraviolet light (UV, 254 nm) and/or staining with either a 1:1 mixture of 1 M H_2SO_4 in EtOH and 3 % 4-methoxyphenol solution in EtOH (Carbohydrates) or Seebach reagent (Cerium phosphomolybdic acid (5.0 g) concentrated sulfuric acid (16 ml), water (200 ml) and Cerium(IV) sulphate (2.0 g)). If not otherwise stated, purification of substances was achieved by standard column chromatography on silica (35-70 µm particle size) from *Acros organics.*

NMR spectroscopy:

Proton nuclear magnetic resonance (^{1}H-NMR) were recorded in deuterated solvents ($CDCl_3$, CD_2Cl_2, DMSO-d_6) on a *Bruker Avance III HD 400 MHz* spectrometer equipped with a CyroProbe™, a *Varian VXR 400 S* spectrometer, a *Bruker AMX600* spectrometer or a *Bruker Avance III HD 800 MHz* spectrometer equipped with a Cyro Probe™. Chemical Shifts (δ scale) were reported in parts per million (ppm) and calibration was carried out with residual protic solvents as internal reference. For indication of multiplicities the following abbreviations were used: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) as well as combinations thereof. Carbon nuclear magnetic resonance spectra (^{13}C-NMR) were recorded on the aforementioned spectrometers with 100 MHz, 150 MHz and 200 MHz and chemical shifts (δ) were also given in ppm referenced to the central carbon signal of the solvents. For further assignment of the ^{1}H and ^{13}C-NMR signals, 2D-NMR experiments (COSY, HSQC, HMBC) were used. If necessary, anomeric configuration of carbohydrate building blocks were proved by using proton coupled HSQC experiments. Numbering of proton and carbon atoms used herein does not necessarily correspond to the IUPAC and are thus further described in the respective section. If necessary, ^{1}H and ^{13}C spectra were processed in *MestreNova* using the Auto Phase Correction and Auto Baseline Correction function.

High-resolution mass spectrometry (HRMS) and Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF)

High resolution (HR-ESI) mass spectra were recorded on a *Thermo Finnigan LTQ FT* spectrometer either in positive or negative ionization mode. MALDI-TOF spectra were recorded on a *Bruker Daltonics Autoflex II* time of flight spectrometer equipped with a N_2-Laser (λ = 337 nm) using either 6-Azo-2-thiothymin (ATT) or Super-DHB (SDHB) as matrix.

High performance liquid chromatography (HPLC):

Analytical RP-HPLC was conducted at a *JASCO* system (PU-2080 Plus, LG-2080-02-S, DG-2080-53 and MD-2010 Plus) on a *Phenomenex Luna* column (C18, 5 µm, 250 mm × 4.6 mm; later referred to as "*Luna*"). A gradient

of water (A)/acetonitrile (B) containing 0.1 % TFA with a flow-rate of 1 ml/min was used as eluent. Exact compositions of gradients are given in brackets at the respective section.

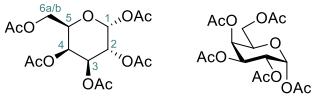
Optical rotation:

Optical rotations were measured on a Perkin-Elmer polarimeter 241 at the Sodium-D-line (589 nm) at the given temperature in °C. Concentration c is given in g/100 ml in the solvent stated in brackets ($CHCl_3$).

Synthesis of monosaccharide building blocks

Synthesis of galactosyl acceptor 23

Penta-*O*-acetyl-α-D-galactopyranoside (**33**)[387]



To a magnetically stirred solution of D-galactose (20.0 g, 111 mmol, 1.0 eq.) in pyridine (150 ml), acetic anhydride (104 ml, 1.11 mmol, 10 eq.) and 4-DMAP (1.23 g, 1.10 mmol, 0.1 eq.) was added. The reaction was stirred for 17 h at room temperature. The solution was concentrated under reduced pressure and the crude residue was poured into ice-cooled water. The mixture was stirred for 6 h at ambient temperature, filtered and the precipitate was washed thoroughly with water and dried overnight. Subsequently, the crude product was dissolved in CH_2Cl_2 (300 ml) and washed sat. aq. $NaHCO_3$ (200 ml), 1 M HCl (200 ml) and brine (150 ml) and dried with $MgSO_4$. Solvents were removed under reduced pressure to afford **33** (40.4 g, 103 mmol, 93 %) as colourless solid.

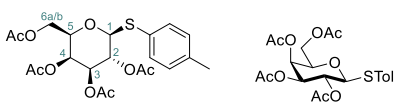
R_f = 0.43 (cHex/EtOAc = 1:1)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 6.37 (d, $J_{H1,H2}$ = 1.8 Hz, 1H, 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 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.99 (s, 3H, OAc).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 170.5, 170.3, 170.2, 170.0, 169.0 (5 × C=O), 89.9 (C-1), 68.9 (C-5), 67.6 (C-4), 67.5 (C-2/C-3), 66.6 (C-2/C-3), 61.4 (C-6), 21.0, 20.8 (2C), 20.7 (2C, 5 × OAc).

HRMS (ESI$^+$): Calculated 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 (**34**)[293]



To an ice-cooled solution of **33** (20.0 g, 51.2 mmol, 1.0 eq.) and *p*-thiocresol (9.55 g, 76.9 mmol, 1.5 eq.) in dry CH_2Cl_2 (125 ml), $BF_3{\cdot}OEt_2$ (13.2 ml, 104 mmol, 2.0 eq.) was added slowly. The reaction mixture was warmed to ambient temperature and stirred for 24 h, before being neutralized by careful addition of solid $NaHCO_3$. The

organic layer was washed with sat. aq. $NaHCO_3$ (100 ml) and brine (100 ml) and dried with $MgSO_4$. Organic solvents were removed under reduced pressure and the crude product was purified by column chromatography (^{c}Hex/EtOAc *v/v* = 2:1) to afford thioglycoside **34** (18.6 g, 40.9 mmol, 80 %) as a colourless solid.

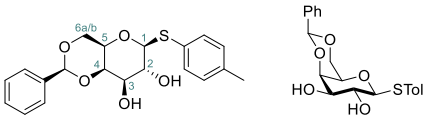
R$_f$ = 0.46 (^{c}Hex/EtOAc = 1:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.43 − 7.37 (m, 2H, Ar-H), 7.12 (d, $J_{CH,CH}$ = 7.9 Hz, 2H, Ar-H), 5.40 (dd, $J_{H4,H3}$ = 3.4 Hz, $J_{H4,H5}$ = 1.2 Hz, 1H, H-4), 5.22 (t, $J_{H2,H1}$ = $J_{H2,H3}$ = 10.0 Hz, 1H, H-2), 5.03 (dd, $J_{H3,H2}$ = 10.0 Hz, $J_{H3,H4}$ = 3.4 Hz, 1H, H-3), 4.64 (d, $J_{H1,H2}$ = 9.9 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), 3.91 (td, $J_{H5,H6a}$ = $J_{H5,H6b}$ = 6.6 Hz, $J_{H5,H4}$ = 1.1 Hz, 1H, H-5), 2.34 (s, 3H, Ar-CH_3), 2.11 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.96 (s, 3H, OAc).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 170.5, 170.3, 170.2, 169.6 (4 × C=O), 138.6, 133.2 (2C), 129.8 (2C), 128.8 (6 × C-Ar), 87.1 (C-1), 74.5 (C-5), 72.2 (C-3), 67.4 (2C, C-2, C-4), 61.7 (C-6), 21.3 (Ar-CH_3), 21.0 (OAc), 20.8 (2C, 2 × OAc), 20.7 (OAc).

HRMS (ESI$^+$): Calculated for $C_{21}H_{30}NO_9S^+$ $[M+NH_4]^+$: 472.1636; found: 472.1638.

4-Methylphenyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**35**)[295]



To a magnetically stirred mixture of **34** (5.58 g, 12.3 mmol, 1.0 eq.) in methanol (50 ml), catalytic amounts of a freshly prepared NaOMe/MeOH solution were added. The reaction was stirred for 17 h at ambient temperature, before being neutralized by addition of *Amberlite® IR 120*, filtered and concentrated under reduced pressure. The thus obtained tetrol was dissolved in dry DMF (40 ml) and BADA (2.41 ml, 16.0 mmol, 1.3 eq.) and camphorsulfonic acid (610 mg, 2.63 mmol, 0.2 eq.) were added. The reaction was stirred for 20 h at ambient temperature, before being neutralized by addition of NEt_3 (2 ml) and concentrated to dryness. The crude product was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 1:1 → 0:1) to afford **35** (3.73 g, 9.96 mmol, 81 % over two steps) as a colorless solid.

R$_f$ = 0.48 (EtOAc).

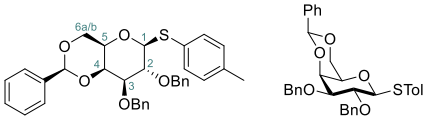
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.61-7.54 (m, 2H, Ar-H), 7.43-7.31 (m, 5H, Ar-H), 7.14-7.08 (m, 2H, Ar-H), 5.50 (s, 1H, CH-Ar), 4.46 (d, $J_{H1,H2}$ = 9.1 Hz, 1H, H-1), 4.38 (dd, $J_{H6a,H6b}$ = 12.4 Hz, $J_{H6a,H5}$ = 1.6 Hz, 1H, H-6a), 4.21 (dd, $J_{H4,H3}$ = 3.5 Hz, $J_{H4,H5}$ = 1.7 Hz, 1H, H-4), 4.03 (dd, $J_{H6b,H6a}$ = 2.5 Hz, $J_{H6b,H5}$ = 1.8 Hz, 1H, H-6b), 3.77-3.64 (m, 1H, H-3), 3.63 (td, $J_{H2,H1}$ = $J_{H2,H3}$ = 9.1 Hz, $J_{H2,OH}$ = 1.7 Hz, 1H, H-2), 3.54 (m, 1H, H-5), 2.54 − 2.47 (m, 2H, 2 × OH), 2.36 (s, 3H, Ar-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 138.7, 137.8, 134.5, 129.9, 129.5, 128.3, 126.7 (7 × C-Ar), 101.6 (CH-Ar), 87.2 (C-1), 75.5 (C-4), 73.9 (C-3), 70.2 (C-5), 69.5 (C-6), 68.9 (C-2), 21.4 (Ar-CH_3). Due to signal overlap 15 out of 20 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{20}H_{26}NO_5S^+$ $[M+NH_4]^+$: 392.1526; found: 392.1527.

Further analytical data see reference.[388]

4-Methylphenyl-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**36**)[295]



To a magnetically stirred solution of **35** (3.60 g, 9.61 mmol, 1.0 eq.) in dry DMF (50 ml), sodium hydride (60 % dispersion in mineral oil, 0.96 g, 24.0 mmol, 2.5 eq.) was added at 0 °C. The reaction mixture was stirred for 0.5 h, before benzyl bromide (2.85 ml, 24.0 mmol, 2.5 eq.) was added dropwise. The reaction was slowly warmed to ambient temperature and stirred for 17 h. Subsequently the reaction was stopped by careful addition of H_2O (2 ml) and concentrated under reduced pressure. The crude product was solved in CH_2Cl_2 (150 ml) and washed with H_2O (25 ml) and brine (15 ml) and dried with $MgSO_4$. Organic solvents were removed under reduced pressure and the crude product was crystallized from EtOH to afford **36** (4.41 g, 7.95 mmol, 83 %) as a colourless solid.

$\mathbf{R}_f$ = 0.73 (^{c}Hex/EtOAc = 1:1).

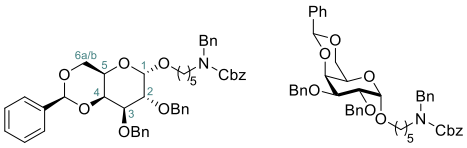
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.65 − 7.27 (m, 17H, Ar-H), 7.01 (d, $J_{CH,CH}$ = 7.9 Hz, 2H, Ar-H), 5.49 (s, 1H, CH-Ar), 4.75-4.68 (m, 4H, 4 × CH_{Bn}), 4.58 (d, $J_{H1,H2}$ = 9.5 Hz, 1H, H-1), 4.38 (dd, $J_{H6a,H6b}$ = 12.3 Hz, $J_{H6a,H5}$ = 1.7 Hz, 1H, H-6a), 4.15 (d, $J_{H4,H3}$ = 3.4 Hz, 1H, H-4), 3.99 (dd, $J_{H6b,H6a}$ = 12.3 Hz, $J_{H6b,H5}$ = 1.7 Hz, 1H, H-6b), 3.85 (t, $J_{H2,H1}$ = $J_{H2,H3}$ = 9.3 Hz, 1H, H-2), 3.63 (dd, $J_{H3,H2}$ = 9.2 Hz, $J_{H3,H4}$ = 3.4 Hz, 1H, H-3), 3.41 (s, 1H, H-5), 2.31 (s, 3H, Ar-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 138.7, 138.2, 138.0, 137.8, 133.6, 129.8, 129.2, 128.7, 128.5 (2C), 128.3 (2C), 128.0, 127.9, 127.8, 126.8 (16 × C-Ar), 101.5 (CH-Ar), 86.7 (C-1), 81.6 (C-3), 75.6 (CH_{Bn}), 75.4 (C-2) 73.8 (C-4), 72.0 (CH_{Bn}), 69.9 (C-5), 69.6 (C-6), 21.3 (Ar-CH_3). Due to signal overlap 26 out of 34 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{34}H_{38}NO_5S^+$ $[M+NH_4]^+$: 572.2465; found: 572.2468.

For further analytical data see references.[388, 389]

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranoside (**38**)



Diethyl ether directed glycosylation:

Thioglycoside donor **36** (1.00 g, 1.80 mmol, 1.0 eq.) and **37**[301, 302] (766 mg, 2.34 mmol, 1.3 eq.) were combined and co-evaporated with toluene (2 × 20 ml) and dried under high vacuum for 1 h. Subsequently, the starting materials were dissolved in a mixture of Et_2O/CH_2Cl_2 (v/v = 3:1, 60 ml) and freshly activated 4 Å MS was added. The mixture was stirred for 1 h at ambient temperature and cooled to – 10 °C before NIS (511 mg, 2.34 mmol, 1.3 eq.) and TMSOTf (414 µl, 2.34 mmol, 1.3 eq.) was added. After TLC monitoring indicated complete conversion of galactosyl donor **36**, the reaction was neutralized by addition of NEt_3 (1.5 ml) and filtered through pad of *Hyflo*® The organic layer was washed with sat. aq. $Na_2S_2O_3$ (2 × 25 ml), sat. aq. $NaHCO_3$ (25 ml) and brine (15 ml) and dried with $MgSO_4$. Organic solvents were removed under reduced pressure and the crude residue was subjected to column flash chromatography (^{c}Hex/EtOAc *v/v* = 5:1 → 3:1) to afford **38** (150 mg, 0.20 mmol, 11 %) and the corresponding β-anomer **38-β** (1.00 g, 1.31 mmol, 73 %) as colourless oils.

NFM-modulated glycosylation:

In accordance to *Ingle et al.*[306]

Donor **36** (2.00 g, 3.60 mmol, 1.0 eq.) was co-evaporated with toluene (2 ×25 ml) and dried 1 h under high vacuum, before being dissolved in dry CH_2Cl_2 (50 ml). *N*-formyl morpholine (5.78 mL, 57.6 mmol, 16 eq.; stored 17 h over freshly activated 4 Å MS prior to use) and freshly activated 4 Å MS were added and the resulting mixture was stirred for 1 h at room temperature. *N*-Iodosuccinimide (1.21 g, 5.40 mmol, 1.5 eq.) was added and the reaction mixture was cooled to – 20 °C. Subsequently, TMSOTf (976 µL, 5.40 mmol, 1.5 eq.) was added dropwise over 10 min and the reaction mixture was allowed to warm to – 10 °C. Upon complete conversion of the starting material to the NFM-imidinium adduct, amino pentanol-linker **37**[301, 302] (2.12 g, 6.49 mmol, 1.8 eq.; co-evaporated with toluene and dried under high vacuum prior to use) dissolved in dry CH_2Cl_2 was added slowly over 0.5 h. The reaction mixture was stirred at – 10 °C until TLC monitoring indicated complete conversion of the imidinium adduct. The reaction was stopped by addition of NEt_3 (3 ml), diluted with CH_2Cl_2, filtered through a pad of *Hyflo*® and washed with sat. aq. $Na_2S_2O_3$ (2 × 25 ml) and brine (15 ml). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was subjected to column flash chromatography (^{c}Hex/EtOAc *v/v* = 1:0 → 6:1) to afford **38** (2.19 g, 2.89 mmol, 80 %) and the corresponding β-anomer **38-β** (261 mg, 0.34 mmol, 9 %) as colourless oils.

α-Anomer (**38**):

$\mathbf{R}_f$ = 0.43 (^{c}Hex/EtOAc *v/v* = 3:1)

Optical Rotation: $[\alpha]_D^{22}$ = + 60.8 ° (c = 0.33; $CHCl_3$)

^{1}H-NMR (800 MHz, $CDCl_3$, α-anomer): δ [ppm] = 7.54 – 7.52 (m, 2H, Ar-H), 7.40 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, Ar-H), 7.38 – 7.23 (m, 20H, Ar-H), 7.17 (d, $J_{CH,CH}$ = 7.4 Hz, 1H, Ar-H), 5.47 (s, 1H, CH-Ar), 5.18 (d, $J_{CH,CH}$ = 25.5 Hz, 2H, CH_{Cbz}), 4.88 – 4.84 (m, 2H, H-1, CH_{Bn}), 4.81 (d, $J_{CH,CH}$ = 12.1 Hz, 1H, CH_{Bn}), 4.73 (d, $J_{CH,CH}$ = 12.2 Hz, 1H, CH_{Bn}), 4.66 – 4.62 (m, 1H, CH_{Bn}), 4.52 – 4.46 (m, 2H, NCH_{Bn}), 4.21 – 4.15 (m, 2H, H-6a, H-4), 4.05 (dd, $J_{H2,H3}$ = 9.2 Hz, $J_{H2,H1}$ = 3.6 Hz, 1H, H-2), 4.02 – 3.95 (m, 2H, H-3, H-6b), 3.64 – 3.52 (m, 2H, H-5, CH_{Linker}), 3.44 – 3.35 (m, 1H, CH_{Linker}), 3.30 – 3.17 (m, 2H, 2 × CH_{Linker}), 1.66 – 1.47 (m, 4H, 4 × CH_{Linker}), 1.35 – 1.21 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (200 MHz, $CDCl_3$, α-anomer): δ [ppm] = 156.9/156.3 (C=O-Cbz), 139.0, 138.9, 138.0 (2C),137.0/136.9 (5 × Cq), 129.0, 128.7, 128.6 (2C), 128.4, 128.2, 128.1, 128.0 (2C), 127.7, 127.6, 127.5, 127.4, 127.3, 126.5 (15 × C-Ar), 101.2 (CH-Ar), 98.3 (C-1), 76.3 (C-3), 75.8 (C-2), 74.9 (C-4), 73.7 (CH_{Bn}), 72.2 (CH_{Bn}), 69.6 (C-6), 68.3 (CH_{Linker}), 67.3 (CH_{Cbz}), 62.7 (C-5), 50.6/50.3 (N-CH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.3 (CH_{Linker}),, 28.1/27.7 (CH_{Linker}), 23.6 (CH_{Linker}). Due to signal overlap 37 out of 47 carbon atoms were assigned in the ^{13}C spectrum.

^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{H1,C1}$ = 169 Hz

HRMS (ESI^+): Calculated for $C_{47}H_{51}O_8NNa^+$ $[M+Na]^+$: 780.3507; found: 780.3493.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 14.72, λ = 230 nm

β-Anomer (38-β):

R$_f$ = 0.18 (^{c}Hex/EtOAc *v/v* = 3:1)

^{1}H-NMR (600 MHz, $CDCl_3$, β-anomer): δ [ppm] = 7.57 – 7.54 (m, 2H, Ar-H), 7.40 – 7.12 (m, 23H), 5.49 (s, 1H, CH-Ar), 5.16 (d, $J_{CH,CH}$ = 17.7 Hz, 2H, CH_{Cbz}), 4.92 – 4.73 (m, 4H, 4 × CH_{Bn}), 4.47 (d, $J_{CH,CH}$ = 16.5 Hz, 2H, NCH_{Bn}), 4.37 – 4.31 (m, 1H, H-1), 4.28 (d, $J_{H6a,H6b}$ = 12.3 Hz, 1H, H-6a), 4.10 (d, $J_{H4,H3}$ = 3.7 Hz, 1H, H-4), 4.00 (dd, $J_{H6b,H6a}$ = 12.3 Hz, $J_{H6b,H5}$ = 1.8 Hz, 1H, H-6b), 3.97 – 3.88 (m, 1H, CH_{Linker}), 3.82 (dd, $J_{H2,H3}$ = 9.7 Hz, $J_{H2,H1}$ = 7.8 Hz, 1H, H-2), 3.54 (dd, $J_{H3,H2}$ = 9.7 Hz, $J_{H3,H4}$ = 3.7 Hz, 1H, H-3), 3.49 – 3.39 (m, 1H, CH_{Linker}), 3.28 (s, 1H, H-5), 3.25 – 3.13 (m, 2H, 2 × CH_{Linker}), 1.72 – 1.46 (m, 4H, 4 × CH_{Linker}), 1.41 – 1.26 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (150 MHz, $CDCl_3$, β-anomer): δ [ppm] = 156.8/156.3 (C=O-Cbz), 139.0, 138.6, 138.0 (2C), 137.0/136.9 (5 × Cq), 129.0, 128.6 (2C), 128.5, 128.4 (2C), 128.2, 128.0, 127.9, 127.8 (3C), 127.4, 127.3, 126.6 (15 × C_{Ar}), 103.8 (C-1), 101.4 (CH-Ar), 79.3 (C-3), 78.6 (C-2), 75.3 (CH_{Bn}), 74.1 (C-4), 72.1 (CH_{Bn}), 69.8/69.7 (CH_{Linker}), 69.4 (C-6), 67.2 (CH_{Cbz}), 66.5 (C-5), 50.7/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.5 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}).

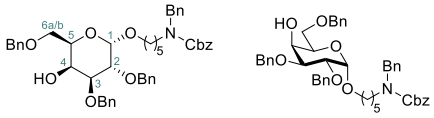
Due to signal overlap 37 out of 47 carbon atoms were assigned in the ^{13}C spectrum.

^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{H1,C1}$ = 159 Hz

HRMS (ESI^+): Calculated for $C_{47}H_{55}O_8N_2^+$ $[M+NH_4]^+$: 775.3953; found: 775.3962.

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 14.60, λ = 230 nm

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**23**)



Compound **23** was synthesized according to a modified procedure.[322]

To a magnetically stirred solution of **38** (1.75 g, 2.31 mmol, 1.0 eq.) in dry CH_2Cl_2 (20 ml), freshly activated 4 Å molecular sieve and triethylsilane (2.58 ml, 16.2 mmol, 7.0 eq.) was added. The reaction mixture was stirred 0.5 h at room temperature, before being cooled to – 78 °C. Subsequently trifluoromethanesulfonic acid (1.23 ml, 13.9 mmol, 6.0 eq.) was added slowly and the reaction mixture was stirred for 4.5 h. After complete conversion of the starting material was observed by TLC monitoring, NEt_3 (3.0 ml) was added. The mixture was filtered through a pad of *Hyflo*® and concentrated under reduced pressure. The crude product was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 4:1) to afford **23** as well as silyl ether **23a** as side-product. For cleavage of the silyl ether compound **23a** was dissolved in MeOH/CH_2Cl_2 (v/v = 1:1, 20 ml) and camphorsulfonic acid (500 mg) was added. The reaction was stirred for 45 min at ambient temperature, neutralized by addition of NEt_3 and solvents were removed under reduced pressure. The thus obtained product was subjected to flash chromatography (^{c}Hex/EtOAc *v/v* = 4:1). Products were combined to afford 1.47 g (1.93 mmol, 84 %) of galactosyl acceptor **23** as colourless oil.

R$_f$ = 0.42 (^{c}Hex/EtOAc *v/v* = 2:1)

Optical rotation: $[\alpha]_D^{22}$ = + 34.1 ° (c = 0.66; $CHCl_3$)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.43 – 7.14 (m, 25H, Ar-H), 5.19 (d, $J_{CH,CH}$ = 16.3 Hz, 2H, CH_{Cbz}), 4.83 – 4.77 (m, 3H, H-1, CH_{Bn}), 4.72 (d, $J_{CH,CH}$ = 11.5 Hz, 1H, CH_{Bn}), 4.65 (d, $J_{CH,CH}$ = 12.2 Hz, 1H, CH_{Bn}), 4.62 – 4.54 (m, 2H, CH_{Bn}), 4.50 (d, $J_{CH,CH}$ = 17.4 Hz, 2H, CH_{Bn}), 4.09 (s, 1H, H-4), 3.93 (bs, 1H, H-5), 3.90 – 3.85 (m, 2H, H-2, H-3), 3.74 (dd, $J_{H6a,H6b}$ = 10.0 Hz, $J_{H6a,H5}$ = 5.5 Hz, 1H, H-6a), 3.67 (dd, $J_{H6b,H6a}$ = 10.0 Hz, $J_{H6b,H5}$ = 6.3 Hz, 1H, H-6b), 3.66 – 3.57 (m, 1H, CH_{Linker}), 3.44 – 3.33 (m, 1H, CH_{Linker}), 3.30-3.17 (m, 2H, CH_{Linker}), 1.69 – 1.48 (m, 4H, CH_{Linker}), 1.40-1.22 (m, 2H, CH_{Linker}).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 156.8/156.3 (C=O-Cbz), 138.7, 138.4, 138.2, 138.1, 137.0/136.9 (5 × Cq), 128.7, 128.6, 128.5 (2C), 128.0 (2C), 127.9 (3C), 127.8 (3C), 127.4 (2C), 127.3 (25 × C_{Ar}), 97.5 (C-1), 77.8 (C-2/C-3), 76.0 (C-2/C-3), 73.7 (CH_{Bn}), 73.4 (CH_{Bn}), 72.8 (CH_{Bn}), 69.7 (C-6), 68.6 (C-5), 68.1 (2C, C-4, CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.6 (CH_{Linker}).

Due to signal overlap 37 out of 47 carbon atoms were assigned in the ^{13}C spectrum.

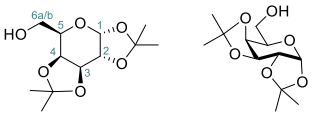
^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{H1,C1}$ = 168 Hz

HRMS (ESI$^+$): Calculated for $C_{47}H_{57}N_2O_8^+$ $[M+NH_4]^+$: 777.4109; found: 777.4098.

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 14.52 min, λ = 230 nm.

Synthesis of fluorinated galactosyl acceptor 24

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranoside (**39**)[323]



To a magnetically stirred mixture of D-galactose (5.00 g, 27.8 mmol, 1.0 eq.) and anhydrous $CuSO_4$ (11.1 g, 69.5 mmol, 2.5 eq.) in acetone (150 ml), conc. H_2SO_4 (1.0 ml) was added. The reaction was stirred for 18 h at ambient temperature, filtered and neutralized by addition of NEt_3 (2 ml). Solvents were removed under reduced pressure and the crude residue was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 2:1) to afford **39** (5.71 g, 22.0 mmol, 79 %) as a colourless oil.

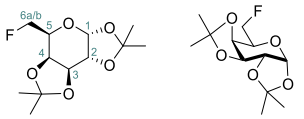
R$_f$ = 0.45 (^{c}Hex/EtOAc *v/v* = 1:1)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 5.56 (d, $J_{H1,H2}$ = 5.0 Hz, 1H, H-1), 4.61 (dd, $J_{H3,H4}$ = 7.9 Hz, $J_{H3,H2}$ = 2.4 Hz, 1H, H-3), 4.33 (dd, $J_{H2,H1}$ = 5.0 Hz, $J_{H2,H3}$ = 2.4 Hz, 1H, H-2), 4.27 (dd, $J_{H4,H3}$ = 8.0 Hz, $J_{H4,H5}$ = 1.5 Hz, 1H, H-4), 3.90 – 3.81 (m, 2H, H-5, H-6a), 3.78 – 3.68 (m, 1H, H-6b), 1.52 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.33 (s, 6H, 2 × CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 109.6 (Cq), 108.8 (Cq), 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 (CH_3), 26.1 (CH_3), 25.1 (CH_3), 24.4 (CH_3).

HRMS (ESI$^+$): Calculated for $C_{12}H_{21}O_6^+$ $[M+H]^+$: 261.1333; found: 261.1335.

1,2:3,4-Di-*O*-isopropylidene-6-deoxy-6-fluoro-α-D-galactopyranoside (**40**)[285, 324, 325]



To a magnetically stirred solution of compound **39** (1.50 g, 5.76 mmol, 1.0 eq.) in dry CH_2Cl_2 (2.5 ml), 2,4,6-collidine (1.53 ml, 11.5 mmol, 2.0 eq.) and DAST (910 µl, 6.92 mmol, 1.2 eq.) were added at ambient temperature. The reaction was heated under microwave irradiation (100W, 80 °C, 1h) and subsequently poured into MeOH (25 ml).* The organic solvents were removed under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (100 ml) and washed with 1 M HCl (25 ml) and brine (25 ml) and dried with $MgSO_4$. Solvents were removed

under reduced pressure and the crude product was subjected to column chromatography (ᶜHex/EtOAc *v/v* = 5:1) to afford product **40** (2.50 g, 9.53 mmol, 83 %) as slightly yellow oil.

* Reaction was conducted in a replicate and reaction mixtures were combined for aqueous work-up and purification.

R*f* = 0.69 (ᶜHex/EtOAc *v/v* = 3:1)

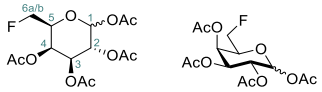
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 5.55 (d, $J_{H1,H2}$ = 5.0 Hz, 1H, H-1), 4.66 – 4.55 (m, 2H, H-6a, H-3), 4.54 – 4.45(m, 1H, H-6b), 4.34 (dd, $J_{H1,H2}$ = 5.0 Hz, $J_{H2,H3}$ = 2.5 Hz, 1H, H-2), 4.26 (dd, $J_{H4,H3}$ = 7.9 Hz, $J_{H4,H5}$ = 2.0 Hz, 1H, H-4), 4.12 – 4.03 (m, 1H, H-5), 1.54 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.33 (s, 6H, 2× CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 109.8 (Cq), 108.9 (Cq), 96.3 (C-1), 82.2 (d, $J_{C6,F}$ = 168.0 Hz, C-6), 70.7 (C-2/C-3/C-4), 70.6 (C-2/C-3/C-4), 70.5 (C-2/C-3/C-4), 66.7 (d, $J_{C5,F}$ = 22.6 Hz, C-5), 26.2 (CH_3), 26.0 (CH_3), 25.0 (CH_3), 24.5 (CH_3).

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 231.2 (ddd, $J_{F,H6a}$ = 47.7 Hz, $J_{F,H6b}$ = 46.1 Hz, $J_{F,H6}$ = 13.3 Hz).

HRMS (ESI^+): Calculated for $C_{12}H_{23}O_5NF^+$ $[M+NH_4]^+$: 280.1555; found: 280.1556.

1,2,3,4-Tetra-*O*-acetyl-6-deoxy-6-fluoro-α/β-D-galactopyranoside (**41**)[285, 324]



A solution of compound **40** (2.50 g 9.53 mmol, 1.0 eq.) in 80 % aq. AcOH (100 ml) was stirred for 18 h at 100 °C. Subsequently, solvents were removed under reduced pressure and the crude residue was co-evaporated with toluene (2 × 50 ml). The crude product was dissolved in pyridine (50 ml) and acetic acid anhydride (9.01 ml, 95.3 mmol, 10 eq.) and 4-DMAP (116 mg, 0.95 mmol, 0.1 eq.) were added at ambient temperature. After the reaction was deemed complete by TLC monitoring, solvents were removed under reduced pressure and the residue was co-evaporated with toluene (2 × 50 ml). The crude product was subjected to column chromatography (ᶜHex/EtOAc *v/v* = 1:1) to afford **41** (3.27 g, 9.33 mmol, 98 % over two steps) as colourless oil.

R*f* = 0.79 (ᶜHex/EtOAc *v/v* = 1:1)

α-Anomer:

^{1}H-NMR (400 MHz, $CDCl_3$, selected signals): δ [ppm] = 6.39 (s, 1H, H-1), 5.55 (bs, 1H, H-4), 5.38 – 5.31 (m, 1H, H-2), 4.69 – 4.27 (m, 3H, H-6a, H-6b, H-5).

^{13}C-NMR (100 MHz, $CDCl_3$, selected signals): δ [ppm] = 89.8 (C-1), 67.6(d, $J_{C4,F}$ = 5.4 Hz, C-4).

β-Anomer:

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 5.72 (d, $J_{H1,H2}$ = 8.3 Hz, 1H, H-1) 5.48 (dd, $J_{H4,H3}$ = 3.4 Hz, $J_{H4,H5}$ = 1.2 Hz, 1H, H-4), 5.38 – 5.31 (m, 1H, H-2), 5.09 (dd, $J_{H3,H2}$ = 10.4 Hz, $J_{H3,H4}$ = 3.4 Hz, 1H, H-3), 4.69 – 4.27 (m, 2H, H-6a, H-6b), 4.15 – 4.05 (m, 1H, H-5), 2.21 – 1.80 (m, 12H, 4 × OAc).

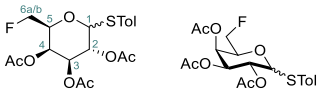
^{13}C-NMR (100 MHz, $CDCl_3$, β-anomer): δ [ppm] = 92.2 (C-1), 80.8 (d, $J_{C6,F}$ = 171.9 Hz, C-6), 72.3 (d, $J_{C5,F}$ = 23.8 Hz, C-5), 70.9 (C-3), 66.9 (d, $J_{C4,F}$ = 5.1 Hz, C-4), 21.0 (OAc), 20.9 (OAc), 20.8(OAc), 20.7 (OAc).

Note: Due to signal overlapping, C-2 could not unambiguously be assigned from the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 232.1 - − 231.8 (m).

HRMS (ESI^+): Calculated for $C_{14}H_{19}FO_9Na^+$ $[M+Na]^+$: 373.0906; found: 373.0904.

4-Methylphenyl-2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-1-thio-α/β-D-galactopyranoside (**42**)[248, 285]



A magnetically stirred solution of 6-fluorinated galactosyl derivative **41** (3.20 g, 9.14 mmol, 1.0 eq.) in dry CH_2Cl_2 (120 ml) was added *p*-thiocresol (1.70 g, 13.7 mmol, 1.5 eq.). The reaction was cooled to 0 °C before boron trifluoride diethyl etherate (2.32 ml, 18.3 mmol, 2.0 eq.) was added slowly. The reaction was allowed to warm to ambient temperature and was stirred for 17 h. Subsequently another portion of *p*-thiocresol (560 mg, 4.51 mmol, 0.5 eq.) and $BF_3{\cdot}OEt_2$ (0.55 ml, 4.51 mmol, 0.5 eq.) were added. The reaction was stirred for further 8 h and neutralized by addition of solid $NaHCO_3$. The reaction was diluted with CH_2Cl_2 (150 ml) and the organic layer was washed with sat. aq. $NaHCO_3$ (50 ml) and brine (50 ml) and dried with $MgSO_4$. The crude product was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 4:1) to afford the thioglycoside **42** (2.10 g, 5.07 mmol, 55 %) as a colorless foam.

R_f = 0.58 (α-anomer), 0.53 (β-anomer) (^{c}Hex/EtOAc v/v = 2:1).

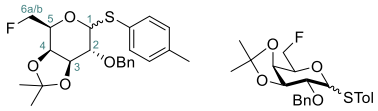
^{1}H-NMR (400 MHz, $CDCl_3$, β-Anomer): δ [ppm] = 7.43 – 7.38 (m, 2H, Ar-H), 7.17 – 7.09 (m, 2H, Ar-H), 5.45 (dd, $J_{H4,H3}$ = 3.3 Hz, $J_{H4,H5}$ = 1.1 Hz, 1H, H-4), 5.23 (t, $J_{H2,H1}$ = $J_{H2,H3}$ = 10.0 Hz, 1H, H-2), 5.05 (dd, $J_{H3,H2}$ = 10.0 Hz, $J_{H3,H4}$ = 3.3 Hz, 1H, H-3), 4.67 (d, $J_{H1,H2}$ = 9.9 Hz, 1H, H-1), 4.60 – 4.30 (m, 2H, H-6a, H-6b), 4.00 – 3.92 (m, 1H, H-5), 2.34 (s, 3H, Ar-CH_3), 2.11 (s, 3H, OAc), 2.10 (s, 3H, OAc), 1.97 (s, 3H, OAc).

^{13}C-NMR (100 MHz, $CDCl_3$, β-Anomer): δ [ppm] = 170.3 (C=O), 170.2 (C=O), 169.6 (C=O), 138.6 (Cq), 133.2 (2C), 129.9 (2C, 4 × C-Ar), 128.8 (Cq), 87.3 (C-1), 80.9 (d, $J_{C6,F}$ = 172.3 Hz, C-6), 75.3 (d, $J_{C5,F}$ = 23.1 Hz, C-5), 72.1 (C-3), 67.4 (C-2), 67.3 (d, $J_{C4,F}$ = 5.8 Hz, C-4), 21.3 (Ar-CH_3), 21.0 (OAc), 20.7 (2C, 2 × OAc).

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 230.8 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 46.5 Hz, $J_{F,H5}$ = 12.0 Hz).

HRMS (ESI^+): Calculated for $C_{19}H_{27}NFS^+$ $[M+NH_4]^+$: 432.1487; found: 432.1482.

4-Methylphenyl-2-*O*-benzyl-3:4-*O*-isopropylidene-6-deoxy-6-fluoro-1-thio-α/β-D-galactopyranoside (**43**)



Compound **43** was synthesized according to previously reported procedures.[326, 327]

To a magnetically stirred solution of **42** (3.10 g, 7.48 mmol, 1.0 eq.) in MeOH (150 ml) catalytic amounts of a freshly prepared sodium methoxide solution (in MeOH) was added. The reaction mixture was stirred for 1 h at ambient temperature, before being neutralized by addition of *Amberlite® IR120*. Subsequently the resin was filtered off and organic solvents were removed under reduced pressure to afford the crude triol.

This triol was subsequently dissolved in acetone (125 ml) and 2,2-dimethoxy propane (9.0 ml, 73.4 mmol, 9.8 eq.) and catalytic amounts of *p*-TsOH were added. The reaction was stirred at ambient temperature until complete conversion of the starting material was observed by TLC monitoring. The reaction was neutralized by addition of NEt_3 (1.5 ml) and concentrated under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (100 ml) washed with 1M HCl (50 ml), sat. aq. $NaHCO_3$ (50 ml) and Brine and was dried with $MgSO_4$.

The crude intermediate was dissolved in dry DMF (50 ml) and cooled to 0°C, before sodium hydride (60 % in mineral oil, 750 mg, 18.7 mmol, 2.5 eq.) was added in portions. The reaction mixture was warmed to ambient temperature and stirred for 10 min at that temperature. Subsequently, benzyl bromide (1.60 ml, 13.5 mmol, 1.8 eq.) was added and the reaction was stirred for another 1h, before sat. aq. NH_4Cl solution (15 ml) was added. The aqueous mixture was concentrated under reduced pressure and the crude residue was dissolved in CH_2Cl_2 and washed with water (20 ml) and brine (25 ml) and dried with $MgSO_4$. The crude product was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 9:1) to afford **43** (2.35 g, 5.62 mmol, 75 % over three steps) as a yellow oil.

R_f = 0.38 (α-anomer), 0.31 (β-anomer) (^{c}Hex/EtOAc v/v = 1:1).

β-Anomer:

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.47 – 7.41 (m, 4H, Ar-H), 7.39 – 7.33 (m, 2H, Ar-H), 7.32 – 7.28 (m, 1H, Ar-H), 7.11 (d, $J_{CH,CH}$ = 7.9 Hz, 2H, Ar-H), 4.81 (d, $J_{CH,CH}$ = 11.3 Hz, 1H, CH_{Bn}), 4.75 – 4.65 (m, 2H, CH_{Bn}, H-6a), 4.63 – 4.53 (m, 2H, H-1, H-6b), 4.30 (t, $J_{H3,H2} = J_{H3,H4}$ = 6.0 Hz, 1H, H-3), 4.20 (dd, $J_{H4,H3}$ = 5.8, $J_{H4,H5}$ = 2.1 Hz, 1H, H-4), 4.01 (dddd, $J_{H5,F}$ = 13.7 Hz, $J_{H5,H6a/b}$ = 6.9 Hz, $J_{H5,H6a/b}$ = 4.6 Hz, $J_{H5,H4}$ = 2.1 Hz, 1H, H-5), 3.53 (dd, $J_{H2,H1}$ = 9.2 Hz, $J_{H2,H3}$ = 6.2 Hz, 1H, H-2), 2.33 (s, 3H, Ar-CH_3), 1.41 (s, 3H, CH_3), 1.35 (s, 3H, CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 137.9 (2C, 2 × Cq), 132.8 (2C), 129.8 (Cq), 129.7 (2C), 128.5 (2C), 128.4 (2C), 127.9 (9 × C-Ar), 110.6 (Cq), 86.7 (C-1), 82.3 (d, $J_{C6,F}$ = 170.1 Hz, C-6), 79.4 (C-3), 78.1 (C-2), 74.7 (d, $J_{C5,F}$ = 22.0 Hz, C-5), 73.6 (CH_{Bn}), 73.1 (d, $J_{C4,F}$ = 6.5 Hz, C-4), 27.7 (CH_3), 26.4 (CH_3), 21.3 (Ar-CH_3).

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 228.4 (ddd, $J_{F,H6a}$ = 47.7 Hz $J_{F,H6b}$ = 46.0 Hz, $J_{F,H5}$ = 13.5 Hz).

α-Anomer:

^{1}H-NMR (400 MHz, $CDCl_3$, selected signals): δ [ppm] = 5.58 (d, $J_{H1,H2}$ = 5.2 Hz, 1H, H-1), 4.40 (t, $J_{H3,H2} = J_{H3,H4}$ = 6.2 Hz, 1H, H-3), 3.95 (dd, $J_{H2,H3}$ = 6.3 Hz, $J_{H2,H1}$ = 5.2 Hz, 1H, H-2).

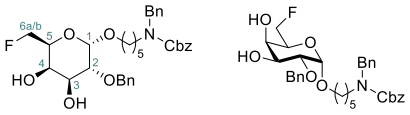
^{13}C-NMR (100 MHz, $CDCl_3$, selected signals): δ [ppm] = 86.5 (C-1), 75.6 (C-2), 74.3 (C-3).

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 230.0 (td, $J_{F,H6a} = J_{F,H6b}$ = 47.0 Hz, $J_{F,H5}$ = 13.8 Hz).

HRMS (ESI^+): Calculated for $C_{23}H_{31}O_4NFS^+$ $[M+NH_4]^+$: 436.1953; found: 436.1954

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 12.72 min, λ = 230 nm

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-2-*O*-benzyl-6-deoxy-6-fluoro-α-D-galactopyranoside (**44**)



Compound **44** was synthesized in accordance to literature known protocols.[306, 324]

Donor **43** (800 mg, 1.91 mmol, 1.0 eq.) was co-evaporated with dry toluene (20 ml) and dried under high vacuum for 1.5 h. Subsequently, starting material was dissolved in dry CH_2Cl_2 (20 ml) and freshly activated 4 Å molecular sieve and NFM (3.10 ml, 30.6 mmol, 16 eq.) were added. The heterogenous mixture was stirred for 1.5 h at ambient temperature before being cooled to − 10°C. After stirring 0.5 h at that temperature, NIS (646 mg, 2.87 mmol, 1.5 eq.) and TMSOTf (519 µl, 2.87 mmol, 1.5 eq.) were added. The reaction was stirred at – 10 °C for 1 h before another portion of NIS (220 mg, 0.89 mmol, 0.5 eq.) and TMSOTf (150 µl, 0.83 mmol, 0.4 eq.) was added. After pre-activation of thiodonor **43** was deemed complete, linker **37**[301, 302] (1.13 g, 3.44 mmol, 1.8 eq.; co-evaporated with toluene and dried under high vacuum prior to use) dissolved in dry CH_2Cl_2 (5 ml) was added. The reaction was stepwise warmed to 0 °C and subsequently to ambient temperature and stirred for 3d before being stopped by addition of NEt_3 (3 ml). The reaction mixture was diluted with CH_2Cl_2 and filtered through a short pad of *Hyflo*®. The organic layer was washed with sat. aq. $Na_2S_2O_3$ (25 ml), 1 M HCl (25 ml) and brine (25 ml) and dried with $MgSO_4$. Organic solvents were removed under reduced pressure and the crude residue was subjected to flash column chromatography (^{c}Hex/EtOAc *v/v* = 5:1). Product containing fractions were pooled and used in the subsequent reaction. The thus obtained glycosylation product was dissolved in 80 % AcOH (50 ml) and stirred for 2 h at 80°C. After complete conversion of the starting material, the reaction was concentrated under reduced pressure and co-evaporated with toluene (2 × 50 ml). The crude residue was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 3:1 → 1:1) to afford pure diol **44** (470 mg, 0.81 mmol, 42 % over two steps) as a colourless oil.

R_f = 0.13 (^{c}Hex/EtOAc v/v = 3:1).

Optical rotation: $[\alpha]_D^{22}$ = + 59.2° (c = 0.5; $CHCl_3$)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.38 – 7.12 (m, 15H, Ar-H), 5.17 (d, $J_{CH,CH}$ = 11.5 Hz, 2H, CH_{Cbz}), 4.83 (s, 1H, H-1), 4.65 (d, $J_{H6a,H6b}$ = 6.0 Hz, 1H, H-6a), 4.62 (s, 2H, CH_{Bn}), 4.53 (d, $J_{H6b,H6a}$ = 6.4 Hz, 1H, H-6b), 4.49 (s, $J_{CH,CH}$ = 8.5 Hz, 2H, NCH_{Bn}), 4.01 (bs, 3H, H-3, H-4, H-5), 3.71 (dd, $J_{H2,H3}$ = 9.2 Hz, $J_{H2,H1}$ = 3.5 Hz, 1H, H-2), 3.68 – 3.5 (m, 1H, 1H, CH_{Linker}), 3.38 – 3.10 (m, 3H, 3 × CH_{Linker}), 1.65 – 1.42 (m, 4H, 4 × CH_{Linker}), 1.39 – 1.25 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 156.9/156.3 (C=O-Cbz), 138.0 (2C), 137.0/136.8 (3 × Cq), 128.8, 128.7, 128.3, 128.2, 128.1, 128.0, 127.4, 127.3 (8 × C-Ar), 96.6 (C-1), 83.0 (d, $J_{C6,F}$ = 168.3 Hz, C-6), 76.7 (C-2), 72.8 (CH_{Bn}), 68.8 – 68.3 (4C, C3, C4, C5, CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.3 (CH_{Linker}), 28.0/27.6 (CH_{Linker}), 23.6 (CH_{Linker}). Due to signal overlapping only 26 out of 33 carbon atoms were assigned.

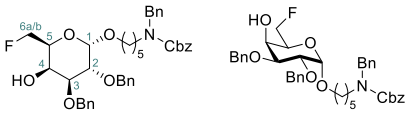
^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 229.7 - − 229.0 (m).

^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{H1,C1}$ = 171 Hz

HRMS (ESI^+): Calculated for $C_{33}H_{44}FN_2O_7^+$ $[M+NH_4]^+$: 599.3127; found: 599.3126.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 10.32 min, λ = 230 nm

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-2,3-di-*O*-benzyl-6-deoxy-6-fluoro-α-D-galactopyranoside (**24**)



Compound **24** was synthesized according to a procedure described by *Kawai et al.*[330]

A magnetically stirred solution of compound **44** (400 mg, 0.69 mmol, 1.0 eq.) in dry toluene (25 ml), dibutyltin oxide (177 mg, 0.71 mmol, 1.0 eq.) was added and the reaction was stirred for 5 h at 85 °C. Subsequently, Benzyl bromide (178 µl, 1.50 mmol, 2.2 eq.) and TBAB (222 mg, 0.69 mmol, 1.0 eq.) were added and the reaction was heated for another 6 h at 100 °C. After the reaction was deemed complete by TLC analysis, solvents were removed under reduced pressure and the crude residue was dissolved in CH_2Cl_2 (50 ml). The organic layer was washed with water (25 ml), sat. aq. $NaHCO_3$ solution (25 ml) and brine and dried with $MgSO_4$. Solvents were removed under reduced pressure and the crude residue was subjected to flash column chromatography (^{c}Hex/EtOAc *v/v* = 1:0 → 5:1) to afford **24** (397 mg, 0.59 mmol, 86 %) as a colourless oil.

R_f = 0.66 (^{c}Hex/EtOAc *v/v* = 1:1)

Optical rotation: $[\alpha]_D^{24}$ = + 41.7° (c = 1.0; $CHCl_3$)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.40 – 7.14 (m, 20H, Ar-H), 5.18 (d, $J_{CH,CH}$ = 19.5 Hz, 2H, CH_{Cbz}), 4.83 – 4.76 (m, 3H, H-1, 2 × CH_{Bn}), 4.69 (d, $J_{CH,CH}$ = 11.5 Hz, 1H, CH_{Bn}), 4.65 – 4.60 (m, 2H, H-6a, CH_{Bn}), 4.55 – 4.46 (m, 3H, H-6b, NCH_{Bn}), 4.06 – 3.95 (m, 2H, H-4, H-5), 3.91 – 3.85 (m, 1H, H-3), 3.81 (dd, $J_{H2,H3}$ = 9.7 Hz, $J_{H2,H1}$

= 3.6 Hz, 1H, H-2), 3.67 – 3.56 (m, 1H, CH_{Linker}), 3.43 – 3.33 (m, 1H, CH_{Linker}), 3.29 – 3.16 (m, 2H, 2 × CH_{Linker}), 1.67 – 1.49 (m, 4H, 4 × CH_{Linker}), 1.35 – 1.24 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 156.9/156.3 (C=O-Cbz), 138.5, 138.2, 138.1/138.0, 137.1/136.9 (4 × Cq), 128.7, 128.5, 128.1, 128.0, 128.0, 127.9 (6 × C-Ar), 97.4 (C-1), 82.9 (d, $J_{C6,F}$ = 168.3 Hz, C-6), 77.4 (C-3), 75.9 (C-2), 73.4 (CH_{Bn}), 73.1 (CH_{Bn}), 68.4 – 68.3 (2C, CH_{Linker}, C-5,), 67.6 (d, $J_{C4,F}$ = 6.6 Hz, C-4), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.6 (CH_{Linker}). Due to signal overlapping only 26 out of 40 carbon atoms were assigned.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 228.9 (dtd, $J_{F,H6a}$ = 61.3 Hz, $J_{F,H6a}$ = 46.9 Hz, $J_{F,H5}$ = 14.8 Hz).

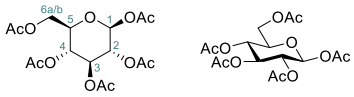
^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{H1,C1}$ = 171 Hz

HRMS (ESI$^+$): Calculated for $C_{40}H_{50}FN_2O_7^+$ $[M+NH_4]^+$: 689.3587; found: 689.3593.

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 13.25 min, λ = 230 nm

Synthesis of glucosyl donor building blocks 29 and 30

1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranoside (**45**)[336]



A magnetically stirred mixture of sodium acetate (12.5 g, 152 mmol, 1.1 eq.) in acetic anhydride (150 ml) was heated to 100 °C. Subsequently, D-glucose (25.0 g, 138 mmol, 1.0 eq.) was added in portions. After complete addition, the reaction was stirred for 2 h at 100 °C. Subsequently, the solution was poured over ice and stirred until a colorless precipitate was formed. The precipitate was filtered off and washed thoroughly with water (5 l) and dried for 24 h under high vacuum. Compound **45** (36.0 g, 92.3 mmol, 67 %) was obtained as a colourless solid.

R_f = 0.49 (^{c}Hex/EtOAc = 1:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 5.71 (d, $J_{H1,H2}$ = 8.3 Hz, 1H, H-1), 5.25 (t, , $J_{H3,H2}$ = $J_{H3,H4}$ = 9.4 Hz, 1H, H-3), 5.17 – 5.08 (m, 2H, H-2, H-4), 4.29 (dd, $J_{H6a,H6b}$ = 12.5 Hz, $J_{H6a,H5}$ = 4.5 Hz, 1H, H-6a), 4.11 (dd, $J_{H6b,H6a}$ = 12.5 Hz, $J_{H6b,H5}$ = 2.2 Hz, 1H, H-6b), 3.84 (ddd, $J_{H5,H4}$ = 10.0 Hz, $J_{H5,H6a}$ = 4.5 Hz, $J_{H5,H6b}$ = 2.2 Hz, 1H, H-5), 2.11 (3H, OAc), 2.09 (3H, OAc), 2.03 (s, 6H, 2 × OAc), 2.01 (s, 3H, OAc).

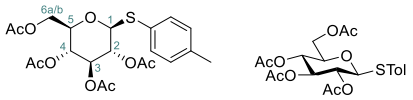
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 170.8, 170.2, 169.5, 169.4, 169.1 (5 × C=O), 91.8 (C-1), 72.9 (2C, C-5, C-3), 70.4 (C2/C4), 67.9 (C2/C4), 61.6 (C-6), 21.0 (OAc), 20.9 (OAc), 20.7 (OAc).

Due to signal overlap 14 out of 16 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{16}H_{26}O_{11}N^+$ $[M+NH_4]^+$: 408.1500; found: 408.1503.

For further analytical data see reference.[390]

4-Methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**46**)[337]



To a magnetically stirred solution of compound **45** (10.0 g, 25.6 mmol, 1.0 eq.) in dry CH_2Cl_2 (150 ml), *p*-thiocresol (4.77 g, 38.4 mmol, 1.5 eq.) was added. The reaction was cooled to 0 °C before $BF_3{\cdot}OEt_2$ (3.48 ml, 28.2 mmol, 1.5 eq.) was added dropwise. After complete addition, the solution was allowed to warm to ambient temperature and was stirred for 48 h. Subsequently, water was added carefully (50 ml) and the reaction was neutralized by addition of solid $NaHCO_3$. This mixture was diluted with CH_2Cl_2 (150 ml) and washed with sat. aq. $NaHCO_3$ (2 × 75 ml) and brine (25 ml) and dried with $MgSO_4$. The organic solvents were removed under reduced pressure and the crude product was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 3:1) to afford thioglycoside **45** (9.45 g, 20.8 mmol, 81 %) as a colourless solid.

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

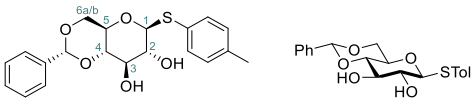
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.39 (d, $J_{CH,CH}$ = 8.2 Hz, 2H, Ar-H), 7.12 (d, $J_{CH,CH}$ = 7.9 Hz, 2H, Ar-H), 5.20 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.4 Hz, 1H, H-3), 5.02 (t, $J_{H4,H3} = J_{H4,H5}$ = 9.7 Hz, 1H, H-4), 4.93 (t, $J_{H2,H1}$ = 9.9 Hz, $J_{H2,H3}$ = 9.2 Hz, 1H, H-2), 4.63 (d, $J_{H1,H2}$ = 10.0 Hz, 1H, H-1), 4.25-4.12 (m, 2H, H-6a, H-6b), 3.76-3.65 (m, 1H, H-5), 2.35 (s, 3H, Ar-CH_3), 2.09 (3H, OAc), 2.08 (3H, OAc), 2.01 (3H, OAc), 1.98 (3H, OAc).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 170.7, 170.3, 169.5, 169.4 (4 × C=O), 139.9, 134.0, 129.8, 127.7 (4 × C-Ar), 86.0 (C-1), 75.9 (C-5), 74.2 (C-3), 70.1 (C-2), 68.4 (C-4), 62.3 (C-6), 21.3 (Ar-CH_3), 20.9 (2C, 2 × OAc), 20.8 (OAc), 20.7 (OAc). Due to signal overlap 19 out of 21 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{21}H_{30}NO_9S^+$ $[M+NH_4]^+$: 472.1636, found: 472.1637.

For further analytical data see reference.[391, 392]

4-Methylphenyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**47**)[294, 338]



To a magnetically stirred mixture of compound **46** (9.10 g, 20.0 mmol, 1.0 eq.) in MeOH (250 ml), catalytic amounts of a freshly prepared sodium methoxide solution (in MeOH) was added. The reaction was stirred at ambient temperature for 20 h and subsequently neutralized by addition of *Amberlite® IR 120*. The resin was filtered off and the filtrate was concentrated under reduced pressure to afford the crude tetrol intermediate. The thus obtained tetrol was dissolved in dry DMF (40 ml) and BADA (3.60 ml, 23.9 mmol, 1.3 eq.) and CSA (0.93 g, 4.00 mmol, 0.2 eq.) were added. The reaction was stirred for 24 h at ambient temperature, before being neutralized by addition of NEt_3 (2 ml). The organic solvents were removed under reduced pressure. The crude product was

subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 2:1 → 1:1), affording **47** (6.91 g, 18.4 mmol, 92 % over two steps) as a colorless solid.

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

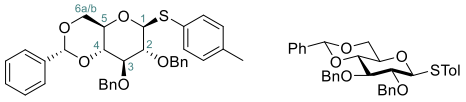
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.54 – 7.32 (m, 7H, Ar-H), 7.15 (d, $J_{CH,CH}$ = 7.9 Hz, 2H, Ar-H), 5.51 (s, 1H, CH-Ar), 4.56 (d, $J_{H1,H2}$ = 9.7 Hz, 1H, H-1), 4.36 (dd, $J_{H6a,H6b}$ = 10.5 Hz, $J_{H6a,H5}$ = 4.3 Hz, 1H, H-6a), 3.88 – 3.72(m, 2H, H-3, H-6b), 3.50 – 3.46 (m, 2H, H-4, H-5), 3.42 (td, $J_{H2,H1}$ = $J_{H2,H3}$ = 9.7 Hz, $J_{H2,OH}$ = 2.3 Hz, 1H, H-2), 2.96 (bs, 1H, OH), 2.80 (d, 1H, $J_{OH,H2}$ = 2.3 Hz, OH), 2.36 (s, 3H, Ar-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 139.0, 137.0, 133.8, 130.0, 129.4, 129.1, 128.5, 127.4, 126.4 (9 × C-Ar), 102.0 (CH-Ar), 88.8 (C-1), 80.4 (C-4/5), 74.7 (C-3), 72.6 (C-2), 70.7 (C-4/5), 68.7 (C-6), 21.3 (Ar-CH_3).

Due to signal overlap 17 out of 20 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{20}H_{26}O_5NS^+$ $[M+NH_4]^+$: 392.1526; found: 392.1529.

4-Methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**29**)[339]



A magnetically stirred solution of **47** (6.80 g, 18.2 mmol, 1.0 eq.) in DMF (150 ml) was cooled to 0 °C before sodium hydride (60 % dispersion in mineral oil, 1.82 g, 45.5 mmol, 2.5 eq.) was added slowly. The reaction mixture was stirred for 30 min at 0 °C before benzyl bromide (5.40 ml, 45.5 mmol, 2.5 eq.) was added slowly. The reaction was allowed to warm to ambient temperature and stirred for further 17 h. After complete conversion of the starting material was observed by TLC monitoring, the reaction was stopped by addition of H_2O (5 ml). Subsequently all organic solvents were removed under reduced pressure and the crude product was dissolved in CH_2Cl_2 (200 ml). The organic layer was washed with H_2O (2 × 250 ml) and brine (250 ml) and dried with $MgSO_4$. Organic volatiles were removed under reduced pressure and the crude residue was crystallized from EtOH, yielding **29** (9.10 g, 16.4 mmol, 90 %) as a colorless solid.

R_f = 0.68 (^{c}Hex/EtOAc = 3:1).

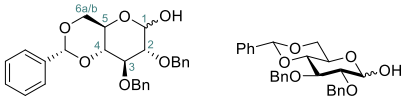
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.52 – 7.26 (m, 17H, Ar-H), 7.16-7.09 (m, 2H, Ar-H), 5.59 (s, 1H, CH-Ar), 4.95 (d, $J_{CH,CH}$ = 11.1 Hz, 1H, CH_{Bn}), 4.89 (d, $J_{CH,CH}$ = 10.3 Hz, 1H, CH_{Bn}), 4.83 (d, $J_{CH,CH}$ = 10.3 Hz, 1H, CH_{Bn}), 4.79 (d, $J_{CH,CH}$ = 11.1 Hz, 1H, CH_{Bn}), 4.71 (d, $J_{H1,H2}$ = 9.8 Hz, 1H, H-1), 4.39 (dd, $J_{H6a,H6b}$ = 10.5 Hz, $J_{H6a,H5}$ = 5.0 Hz, 1H, H-6a), 3.88 – 3.76 (m, 2H, H-3, H-6b), 3.70 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.4 Hz, 1H, H-4), 3.53 – 3.42 (m, 2H, H-2, H-5), 2.35 (s, 3H, Ar-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 138.5, 138.3, 138.2, 137.4, 133.2, 129.9, 129.3, 129.1, 128.5 (2C), 128.4, 128.3 (2C), 128.0, 127.9, 126.1 (16 × C-Ar), 101.3 (CH-Ar), 88.7 (C-1), 83.2 (C-3), 81.6 (C-4), 80.6 (C-2), 76.0

(CH_{Bn}), 75.5 (CH_{Bn}), 70.4 (C-5), 68.9 (C-6), 21.3 (Ar-CH_3). Due to signal overlap 26 out of 34 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{34}H_{38}NO_5S^+$ $[M+NH_4]^+$: 572.2468; found: 572.2470.

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α/β-D-glucopyranoside (**48**)[340]



To a magnetically stirred solution of **29** (600 mg, 1.08 mmol, 1.0 eq.) in acetone (18 ml), water (2 ml) and NBS (732 mg, 4.11 mmol, 3.8 eq.) were added. The reaction was stirred at ambient temperature until complete conversion of the starting material was observed. The solution was concentrated (without warming) until turbidity arose and subsequently sat. aq. $Na_2S_2O_3$ (50 ml) was added. The aqueous mixture was extracted with EtOAc (3 × 100 ml) and the combined organic layers were washed with sat. aq. $NaHCO_3$ (15 ml) and brine (10 ml) and dried with $MgSO_4$. Solvents were removed under reduces pressure and the white solid residue was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 3:1), affording **48** (400 mg, 0.89 mmol, 83 %) as an anomeric mixture (α/β = 1:1.1).

R_f = 0.30 (^{c}Hex/EtOAc = 3:1).

α-Anomer:

^{1}H-NMR (400 MHz, $CDCl_3$, selected signals): δ [ppm] = 5.58 (s, 1H, CH-Ar), 5.19 (d, $J_{H1,H2}$ = 3.7 Hz, 1H, H-1), 4.02 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.2 Hz, 1H, H-3), 3.69 – 3.60 (m, 1H, H-4), 3.60 (dd, $J_{H2,H3}$ = 8.4 Hz, $J_{H2,H1}$ = 3.1 Hz, 1H, H-2).

^{13}C-NMR (100 MHz, $CDCl_3$, selected signals): δ [ppm] = 101.3 (CH-Ar), 92.3 (C-1), 82.1 (C-4), 79.4 (C-2), 78.5 (C-3).

β-Anomer:

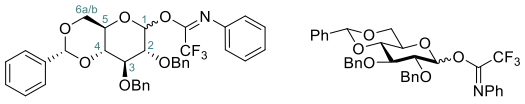
^{1}H-NMR (400 MHz, $CDCl_3$, selected signals): δ [ppm] = 5.57 (s, 1H, CH-Ar), 3.42 (t, $J_{H2,H1} = J_{H2,H3}$ = 7.3 Hz, 1H, H-2).

^{13}C-NMR (100 MHz, $CDCl_3$, selected signals): δ [ppm] = 101.4 (CH-Ar), 97.9 (C-1), 83.1 (C-2).

HRMS (ESI$^+$): Calculated for $C_{27}H_{32}NO_6^+$ $[M+NH_4]^+$: 466.2224, found: 466.2223.

Further analytical data see reference.[393]

2,3-Di-*O*-benzyl-4,6-*O*-benzyliden-α/β-D-glucopyranosyl *N*-(phenyl) trifluoroacetimidate (**30**)



Compound **30** was synthesized according to a modified procedure.[341]

To a magnetically stirred solution of **48** (0.50 g, 1.11 mmol, 1.0 eq.) in dry CH_2Cl_2 (25 ml), 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (**49**) (1.15 g, 5.55 mmol, 5.0 eq.) and caesium carbonate (1.09 g, 3.33 mmol, 3.0 eq.) were added. The reaction mixture was stirred until complete conversion of the starting material was indicated by TLC monitoring. The heterogenous mixture was filtered through a short plug of *Hyflo*® and solvents were removed under reduced pressure. The crude product was subjected to column chromatography (^{c}Hex/EtOAc *v/v* = 1:0 + 1 % NEt_3 → ^{c}Hex/EtOAc *v/v* = 6:1 + 1 % NEt_3) affording **30** (599 mg, 0.97 mmol, 87 %) as a mixture of anomers.

R_f = 0.74, 0.83 (^{c}Hex/EtOAc = 3:1 + 1 % NEt_3).

^{1}H-NMR (400 MHz, $CDCl_3$, characteristic signals): δ [ppm] = 7.55 – 7.46 (m, 2H, H-$Ar_{α+β}$), 7.44 – 7.26 (m, 15H, H-$Ar_{α+β}$), 7.16 – 7.09 (m, 1H, H-$Ar_{α+β}$), 6.86 – 6.74 (m, 2H, H-$Ar_{α+β}$), 5.63 – 5.57 (m, 1H, CH-$Ar_{α+β}$), 5.00 – 4.74 (m, 4H, 4 × CH_{Bn}), 4.36 (dd, $J_{H6a,H6b}$ = 10.4 Hz, $J_{H6a,H5}$ = 4.9 Hz, 1H, H-$6a_{α+β}$), 4.12 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.3 Hz, 1H, H-$3_α$), 4.03 (bs, 1H, H-$5_α$), 3.87 – 3.68 (m, 4H, H-$6b_{α+β}$, H-$2_{α+β}$, H-$4_{α+β}$, H-$3_β$).

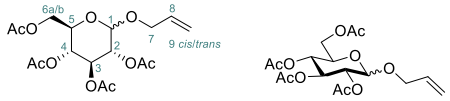
^{13}C-NMR (100 MHz, $CDCl_3$, characteristic Signals): δ [ppm] = 101.5 (CH-$Ar_β$), 101.4 (CH-$Ar_α$), 78.3 (C-$3_α$), 75.7 ($CH_{Bnβ}$), 75.5 ($CH_{Bnα}$), 75.2 ($CH_{Bnβ}$), 74.0($CH_{Bnα}$), 68.8 (C-$6_α$), 68.6 (C-$6_β$), 65.2 (C-$5_α$).

HRMS (ESI$^+$): Calculated for $C_{35}H_{33}NO_6F_3^+$[M+H]$^+$: 620.2254; found:620.2264.

For further analytical data see reference.[342]

Synthesis of fluorinated glucose donor 31

Allyl 2,3,4,6-tetra-*O*-acetyl-α/β-D-glucopyranoside (**50**)



Compound **50** was synthesized according to slightly modified protocols.[363, 364]

To a magnetically stirred solution of **45** (6.50 g, 16.7 mmol, 1.0 eq.) in dry CH_2Cl_2 (125 ml), freshly activated 3 Å molecular sieve and allylic alcohol (2.28 ml, 33.4 mmol, 2.0 eq.) were added. The reaction mixture was stirred for 0.5 h at ambient temperature, before being chilled to 0 °C. Subsequently, TfOH (1.48 ml, 16.7 mmol, 1.0 eq.) was added dropwise. The reaction was stirred for 2 h at 0 °C, neutralized by addition of NEt_3 (10 ml), filtered through a pad of *Hyflo*® and concentrated to dryness. The brown residue was dissolved in pyridine (100 ml) and acetic anhydride (10 ml) and catalytic amounts of 4-DMAP were added. The brown solution was stirred for 2 h at ambient

temperature. Solvents were removed under reduced pressure and the crude residue was dissolved in CH_2Cl_2 (200 ml). The organic layer was washed with sat. aq. $NaHCO_3$ (25 ml) and brine (30 ml) and dried over $MgSO_4$. The crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **50** (4.24 g, 10.9 mmol, 65 %) as a mixture of anomers.

R_f = 0.62 (α-Anomer); 0.57 (β-Anomer) (^{c}Hex/EtOAc v/v = 1:1).

β-Anomer:

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 5.92 – 5.79 (m, 1H, H-8), 5.27 (dq, $J_{H9trans, H8}$ = 17.2 Hz, $J_{H9trans, H9cis}$ = $J_{H9trans,H7a}$ = $J_{H9trans,H7b}$ = 1.7 Hz, 1H, H-9_{trans}), 5.24 – 5.17 (m, 2H, H-3, H-9_{cis}), 5.09 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 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, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1), 4.37 – 4.30 (m, 1H, H-7a), 4.26 (dd, $J_{H6a,H6b}$ = 12.3 Hz, $J_{H6a,H5}$ = 4.8 Hz, 1H, H-6a), 4.17 – 4.06 (m, 2H, H-6b, H-7b), 3.68 (ddd, $J_{H5,H4}$ = 9.9 Hz, $J_{H5,H6a}$ = 4.7 Hz, $J_{H5,H6b}$ = 2.5 Hz, 1H, H-5), 2.09 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, -OAc), 2.00 (s, 3H, OAc).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 170.8, 170.4, 169.5 (2C, 4 × C=O), 133.5 (C-8), 117.8 (C-9), 99.7 (C-1), 73.0 (C-3), 72.0 (C-5), 71.5 (C-2), 70.2 (C-7), 68.6 (C-4), 62.1 (C-6), 20.9, 20.8 (2C), 20.7 (4 × OAc).

α-Anomer:

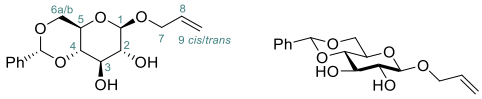
^{1}H-NMR (400 MHz, $CDCl_3$, selected signals): δ [ppm] = 5.45 (dd, $J_{H3,H2}$ = 10.3 Hz, $J_{H3,H4}$ = 9.3 Hz, 1H, H-3), 5.04 (d, $J_{H1,H2}$ = 3.8 Hz, 1H, H-1), 4.83 (dd, $J_{H2,H3}$ = 10.3 Hz, $J_{H2,H1}$ = 3.7 Hz, 1H, H-2).

^{13}C-NMR (100 MHz, $CDCl_3$, selected signals): δ [ppm] = 95.0 (C-1), 70.9 (C-2), 70.3 (C-3).

HRMS (ESI^+): Calculated for $C_{17}H_{28}O_{10}N^+$ $[M+NH_4]^+$: 406.1708; 406.1710.

For further analytical data see reference.[394]

Allyl 4,6-*O*-benzyliden-β-D-glucopyranoside (**51**)



Compound **51** was synthesized according to previously reported protocols.[294, 365]

To a magnetically stirred solution of **50** (β-anomer) (1.90 g, 3.09 mmol, 1.0 eq.) in MeOH, a sodium methoxide solution (5 M in MeOH, 100 µl, 0.5 mmol, 0.1 eq.) was added at ambient temperature. The reaction was stirred until complete conversion of starting material was observed. Subsequently the reaction was neutralized by addition of *Amberlite® IR 120*. After filtration, organic solvents were removed under reduced pressure. The crude product was obtained as a colorless solid which was used in the subsequent steps without further purification.

The crude tetrol was dissolved in dry DMF (25 ml) and benzaldehyde dimethyl acetal (1.16 ml, 7.55 mmol, 1.5 eq.) was added in one portion. The reaction solution was adjusted to pH = 3.5 by addition of camphorsulfonic acid and

stirred at 50 °C and 39 mbar for 3 h. After TLC monitoring indicated complete conversion of the starting material, the reaction was neutralized by addition of NEt_3 (10 ml) and concentrated to dryness under reduced pressure. The crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 3:1 → 1:2) affording **51** (1.39 g, 4.51 mmol, 92 % over two steps) as a colorless solid.

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

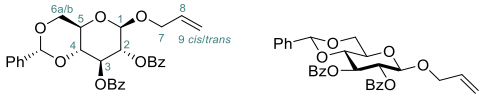
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.55 – 7.45 (m, 2H, H-Ar), 7.41 – 7.31 (m, 3H, H-Ar), 5.94 (dddd, $J_{H8,H9trans}$ = 17.0 Hz, $J_{H8,H9cis}$ = 10.3 Hz, $J_{H8,H7b}$ = 6.4 Hz, $J_{H8,H7a}$ = 5.3 Hz, 1H, H-8), 5.52 (s, 1H, $CH_{Benzyliden}$), 5.34 (dq, $J_{H9trans,\ H8}$ = 17.2 Hz, $J_{H9trans,H9cis}$ = $J_{H9trans,H7a}$ = $J_{H9trans,H7b}$ = 1.6 Hz, 1H, H-9_{trans}), 5.24 (dq, $J_{H9cis,H8}$ = 10.4 Hz, $J_{H9cis,H9trans}$ = $J_{H9cis,H7a}$ = $J_{H9cis,H7b}$ = 1.3 Hz, 1H, H-9_{cis}), 4.44 (d, $J_{H1,H2}$ = 7.7 Hz, 1H, H-1), 4.42 – 4.30 (m, 2H, H-6a, H-7a), 4.14 (ddt, $J_{H7b,H7a}$ = 12.5 Hz, $J_{H7b,H8}$ = 6.4 Hz, $J_{H7b,H9cis}$ = $J_{H7b,\ H9trans}$ = 1.3 Hz, 1H, H-7b), 3.84 – 3.73 (m, 2H, H-3, H-6b), 3.59 – 3.48 (m, 2H, H-2, H-4), 3.47 – 3.40 (m,1H, H-5).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 137.1 (C-Ar), 133.6 (C-8), 129.4 (C-Ar), 128.5 (2C, C-Ar), 126.4 (2C, C-Ar), 118.5 (C-9), 102.2 (C-1), 102.0 (CH-Ar), 80.6 (C-4), 74.6 (C-2), 73.2 (C-3), 70.8 (C-7), 68.8 (C-6), 66.5 (C-5).

HRMS (ESI^+): Calculated for $C_{16}H_{21}O_6^+$ $[M+H]^+$: 309.1333; found: 309.1334.

For further analytical data see reference.[395]

Allyl 2,3-di-*O*-benzoyl-4,6-*O*-benzyliden-β-D-glucopyranoside (**52**)[366]



To a magnetically stirred solution of **51** (4.71 g, 15.3 mmol, 1.0 eq.) dry CH_2Cl_2 (250 ml), pyridine (20 ml) and benzoyl chloride (4.65 ml, 39.7 mmol, 2.6 mmol) were added. The reaction was stirred at ambient temperature until TLC monitoring indicated complete conversion of the starting material. The reaction was stopped by addition of H_2O, and diluted with CH_2Cl_2 (50 ml). The organic layer was washed with sat. aq. $NaHCO_3$ (25 ml) and brine (30 ml) and dried over $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 5:1) and further purified by crystallization from EtOH. This furnished **52** (6.10 g, 11.8 mmol, 77 %) as a colorless solid.

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

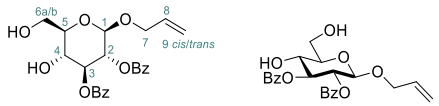
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.97 (m, 4H, H-Ar), 7.54 – 7.46 (m, 2H, H-Ar), 7.45 – 7.29 (m, 9H, H-Ar), 5.85 – 5.71 (m, 2H, H-8, H-3), 5.55 (s, 1H, CH-Ar) 5.51 (dd, $J_{H2,H3}$ = 9.5 Hz, $J_{H2,H1}$ = 7.8 Hz, 1H, H-2), 5.25 (dq, $J_{H9trans,\ H8}$ = 17.2 Hz, $J_{H9trans,H9cis}$ = $J_{H9trans,H7a}$ = $J_{H9trans,H7b}$ = 1.6 Hz, 1H, H-9_{trans}), 5.14 (dq, $J_{H9cis,H8}$ = 10.4 Hz, $J_{H9cis,H9trans}$ = $J_{H9cis,H7a}$ = $J_{H9cis,H7b}$ = 1.4 Hz, 1H, H-9_{cis}), 4.86 (d, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1), 4.44 (dd, $J_{H6a,H6b}$ =

10.5 Hz, $J_{H6a,H5}$ = 4.9 Hz, 1H, H-6a), 4.37 (ddt, $J_{H7a,H7b}$ = 13.2 Hz, $J_{H7a,H8}$ = 4.9 Hz, $J_{H7a,\ H9cis}$ = $J_{H7a,H9trans}$= 1.6 Hz, 1H, H-7a), 4.14 (ddt, $J_{H7b,H7a}$ = 13.2 Hz, $J_{H7b,H8}$ = 6.2 Hz, $J_{H7b,H9cis}$ = $J_{H7b,H9trans}$ = 1.4 Hz, 1H, H-7b), 3.99 – 3.86 (m, 2H, H-4, H-6b), 3.70 (m, 1H, H-5).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 165.8, 165.4 (2 × C=O), 136.9 (C-Ar), 133.4 (C-8), 133.3, 133.2, 130.0 (4C), 129.6, 129.5, 129.2, 128.5, 128.4, 128.3, 126.3 (13 × C-Ar), 118.0 (C-9), 101.6 (CH-Ar), 100.6 (C-1), 79.0 (C-4), 72.6 (C-2), 72.3 (C-3), 70.5 (C-7), 68.8 (C-6), 66.8 (C-5). Due to signal overlap 26 out of 30 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{30}H_{28}O_8K^+$ [M+K]$^+$: 555.1416; found: 555.1420

Allyl 2,3-di-*O*-benzoyl-β-D-glucopyranoside (**53**)[366]



To a magnetically stirred solution of **52** (1.70 g, 3.29 mmol, 1.0 eq.) in CH_2Cl_2 (40 ml), H_2O (220 µl) and TFA (1.70 ml) were added. The reaction was stirred at ambient temperature until TLC-monitoring indicated complete conversion of the starting material. Organic solvents were removed under reduced pressure and the crude residue was co-evaporated with toluene (2 × 20 ml). The crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 1:1), affording **53** (1.15 g, 2.68 mmol, 82 %) as a colorless foam.

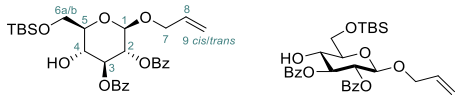
R_f = 0.17 (^{c}Hex/EtOAc v/v = 1:1)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.99 – 7.92 (m, 4H, Ar-H), 7.55 – 7.48 (m, 2H, Ar-H), 7.42 – 7.32 (m, 4H, Ar-H), 5.78 (dddd, $J_{H8,H9trans}$ = 16.8 Hz, $J_{H8,H9cis}$ = 10.7 Hz, $J_{H8,H7b}$ = 6.1 Hz, $J_{H8,H7a}$ = 5.0 Hz, 1H, H-8), 5.48 – 5.38 (m, 2H, H-2, H-3), 5.23 (dq, $J_{H9trans,H8}$ = 17.2 Hz, $J_{H9trans,\ H9cis}$ = $J_{H9trans,H7a}$ = $J_{H9trans,H7b}$ =1.6 Hz, 1H, H-9$_{trans}$), 5.13 (dq, $J_{H9cis,H8}$ = 10.4 Hz, $J_{H9cis,H9trans}$ = $J_{H9cis,H7a}$ = $J_{H9cis,H7b}$ = 1.4 Hz, 1H, H-9$_{cis}$), 4.78 (d, $J_{H1,H2}$ = 7.5 Hz, 1H, H-1), 4.34 (ddt, $J_{H7a,H7b}$ = 13.3 Hz, $J_{H7a,H8}$ = 5.0 Hz, $J_{H7a,H9cis}$ = $J_{H7a,H9trans}$= 1.6 Hz, 1H, H-7a), 4.14 (ddt, $J_{H7b,H7a}$ = 13.2 Hz, $J_{H7b,H8}$ = 6.1 Hz, $J_{H7b,H9cis}$ = $J_{H7b,H9trans}$ = 1.5 Hz, 1H, H-7b), 4.05 – 3.86 (m, 3H, H-4, H-6a, H-6b), 3.62 – 3.52 (m, 1H, H-5), 3.36 (d, $J_{OH,H4}$ = 4.5 Hz, 1H, OH), 2.27 (t, $J_{OH,H6a}$ = $J_{OH,H6b}$ = 6.6 Hz, 1H, OH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 167.7, 165.4 (2 × C=O), 133.7 (C-Ar), 133.6 (C-Ar), 133.4 (C-8), 130.1, 129.9, 129.5, 128.9, 128.6, 128.5 (6 × C-Ar), 117.9 (C-9), 100.0 (C-1), 77.3 (C-2/C-3), 75.9 (C-5), 71.5 (C-2/C-3), 70.4 (C-7), 70.1 (C-4), 62.4 (C-6). Due to signal overlap 19 out of 23 carbon atoms were assigned in the ^{13}C spectrum

HRMS (ESI$^+$): Calculated for $C_{23}H_{28}NO_8^+$ [M+NH_4]$^+$: 446.1810; found: 446.1811.

Allyl 2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl- β-D-glucopyranoside (**28**)



Building block **28** was synthesized in accordance with a slightly modified procedure.[361]

To a magnetically stirred solution of **53** (800 mg, 1.87 mmol, 1.0 eq.) in dry DMF (40 ml), imidazole (636 mg, 9.34 mmol, 5.0 eq.) and *tert*-butyldimethylsilyl chloride (704 mg, 4.67 mmol, 2.5 eq.) was added. The reaction was stirred for 45 min at ambient temperature. Upon completion, MeOH (2.5 ml) was added and the reaction was evaporated to dryness. The crude product was dissolved in CH_2Cl_2 (100 ml) and washed with 1 M HCl (50 ml), 10 % aq. LiCl (50 ml) and brine (25 ml) and dried over $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 7:1) affording **28** (961 mg, 1.77 mmol, 95 %) as a colorless oil.

R_f = 0.19 (^{c}Hex/EtOAc v/v = 7:1)

Optical rotation: $[\alpha]_D^{24}$ = + 16.8 ° (c = 0.5, $CHCl_3$)

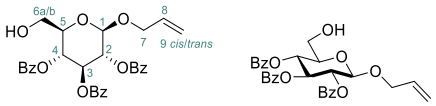
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.97 (m, 4H, H-Ar), 7.56 – 7.44 (m, 2H, H-Ar), 7.37 (m, 4H, H-Ar), 5.78 (dddd, $J_{H8,H9trans}$ =16.9 Hz, $J_{H8,H9cis}$ =10.7 Hz, $J_{H8,H7b}$ = 6.2 Hz, $J_{H8,H7a}$ = 4.8 Hz, 1H, H-8), 5.50 (dd, $J_{H3,H2}$ = 9.8 Hz, $J_{H3,H4}$ = 9.0 Hz, 1H, H-3), 5.41 (dd, $J_{H2,H3}$ = 9.9 Hz, $J_{H2,H1}$ = 7.8 Hz, 1H, H-2), 5.22 (dd, $J_{H9trans,H8}$ = 17.2 Hz, $J_{H9trans,H9cis}$ = 1.7 Hz, 1H, H-9_{trans}), 5.14 – 5.10 (m, 1H, H-9_{cis}), 4.74 (d, $J_{H1,H2}$ = 7.8 Hz, 1H, H-1), 4.33 (ddt, $J_{H7a,H7b}$ = 13.3 Hz, $J_{H7a,H8}$ = 4.9 Hz, $J_{H7a,H9cis}$ = $J_{H7a,H9trans}$ = 1.6 Hz, 1H, H-7a), 4.12 (ddt, $J_{H7b,H7a}$ = 13.3 Hz, $J_{H7b,H8}$ = 6.2 Hz, $J_{H7b,H9cis}$ = $J_{H7b,H9trans}$ =1.4 Hz, 1H, H-7b), 4.04 – 3.91 (m, 3H, H-4, H-6a, H-6b), 3.58 (dt, $J_{H5,H4}$ = 9.3 Hz, $J_{H5,H6a}$ = $J_{H5,H6b}$ = 5.3 Hz, 1H, H-5), 3.52 (d, $J_{OH,H4}$ = 2.5 Hz, 1H, OH), 0.92 (s, 9H, Si-^{t}Bu), 0.13 (s, 3H, Si-CH_3), 0.12 (s, 3H, Si-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 167.1 (C=O), 165.5 (C=O), 133.6 (C-8), 133.5, 133.3, 130.1 (2C), 129.9 (2C), 129.6 (C_q), 129.3 (C_q), 128.5 (2C, 10 × C-Ar), 117.7 (C-9), 99.7 (C-1), 76.4 (C-3), 74.8 (C-5), 72.2 (C-4), 71.6 (C-2), 69.9 (C-7), 64.6 (C-6), 26.0 (3C, Si-^{t}Bu), 18.4 (C_q, Si-^{t}Bu) , − 5.30 (2C, 2× CH_3). Due to signal overlap 27 out of 29 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{29}H_{42}O_8NSi^+$ $[M+NH_4]^+$: 560.2674; found: 560.2683.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 13.85 min, λ = 230 nm.

Allyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**54**)



Compound **54** was synthesized according to modified synthetic protocols.[322, 367, 373]

To a magnetically stirred solution of **28** (920 mg, 1.70 mmol, 1.0 eq.) in dry CH_2Cl_2 (50 ml), NEt_3 (1.18 ml, 8.50 mmol, 5.0 eq.) and benzoyl chloride (588 µl, 5.10 mmol, 3.0 eq.) was added. The reaction was stirred for 17 h at ambient temperature before DMAPA (1.06 ml, 8.50 mmol, 5.0 eq.) was added. The resulting mixture was diluted with CH_2Cl_2 (100 ml) and washed with 1 M HCl (2 × 50 ml) and Brine (25 ml) and dried with $MgSO_4$.

The crude benzoyl ester was dissolved in a mixture of CH_2Cl_2/MeOH (v/v = 1:1, 40 ml) and *p*-TsOH·H_2O (755 mg, 3.97 mmol, 2.3 eq.) was added. The reaction was stirred at ambient temperature until TLC monitoring indicated complete conversion of the starting material. The reaction was subsequently neutralized by addition of NEt_3 (4 ml) and the organic solvents were removed under reduced pressure. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 4:1) affording **54** (752 mg, 1.41mmol, 83 % over two steps) as a colorless oil.

R_f = 0.12 (^{c}Hex/EtOAc v/v = 4:1).

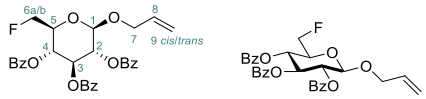
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.95 (m, 4H, H-Ar), 7.87 – 7.81 (m, 2H, H-Ar), 7.56 – 7.48 (m, 2H, H-Ar), 7.45 – 7.34 (m, 5H, H-Ar), 7.31 – 7.24 (m, 2H, H-Ar), 5.94 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.7 Hz, 1H, H-3), 5.81 (dddd, $J_{H8,H9trans}$ = 16.8 Hz, $J_{H8,H9cis}$ = 10.8 Hz, $J_{H8,H7b}$ = 6.1 Hz, $J_{H8,H7a}$ = 4.9 Hz, 1H, H-8), 5.57 – 5.47 (m, 2H, H-2, H-4), 5.26 (dq, $J_{H9trans,H8}$ = 17.2 Hz, $J_{H9trans,H9cis} = J_{H9trans,H7a} = J_{H9trans,H7b}$ = 1.6 Hz, 1H, H-9_{trans}), 5.15 (dq, $J_{H9cis,H8}$ = 10.5 Hz, $J_{H9cis,H9trans} = J_{H9cis,H7a} = J_{H9cis,H7b}$ = 1.4 Hz, 1H, H-9_{cis}), 4.89 (d, $J_{H1,H2}$ = 8.0 Hz, 1H, H-1), 4.39 (ddt, $J_{H7a,H7b}$ = 13.3 Hz, $J_{H7a,H8}$ = 5.0 Hz, $J_{H7a,\ H9trans} = J_{H7a,\ H9cis}$ = 1.6 Hz, 1H, H-7a), 4.19 (ddt, $J_{H7b,H7a}$ = 13.3 Hz, $J_{H7b,H8}$ = 6.1 Hz, $J_{H7b,\ H9trans} = J_{H7b,\ H9cis}$ = 1.4 Hz, 1H, H-7b), 3.87 (ddd, $J_{H6a,H6b}$ = 12.2 Hz, $J_{H6a,OH}$ = 8.5 Hz, $J_{H6a,H5}$ = 1.8 Hz, 1H, H-6a), 3.82 – 3.72 (m, 2H, H-5, H-6b), 2.57 (dd, $J_{OH,H6a}$ = 8.7 Hz, $J_{OH,H6b}$ = 5.2 Hz, 1H, OH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 166.2, 166.0, 165.2 (3 × C=O), 133.8 (C-Ar), 133.6 (C-8), 133.4, 133.3, 130.1, 129.9 (2C), 129.5, 129.0, 128.7 (2C), 128.5, 128.4 (11 × C-Ar), 117.9 (C-9), 100.1 (C-1), 74.8 (C-5), 72.9 (C-3), 72.0 (C-2/C-4), 70.4 (C-7), 69.7(C-2/C-4), 61.5 (C-6). Due to signal overlap 24 out of 30 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI^+): Calculated for $C_{30}H_{33}O_9N^+$ $[M+NH_4]^+$: 550.2072; found: 550.2074.

For further analytical data, see reference.[396]

Allyl 2,3,4-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-glucopyranoside (**55**)



Compound **55** was synthesized in accordance with a previously reported fluorination protocol.[324, 325]

In a flame-dried microwave vessel, **54** (600, mg 1.13 mmol, 1.0 eq.) were solved in dry CH_2Cl_2 (4 ml) before 2,4,6-collidine (452 µl, 3.39 mmol, 3.0 eq.) and DAST (225 µl,1.70 mmol, 1.5 eq.) were added. The reaction was stirred for 2 min at ambient temperature and then heated in a microwave oven (80 °C, 100 W, 60 min). After complete conversion of the starting material was observed, the reaction was stopped by addition of MeOH (500 µl) and diluted with CH_2Cl_2.* The organic layer was washed with sat. aq. $NaHCO_3$ (2 × 45 ml) and brine (30 ml) and dried over $MgSO_4$. Organic solvents were removed under reduced pressure and the crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 5:1) affording **55** (1.46 g, 2.73 mmol, 81 %) as colorless foam.

* Reaction was conducted in a replicate and reaction mixtures were combined for aqueous work-up and purification.

R_f = 0.33 (^{c}Hex/EtOAc v/v = 5:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.98 – 7.91 (m, 4H, Ar-H), 7.85 – 7.80 (m, 2H, Ar-H), 7.56 – 7.48 (m, 2H, Ar-H), 7.46 – 7.35 (m, 5H, Ar-H), 7.32 – 7.24 (m, 2H, Ar-H), 5.90 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.6 Hz, 1H, H-3), 5.81 (dddd, $J_{H8,H9trans}$ = 17.0 Hz, $J_{H8,H9cis}$ =10.4 Hz, $J_{H8,H7b}$ = 6.4 Hz, $J_{H8,H7a}$ = 4.8 Hz, 1H, H-8), 5.56 – 5.46 (m, 2H, H-2, H-4), 5.27 (dq, $J_{H9trans,H8}$ = 17.2 Hz, $J_{H9trans,H9cis} = J_{H9trans,H7a}$ = 1.6 Hz, 1H, H-9_{trans}), 5.16 (dq, $J_{H9cis,H8}$ = 10.5 Hz, $J_{H9cis,H9trans}$ = 1.4 Hz, 1H, H-9_{cis}), 4.90 (d, $J_{H1,H2}$ = 7.8 Hz, 1H, H-1), 4.68 – 4.53 (m, 2H, H-6a, H-6b), 4.41 (ddt, $J_{H7a,H7b}$ = 13.2 Hz, $J_{H7a,H8}$ = 4.8 Hz, $J_{H7a,H9trans} = J_{H7a,H9cis}$ = 1.6 Hz, 1H, H-7a), 4.19 (ddt, $J_{H7b,H7a}$ = 13.2 Hz, $J_{H7b,H8}$ = 6.3 Hz, $J_{H7b,H9trans} = J_{H7b,H9cis}$ = 1.4 Hz, 1H, H-7b), 4.04 (dddd, $J_{H5,F}$ = 19.8 Hz, $J_{H5,H4}$ = 10.0 Hz, $J_{H5,6a/b}$ = 4.8 Hz, $J_{H5,6a/b}$ = 3.2 Hz, 1H, H-5).

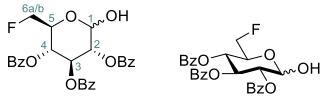
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 165.9, 165.4, 165.2 (3 × C=O), 133.7 (C-Ar), 133.4 (3C, 2 ×C-Ar, C-8), 130.0, 129.9 (2C), 129.4, 128.9, 128.8, 128.6, 128.5 (2C, 9 × C-Ar), 118.1 (C-9), 99.8 (C-1), 81.8 (d, $J_{C6,F}$ = 176 Hz, C-6), 73.4 (d, $J_{C5,F}$ = 19.6 Hz, C-5), 73.0 (C-3), 71.9 (C-2), 70.2 (C-7), 69.0 (d, $J_{C4,F}$ = 6.9 Hz, C-4). Due to signal overlap 24 out of 30 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 230.2 (td, $J_{F,H6a} = J_{F,H6b}$ = 47.0 Hz, $J_{F,H5}$ = 19.7 Hz).

HRMS (ESI^+): Calculated for $C_{30}H_{31}O_8NF^+$ $[M+NH_4]^+$: 552.2028; found: 552.2031.

For further analytical data, see reference.[368]

2,3,4-Tri-*O*-benzoyl-6-deoxy-6-fluoro-α/β-D-glucopyranoside (**56**)



Deallylation was conducted according to a modified procedure.[370]

To a magnetically stirred solution of **55** (1.20 g, 2.25 mmol, 1.0 eq.) in MeOH/THF (v/v = 4:1, 50 ml), palladium(II) chloride (199 mg, 1.12 mmol, 0.6 eq.) were added. The reaction mixture was stirred at 40 °C until complete conversion of the starting material was observed. The reaction was diluted with THF and filtered through *Hyflo*®. Organic solvents were removed under reduced pressure and the crude residue was dissolved in CH_2Cl_2 (50 ml), washed with water (15 ml) and brine (15 ml) and dried over $MgSO_4$. Organic solvents were removed under reduced pressure and the crude product was subjected to f column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **56** (850 mg, 1.72 mmol, 76 %, α/β = 9:1) as an amorphous solid.

R_f = 0.19 (^{c}Hex/EtOAc v/v = 4:1).

α-Anomer:

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.98 (td, $J_{CH,CH}$ = 8.2 Hz, $J_{CH,CH}$ = 1.3 Hz, 4H, Ar-H), 7.91 – 7.85 (m, 2H, Ar-H), 7.57 – 7.47 (m, 2H, Ar-H), 7.47 – 7.36 (m, 5H, Ar-H), 7.29 (dd, $J_{CH,CH}$ = 8.4 Hz, $J_{CH,CH}$ = 7.2 Hz, 2H, Ar-H), 6.25 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.9 Hz, 1H, H-3), 5.79 (t, $J_{H1,H2} = J_{H1,OH}$ = 3.7 Hz, 1H, H-1), 5.59 (t, $J_{H4,H3} = J_{H4,H5}$ = 9.9 Hz, 1H, H-4), 5.31 (ddd, $J_{H2,H3}$ = 10.2 Hz, $J_{H2,H1}$ = 3.6 Hz, $J_{H2,OH}$ = 1.1 Hz, 1H, H-2), 4.69 – 4.49 (m, 3H, H-5, H-6a, H-6b), 3.39 (dd, $J_{OH,H1}$ = 3.9 Hz, $J_{OH,H2}$ = 1.3 Hz, 1H, OH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 166.0, 165.9, 165.5 (3 × C=O), 133.7, 133.6, 133.3, 130.1, 130.0, 129.8, 129.2, 129.0, 128.9, 128.6, 128.5 (11 × C-Ar), 90.6 (C-1), 81.7 (d, $J_{C6,F}$ = 175 Hz, C-6), 72.2 (C-2), 70.1 (C-3), 68.8 (d, $J_{C5,F}$ = 18.8 Hz, C-5), 68.60 (d, $J_{C4,F}$ = 6.9 Hz, C-4). Due to signal overlap 20 out of 27 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 232.0 (td, $J_{F,H6a} = J_{F,H6b}$ = 47.2 Hz, $J_{F,H5}$ = 23.2 Hz).

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 10.24 min (α) λ = 230 nm

β-Anomer:

^{1}H-NMR (400 MHz, $CDCl_3$, selected signals): δ [ppm] = 5.96 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.7 Hz, 1H, H-3), 5.37 – 5.33 (m, 1H, H-2), 5.06 (t, $J_{H1,H2} = J_{H1,OH}$ = 8.3 Hz, 1H, H-1).

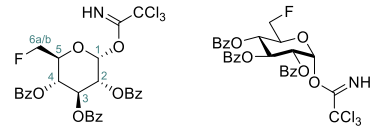
^{13}C-NMR (100 MHz, $CDCl_3$, selected signals): δ [ppm] = 96.2 (C-1), 74.3 (C-2), 72.3 (C-3).

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 231.3 (td, $J_{F,H6a} = J_{F,H6b}$ = 46.9 Hz, $J_{F,H5}$ = 20.9 Hz).

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 9.55 min (β), λ = 230 nm

HRMS (ESI$^+$): Calculated for $C_{27}H_{27}O_8NF^+$ $[M+NH_4]^+$: 512.1715; found: 512.1716.

2,3,4-Tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-glucopyranosyl trichloroacetimidate (**31**)



Compound **31** was synthesized in accordance with a literature known protocol.[371]

To an ice-cooled solution of lactol **56** (500 mg, 1.01 mmol, 1.0 eq.) in dry CH_2Cl_2 (20 ml), trichloroacetonitril (1.00 ml, 10.1 mmol, 10 eq.) and DBU (165 µl, 1.11 mmol, 1.1 eq.) were added. The reaction was allowed to warm to ambient temperature and was stirred for 18 h. Subsequently, the reaction was concentrated under reduced pressure and the crude residue was subjected to column chromatography (^{c}Hex/EtOAc v/v = 5:1 + 1 % NEt_3) affording **31** (400 mg, 0.63 mmol, 62 %) as a colorless oil.

R_f = 0.41 ^{c}Hex/EtOAc v/v = 4:1 + 1% NEt_3).

Optical rotation: $[\alpha]_D^{22}$ = + 46.1 ° (c = 0.5; $CHCl_3$)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 8.68 (s, 1H, C=NH), 7.97 (ddd, $J_{CH,CH}$ = 11.9 Hz, $J_{CH,CH}$ = 8.3 Hz, $J_{CH,CH}$ = 1.4 Hz, 4H, Ar-H), 7.90 – 7.85 (m, 2H, Ar-H), 7.57 – 7.27 (m, 9H, Ar-H), 6.87 (d, $J_{H1,H2}$ = 3.6 Hz, 1H, H-1), 6.29 (t, $J_{H3,H2}$ = $J_{H3,H4}$ = 10.0 Hz, 1H, H-3), 5.75 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 10.0 Hz, 1H, H-4), 5.60 (dd, $J_{H2,H3}$ = 10.2 Hz, $J_{H2,H1}$ = 3.6 Hz, 1H, H-2), 4.70 – 4.54 (m, 2H, H-6a, H-6b), 4.49 (dddd, $J_{H5,F}$ = 23.5 Hz, $J_{H5,H4}$ = 10.5 Hz, $J_{H5,6a/b}$ = 4.1 Hz, $J_{H5,6a/b}$ = 2.3 Hz, 1H, H-5).

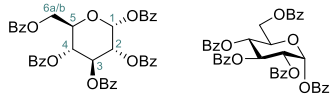
^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 165.8, 165.5, 165.2 (3 × C=O), 160.6 (C=NH), 133.8, 133.7, 133.4, 130.0 (2C), 129.8, 129.0, 128.7 (2C), 128.6, 128.5 (2C, 12 × C-Ar), 93.3 (C-1), 90.8 (CCl_3), 81.0 (d, $J_{C6,F}$ = 177 Hz, C-6), 71.6 (d, $J_{C5,F}$ = 19.3 Hz, C-5), 70.7 (C-2), 70.2 (C-3), 67.9 (d, $J_{C4,F}$ = 6.6 Hz, C-4). Due to signal overlap 23 out of 29 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 232.6 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 47.0 Hz, $J_{F,H5}$ = 23.5 Hz).

HRMS (ESI^+): Calculated for $C_{27}H_{22}FO_7^+$ $[M\text{-}OC(NH)CCl_3]^+$: 477.1345; found: 477.1343.

Synthesis of non-fluorinated glucose donor 32

1,2,3,4,6-Penta-*O*-benzoyl-α-D-glucopyranoside (**57**)



Compound **57** was synthesized according to a modified procedure.[372, 373]

To a magnetically stirred solution of D-glucose (2.50 g, 12.6 mmol, 1.0 eq.) in pyridine (100 ml), benzoyl chloride (8.70 ml, 75.5 mmol, 6.0 eq.) was added at 0 °C. The reaction was allowed to warm to ambient temperature and was stirred for 17 h. After the reaction was deemed complete, it was cooled to 0 °C and stopped by addition of

DMAPA (1.59 ml,12.6 mmol, 1.0 eq.). Subsequently, solvents were removed under reduced pressure and the crude residue was dissolved in CH_2Cl_2 (150 ml) and washed with 1 M HCl (100 ml) and brine (75 ml) and dried with $MgSO_4$. Organic solvents were removed under reduced pressure and the crude product was crystallized from EtOH to afford **57** (8.34 g, 11.9 mmol, 94 %) as a colorless solid.

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

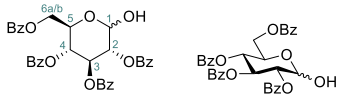
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.24 – 8.08 (m, 2H, Ar-H), 8.06 – 7.99 (m, 2H, Ar-H), 7.97 – 7.92 (m, 2H, Ar-H), 7.91 – 7.83 (m, 4H, Ar-H), 7.71 – 7.63 (m, 1H, Ar-H), 7.60 – 7.28 (m, 14H, Ar-H), 6.86 (d, $J_{H1,H2}$ = 3.7 Hz, 1H, H-1), 6.32 (t, $J_{H3,H2} = J_{H3,H4}$ = 10.0 Hz, 1H, H-3), 5.87 (t, $J_{H4,H3} = J_{H4,H5}$ = 9.8 Hz, 1H, H-4), 5.69 (dd, $J_{H2,H3}$ = 10.3 Hz, $J_{H2,H1}$ = 3.7 Hz, 1H, H-2), 4.66 – 4.59 (m, 2H, H-6a, H-5), 4.52 – 4.46 (m, 1H, H-6b).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 166.2, 166.1, 165.5, 165.3, 164.6 (5 × C=O), 134.1, 133.7, 133.6, 133.5, 133.3, 130.2, 130.0, 129.9 (2C), 129.7, 129.1, 129.0, 128.8, 128.7, 128.6 (2C), 128.5 (17 × C-Ar), 90.2 (C-1), 70.6 (3C, C-2, C-3, C-5), 69.0 (C-4), 62.6 (C-6). Due to signal overlapping only 28 out of 41 carbon signals were assigned.

HRMS (ESI$^+$): Calculated for $C_{41}H_{36}O_{11}N^+$ $[M+NH_4]^+$: 718.2283; found: 718.2283.

For further analytical data see reference.[397]

2,3,4,6-Tetra-*O*-benzoyl-α/β-D-glucopyranoside (**58**)



Compound **58** was synthesized according to modified procedures.[374, 375]

To a magnetically stirred solution of **57** (1.60 g, 2.28 mmol, 1.0 eq.) in pyridine (30 ml), a solution of dimethylamine (2 M in THF) was added. The reaction was stirred until complete conversion of the starting material was observed by TLC monitoring. Subsequently, the reaction was diluted with CH_2Cl_2 (150 ml) and washed with 1 M HCl (50 ml) and brine (30 ml) and dried with $MgSO_4$. Subsequently, the reaction was concentrated under reduced pressure and the crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 4:1 → 3:1) affording **58** (1.10 g, 1.84 mmol, 81 %) as a colorless foam.

R_f = 0.50 (^{c}Hex/EtOAc v/v = 2:1)

α-Anomer:

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] =8.06 – 8.04 (m, 2H, Ar-H), 8.01 – 7.98 (m, 3H, Ar-H), 7.94 (dt, $J_{CH,CH}$ = 8.4 Hz, $J_{CH,CH}$ = 1.7 Hz, 2H, Ar-H), 7.88 (dd, $J_{CH,CH}$ = 8.3 Hz, $J_{CH,CH}$ = 1.4 Hz, 2H, Ar-H), 7.44 – 7.33 (m, 9H, Ar-H), 7.32 – 7.27 (m, 2H, Ar-H), 6.27 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.9 Hz, 1H, H-3), 5.79 – 5.72 (m, 2H, H-1, H-4), 5.32 (dd,

$J_{H2,H3}$ = 10.3 Hz, $J_{H2,H1}$ = 3.6 Hz, 1H, H-2), 4.70 – 4.63 (m, 2H, H-5, H-6a), 4.44 (dd, $J_{H6b,H6a}$ = 12.1 Hz, $J_{H6b,H5}$ = 4.2 Hz, 1H, H-6b).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 166.5, 166.0 (2C), 165.4 (4 × C=O), 133.6, 133.5, 133.3 (2C), 130.1, 130.0, 129.9, 129.8, 129.3, 129.1 (2C), 128.6 (2C), 128.5, 128.4 (15 × C-Ar), 90.6 (C-1), 72.4 (C-2), 70.3 (C-3), 69.6 (C-4), 67.9 (C-5), 63.0 (C-6). Due to signal overlapping 25 out of 34 carbon atoms were assigned in the ^{13}C spectrum.

β-Anomer:

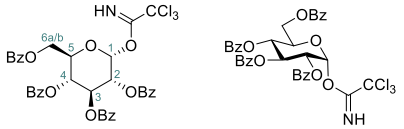
^{1}H-NMR (600 MHz, $CDCl_3$, selected signals): δ [ppm] = 5.08 (t, $J_{H1,H2} = J_{H1,OH}$ = 7.8 Hz, 1H, H-1), 5.37 (dd, $J_{H2,H3}$ = 9.9 Hz, $J_{H2,H1}$ = 8.0 Hz, 1H, H-2), 5.97 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.7 Hz, 1H, H-3).

^{13}C-NMR (150 MHz, $CDCl_3$, selected signals): δ [ppm] = 96.3 (C-1), 74.5 (C-2), 72.5 (C-3).

HRMS (ESI$^+$): Calculated for $C_{34}H_{32}O_{10}N^+$ $[M+NH_4]^+$: 614.2021; found: 614.2024.

For further analytical data, see reference.[398]

2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**32**)[371]



To a solution of hemiacetal **58** (2.18 g, 3.66 mmol, 1.0 eq.) in dry CH_2Cl_2 (75 ml), trichloroacetonitrile (1.84 ml, 18.3 mmol, 5.0 eq.) was added. The solution was cooled to 0 °C, before DBU (279 µl, 1.83 mmol, 0.5 eq.) was added in one portion. The reaction was slowly warmed to ambient temperature and stirred for 17 h. Another portion of trichloroacetonitrile (0.50 ml, 4.97 mmol, 1.4 eq.) and DBU (0.10 ml, 0.66 mmol, 0.2 eq.) were added at 0 °C. The reaction was stirred for another 5 h at ambient temperature and concentrated under reduced pressure. The resulting black solution was directly subjected to column chromatography (^{c}Hex/EtOAc v/v = 1:0 + 1 % NEt_3 → ^{c}Hex/EtOAc v/v = 4:1 + 1 % NEt_3), affording **32** (1.60 g, 2.16 mmol, 59 %) as a white foam.

R_f = 0.61 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.64 (s, 1H, C=NH), 8.07 – 8.01 (m, 2H, Ar-H), 7.98 – 7.93 (m, 4H, Ar-H), 7.89 – 7.85 (m, 2H, Ar-H), 7.60 – 7.27 (m, 12H, Ar-H), 6.84 (d, $J_{H1,H2}$ = 3.7 Hz, 1H, H-1), 6.28 (t, $J_{H3,H2} = J_{H3,H4}$ = 10.0 Hz, 1H, H-3), 5.82 (t, $J_{H4,H3} = J_{H4,H5}$ = 9.8 Hz, 1H, H-4), 5.63 (dd, $J_{H2,H3}$ = 10.2 Hz, $J_{H2,H1}$ = 3.7 Hz, 1H, H-2), 4.71 – 4.56 (m, 2H, H-5, H-6a), 4.54 – 4.44 (m, 1H, H-6b).

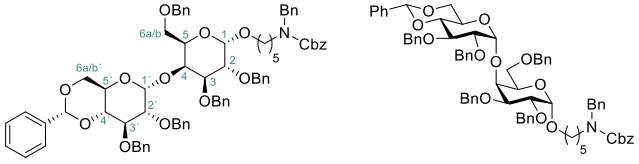
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 166.2, 165.8, 165.6, 165.3 (4 × C=O), 160.7 (C=NH), 133.7, 133.5, 133.3, 130.1, 129.9 (2C), 128.6, 128.5 (2C, 9 × C-Ar), 93.2 (C-1), 90.8 (CCl_3) 70.8 (2C, C-2, C-5), 70.3 (C-3), 68.7 (C-4), 62.6 (C-6). Due to signal overlapping only 21 out of 36 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI$^+$): Calculated for $C_{27}H_{22}FO_7^+$ $[M - OC(CCl_3)=NH]^+$: 477.1344; found: 477.1343

Synthesis of disaccharides

Synthesis of disaccharide acceptors 18, 19 and 20

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3-di-*O*-benzyl-4,6-*O*-benzyliden-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**21**)



Method A: PTFA Donor Glycosylation

N-phenyltrifluoroacetimidate donor **30** (459 mg, 0.74 mmol, 1.4 eq.) and acceptor **23** (400 mg, 0.53 mmol, 1.0 eq.) were combined, co-evaporated with toluene (2 × 10 ml) and dried under high vacuum for 1 h. Starting materials were dissolved in dry Et_2O (25 ml) and stirred for 1 h with freshly activated 4 Å molecular sieve. Subsequently the reaction mixture was cooled to 0 °C and TMSOTf (9.00 µl, 0.05 mmol, 0.1 eq.) was added. The reaction mixture was stirred for 1 h at 0 °C until TLC monitoring indicated complete conversion of the galactosyl acceptor. The reaction was stopped by addition of NEt_3 (1 ml), diluted with CH_2Cl_2 and filtered through a pad of *Hyflo*®. The organic layer was washed with 1 M HCl (10 ml), sat. aq. $NaHCO_3$ (20 ml) and brine (15 ml) and dried over $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 7:1) affording **21** (0.54 g, 0.45 mmol, 85 %) as a colorless oil.

Method B: **Thioglycoside glycosylation**

Glycosylation was conducted in accordance with literature known protocols, with minor modifications.[359]

Donor **29** (321 mg, 0.58 mmol, 1.4 eq.) and galactosyl acceptor **23** (310 mg, 0.41 mmol, 1.0 eq.) were combined, co-evaporated with toluene (10 ml) and dried under high vacuum for 1 h. Starting materials were dissolved in dry CH_2Cl_2 (4 ml) and stirred for 1 h with freshly activated 4 Å molecular sieve. Subsequently the reaction mixture was cooled to − 30 °C and NIS (191 mg, 0.85 mmol, 1.6 eq.) and TMSOTf (2.30 µl, 12.3 µmol, 0.03 eq.) was added. The reaction was stirred for 1 h at − 30 °C before another portion of TMSOTf (2.30 µl, 12.3 µmol, 0.03 eq.) was added. The reaction was stirred for another 2 h at − 30 °C before another portion of NIS (50 mg, 0.23 mmol, 0.6 eq.) and TMSOTf (2.30 µl, 12.3 µmol, 0.03 eq.) was added. After the reaction was deemed complete by TLC monitoring, it was neutralized by addition of NEt_3 (100 µl), diluted with CH_2Cl_2 and filtered through a pad of *Hyflo*®. The organic layer was washed with sat. aq. $Na_2S_2O_3$ (15 ml), 1 M HCl (10 ml), saturated aqueous $NaHCO_3$ solution (10 ml) and brine (10 ml) and dried over $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 7:1) affording **21** (450 mg, 0.38 mmol, 93 %) as a colorless oil.

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

Optical rotation: $[\alpha]_D^{22}$ = + 22.7 ° (c = 1.1; $CHCl_3$)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.54 – 7.13 (m, 40H, Ar-H), 5.51 (s, 1H, CH-Ar), 5.17 (d, $J_{CH,CH}$ = 9.6 Hz, 2H, CH_{Cbz}), 4.98 – 4.92 (m, 2H, H-1´, CH_{Bn}), 4.90 – 4.84 (m, 2H, 2 × CH_{Bn}), 4.80 – 4.72 (m, 5H, H-1, 4 × CH_{Bn}), 4.70 (d, $J_{CH,CH}$ = 11.9 Hz, 1H, CH_{Bn}), 4.48 (d, $J_{CH,CH}$ = 10.6 Hz, 2H, NCH_{Bn}), 4.31 – 4.19 (m, 3H, H-5´,2 × CH_{Bn}), 4.08 (bs, 1H, H-4), 4.03 (t, $J_{H3´,H2´}$ = $J_{H3´,H4´}$ = 9.3 Hz, 1H, H-3´), 3.96 – 3.83 (m, 4H, H-2, H-3, H-5, H-6a), 3.73 (dd, $J_{H6a´,H6b´}$ = 10.1 Hz, $J_{H6a´,H5´}$ = 4.9 Hz, 1H, H-6a´), 3.63 – 3.33 (m, 6H, H-6b´, H-2´, H-4´, H-6b, 2 × CH_{Linker}), 3.29 – 3.14 (m, 2H, 2 × CH_{Linker}), 1.66 – 1.43 (m, 4H, 4 × CH_{Linker}), 1.35 – 1.17 (m, 2H, 2 × CH_{Linker}).

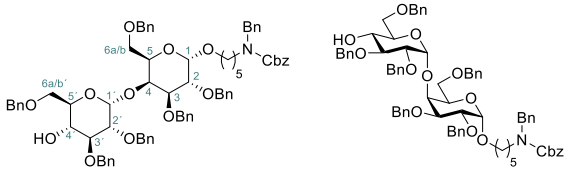
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 156.8/156.3 (C=O-Cbz), 139.0, 138.8, 138.6, 138.5, 138.4, 138.1, 137.9, 137.0/136.9 (8 × Cq), 128.9, 128.7, 128.5 (3C), 128.4 (2C), 128.3, 128.2, 128.1, 128.0, 127.8, 127.7 (3C), 127.6, 127.5, 126.2 (18 × C-Ar), 101.2 (CH-Ar), 100.6 (C-1´), 97.9 (C-1), 83.0 (C-4´), 79.7 (C-2´), 79.3 (C-3´), 78.0 (C-2/C-3/C-5), 77.4 (C-4), 75.3 (CH_{Bn}), 74.7 (C-2/C-3/C-5), 74.5 (CH_{Bn}), 73.4 (CH_{Bn}), 73.0 (CH_{Bn}), 73.0 (CH_{Bn}), 69.5 (C-2/C-3/C-5), 69.1 (C-6´), 68.2 (2C, C-6, CH_{Linker}), 67.3 (CH_{Cbz}), 63.1 (C-5´), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.6 (CH_{Linker}). Due to signal overlap 52 out of 74 carbon atoms were assigned in the ^{13}C spectrum.

^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 166 Hz, $J_{C1´H1´}$ = 170 Hz.

HRMS (ESI^+): Calculated for $C_{74}H_{83}O_{13}N_2^+$ $[M+NH_4]^+$:1207.5890; found: 1207.5919.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 20.40 min, λ = 230 nm.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**18**)



The reaction was conducted following a modified procedure of *Danieli et al*.[360]

A magnetically stirred solution of disaccharide **21** (470 mg, 0.40 mmol, 1.0 eq.) in a mixture of dry MeCN/CH_2Cl_2 (v:v = 3:1, 40 ml) was cooled to – 10 °C. Sequentially borane trimethylamine complex (216 mg, 2.96 mmol, 7.4 eq.) and $BF_3{\cdot}OEt_2$ (375 µl, 2.96 mmol, 7.4 eq.) were added. The reaction was stirred at – 10 °C for 1.5 h before another portion of borane trimethylamine complex (60 mg, 0.82 mmol, 2.0 eq.) and $BF_3{\cdot}OEt_2$ (104 µl, 0.82 mmol, 2.0 eq.) was added. After the reaction was deemed complete by TLC monitoring, MeOH (10 ml) and NEt_3 (5 ml) was added and it was stirred for further 20 min at ambient temperature. Subsequently, solvents were removed under reduced pressure and the crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 7:1 → 5:1 → 3:1) affording **18** (376 mg, 0.32 mmol, 80 %) as a colorless oil.

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

Optical rotation: $[\alpha]_D^{24}$ = + 46.7 ° (c = 1.0; $CHCl_3$)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.42 – 7.13 (m, 40H, Ar-H), 5.18 (d, $J_{CH,CH}$ = 14.2 Hz, 2H, CH_{Cbz}), 5.01 (d, $J_{H1´,H2´}$ = 3.4 Hz, 1H, H-1´), 4.93 (d, $J_{CH,CH}$ = 11.3 Hz, 1H, CH_{Bn}), 4.86 – 4.75 (m, 5H, H-1, 4 × CH_{Bn}), 4.71 – 4.66 (m, 3H, 3 × CH_{Bn}), 4.48 (d, $J_{CH,CH}$ = 15.5 Hz, 2H, NCH_{Bn}), 4.36 (d, $J_{CH,CH}$ = 12.2 Hz, 1H, CH_{Bn}), 4.30 – 4.22 (m, 2H, CH_{Bn}), 4.20 (d, $J_{CH,CH}$ = 12.2 Hz, 1H, CH_{Bn}), 4.17 (dt, $J_{H5´,H4´}$ = 9.9 Hz, $J_{H5´,H6a´}$ = $J_{H5´,H6b´}$ = 3.5 Hz, 1H, H-5´), 4.11 (d, $J_{H4,H3/H5}$ = 2.7 Hz, 1H, H-4), 3.96 – 3.81 (m, 5H, H-2, H-3, H-5, H-3´, H-6a), 3.75 (dd, $J_{H4´,H5´}$ = 9.8 Hz, $J_{H4´,H3´}$ = 8.9 Hz, 1H, H-4´), 3.62 – 3.48 (m, 3H, H-2´, H-6b, CH_{Linker}), 3.45 – 3.35 (m, 1H, CH_{Linker}), 3.32 (dd, $J_{H6a´,H6b´}$ = 10.3 Hz, $J_{H6a´,H5´}$ = 3.0 Hz Hz, 1H, H-6a´), 3.28 – 3.15 (m, 2H, 2 × CH_{Linker}), 3.12 (dd, $J_{H6b´,H6a´}$ = 10.3 Hz, $J_{H6b´,H5´}$ = 4.0 Hz, 1H, H-6b´), 1.68 – 1.46 (m, 4H, 4 × CH_{Linker}), 1.36 – 1.19 (m, 2H, 2 × CH_{Linker}).

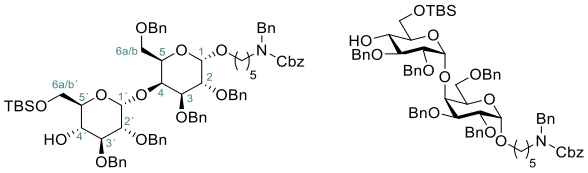
^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 156.8/156.3 (C=O-Cbz), 139.1, 139.0, 138.6, 138.4, 138.3, 138.2, 138.0, 137.0/136.9 (8 × Cq), 128.7, 128.6, 128.5 (2C), 128.4, 128.3, 128.1, 128.0, 127.9, 127.8 (2C), 127.7 (2C), 127.6, 127.5 (2C), 127.3 (17 × C-Ar), 99.8 (C-1´), 97.6 (C-1), 81.8 (C-2/C-3/C-3´/C-5), 80.1 (C-2´), 78.0 (C-2/C-3/C-3´/C-5), 76.6 (C-4), 75.4 (2C, CH_{Bn}, C-2/C-3/C-3´/C-5), 73.9 (CH_{Bn}), 73.4 (2C, 2 × CH_{Bn}) , 73.1 (CH_{Bn}), 72.9 (CH_{Bn}), 71.8 (C-4´), 70.3 (C-5´), 69.5(C-2/C-3/C-3´/C-5), 69.1 (C-6´), 68.1 (2C, C-6, CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.6 (CH_{Linker}). Due to signal overlap 51 out of 74 carbon atoms were assigned in the ^{13}C spectrum.

^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 169 Hz, $J_{C1´H1´}$ = 169 Hz.

HRMS (ESI^+): Calculated for $C_{74}H_{85}O_{13}N_2^+$ $[M+NH_4]^+$: 1209.6046; found: 1209.6078.

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 18.50 min, λ = 230 nm.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3-di-*O*-benzyl-6-*O*-*tert*-butyl-dimethylsilyl-α-D-gluco-pyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**19**)



Compound **19** was synthesized in accordance with previously described synthetic protocols.[194, 361]

To a magnetically stirred solution of disaccharide **21** (350 mg, 0.29 mmol, 1.0 eq.) in CH_2Cl_2 (16 ml), ethanethiol (212 µl, 2.94 mmol, 10 eq.) and *p*-TsOH (30.0 mg, 0.15 mmol, 0.5 eq.) were added. The reaction was stirred for 1 h at ambient temperature, before being neutralized by addition of NEt_3 (0.5 ml). Solvents were removed under reduced pressure affording the crude diol, which was subjected to flash chromatography (^{c}Hex/EtOAc v/v = 4:1 → 2:1). Product containing fractions were pooled and organic solvents were removed under reduced pressure.

The thus obtained diol was dissolved in dry DMF (10 ml) and imidazole (102 mg, 1.50 mmol, 5.0 eq.) and TBS-Cl (110 mg, 0.73 mmol, 2.5 eq.) were added sequentially. The reaction was stirred at ambient temperature until TLC monitoring indicated complete conversion of the starting material. The reaction was stopped by addition of MeOH (2 ml) and was stirred for 10 min at ambient temperature. Subsequently, organic solvents were removed

under reduced pressure and the crude residue was co-evaporated with toluene (2 × 15 ml). The crude product was dissolved in CH_2Cl_2 (20 ml) and washed with 1 M HCl (15 ml) and brine (15 ml) and dried with $MgSO_4$. Solvents were removed under reduced pressure and the crude silyl ether was subjected to column chromatography (^{c}Hex/EtOAc v/v = 5:1) affording **19** (296 mg, 0.24 mmol, 83 % over two steps) as a colorless oil.

R_f = 0.33 (^{c}Hex/EtOAc v/v = 4:1)

Optical rotation: $[\alpha]_D^{24}$ = + 45.0 ° (c = 1.0; $CHCl_3$)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.44 – 7.11 (m, 35, Ar-H), 5.17 (d, $J_{CH,CH}$ = 13.4 Hz, 2H, CH_{Cbz}), 4.98 (d, $J_{H1´,H2´}$ = 3.3 Hz, 1H, H-1´), 4.93 – 4.90 (m, 1H, CH_{Bn}), 4.88 – 4.83 (m, 2H, 2 ×CH_{Bn}), 4.82 – 4.76 (m, 3H, H-1, 2 ×CH_{Bn}), 4.71 – 4.63 (m, 3H, 3 ×CH_{Bn}), 4.47 (d, $J_{CH,CH}$ = 15.0 Hz, 2H, NCH_{Bn}), 4.29 – 4.20 (m, 2H, 2 ×CH_{Bn}), 4.14 – 4.07 (m, 2H, H-5´, H-4), 3.95 (dd, $J_{H2,H3}$ = 10.3 Hz, $J_{H2,H1}$ = 3.6 Hz, 1H, H-2), 3.93 – 3.83 (m, 4H, H-3, H-3´,H-5,H-6a), 3.72 (t, $J_{H4´,H3´}$ = $J_{H4´,H5´}$ = 9.3 Hz, 1H, H-4´), 3.61 – 3.52 (m, 2H, H-6a´, CH_{Linker}), 3.51 – 3.49 (m, 1H, H-6b) 3.47 (dd, $J_{H2´,H3´}$ = 9.9 Hz, $J_{H2´,H1´}$ = 3.4 Hz, 1H, H-2´), 3.44 – 3.35 (m, 1H, CH_{Linker}), 3.33 (dd, $J_{H6b´,H6a´}$ = 10.7 Hz, $J_{H6b´,H5´}$ = 4.7 Hz, 1H, H-6b´), 3.28 – 3.13 (m, 2H, 2 × CH_{Linker}), 2.77 (s, 1H, OH), 1.65 – 1.41 (m, 4H, 4 × CH_{Linker}), 1.35 – 1.18 (m, 2H, 2 × CH_{Linker}), 0.82 (s, 9H, Si-^{t}Bu), − 0.05 (s, 3H, Si-CH_3), − 0.07 (s, 3H, Si-CH_3).

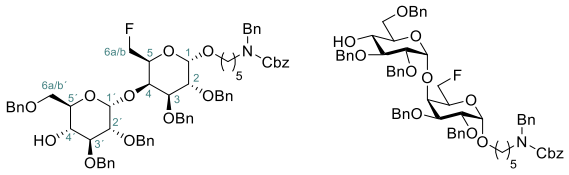
^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 156.8/156.3 (C=O-Cbz), 139.2, 139.0, 138.8, 138.5, 138.4, 138.1, 137.0/136.9 (7 × Cq), 128.7, 128.6, 128.5 (3C), 128.4, 128.3, 128.0 (2C), 127.9, 127.8, 127.7 (2C), 127.5 (2C), 127.3 (16 × C-Ar), 99.7 (C-1´), 97.7 (C-1), 81.7 (C-3/C-3´/C-5), 80.2 (C-2´), 78.2 (C-3/C-3´/C-5), 76.6 (C-4), 75.6 (C-2), 75.5 (CH_{Bn}), 73.9 (CH_{Bn}), 73.5 (CH_{Bn}), 73.1 (CH_{Bn}), 72.7 (CH_{Bn}), 72.5 (C-4´), 70.9 (C-5´), 69.6 (C-3/C-3´/C-5), 68.4 (C-6), 68.2 (CH_{Linker}), 67.3 (CH_{Cbz}), 63.7 (C-6´), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.3 (CH_{Linker}), 28.1/27.7(CH_{Linker}), 26.1 (3C, Si-^{t}Bu), 23.6 (CH_{Linker}), 18.5 (Cq, Si-^{t}Bu), − 5.3 (Si-CH_3), − 5.4 (Si-CH_3). Due to signal overlap 54 out of 73 carbon atoms were assigned in the ^{13}C spectrum.

^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 173 Hz, $J_{C1´H1´}$ = 172 Hz.

HRMS (ESI^+): Calculated for $C_{73}H_{93}O_{13}N_2Si^+$ $[M+NH_4]^+$: 1233.6441; found: 1233.6473.

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 25.48 min, λ = 230 nm.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl) -(1→4)-2,3-di-*O*-benzyl-6-deoxy-6-fluoro-α-D-galactopyranoside (**20**)



Glycosylation:

Glycosylation was conducted in accordance with a previously described synthetic procedure.[359]

Thioglycoside **29** (458 mg, 0.83 mmol, 1.5 eq.) and acceptor **24** (370 mg, 0.55 mmol, 1.0 eq.) were combined, co-evaporated with toluene (50 ml) and dried under high vacuum for 1 h. Starting materials were dissolved in dry CH_2Cl_2 (6 ml) and stirred for 1 h with freshly activated 4 Å MS. Subsequently the reaction mixture was cooled to − 30 °C and NIS (198 mg, 0.88 mmol, 1.6 eq.) and TMSOTf (9.90 µl, 60.0 µmol, 0.1 eq.) was added. The reaction was stirred for 2 h at − 30 °C before another portion of TMSOTf (9.90 µl, 60.0 µmol, 0.1 eq.) was added. The reaction was slowly warmed to − 10 °C and further to 0 °C and stirred for another 2 h. After the reaction was deemed complete by TLC monitoring, it was neutralized by addition of NEt_3 (1 ml), diluted with CH_2Cl_2 and filtered through a pad of *Hyflo*®. The organic layer was washed with sat. aq. $Na_2S_2O_3$ (15 ml), 1 M HCl (10 ml), and brine (10 ml) and dried over $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 7:1) affording **22** (480 mg*) as a colorless oil.

* NMR revealed an inseparable impurity most likely arising from donor decomposition (~ 10 % as determined from integral of benzylidene proton)

R_f = 0.48 (^{c}Hex/EtOAc v/v = 3:1).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm]= 7.54 – 7.13 (m, 35H, Ar-H), 5.51 (s, 1H, CH-Ar), 5.18 (d, $J_{CH,CH}$ = 10.6 Hz, 2H, CH_{Cbz}), 4.96 (d, $J_{CH,CH}$ = 11.1 Hz, 1H, CH_{Bn}), 4.91 – 4.68 (m, 10H, H-1, H-1´, H-6a, 7 × CH_{Bn}), 4.50 (d, $J_{CH,CH}$ = 8.6 Hz, 2H, NCH_{Bn}), 4.36 (dt, $J_{H6b,F}$ = 46.4 Hz, $J_{H6b,H6a}$ = 7.4 Hz, 2H, H-6b), 4.20 (td, $J_{H5´,H4´}$ = 9.9 Hz, $J_{H5´,H6a´}$ = 4.9 Hz, 1H, H-5´), 4.07 – 3.85 (m, 5H, H-2, H-3, H-4, H-5, H-3´), 3.74 (dd, $J_{H6a´,H6b´}$ = 10.1 Hz, $J_{H6a´,H5´}$ = 4.9 Hz, 1H, H-6a´), 3.64 – 3.50 (m, 3H, H-2´, H-4´, CH_{Linker}), 3.50 – 3.37 (m, 2H, H-6b´, CH_{Linker}), 3.31 – 3.12 (m, 2H, 2 × CH_{Linker}), 1.68 – 1.46 (m, 4H, 4 × CH_{Linker}), 1.37 – 1.22 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 156.8/156.3 (C=O-Cbz), 139.0, 138.6, 138.4, 138.0, 137.8 (5 × Cq), 137.0/136.9 (Cq_{Cbz}), 128.9, 128.7, 128.6, 128.5 (3C), 128.4, 128.3, 128.1, 128.0 (2C), 127.9, 127.8, 127.7, 126.2 (15 × C-Ar), 101.2 (CH-Ar), 100.9 (C-1´), 98.0 (C-1), 82.8 (C-4´), 81.0 (d, $J_{C6,F}$ = 162.4 Hz, C-6), 79.6 (C-2´), 79.1 (C-3´), 77.6 (C-2/C-3), 77.2 (C-4), 75.3 (CH_{Bn}), 74.7 (CH_{Bn}), 74.5 (C-2/C-3), 73.5 (CH_{Bn}), 73.0 (CH_{Bn}), 69.0 (C-6´), 68.9 − 68.3 (2C, C-5, CH_{Linker}), 67.3 (CH_{Cbz}), 63.3 (C-5´), (50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.5 (CH_{Linker}). Due to signal overlap 46 out of 67 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 235.1 - −235.6 (m).

^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 170 Hz, $J_{C1´H1´}$ = 171 Hz.

HRMS (ESI^+): Calculated for $C_{67}H_{73}FNO_{12}^+$ $[M+H]^+$: 1102.5112; found: 1102.5139.

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 18.13 min, λ = 230 nm.

Regioselective benzylidene opening:

Regioselective benzylidene opening was conducted according to a modified procedure from *Danieli et al.*[360]

A magnetically stirred solution of the thus obtained glycosylation product **22** (480 mg) in dry $MeCN/CH_2Cl_2$ (v/v = 3:1, 40 ml) was cooled to – 10 °C before $BH_3{\cdot}NMe_3$ (238 mg, 3.26 mmol, 7.4 eq.) and $BF_3{\cdot}OEt_2$ (413 µl, 3.26 mmol, 7.4 eq.) were added. The reaction was stirred for 1.5 h at – 10 °C, before another portion of $BH_3{\cdot}NMe_3$ (32 mg, 0.44 mmol, 1.0 eq.) and $BF_3{\cdot}OEt_2$ (62 µl, 0.44 mmol, 1.0 eq.) was added. After TLC monitoring indicated complete conversion of the starting material, the reaction was stopped by addition of a methanolic NEt_3 solution (33 % vol.) and warmed to ambient temperature (over 0.5 h). Subsequently, organic solvents were removed under reduced pressure and the crude residue was directly subjected to column chromatography (^{c}Hex/EtOAc v/v = 7:1) affording **20** (375 mg, 0.34 mmol, 62 % over two steps) as a colorless oil.

$\mathbf{R}_f$ = 0.39 (^{c}Hex/EtOAc v/v = 3:1)

Optical rotation: $[\alpha]_D^{22}$ = + 51.8 ° (c = 1.1; $CHCl_3$)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.44 – 7.13 (m, 35H, Ar-H), 5.18 (d, $J_{CH,CH}$ = 17.1 Hz, 2H, CH_{Cbz}), 4.96 (d, $J_{CH,CH}$ = 11.4 Hz, 1H, CH_{Bn}), 4.91 (d, $J_{H1´,H2´}$ = 3.4 Hz, 1H, H-1´), 4.86 – 4.64 (m, 9H, H-1, H-6a, 7 × CH_{Bn}), 4.50 (d, $J_{CH,CH}$ = 16.0 Hz, 2H, NCH_{Bn}), 4.47 – 4.34 (m, 2H, H-6b, CH_{Bn}), 4.21 (d, $J_{CH,CH}$ = 12.2 Hz, 1H, CH_{Bn}), 4.10 (dt, $J_{H5´,H4´}$ = 9.6 Hz, $J_{H5´,H6a´}$ = $J_{H5´,H6b´}$ = 3.6 Hz, 1H, H-5´), 4.08 – 4.04 (m, 1H, H-4), 4.00 – 3.93 (m, 1H, H-5), 3.92 – 3.84 (m, 2H, H-2, H-3), 3.79 (t, $J_{H3´,H2´}$ = $J_{H3´,H4´}$ = 9.1 Hz, 1H, H-3´), 3.75 (td, $J_{H4´,H3´}$ = $J_{H4´,H5´}$ = 9.2 Hz, $J_{H4´,OH}$ = 2.5 Hz, 1H, H-4´), 3.63 – 3.55 (m, 1H, CH_{Linker}), 3.53 (dd, $J_{H2´,H3´}$ = 9.4 Hz, $J_{H2´,H1´}$ =3.4 Hz, 1H, H-2´), 3.46 – 3.37 (m, 1H, CH_{Linker}), 3.36 (dd, $J_{H6a´,H6b´}$ = 10.3 Hz, $J_{H6a´,H5´}$ = 3.0 Hz, 1H, H-6a´), 3.29 – 3.17 (m, 2H, 2 × CH_{Linker}), 3.14 (dd, $J_{H6b´,H6a´}$ = 10.3 Hz, $J_{H6b´,H5´}$ = 4.0 Hz, 1H, H-6b´), 2.46 (d, $J_{OH,H4´}$ = 2.6 Hz, 1H, OH), 1.66 – 1.47 (m, 4H, 4 × CH_{Linker}), 1.39 – 1.20 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 156.9/156.3 (C=O-Cbz), 139.1, 138.8, 138.5, 138.2, 138.0 (2C), 137.0/ 136.9 (7 × Cq), 128.7, 128.6 (2C), 128.5, 128.4 (2C), 128.3, 128.2, 128.0 (3C), 127.9 (2C), 127.8, 127.7 (2C), 127.6 (2C), 127.5, 127.4, 127.3 (21 × C-Ar), 100.1 (C-1´), 97.8 (C-1), 81.6 (C-3´), 80.5 (C-6, d, $J_{C6,F}$ = 166 Hz), 80.1 (C-2´), 77.5 (C-2/C-3), 76.7 (d, $J_{C4,F}$ = 2.9 Hz, C-4), 75.4 (2C, C-2/C-3, CH_{Bn}), 74.1 (CH_{Bn}), 73.5 (2C, 2 × CH_{Bn}), 72.9 (CH_{Bn}), 71.7 (C-4´), 70.5 (C-5´), 69.2 (C-6´), 68.9 (d, $J_{C5,F}$ = 25 Hz, C-5), 68.3 (CH_{Linker}), 67.3 (CH_{Cbz}), 50.6/50.3 (NCH_{Bn}), 47.3/46.2 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.5 (CH_{Linker}). Due to signal overlap 53 out of 67 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 230.0 - − 231.4 (m).

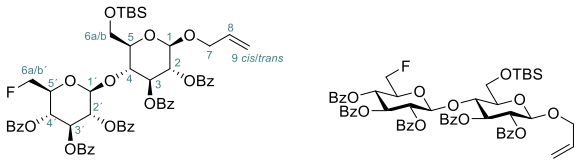
^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 174 Hz, $J_{C1´H1´}$ = 170 Hz.

HRMS (ESI^+): Calculated for $C_{67}H_{74}O_{12}NFNa^+[M+Na]^+$: 1126.5087; found: 1126.5118.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 16.73, λ = 230 nm.

Synthesis of cellobiose donor building blocks 25, 26 and 27

Allyl (2,3,4-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (**61**)



Glucosyl donor **31** (450 mg, 0.70 mmol, 1.6 eq.) and acceptor **28** (239 mmol, 0.44 mmol, 1.0 eq.) were combined, co-evaporated with toluene (12 ml) and dried under high vacuum for 1 h. Subsequently, starting materials were dissolved in dry CH_2Cl_2 (12 ml) and stirred over freshly activated 4 Å molecular sieve. The reaction mixture was cooled to 0 °C and TMSOTf (7.20 µl, 0.04 mmol, 0.1 eq.) was added. The reaction was stirred for 1 h at 0 °C, before another portion of TMSOTf (7.20 µl, 0.04 mmol, 0.1 eq.) was added. The reaction was slowly warmed to ambient temperature and stirred for another 3.5 h at that temperature, before being neutralized by addition of NEt_3. The reaction mixture was diluted with CH_2Cl_2 and filtered through a pad of *Hyflo*®. The filtrate was washed with 1 M HCl (15 ml) and brine (10 ml) and dried with $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 6:1) affording **61** (330 mg, 0.32 mmol, 74 %) as an amorphous and colorless solid.

R_f = 0.43 (^{c}Hex/EtOAc v/v = 3:1)

Optical rotation: $[\alpha]_D^{22}$ = − 49.1 ° (c = 0.5; $CHCl_3$)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.10 – 8.06 (m, 2H, Ar-H), 7.98 – 7.93 (m, 4H, Ar-H), 7.89 – 7.84 (m, 2H, Ar-H), 7.79 – 7.73 (m, 2H, Ar-H), 7.57 – 7.45 (m, 4H, Ar-H), 7.43 – 7.31 (m, 9H, Ar-H), 7.25 (t, $J_{CH,CH}$ = 7.7 Hz, 2H, Ar-H), 5.80 – 5.65 (m, 3H, H-8, H-3, H-3´), 5.44 (dd, $J_{H2´,H3´}$ = 9.8 Hz, $J_{H2´,H1´}$ =8.0 Hz, 1H, H-2´), 5.37 (dd, $J_{H2,H3}$ = 9.8 Hz, $J_{H2,H1}$ = 7.9 Hz, 1H, H-2), 5.22 (t, $J_{H4´,H3´}$ = $J_{H4´,H5´}$ = 9.7 Hz, 1H, H-4´), 5.20 – 5.13 (m, 1H, H-9_{trans}), 5.10 – 5.04 (m, 2H, H-1´, H-9_{cis}), 4.65 (d, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1), 4.31 – 3.72 (m, 8H, H-7a, H-7b, H-6a´, H-6b´, H-6a, H-6b, H-4, H-5´), 3.37 (ddd, $J_{H5,\ H4}$ = 9.6 Hz, $J_{H5,\ H6a/b}$ = 2.9 Hz, $J_{H5,\ H6a/b}$ = 1.6 Hz, 1H, H-5), 1.02 (s, 9H, Si-^{t}Bu), 0.16 (s, 6H, 2 × Si-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 165.8, 165.7, 165.4, 165.2, 164.6 (5 × C=O), 133.7 (2C, C-Ar, C-8), 133.5, 133.4, 133.2, 133.1 (2C), 130.0, 129.9 (2C), 129.9, 129.8, 129.6, 129.1, 128.7, 128.6 (2C), 128.5, 128.4, 128.3 (19 × C-Ar) , 117.4 (C-9), 100.5 (C-1´), 99.6 (C-1), 81.0 (d, $J_{C6´,F}$ = 175.7 Hz, C-6´), 75.3 (C-5), 75.2 (C-4),

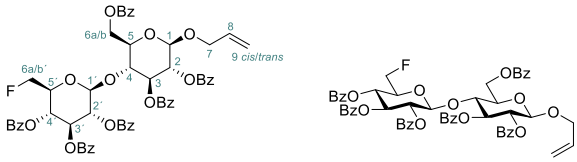
73.2 (2C, C-5´ (d, $J_{C5´,F}$ = 20.7 Hz), C-3´), 73.1 (C-3), 72.1 (C-2), 72.0 (C-2´), 69.5 (C-7), 68.8 (d, $J_{C4´,F}$ = 6.9 Hz, C-4´), 61.0 (C-6), 26.1 (3C, Si-^{t}Bu), 18.5 (C_q, Si-^{t}Bu), − 4.8 (Si-CH_3), − 5.1 (Si-CH_3). Due to signal overlap 45 out of 56 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 228.6 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 46.4 Hz, $J_{F,H5}$ = 19.0 Hz).

HRMS (ESI$^+$): Calculated for $C_{56}H_{63}O_{15}FNSi^+$ $[M+NH_4]^+$: 1036.3946; found: 1036.3968.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 16.61 min, λ = 230 nm.

Allyl (2,3,4-tri-*O*-benzoyl 6-deoxy-6-fluoro-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**62**)



Compound **62** was synthesized in accordance to literature known protocols.[367, 373, 378]

To a magnetically stirred solution of disaccharide **61** (480 mg, 0.47 mmol, 1.0 eq.) in MeOH/CH_2Cl_2 (30 ml, v/v = 1:1), *p*-TsOH (90.0 mg, 0.47 mmol, 1.0 eq.) were added. The reaction was stirred at 50 °C until complete conversion of the starting material was observed by TLC monitoring. The reaction was subsequently neutralized by addition of NEt_3 (200 µl) and concentrated to dryness under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (30 ml), washed with 1 M HCl (15 ml), sat. aq. $NaHCO_3$ (15 ml) and brine (15 ml) and dried with $MgSO_4$. The crude residue was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 2:1) and fractions containing the primary alcohol were combined and used in the subsequent reaction. The thus obtained alcohol was dissolved in dry pyridine (15 ml) and Benzoyl chloride (128 µl, 1.06 mmol, 3.0 eq.) was added dropwise. The reaction was stirred at 50 °C until TCL monitoring indicated complete conversion of the alcohol. Subsequently, DMAPA (140 µl, 1.12 mmol, 2.4 eq.) was added and the reaction was stirred for 20 min at ambient temperature. Organic solvents were removed under reduced pressure and the crude residue was dissolved in CH_2Cl_2 (50 ml) and washed with 1 M HCl (25 ml) and brine (25 ml) and dried over $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **62** (330 mg, 0.33 mmol, 70 % over two steps) as a colorless foam.

R_f = 0.40 (^{c}Hex/EtOAc v/v = 2:1)

Optical rotation: $[\alpha]_D^{22}$ = + 28.3 ° (c = 0.66; $CHCl_3$)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.10 – 7.99 (m, 4H, Ar-H), 7.93 (ddd, $J_{CH,CH}$ = 8.0 Hz, $J_{CH,CH}$ = 6.4 Hz, $J_{CH,CH}$ = 1.4 Hz, 4H, Ar-H), 7.86 – 7.78 (m, 2H, H-Ar), 7.77 – 7.70 (m, 2H, H-Ar), 7.67 – 7.57 (m, 1H, H-Ar), 7.56 – 7.44 (m, 5H, H-Ar), 7.44 – 7.20 (m, 12H, H-Ar), 5.80 – 5.65 (m, 3H, H-8, H-3´, H-3), 5.49 – 5.39 (m, 2H, H-2, H-2´), 5.20 (t, $J_{H4´,H3´}$ = $J_{H4´,H5´}$ = 9.6 Hz, 1H, H-4´), 5.19 – 5.10 (m, 1H, H-9_{trans}), 5.06 (dq, $J_{H9cis,H8}$ = 10.4 Hz, $J_{H9cis,}H_{9trans}$ = $J_{H9cis,H7}$ = 1.4 Hz, 1H, H-9_{cis}), 4.90 (d, $J_{H1´,H2´}$ = 7.9 Hz, 1H, H-1´), 4.73 (d, $J_{H1,H2}$ = 7.8 Hz, 1H, H-

1), 4.60 (dd, $J_{H6a, H6b}$ = 12.1 Hz, $J_{H6a, H5}$ = 1.9 Hz, 1H, H-6a), 4.44 (dd, $J_{H6b, H6a}$ = 12.1 Hz, $J_{H6b, H5}$ = 4.6 Hz 1H, H-6b), 4.33 – 4.19 (m, 2H, H-4, H-7a), 4.10 – 3.61 (m, 5H, H-5, H-5´, H6a´, H-6b´, H-7b).

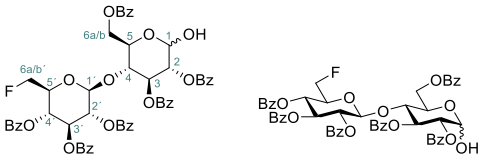
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 166.0, 165.7, 165.6, 165.4, 165.2, 164.8 (6 × C=O), 133.7 (2C, C-Ar, C-8), 133.5, 133.4 (2C), 133.3 (2C), 130.1, 130.0, 129.9 (4C), 129.8, 129.5, 128.8, 128.7 (3C), 128.6 (2C), 128.5 (2C), 128.4 (23 × C-Ar), 117.8 (C-9), 100.9 (C-1´), 99.7 (C-1), 80.9 (d, $J_{C6´,F}$ = 175.8 Hz, C-6´), 76.5 (C-4), 73.5 (d, $J_{C5´,F}$ = 20.3 Hz, C-5´), 73.1 (2C, C-3/C-3´, C-5), 72.9 (C-3/C-3´), 72.0 (2C, C-2, C-2´), 70.1 (C-7), 68.7 (d, $J_{C4´,F}$ = 7.0 Hz, C-4´), 62.6 (C-6). Due to signal overlap 44 out of 57 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 228.7 (td, $J_{F,H6a´} = J_{F,H6b´}$ = 46.6 Hz, $J_{F,H5´}$ = 19.2 Hz).

HRMS (ESI^+): Calculated for $C_{57}H_{57}O_{16}FN^+$ $[M+NH_4]^+$: 1026.3343; found: 1026.3354.

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 13.95 min, λ = 230 nm.

2,3,4-Tri-*O*-benzoyl 6-deoxy-6-fluoro-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α/β-D-glucopyranoside **(63)**



Compound **63** was synthesized in accordance to literature known protocols.[370]

To a magnetically stirred solution of fluorinated disaccharide **62** (330 mg, 0.33 mmol, 1.0 eq.) in MeOH/THF (25 ml, v/v = 4:1), palladium(II) chloride (59.0 mg, 0.33 mmol, 1.0 eq.) was added. The reaction was stirred for 3 h at 60 °C, before organic solvents were removed under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (50 ml) and washed with H_2O (25 ml) and brine (20 ml) and dried with $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 5:2) affording **63** (305 mg, 320 µmol, 97 %, α/β ~ 3:1) as anomeric mixture.

R_f = 0.11 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3).

α-Anomer:

^{1}H-NMR (800 MHz, $CDCl_3$): δ [ppm] = 8.11 – 8.08 (m, 2H, Ar-H), 8.03 – 8.00 (m, 2H, Ar-H), 7.99 – 7.97 (m, 2H, Ar-H), 7.94 – 7.90 (m, 2H, Ar-H), 7.84 – 7.80 (m, 2H, Ar-H), 7.76 – 7.71 (m, 2H, Ar-H), 7.54 – 7.21 (m, 18H, Ar-H), 6.11 (dd, $J_{H3,H4}$ = 10.2 Hz, $J_{H3,H2}$ = 9.1 Hz, 1H, H-3), 5.74 (t, $J_{H3´,H4´} = J_{H3´,H2´}$ = 9.7 Hz, 2H, H-3´), 5.60 (d, $J_{H1,H2}$ = 3.7 Hz, 1H, H-1), 5.47 (dd, $J_{H2´,H3´}$ = 9.7 Hz, $J_{H2´,H1´}$ = 7.9 Hz, 1H, H-2´), 5.26 – 5.18 (m, 2H, H-2, H-4´), 4.96 (d, $J_{H1´,H2´}$ = 7.8 Hz, 1H, H-1´), 4.59 (dd, $J_{H6a,H6b}$ = 12.1 Hz, $J_{H6a,H5}$ = 1.9 Hz, 1H, H-6a), 4.44 (dd, $J_{H6b,H6a}$ = 12.1 Hz, $J_{H6b,H5}$ = 3.8 Hz, 1H, H-6b), 4.35 – 4.31 (m, 1H, H-5), 4.24 (t, $J_{H4,H3} = J_{H4,H5}$ = 9.6 Hz, 1H, H-

4), 4.12 – 4.04 (m, 1H, H-6a´), 3.87 (ddd, $J_{H6b´,F}$ = 47.2 Hz, $J_{H6b´,H6a´}$ = 10.3 Hz, $J_{H6b´,H5´}$ = 5.8 Hz, 1H, H-6b´), 3.74 – 3.68 (m, 1H, H-5´), 3.30 (s, 1H, OH).

^{13}C-NMR (200 MHz, $CDCl_3$): δ [ppm] = 166.1, 166.0, 165.8, 165.5, 165.2, 164.8 (6 × C=O), 133.7, 133.5 (3C), 133.4, 133.2, 130.1, 130.0, 129.9 (2C), 129.8 (2C), 129.1, 128.7 (2C), 128.6 (3C), 128.4 (2C, 20 × C-Ar), 100.8 (C-1´), 90.4 (C-1), 81.0 (d, $J_{C6´,F}$ = 175.5 Hz, C-6´), 76.3 (C-4) , 73.5 (d, $J_{C5´,F}$ = 20.2 Hz, C-5´), 72.9 (C-3´), 72.3 (C-2), 72.0 (C-2´), 70.1 (C-3), 68.7 (d, $J_{C4´,F}$ = 7.0 Hz, C-4´), 68.6 (C-5), 62.4 (C-6). Due to signal overlap 38 out of 54 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 228.6 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 46.7 Hz, $J_{F,H5}$ = 18.9 Hz).

HRMS (ESI^+): Calculated for $C_{54}H_{49}O_{16}NF$ $[M+NH_4]^+$: 986.3030; found: 986.3039.

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 12.91 min, λ = 230 nm.

β-Anomer:

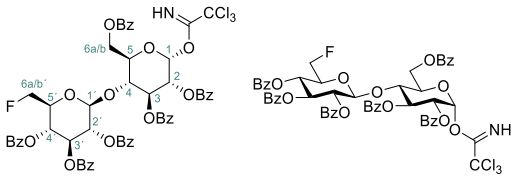
^{1}H-NMR (800 MHz, anomeric signals, $CDCl_3$): δ [ppm] = 4.93 – 4.89 (m, 2H, H-1, H-1´).

^{13}C-NMR (200 MHz, anomeric signals, $CDCl_3$): δ [ppm] = 100.8 (C-1´), 95.9 (C-1).

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 228.3 - – 228.5 (m).

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 12.66 min, λ = 230 nm.

2,3,4-Tri-*O*-benzoyl 6-deoxy-6-fluoro-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**26**)



Compound **26** was synthesized according to a slightly modified synthetic protocol.[379]

To a magnetically stirred solution of **63** (220 mg, 0.23 mmol, 1.0 eq.) in dry CH_2Cl_2 (24 ml), trichloroacetonitrile (231 µl, 2.30 mmol, 10.0 eq.) was added. The reaction was cooled to 0 °C, before DBU (35.0 µl, 0.23 mmol, 1.0 eq.) was added in one portion. The reaction as stirred for 10 min at 0 °C before being slowly warmed to ambient temperature. The reaction was stirred for 17 h at that temperature and concentrated under reduced pressure. The crude residue was dissolved CH_2Cl_2 (500 µl) and subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3) affording **26** (220 mg, 0.20 mmol, 87 %) as a slightly yellow oil.

R_f = 0.27 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3).

Optical rotation: $[\alpha]_D^{23}$ = + 48.7 ° (c = 1.2; $CHCl_3$)

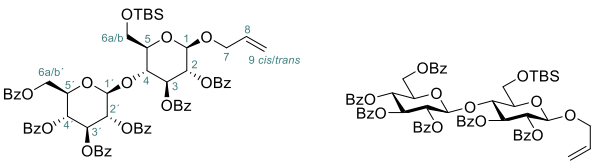
^{1}H-NMR (400 MHz, CD_2Cl_2): δ [ppm] = 8.61 (s, 1H, N=H), 8.12 – 8.07 (m, 2H, Ar-H), 8.02 – 7.97 (m, 2H, Ar-H), 7.95 – 7.88 (m, 4H, Ar-H), 7.85 – 7.80 (m, 2H, Ar-H), 7.75 – 7.70 (m, 2H, Ar-H), 7.67 – 7.60 (m, 1H, Ar-H), 7.60 – 7.32 (m, 13H, Ar-H), 7.32 – 7.23 (m, 4H, Ar-H), 6.66 (d, $J_{H1,H2}$ = 3.8 Hz, 1H, H-1), 6.09 (dd, $J_{H3,H2}$ =10.2 Hz, $J_{H3,H4}$ = 9.0 Hz, 1H, H-3), 5.76 (t, $J_{H3´,H2´} = J_{H3´,H4´}$ = 9.6 Hz, 1H, H-3´), 5.49 (dd, $J_{H2,H3}$ = 10.2 Hz, $J_{H2,H1}$ = 3.8 Hz, 1H, H-2), 5.43 (dd, $J_{H2´,H3´}$ = 9.8 Hz, $J_{H2´,H1´}$ = 7.9 Hz, 1H, H-2´), 5.28 (t, $J_{H4´,H3´} = J_{H4´,H5´}$ = 9.8 Hz, 1H, H-4´), 5.03 (d, $J_{H1´,H2´}$ = 7.9 Hz, 1H, H-1´), 4.63 (dd, $J_{H6a,H6b}$ = 12.3 Hz, $J_{H6a,H5}$ = 1.9 Hz, 1H, H-6a), 4.44 (dd, $J_{H6b,H6a}$ = 12.3 Hz, $J_{H6b,H5}$ = 4.0 Hz, 1H, H-6b), 4.37 (dd, $J_{H4,H5}$ = 10.2 Hz, $J_{H4,H3}$ = 9.0 Hz, 1H, H-4), 4.33 – 4.27 (m, 1H, H-5), 4.09 (ddd, $J_{H6a´,F}$ = 46.8 Hz, $J_{H6a´,H6b´}$ = 10.5 Hz, $J_{H6a´,H5´}$ = 2.3 Hz, 1H, H-6a´), 3.91 (ddd, $J_{H6b´,F}$ = 47.1 Hz, $J_{H6b´,H6a´}$ = 10.5 Hz, $J_{H6b´,H5´}$ = 5.1 Hz, 1H, H-6b´), 3.75 (dddd, $J_{H5´,F}$ = 20.7 Hz, $J_{H5´,H4´}$ = 9.9 Hz, $J_{H5´,H6b´}$ = 5.1 Hz, $J_{H5´,H6a´}$ = 2.2 Hz, 1H, H-5´).

^{13}C-NMR (200 MHz, CD_2Cl_2): δ [ppm] = 166.1, 166.0, 165.9, 165.8, 165.5, 165.4 (6 × C=O), 161.1 (C=NH), 134.1 (2C), 134.0, 133.9, 130.4, 130.3, 130.2 (2C), 130.1, 129.3, 129.2 (2C), 129.1, 129.0 (2C), 128.9 (16 × C_{Ar}), 101.4 (C-1´), 93.5 (C-1), 91.2 (CCl_3), 81.3 (d, $J_{C6´,F}$ = 175.7 Hz, C-6´), 76.4 (C-4), 73.8 (d, $J_{C5´,F}$ = 19.9 Hz, C-5´), 73.4 (C-3´), 72.5 (C-2´), 71.9 (C-5), 71.2 (C-2), 70.8 (C-3), 68.7 ($J_{C4´,F}$ = 6.9 Hz, C-4´), 62.3 (C-6). Due to signal overlap 36 out of 56 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, CD_2Cl_2): δ [ppm] = − 230.4 (td, $J_{F,H6a´} = J_{F,H6b´}$ = 46.9 Hz, $J_{F,H5´}$ = 20.9 Hz).

HRMS (ESI$^+$): Calculated for $C_{56}H_{49}Cl_3FN_2O_{16}^+$ $[M+NH_4]^+$: 1129.2127; found: 1129.2144.

Allyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (**64**)



Donor **32** (1.39 g, 1.88 mmol, 1.7 eq.) and the acceptor **28** (600 mg, 1.10 mmol, 1.0 eq.) were combined, co-evaporated with toluene (2 × 10 ml) and dried under high vacuum for 2 h. Starting materials were dissolved in dry CH_2Cl_2 (15 ml) and stirred for 1 h with freshly activated 4 Å molecular sieve. Subsequently, TMSOTf (18.0 µl, 0.11 mmol, 0.1 eq.) was added at 0°C and the reaction was allowed to slowly warm to room temperature. After complete conversion of the trichloroacetimidate was indicated by TLC monitoring, the reaction was stopped by addition of NEt_3 (1 ml), diluted with CH_2Cl_2 and filtered through a pad of *Hyflo*®. The organic layer was washed with 1 M HCl (20 ml), sat. aq. $NaHCO_3$ (20 ml) and brine (15 ml) and dried with $MgSO_4$. The crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 6:1) affording **64** (1.18 g, 1.05 mmol, 95 %) as a colorless oil.

R_f = 0.39 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3)

Optical rotation: $[\alpha]_D^{24}$ = − 45.2 ° (c = 0.5, $CHCl_3$)

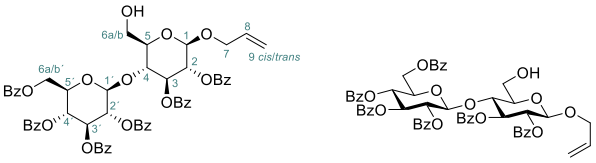
^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 8.01 – 7.97(m, 4H, Ar-H), 7.98 – 7.94 (m, 2H, Ar-H), 7.94 – 7.90 (m, 2H, Ar-H), 7.84 – 7.81 (m, 2H, Ar-H), 7.77 – 7.75 (m, 2H, Ar-H), 7.61 – 7.56 (m, 1H, Ar-H), 7.56 – 7.51 (m, 1H, Ar-H), 7.51 – 7.44 (m, 4H, Ar-H), 7.43 – 7.37 (m, 3H, Ar-H), 7.37 – 7.33 (m, 3H, Ar-H), 7.32 – 7.28 (m, 2H, Ar-H), 7.27 – 7.23 (m, 2H, Ar-H), 7.21 – 7.18 (m, 2H, Ar-H), 5.78 – 5.67 (m, 3H, H-3, H-3´,H-8), 5.47 (dd, $J_{H2´,H3´}$ = 9.7 Hz, $J_{H2´,H1´}$ = 8.0 Hz, 1H, H-2´), 5.35 (dd, $J_{H2,H3}$ = 9.8 Hz, $J_{H2,H1}$ = 7.9 Hz, 1H, H-2), 5.30 (t, $J_{H4´,H3´}$ = $J_{H4´,H5´}$ = 9.7 Hz, 1H, H-4´), 5.16 (dq, $J_{H9trans,H8}$ = 17.3 Hz, $J_{H9trans,H9cis}$ = $J_{H9trans,H7}$ = 1.7 Hz, 1H, H-9_{trans}), 5.11 (d, $J_{H1´,H2´}$ = 8.0 Hz, 1H, H-1´), 5.07 (dq, $J_{H9cis,H8}$ = 10.5 Hz, $J_{H9cis,H9trans}$ = $J_{H9cis,H7}$ = 1.4 Hz, 1H, H-9_{cis}), 4.64 (d, $J_{H1,H2}$ = 8.0 Hz, 1H, H-1), 4.27 – 4.22 (m, 2H, H-4, H-7a), 4.20 (dd, $J_{H6a´, H6b´}$ = 11.9 Hz, $J_{H6a´, H5´}$ = 2.8 Hz, 1H, H-6a´), 4.04 (ddt, $J_{H7b,H7a}$ = 13.4 Hz, $J_{H7b,H8}$ = 6.1 Hz, $J_{H7b,H9cis}$ = $J_{H7b,H9trans}$ = 1.4 Hz, 1H, H-7b), 3.90 (ddd, $J_{H5´, H4´}$ = 9.8 Hz, $J_{H5´, H6b´}$ = 6.8 Hz, $J_{H5´, H6a´}$ =2.8 Hz, 1H, H-5´), 3.84 (dd, $J_{H6a,H6b}$ = 11.9 Hz, $J_{H6a,H5}$ = 2.8 Hz, 1H, H-6a), 3.75 (dd, $J_{H6b,H6a}$ = 11.9 Hz, $J_{H6b,H5}$ = 1.5 Hz, 1H, H-6b), 3.61 (dd, $J_{H6b´,H6a´}$ = 12.0 Hz, $J_{H6b´,H5´}$ = 6.8 Hz, 1H, H-6b´), 3.36 (ddd, $J_{H5,H4}$ = 9.6 Hz, $J_{H5,H6a}$ = 2.8 Hz, $J_{H5,H6b}$ = 1.5 Hz, 1H, H-5), 0.94 (s, 9H, Si-^{t}Bu), 0.13 (s, 3H, Si-CH_3), 0.09 (s, 3H, Si-CH_3). [ppm]

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 165.9 (2C), 165.8, 165.4, 165.3, 164.7 (6 × C=O), 133.8 (C-Ar), 133.5 (2C, C-8, C-Ar), 133.4, 133.3, 133.2 133.1, 130.0 (2C), 129.9 (3C), 129.8 (2C), 129.7 (2C), 129.2, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4 (2C), 128.3 (22 × C-Ar), 117.4 (C-9), 100.6 (C-1´), 99.7 (C-1), 75.4 (C-5), 75.1 (C-4), 73.4 (C3/C-3´), 73.1 (C3/C-3´), 72.6 (C-5´), 72.2 (2C, C-2, C-2´), 69.7 (C-4´), 69.6 (C-7), 63.1 (C-6´), 60.9 (C-6), 26.0 (3C, Si-^{t}Bu), 18.4 (C_q, Si-^{t}Bu), − 4.9 (Si-CH_3), − 5.1 (Si-CH_3). Due to signal overlap 51 out of 63 carbon atoms were assigned in the ^{13}C spectrum

HRMS (ESI$^+$): Calculated for $C_{63}H_{68}O_{17}NSi^+$ $[M+NH_4]^+$: 1138.4251; found: 1138.4263.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 17.91 min, λ = 230 nm

Allyl (2,3,4,6-tetra-*O*-benzoyl- β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-β-D-glucopyranoside (**65**)



Compound **65** was synthesized according a literature known protocol.[380]

Cellobiose derivative **64** (1.00 g, 0.89 mmol, 1.0 eq.) was dissolved in acetonitrile (60 ml) and 80 % aqueous acetic acid (60 ml) and stirred for 20 h at 60 °C. After complete conversion of the starting material was observed by TLC monitoring, solvents were removed under reduced pressure. The crude product was co-evaporated with toluene (2× 50 ml) and subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 4:1 → 1:1) affording **65** (806 mg, 0.80 mmol, 90 %) as a colorless oil.

R_f = 0.12 (^{c}Hex/EtOAc v/v = 2:1)

Optical rotation: $[\alpha]_D^{22}$ = − 8.1 ° (c = 1.1, $CHCl_3$)

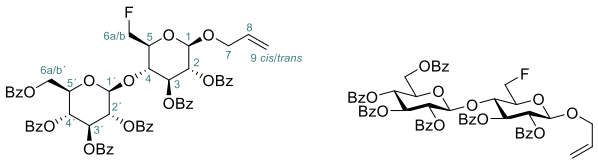
^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.96 (ddd, $J_{CH,CH}$ = 10.8 Hz, $J_{CH,CH}$ = 8.3 Hz, $J_{CH,CH}$ = 1.4 Hz, 4H, Ar-H), 7.91 (dt, $J_{CH,CH}$ = 8.4, $J_{CH,CH}$ = 1.3 Hz, 4H, Ar-H), 7.76 (ddd, $J_{CH,CH}$ = 16.5, $J_{CH,CH}$ = 8.3 Hz, $J_{CH,CH}$ = 1.4 Hz, 4H, Ar-H), 7.57 – 7.51 (m, 2H, Ar-H), 7.51 – 7.46 (m, 1H, Ar-H), 7.46 – 7.31 (m, 9H, Ar-H), 7.30 – 7.26 (m, 2H, Ar-H), 7.26 – 7.20 (m, 2H, Ar-H), 7.20 – 7.10 (m, 2H, Ar-H), 5.81 (t, $J_{H3',H2'}$ = $J_{H3',H4'}$ = 9.7 Hz, 1H, H-3´), 5.76 – 5.68 (m, 2H, H-3, H-8), 5.50 (dd, $J_{H2',H3'}$ = 9.9 Hz, $J_{H2',H1'}$ = 8.0 Hz, 1H, H-2´), 5.43 – 5.36 (m, 2H, H-2, H-4´), 5.18 (dq, $J_{H9trans,H8}$ = 17.3 Hz, $J_{H9trans,H9cis}$ = $J_{H9trans,H7}$ = 1.7 Hz, 1H, H-9_{trans}), 5.09 (dq, $J_{H9cis,H8}$ = 10.4 Hz, $J_{H9cis,H9trans}$ = $J_{H9cis,H7}$ = 1.4 Hz, 1H, H-9_{cis}), 5.02 (d, $J_{H1',H2'}$ =8.0 Hz, 1H, H-1´), 4.70 (d, $J_{H1,H2}$ = 8.0 Hz, 1H, H-1), 4.30 – 4.22 (m, 2H, H-4, H-7a), 4.07 – 4.01 (m, 2H, H-6a´, H-7b), 3.98 (ddd, $J_{H5',H4'}$ = 9.9 Hz, $J_{H5',H6b'}$ = 5.3 Hz, $J_{H5',H6a'}$ = 3.2 Hz, 1H, H-5´), 3.83 (dd, $J_{H6b',H6a'}$ = 11.9 Hz, $J_{H6b',H5'}$ = 5.3 Hz, 1H, H-6b´), 3.80 – 3.77 (m, 2H, H-6a, H-6b), 3.45 (dt, $J_{H5,H4}$ = 9.8 Hz, $J_{H5,H6a}$ = $J_{H5,H6b}$ = 2.4 Hz, 1H, H-5).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 165.9, 165.8, 165.6, 165.3, 165.1, 164.8 (6 × C=O), 133.5 (2C, 2 × C-Ar), 133.4 (C-8), 133.3, 133.2 (2C), 129.9 (4C), 129.8, 129.7 (2C), 129.6, 129.5, 129.1, 128.9, 128.8, 128.7, 128.5 (3C), 128.4, 128.3 (21 × C-Ar), 117.8 (C-9), 101.1 (C-1´), 100.0 (C-1), 75.4 (C-4), 75.2 (C-5), 73.1 (C-3´), 72.9 (C-3), 72.3 (C-5´), 72.1 (2C, C-2, C-2´), 70.4 (C-7), 69.6 (C-4´), 62.9 (C-6´), 60.4 (C-6). Due to signal overlap 44 out of 57 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI^+): Calculated for $C_{57}H_{54}O_{17}N^+$ $[M+NH_4]^+$: 1024.3386; found: 1024.3405.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 13.40 min, λ = 230 nm

Allyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-deoxy-6-fluoro-β-D-gluco-pyranoside (**66**)



Fluorination was conducted in accordance to a previously described synthetic procedure.[324, 325]

To a magnetically stirred solution of **65** (620 mg, 617 µmol, 1.0 eq.) in dry CH_2Cl_2 (6 ml), 2,4,6-collidine (206 µl, 1.55 mmol, 2.5 eq.) and DAST (204 µl, 1.54 mmol, 2.5 eq.) were added slowly. The reaction solution was heated in a microwave oven (100 W, 80 °C) for 1 h. After complete conversion of the starting material was observed by TLC monitoring, the reaction was poured into MeOH (25 ml) and stirred for 0.5 h at ambient temperature. The resulting mixture was concentrated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 ml) and washed with sat. aq. $NaHCO_3$ (15 ml), 1 M HCl (15 ml) and brine (10 ml) and dried with $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **66** (580 mg, 575 µmol, 93 %) as a colorless foam.

R_f = 0.31 (^{c}Hex/EtOAc v/v = 3:1)

Optical rotation: $[\alpha]_D^{22}$ = − 45.3 ° (c = 0.5; $CHCl_3$)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.01 – 7.85 (m, 8H, Ar-H), 7.75 (td, $J_{CH,CH}$ = 8.6 Hz, $J_{CH,CH}$ = 1.4 Hz, 4H, Ar-H), 7.59 – 7.13 (m, 18H, Ar-H), 5.82 – 5.64 (m, 3H, H-3, H-3´, H-8), 5.51 (dd, $J_{H2´,H3´}$ = 9.8 Hz, $J_{H2´,H1´}$ = 7.9 Hz, 1H, H-2´), 5.44 – 5.36 (m, 2H, H-2, H-4´), 5.16 (dt, $J_{H9trans,H8}$ = 17.3 Hz, $J_{H9trans,H7}$ = 1.7 Hz, 1H, H-9_{trans}), 5.07 (dq, $J_{H9cis,H8}$ = 10.5 Hz, $J_{H9cis,H7}$ = 1.5 Hz, 1H, H-9_{cis}), 4.94 (d, $J_{H1´,H2´}$ = 8.0 Hz, 1H, H-1´), 4.67 (d, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1), 4.65 – 4.45 (m, 2H, H-6a, H-6b), 4.28 (ddt, $J_{H7a,H7b}$ = 13.3 Hz, $J_{H7a,H8}$ = 4.8 Hz, $J_{H7a,H9trans}$ = $J_{H7a,H9cis}$ = 1.6 Hz, 1H, H-7a), 4.20 (t, $J_{H4,H5}$ = $J_{H4,H3}$ = 9.6 Hz, 1H, H-4), 4.08 – 3.96 (m, 2H, H-7b, H-6a´), 3.92 (ddd, $J_{H5´,H4´}$ = 9.9 Hz, $J_{H5´,H6b´}$ = 5.1, $J_{H5´,H6a´}$ = 3.1 Hz, 1H, H-5´), 3.77 (dd, $J_{H6b´,H6a´}$ = 11.9 Hz, $J_{H6b´,H5´}$ = 5.1 Hz, 1H, H-6b´), 3.55 (dd, $J_{H5,F}$ = 29.3 Hz, $J_{H5,H4}$ = 9.8 Hz, 1H, H-5).

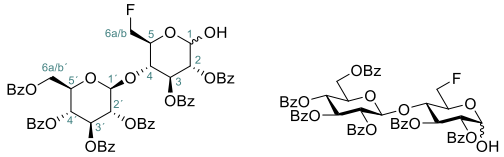
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 165.9, 165.8, 165.5, 165.2, 165.1, 164.8 (6 × C=O), 133.7, 133.5, 133.4, 133.3 (2C, C-8, C-Ar), 129.9 (2C), 129.8, 129.7, 129.6, 129.5, 129.4, 128.9, 128.8, 128.7 (2C), 128.7, 128.6, 128.5 (2C), 128.4 (20 × C-Ar), 117.9 (C-9), 101.2 (C-1´), 99.8 (C-1), 80.8 (d, $J_{C6,F}$ = 174 Hz, C-6), 75.6 (d, $J_{C4,F}$ = 5.6 Hz, C-4), 73.9 (d, $J_{C5,F}$ = 18.9 Hz, C-5), 73.1 (C-3´), 72.8 (C-3), 72.4 (C-5´), 72.0 (C-2´), 71.9 (C-2), 70.1 (C-7), 69.4 (C-4´), 62.7 (C-6´). Due to signal overlap 41 out of 57 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 230.2 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 47.4 Hz, $J_{F,H5}$ = 29.3 Hz).

HRMS (ESI$^+$): Calculated for $C_{57}H_{49}O_{16}FNa^+$ $[M+Na]^+$: 1031.2897; found: 1031.2926.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 13.95 min, λ = 230 nm

2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-deoxy-6-fluoro-α/β-D-glucopyranoside (**67**)



Compound **67** was synthesized in accordance to literature known protocols.[370]

To a magnetically stirred solution of **66** (500 mg, 0.50 mmol, 1.0 eq.) in a mixture of THF/MeOH (90 ml, v/v = 1:4) palladium(II) chloride (89.0 mg, 0.50 mmol, 1.0 eq.) was added. The reaction was stirred for 3 h at 60 °C, before organic solvents were removed under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (50 ml) and washed with H_2O (15 ml) and brine (15 ml) and dried with $MgSO_4$. The crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **67** (470 mg, 490 µmol, 98 %, α/β = 5:1) as a colorless foam.

R_f = 0.14 (^{c}Hex/EtOAc v/v = 3:1)

α-Anomer:

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 8.00 – 7.97 (m, 2H, Ar-H), 7.95 – 7.92 (m, 2H, Ar-H), 7.92 – 7.90 (m, 2H, Ar-H), 7.81 – 7.78 (m, 2H, Ar-H), 7.77 – 7.73 (m, 2H, Ar-H), 7.58 – 7.15 (m, 20H, Ar-H), 6.09 (t, $J_{H3,H2}$ = $J_{H3,H4}$ = 9.7 Hz, 1H, H-3), 5.81 (t, $J_{H3´,H2´}$ = $J_{H3´,H4´}$ = 9.7 Hz, 1H, H-3´), 5.63 (t, $J_{H1,H2}$ = $J_{H1,OH}$ = 3.5 Hz, 1H, H-

1), 5.54 (dd, $J_{H2',H3'}$ = 9.8 Hz, $J_{H2',H1'}$ = 8.0 Hz, 1H, H-2´), 5.46 (t, $J_{H4',H3'} = J_{H4',H5'}$ = 9.4 Hz, 1H, H-4´), 5.16 (dd, $J_{H2,H3}$ = 10.0 Hz, $J_{H2,H1}$ = 3.8 Hz, 1H, H-2), 5.01 (d, $J_{H1',H2'}$ = 7.9 Hz, 1H, H-1´), 4.68 (ddd, $J_{H6a,F}$ = 47.0 Hz, $J_{H6a,H6b}$ = 10.8 Hz, $J_{H6a,H5}$ = 2.3 Hz, 1H, H-6a), 4.46 (dd, $J_{H6b,F}$ = 48.8 Hz, $J_{H6b,H6a}$ = 10.4 Hz, 1H, H-6b), 4.24 – 4.19 (m, 1H, H-4), 4.18 – 4.11 (m, 1H, H-5), 4.07 (dd, $J_{H6a',H6b'}$ = 9.7 Hz, $J_{H6a',H5'}$ = 3.9 Hz, 1H, H-6a´), 3.98 – 3.93 (m, 2H, H-6b´, H-5´), 2.92 (d, $J_{OH,H1}$ = 3.8 Hz, 1H, OH).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 165.9 (3C), 165.5, 165.1, 164.8 (6 × C=O), 133.6 (2C), 133.5, 133.4, 133.3 (2C), 130.0, 129.9 (2C), 129.8, 129.7, 128.6 (2C), 128.5 (2C), 128.4 (2C, 17 × C-Ar), 101.0 (C-1´), 90.5 (C-1), 81.2 (d, $J_{C6,F}$ = 173.2 Hz, C-6), 75.5 (d, $J_{C4,F}$ = 6.0 Hz, C-4), 73.1 (C-3´), 72.4 (2C, C-2, C-5´), 72.1 (C-2´), 69.9 (C-3), 69.5 (d, $J_{C5,F}$ = 18.1 Hz, C-5), 69.4 (C-4´), 62.8 (C-6´). Due to signal overlap 35 out of 54 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 234.6 (td, $J_{F,H6a} = J_{F,H6b}$ = 47.7 Hz, $J_{F,H5}$ = 21.4 Hz).

RP-HPLC (Luna**,** 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 12.97 min, λ = 230 nm.

β-Anomer:

^{1}H-NMR (600 MHz, anomeric signals, $CDCl_3$): δ [ppm] = 4.96 (d, $J_{H1',H2'}$ = 8.0 Hz, H-1´), 4.70 (d, $J_{H1,H2}$ = 8.0 Hz, H-1).

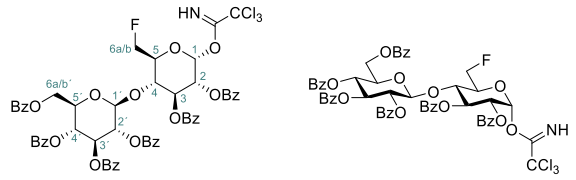
^{13}C-NMR (150 MHz, anomeric signals, $CDCl_3$): δ [ppm] = 100.1 (C-1´), 100.9 (C-1).

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 233.9 - − 233.7 (m).

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 12.65 min, λ = 230 nm.

HRMS (ESI^+): Calculated for $C_{54}H_{45}FKO_{16}^+$ $[M+K]^+$: 1007.2324; found: 1007.2345.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-deoxy-6-fluoro-α-D-glucopyranosyl trichloroacetimidate (**27**)



Compound **27** was synthesized according to a slightly modified protocol.[379]

To an ice-cooled solution of **67** (430 mg, 0.44 mmol, 1.0 eq.) in dry CH_2Cl_2 (20 ml) trichloroacetonitrile (441 µl, 4.40 mmol, 10 eq.) and DBU (60.0 µl, 0.44 mmol, 1.0 eq.) were added. The reaction was allowed to warm to ambient temperature and was stirred for 17 h. After the reaction was deemed complete, it was concentrated under reduced pressure (v ~ 1 ml) and directly subjected to column chromatography (^{c}Hex/EtOAc v/v = 1:0 + 1 % NEt_3 → 7:1 + 1 % NEt_3) affording **27** (430 mg, 0.39 mmol, 89 %) as a colorless oil.

R_f = 0.33 (cHex/EtOAc v/v = 2:1 + 1 % NEt_3)

Optical rotation: $[\alpha]_D^{24}$ = + 28.5 ° (c = 1.9; $CHCl_3$)

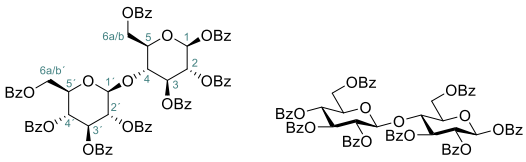
^{1}H-NMR (800 MHz, CD_2Cl_2): δ [ppm] = 8.62 (s, 1H, C=NH), 8.01 – 7.98 (m, 2H, Ar-H), 7.97 – 7.95 (m, 2H, Ar-H), 7.92 – 7.86 (m, 4H, Ar-H), 7.81 – 7.77 (m, 2H, Ar-H), 7.76 – 7.73 (m, 2H, Ar-H), 7.61 – 7.19 (m, 18H, Ar-H), 6.70 (d, $J_{H1,H2}$ = 3.8 Hz, 1H, H-1), 6.09 (t, $J_{H3,H2}$ = $J_{H3,H4}$ = 9.8 Hz, 1H, H-3), 5.84 (t, $J_{H3´,H2´}$ = $J_{H3´,H4´}$ = 9.6 Hz, 1H, H-3´), 5.53 – 5.44 (m, 3H, H-2, H-2´, H-4´), 5.07 (d, $J_{H1´,H2´}$ = 7.9 Hz, 1H, H-1´), 4.68 (ddd, $J_{H6a,F}$ = 46.5 Hz, $J_{H6a,H6b}$ = 10.8 Hz, $J_{H6a,H5}$ = 2.1 Hz, 1H, H-6a), 4.51 (dd, $J_{H6b,F}$ = 48.8 Hz, $J_{H6b,H6a}$ = 10.5 Hz, 1H, H-6b), 4.36 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.8 Hz, 1H, H-4), 4.07 (dd, $J_{H5,F}$ = 30.9 Hz, $J_{H5,H4}$ = 10.1 Hz, 1H, H-5), 4.02 (dd, $J_{H6a´,H6b´}$ = 11.7 Hz, $J_{H6a´,H5´}$ = 3.0 Hz, 1H, H-6a´), 3.98 (ddd, $J_{H5´,H4´}$ = 9.8 Hz, $J_{H5´,H6b´}$ = 4.5 Hz, $J_{H5´,H6a´}$ = 3.0 Hz, 1H, H-5´), 3.94 (dd, $J_{H6b´,H6a´}$ = 11.7 Hz, $J_{H6b´,H5´}$ = 4.5 Hz, 1H, H-6b´).

^{13}C-NMR (200 MHz, CD_2Cl_2): δ [ppm] = 166.1 (2C), 165.9, 165.7, 165.4 (2C, 6 × C=O), 161.1 (N=H), 134.1 (2C), 134.0, 133.8, 133.7, 130.5, 130.3, 130.2, 130.1, 129.4, 129.3, 129.1, 129.0, 128.9 (2C, 15 × C_{Ar}), 101.8 (C-1´), 93.6 (C-1), 91.1 (CCl_3) 81.1 (d, $J_{C6,F}$ = 173.9 Hz, C-6), 75.5 (d, $J_{C4,F}$ = 5.8 Hz, C-4), 73.6 (C-3´), 72.9 (C-5´), 72.7 (d, $J_{C5,F}$ = 18.3 Hz, C-5), 72.6 (C-2´), 71.2 (C-2), 70.6 (C-3), 69.7 (C-4´), 63.0 (C-6´). Due to signal overlapping in the aromatic region, only 35 out 56 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, CD_2Cl_2): δ [ppm] = − 234.9 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 47.6 Hz, $J_{F,H5}$ = 30.7 Hz).

HRMS (ESI^+): Calculated for $C_{56}H_{49}Cl_3FN_2O_{16}^+$ $[M+NH_4]^+$: 1129.2127; found: 1129.2160.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-β-D-glucopyranoside (**68**)



Compound **68** was synthesized according to a previously described synthetic protocol.[381]

To a magnetically stirred solution of D-cellobiose (1.00 g, 2.92 mmol, 1.0 eq.) in pyridine (15 ml) benzoyl chloride (5.75 ml, 49.7 mmol, 17.0 eq.) was added slowly. The reaction was stirred for 16 h at 60 °C and subsequently poured onto ice. The aqueous mixture was extracted with CH_2Cl_2 (2 × 25 ml) and the combined organic layers were washed with 1 M HCl (25 ml) and brine (25 ml) and dried with $MgSO_4$. Organic solvents were removed under reduced pressure. The thus obtained product was purified by crystallization from EtOH, affording compound **68** as a colorless solid (3.28 g, 2.79 mmol, 96 %).

R_f = 0.56 (cHex/EtOAc v/v = 2:1)

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.04 – 7.93 (m, 10H, Ar-H), 7.92 – 7.86 (m, 2H, Ar-H), 7.76 – 7.73 (m, 4H, Ar-H), 7.59 – 7.49 (m, 2H, Ar-H), 7.48 – 7.35 (m, 12H, Ar-H), 7.34 – 7.20 (m, 10H, Ar-H), 6.12 (d, $J_{H1,H2}$ = 8.0 Hz, 1H, H-1), 5.94 (t, $J_{H3,H2}$ = $J_{H3,H4}$ = 9.2 Hz, 1H, H-3), 5.79 – 5.71 (m, 2H, H-2, H-3´), 5.58 – 5.52 (m, 1H,

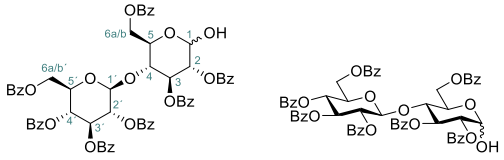
H-2´), 5.41 (t, $J_{H4´,H3´} = J_{H4´,H5´} = 9.5$ Hz, 1H, H-4´), 4.96 (d, $J_{H1´,H2´} = 7.9$ Hz, 1H, H-1´), 4.61 (dd, $J_{H6a,H6b} = 12.4$ Hz, $J_{H6a,H5} = 1.9$ Hz, 1H, H-6a), 4.50 (dd, $J_{H6b,H6a} = 12.4$ Hz, $J_{H6b,H5} = 4.0$ Hz, 1H, H-6b), 4.38 (t, $J_{H4,H3} = J_{H4,H5} = 9.3$ Hz, 1H, H-4), 4.10 – 4.01 (m, 2H, H-5, H-6a´), 3.86 – 3.77 (m, 2H, H-5´, H-6b´).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 165.8 (2C), 165.7, 165.4, 165.3, 165.1, 164.9, 164.6 (8 × C=O), 133.9, 133.6, 133.5 (3C), 133.4 (3C), 130.7, 130.2, 129.9, 129.8 (2C), 129.7, 129.0, 128.7, 128.6 (2C), 128.5, 128.4 (2C, 21 × C-Ar), 101.2 (C-1´), 92.6 (C-1), 76.1 (C-4), 73.9 (C-5), 72.9 (C-3), 72.8 (C-3´), 72.5 (C-5´), 71.9 (C-2´), 70.9 (C-2), 69.5 (C-4´), 62.7 (C-6´), 62.2 (C-6). Due to signal overlapping only 41 out of 68 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI^+): Calculated for $C_{68}H_{58}NO_{19}^+$ $[M+NH_4]^+$: 1192.3598; found: 1192.3625.

For further analytical data see reference.[399]

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α/β-D-glucopyranoside (**69**)



Compound **69** was synthesized according to a modified procedure.[374, 375]

To a magnetically stirred solution of benzoyl ester **68** (1.00 g, 0.86 mmol, 1.0 eq.) in pyridine (10 ml), dimethylamine (2 M in THF, 12 ml) was added. The reaction was stirred for 30 h at ambient temperature before being diluted with CH_2Cl_2. The organic layer was washed with 1M HCl (15 ml) and brine (15 ml) and dried with $MgSO_4$. The crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 3:1 → 2:1) affording **69** (614 mg, 0.58 mmol, 67 %) as a mixture of anomers (α/β ~ 2:1).

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

α-Anomer:

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 8.03 – 7.19 (m, 35H, Ar-H), 6.14 (dd, $J_{H3,H2} = 10.1$ Hz, $J_{H3,H4} = 9.2$ Hz, 1H, H-3), 5.76 (t, $J_{H3´,H2´} = J_{H3´,H4´} = 9.7$ Hz, 1H, H-3´), 5.60 (d, $J_{H1,H2} = 3.5$ Hz, 1H, H-1), 5.54 (dd, $J_{H2´,H3´} = 9.9$ Hz, $J_{H2´,H1´} = 7.9$ Hz, 1H, H-2´), 5.42 (t, $J_{H4´,H3´} = J_{H4´,H5´} = 9.6$ Hz, 1H, H-4´), 5.19 (dd, $J_{H2,H3} = 10.2$ Hz, $J_{H2,H1} = 3.6$ Hz, 1H, H-2), 5.02 (d, $J_{H1´,H2´} = 8.0$ Hz, 1H, H-1´), 4.61 (dd, $J_{H6a,H6b} = 12.2$ Hz, $J_{H6a,H5} = 2.0$ Hz, 1H, H-6a), 4.47 (dd, $J_{H6b,H6a} = 12.1$, $J_{H6b,H5} = 3.7$ Hz, 1H, H-6b), 4.37 (ddd, $J_{H5,H4} = 10.1$ Hz, $J_{H5,H6b} = 3.7$ Hz, $J_{H5,H6a} = 1.9$ Hz, 1H, H-5), 4.24 (t, $J_{H4,H3} = J_{H4,H5} = 9.8$ Hz, 1H, H-4), 4.14 – 4.09 (m, 1H, H-6a´), 3.89 (dd, $J_{H6a´,H6b´} = 11.9$ Hz, $J_{H6a´,H5´} = 5.4$ Hz, 1H, H-6b´), 3.86 – 3.82 (m, 1H, H-5´).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 166.1, 166.0, 165.9, 165.8, 165.5, 165.1, 164.9 (7 × C=O), 133.5 (3C), 133.4, 133.3 (3C), 130.3, 130.0, 129.9 (2C), 129.8 (2C), 129.7, 129.6, 129.1, 128.6 (3C), 128.5 (2C), 128.4 (2C, 23 × C-Ar), 100.9 (C-1´), 90.4 (C-1), 76.5 (C-4), 73.0 (C-3´), 72.5 (C-5´), 72.4 (C-2), 72.1 (C-2´), 69.9 (C-3), 69.6

(C-4´), 68.7 (C-5), 62.8 (C-6´), 62.4 (C-6). Due to signal overlapping in the aromatic region, only 42 out of 61 carbon atoms were assigned in the ^{13}C-Spectrum.

HRMS (ESI$^+$): Calculated for $C_{61}H_{54}O_{18}N^+$ $[M+NH_4]^+$: 1088.3336; found: 1088.3364.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 13.64 min, λ = 230 nm

β-Anomer:

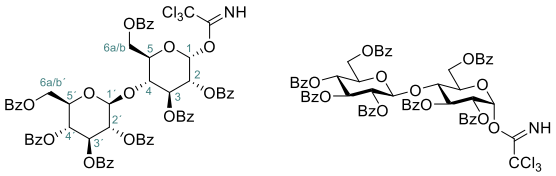
^{1}H-NMR (600 MHz, $CDCl_3$, selected signals): δ [ppm] = 5.85 (t, $J_{H3,H2} = J_{H3,H4}$ = 9.5 Hz, 1H, H-3), 5. 47 (t, $J_{H3´,H2´} = J_{H3´,H4´}$ = 9.7 Hz, 1H, H-3´), 5.22 (dd, $J_{H2,H3}$ = 10.0 Hz, $J_{H2,H1}$ = 8.0 Hz, 1H, H-2), 4.96 (d, $J_{H1´,H2´}$ = 7.9 Hz, 1H, H-1´), 4.89 (d, $J_{H1,H2}$ = 8.0 Hz, H-1).

^{13}C-NMR (150 MHz, $CDCl_3$, selected signals): δ [ppm] = 167.1, 166.0, 165.8 (2C), 165.5, 165.1 (2C, 7 × C=O), 133.8, 133.7, 133.4, 130.1, 129.7, 129.6, 129.1, 128.8 (2C), 128.7 (10 × C-Ar), 101.1 (C-1´), 95.9 (C-1), 76.5 (C-4), 74.5 (C-2), 73.4 (C-5´), 72.9 (C-2´), 72.5 (C-3´/C-5´), 72.2 (C-3), 72.0 (C-3´/C-5´), 69.5 (C-4´), 62.8 (C-6´), 62.5 (C-6).

HRMS (ESI$^+$): Calculated for $C_{61}H_{54}O_{18}N^+$ $[M+NH_4]^+$: 1088.3336; found: 1088.3364.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 13.30 min, λ = 230 nm.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**25**)



Compound **25** was synthesized in accordance with a slightly modified procedure.[379]

To a magnetically stirred solution of disaccharide **69** (730 mg, 0.68 mmol, 1.0 eq.) in dry CH_2Cl_2 (20 ml), trichloroacetonitrile (682 µl, 6.80 mmol, 10.0 eq.) was added. The reaction was cooled to 0 °C before DBU (102 µl, 0.68 mmol, 1.0 eq.) was added. The reaction was slowly warmed to ambient temperature and stirred for 24 h. Subsequently the reaction was concentrated to dryness and the crude residue was dissolved in CH_2Cl_2 (500 µl) and directly subjected to column chromatography (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3), affording **25** (500 mg, 0.41 mmol, 60 %) as a colorless oil.

R_f = 0.39 (^{c}Hex/EtOAc v/v = 2:1 + 1 % NEt_3)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 8.55 (s, 1H, N=H), 8.02 – 8.00 (m, 2H, Ar-H), 7.97 (dd, $J_{CH,CH}$ = 8.3 Hz, 1.4 Hz, 2H, Ar-H), 7.94 – 7.91 (m, 6H, Ar-H), 7.77 (dd, $J_{CH,CH}$ = 8.3 Hz, $J_{CH,CH}$ = 1.4 Hz, 2H, Ar-H), 7.75 – 7.73 (m, 2H, Ar-H), 7.58 – 7.21 (m, 21H, Ar-H), 6.69 (d, $J_{H1,H2}$ = 3.8 Hz, 1H, H-1), 6.16 – 6.09 (m, 1H, H-3), 5.75 (t, $J_{H3´,H2´} = J_{H3´,H4´}$ = 9.6 Hz, 1H, H-3´), 5.53 (dd, $J_{H3´,H2´}$ = 9.8 Hz, $J_{H2´,H1´}$ = 7.9 Hz, 1H, H-2´), 5.48 (dd, $J_{H2,H3}$ =

10.1 Hz, $J_{H2,H1}$ = 3.8 Hz, 1H, H-2), 5.42 (t, $J_{H4´,H3´} = J_{H4´,H5´}$ = 9.7 Hz, 1H, H-4´), 5.02 (d, $J_{H1´,H2´}$ = 7.9 Hz, 1H, H-1´), 4.59 (dd, $J_{H6a,H6b}$ = 12.4 Hz, $J_{H6a,H5}$ = 1.8 Hz, 1H, H-6a), 4.50 (dd, $J_{H6b,H6a}$ = 12.2 Hz, $J_{H6b,H5}$ = 3.4 Hz, 1H, H-6b), 4.36 – 4.31 (m, 2H, H-4, H-5), 4.07 (dd, $J_{H6a´,H6b´}$ = 12.0 Hz, $J_{H6a´,H5´}$ =3.3 Hz, 1H, H-6a´), 3.88 (dd, $J_{H6b´,H6a´}$ = 12.0 Hz, $J_{H6b´,H5´}$ = 5.1 Hz, 1H, H-6b´), 3.84 – 3.80 (m, 1H, H-5´).

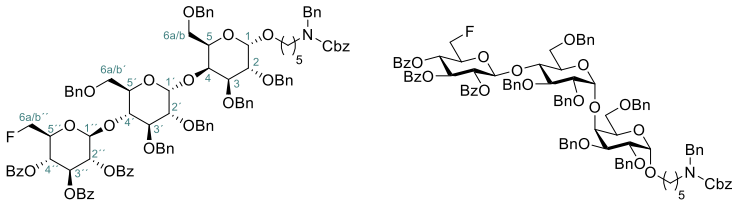
^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 165.8 (3C), 165.6, 165.3, 165.1, 164.9 (7 × C=O), 160.8 (C=NH), 133.7, 133.5 (2C), 133.4 (2C), 133.3, 130.0, 129.9 (2C), 129.8 (4C), 129.7 (2C), 129.6, 129.5, 128.6 (2C), 128.5 (3C), 128.4 (23 × C_{Ar}), 101.3 (C-1´), 93.1 (C-1), 90.8 (CCl_3), 76.1 (C-4), 73.0 (C-3´), 72.6 (C-5´), 72.2 (C-2´), 71.4 (C-5), 70.8 (C-2), 70.2 (C-3), 69.5 (C-4´), 62.8 (C-6´), 62.0 (C-6). Due to signal overlapping in the aromatic region, only 44 out of 63 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI^+): Calculated for $C_{63}H_{54}Cl_3N_2O_{18}^+$ $[M+NH_4]^+$: 1231.2432; found: 1231.2478.

For further analytical data see reference.[379]

Synthesis of trisaccharides

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-glucopyranosyl) (1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**70**)



Acceptor **18** (280 mg, 235 µmol, 1.0 eq.) and fluorinated glucosyl donor **31** (210 mg, 329 µmol, 1.4 eq.) were combined and co-evaporated with toluene (2 × 10 ml). Educts were dried for 1 h under high vacuum and subsequently dissolved in dry CH_2Cl_2 (15 ml). Freshly activated 4 Å molecular sieve was added, and the mixture was stirred for 1 h at ambient temperature. The reaction was chilled to 0 °C and TMSOTf (4.00 µl, 24.0 µmol, 0.1 eq.) was added. After the reaction was deemed complete (approximately 2.5 h) it was neutralized by addition of NEt_3 (300 µl) and filtered through a pad of *Hyflo*®. Solvents were removed under reduced pressure and the crude product was subjected to flash chromatography (^{c}Hex/EtOAc v/v = 5:1) affording **70** (300 mg, 0.18 mmol, 77 %) as a colorless oil.

R_f = 0.28 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3)

Optical rotation: $[\alpha]_D^{24} = -9.9°$ (c = 0.6; $CHCl_3$)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.97 – 7.92 (m, 2H, Ar-H), 7.78 – 7.76 (m, 2H, Ar-H), 7.68 – 7.64 (m, 2H, Ar-H), 7.58 – 7.11 (m, 49H, Ar-H), 5.53 (t, $J_{H3´´,H2´´} = J_{H3´´,H4´´}$ = 9.5 Hz, 1H, H-3´´), 5.47 – 5.41 (m, 2H, H-2´´, H-4´´), 5.20 – 5.11 (m, 3H, 2 × $CH_{Cbz,}$, CH_{Bn}), 4.98 (d, $J_{H1´,H2´}$ = 3.5 Hz, 1H, H-1´), 4.82 (d, $J_{CH,CH}$ = 11.9 Hz,

1H, CH_{Bn}), 4.78 (d, $J_{CH,CH}$ = 10.6 Hz, 1H, CH_{Bn}), 4.71 – 4.64 (m, 3H, H-1´´,2 × CH_{Bn}), 4.59 (d, $J_{CH,CH}$ = 12.5 Hz, 1H, CH_{Bn}), 4.56 – 4.50 (m, 2H, H-1, CH_{Bn}), 4.50 – 4.38 (m, 3H, H-6a´´, 2 × NCH_{Bn}), 4.37 – 4.25 (m, 2H, H-6b´´, CH_{Bn}), 4.20 – 4.15 (m, 3H, 3 × CH_{Bn}), 4.12 – 4.00 (m, 4H, H-4´, H-5´,H-4, CH_{Bn}), 3.93 – 3.87 (m, 2H, H-3´, H-6a), 3.78 – 3.69 (m, 3H, H-2, H-3, H-5), 3.59 – 3.51 (m, 3H, H-5´´, H-6a´,H-2´), 3.51 – 3.40 (m, 1H, CH_{Linker}), 3.41 – 3.37 (m, 1H, H-6b), 3.35 – 3.24 (m, 1H, CH_{Linker}), 3.23 – 3.11 (m, 2H, 2 × CH_{Linker}), 2.98 (dd, $J_{H6b,H6a}$ = 11.0 Hz, $J_{H6b,H5}$ = 1.6 Hz, 1H, H-6b´), 1.56 – 1.41 (m, 4H, 4 × CH_{Linker}), 1.33 – 1.13 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 165.8 (C=O), 165.2 (C=O), 164.7 (C=O), 156.8/156.3 (C=O-Cbz), 139.5, 139.1, 138.7, 138.4, 138.2, 138.1, 137.0/136.9 (7 × Cq), 133.6, 133.3, 133.2, 129.9, 129.8 (2C), 129.1 (2C), 129.0 (2C), 128.7 (3C), 128.6 (2C), 128.4 (5C), 128.2, 128.0 (2C), 127.7, 127.6, 127.5, 127.4, 127.3 (28 × C-Ar), 100.2 (C-1´´), 100.0 (C-1´), 97.9 (C-1), 81.2 (d, $J_{C6´´,F}$ = 175.8 Hz, C-6´´), 80.2 (C-3´), 79.7 (C-2´), 77.7 (C-3/C-2/C-5), 77.3 (2C, C-4, C-4´)*, 75.5 (CH_{Bn}), 75.0 (C-3/C-2/C-5), 74.3 (CH_{Bn}), 73.7 (CH_{Bn}), 73.6 (CH_{Bn}), 73.3 (C-3´´), 73.0 (CH_{Bn}), 72.9 (d, $J_{C5´´,F}$ = 19.9 Hz, C-5´´), 72.5 (CH_{Bn}), 72.2 (C-2´´), 70.5 (C-5´), 69.5 (C-3/C-2/C-5), 69.1 (d, $J_{C4´´,F}$ = 6.5 Hz, C-4´´), 68.0 (2C, C-6, CH_{Linker}), 67.3 (CH_{Cbz}), 67.2 (C-6´), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.2 (CH_{Linker}) 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}). * Assigned from HSQC-Spectrum due to signal superimposition with solvent peak. Due to signal overlap 70 out of 101 carbon atoms were assigned in the ^{13}C spectrum.

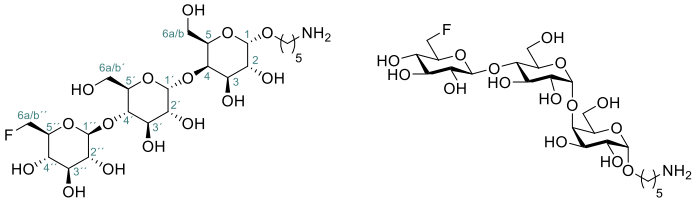
^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 230.5 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 46.9 Hz, $J_{F,H5}$ = 22.1 Hz).

^{1}H, ^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 171 Hz, $J_{C1´H1´}$ = 171 Hz, $J_{C1´´,H1´´}$ = 161 Hz.

HRMS (ESI^+): Calculated for $C_{101}H_{106}O_{20}FN_2F^+[M+NH_4]^+$: 1686.7352; found: 1686.7282.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 22.19 min, λ = 230 nm

5-Aminopentyl (6-deoxy-6-fluoro-β-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→4)-α-D-galacto-pyranoside (**3**)



Compound **3** was synthesized according to literature known protocols.[194, 382]

To a magnetically stirred solution of the fluorinated trisaccharide **70** (95 mg, 56.9 µmol, 1.0 eq.) in a mixture of MeOH/THF (v/v = 1:1, 7 ml), sodium methoxide (1.00 ml, 1.5 M in MeOH) was added. The reaction solution was stirred for 6 h at ambient temperature, before being neutralized by addition of *Amberlite® IR 120*. The reaction mixture was filtered and solvents were removed under reduced pressure. The crude residue was dried for 17 h under high vacuum. Subsequently the crude residue was dissolved in a mixture of CH_2Cl_2/^{t}BuOH/H_2O (v/v = 1:6:2, 25 ml) and $Pd(OH)_2$/C (100 mg) was added under an argon atmosphere. The reaction was then purged three times with hydrogen and stirred for 36 h at ambient temperature. The catalyst was removed by filtration through *Hyflo®* and the solvents were removed under reduced pressure. The crude product was dissolved in H_2O/MeOH (v/v = 4:1) and subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1). Since

hydrolysis was not deemed complete *via* NMR analysis, the thus obtained product was dissolved in mixture of MeOH/THF/H_2O/AcOH (v/v = 19:5:4:1, 15 ml). Pd/C (40 mg) was added under an argon atmosphere and the reaction was purged three times with hydrogen. After stirring for 24 h at ambient temperature, the catalyst was removed by filtration through a short plug of *Hyflo*® and solvents were removed under reduced pressure. The crude product was then dissolved in H_2O/MeOH (v/v = 4:1) and subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1), affording **3** (30 mg, 50.7 µmol, 89 % over two steps) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 8.13 – 8.05 (m, 2H, NH_2), 4.82 (d, $J_{H1´,H2´}$ = 3.7 Hz, 1H, H-1´), 4.65 (d, $J_{H1,H2}$ = 3.6 Hz, 1H, H-1), 4.63– 4.45 (m, 2H, H-6a´´, H-6b´´), 4.35 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H-1´´), 4.15 (dt, $J_{H5´,H4´}$ = 10.1 Hz, $J_{H5´,H6a´}$ = $J_{H5´,H6b´}$ = 3.0 Hz, 1H, H-5´), 3.86 (bs, 1H, H-4), 3.74 – 3.67 (m, 2H, H-6a, H-6a´), 3.67 – 3.64 (m, 2H, H-3, H-5), 3.61 – 3.51 (m, 4H, H-2, H-3´, H-6b/H-6b´, CH_{Linker}), 3.49 – 3.41 (m, 2H, H-5´´, H-6b/H-6b´), 3.38 (t, $J_{H4´,H3´}$ = $J_{H4´,H5´}$ = 9.5 Hz, 1H, H-4´), 3.31 (dt, $J_{CH,CH}$ = 9.6 Hz, $J_{CH,CH}$ = 6.1 Hz, 1H, CH_{Linker}), 3.26 (dd, $J_{H2´,H3´}$ = 9.7 Hz, $J_{H2´,H1´}$ = 3.7 Hz, 1H, H-2´), 3.22 (t, $J_{H3´´,H2´´}$ = $J_{H3´´,H4´´}$ = 8.9 Hz, 1H, H-3´´), 3.11 (t, $J_{H4´´,H3´´}$ = $J_{H4´´,H5´´}$ = 9.4 Hz, 1H, H-4´´), 2.99 (t, $J_{H2´´,H1´´}$ = $J_{H2´´,H3´´}$ = 8.5 Hz, 1H, H-2´´), 2.73 (q, $J_{CH,CH}$ = 6.7 Hz, 2H, CH_{Linker}), 1.60 – 1.46 (m, 4H, CH_{Linker}), 1.41 – 1.32 (m, 2H, CH_{Linker}).

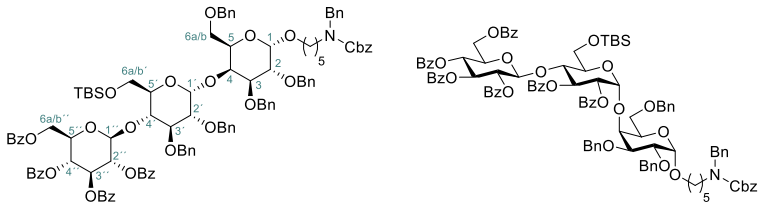
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 102.9 (C-1´´), 99.4 (C-1´), 99.0 (C-1), 82.7 (d, $J_{C6´´,F}$ = 169.1 Hz, C-6´´), 79.6 (C-4´), 77.1 (C-4), 76.3 (C-3´´), 74.6 (d, $J_{C5´´,F}$ = 17.1 Hz, C-5´´), 73.2 (C-2´´), 72.2 (C-2´), 71.2 (C-3´), 70.9 (C-3/C-5), 70.1 (C-5´), 68.8/68.7 (2C, C-4´´, C-3/C-5), 68.4 (C-2), 66.8 (CH_{Linker}), 59.4 (C-6/C-6´), 58.9 (C-6/C-6´), 38.7 (CH_{Linker}), 28.6 (CH_{Linker}), 26.7 (CH_{Linker}), 22.8 (CH_{Linker}).

^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 232.1 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 47.8 Hz, $J_{F,H5}$ = 23.8 Hz).

^{1}H, ^{13}C-coupled HSQC (DMSO-d_6): $J_{C1,H1}$ = 167 Hz, $J_{C1´H1´}$ = 170 Hz, $J_{C1´´,H1´´}$ = 161 Hz.

HRMS (ESI$^+$): Calculated for $C_{23}H_{43}FNO_{15}^+$ [M+H]$^+$: 592.2611; found: 592.2610.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-6-*O*-*tert*-butyl-dimethylsilyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**71**)



Acceptor **19** (170 mg, 0.14 mmol, 1.0 eq.) and donor **32** (156 mg, 0.21 mmol, 1.5 eq.) were combined and co-evaporated with toluene (2 × 10 ml). Educts were dried 1h under high vacuum and subsequently dissolved in dry CH_2Cl_2 (10 ml). Freshly activated 4 Å molecular sieve was added, and the mixture stirred for 1 h at ambient temperature. The reaction was cooled to 0 °C and TMSOTf (3.60 µl, 20.0 µmol, 0.1 eq.) was added in one portion. After the reaction was deemed complete by TLC monitoring, it was neutralized by addition of NEt_3 (100 µl) and filtered through a pad of *Hyflo*®. Solvents were removed under reduced pressure and the crude product was

subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 6:1) affording **71** (240 mg, 0.13 mmol, 93 %) as a colorless oil.

R_f = 0.43 (^{c}Hex/EtOAc v/v = 3:1)

Optical rotation: $[\alpha]_D^{24} = -10.0\ °$ (c = 1.1, $CHCl_3$)

^{1}H-NMR (600 MHz, CD_2Cl_2): δ [ppm] = 8.02 – 7.98 (m, 2H, Ar-H), 7.92 – 7.88 (m, 2H, Ar-H), 7.85 – 7.83 (m, 2H, Ar-H), 7.80 – 7.75 (m, 2H, Ar-H), 7.53 (dtd, $J_{CH,CH}$ = 8.3 Hz, $J_{CH,CH}$ = 7.3 Hz, $J_{CH,CH}$ = 1.4 Hz, 2H, Ar-H), 7.47 – 7.40 (m, 4H, Ar-H), 7.39 – 7.15 (m, 41H, Ar-H), 5.83 (t, $J_{H3'',H4''} = J_{H3'',H2''}$ = 9.6 Hz, 1H, H-3´´), 5.67 – 5.60 (m, 2H, H-2´´, H-4´´), 5.31 – 5.26 (m, 2H, H-1´´, CH_{Bn}), 5.13 (d, $J_{CH,CH}$ = 12.1 Hz, 2H, CH_{Cbz}), 4.91 (d, $J_{H1',H2'}$ = 3.6 Hz, 1H, H-1´), 4.75 (d, $J_{CH,CH}$ = 11.1 Hz, 1H, CH_{Bn}), 4.72 (d, $J_{CH,CH}$ = 11.4 Hz, 1H, CH_{Bn}), 4.63 (s, 1H, H-1), 4.58 – 4.52 (m, 3H, 3× CH_{Bn}), 4.49 (dd, $J_{H6a'',H6b''}$ = 12.0 Hz, $J_{H6a'',H5''}$ = 3.0 Hz, 1H, H-6a´´), 4.45 (s, 2H, NCH_{Bn}), 4.35 – 4.30 (m, 2H, H-6b´´, CH_{Bn}), 4.29 – 4.22 (m, 3H, 3 × CH_{Bn}), 4.13 – 4.07 (m, 3H, H-5´´, H-4´, H-5´), 4.00 (s, 1H, H-4), 3.91 (dd, $J_{H3',H2'/H-4'}$ = 9.8 Hz, $J_{H3',H2'/H-4'}$ = 8.1 Hz, 1H, H-3´), 3.87 – 3.70 (m, 4H, H-6a´, H-6a, H-2, H-3), 3.68 – 3.64 (m, 1H, H-5), 3.51 – 3.45 (m, 1H, CH_{Linker}), 3.42 – 3.34 (m, 3H, H-2´, H-6b´, H-6b), 3.32 – 3.23 (m, 1H, CH_{Linker}), 3.22 – 3.11 (m, 2H, 2× CH_{Linker}), 1.60 – 1.41 (m, 4H, 4× CH_{Linker}), 1.33 – 1.14 (m, 2H, 2× CH_{Linker}), 0.95 (s, 9H, Si-^{t}Bu), 0.09 (s, 3H, Si-CH_3), 0.02 (s, 3H, Si-CH_3).

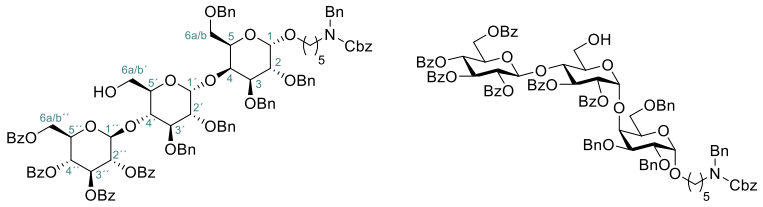
^{13}C-NMR (150 MHz, CD_2Cl_2): δ [ppm] = 166.4, 166.1, 165.7, 165.3 (4 × C=O), 157.1/156.5 (C=O-Cbz), 140.3, 139.4, 139.2, 139.0, 138.9, 137.8/137.7 (6 × Cq), 134.0, 133.9, 133.8, 133.5, 130.4, 130.3, 130.1 (2C), 129.7, 129.6, 129.5, 129.0 (3C), 128.9 (2C), 128.8 (3C), 128.7, 128.5, 128.2, 128.1 (2C), 128.0 (2C), 127.5, 127.4 (28 × C-Ar), 101.0 (C-1´´), 99.7 (C-1´), 98.3 (C-1), 80.6 (2C, C-2´, C-3´), 77.7 (C-4´), 77.6 (C-5), 76.5 (C-4), 75.7 (CH_{Bn}), 75.6 (C-2), 75.1 (CH_{Bn}), 74.2 (C-3´´), 73.6 (CH_{Bn}), 73.4 (CH_{Bn}), 73.0 (C-2´´/C-4´´), 72.8 (C-5´´), 72.3 (CH_{Bn}), 71.9 (C-5´), 70.5 (C-2´´/C-4´´), 69.8 (C-3), 68.4 (2C, C-6, CH_{Linker}), 67.4 (CH_{Cbz}), 63.8 (C-6´´), 61.5 (C-6´), 51.0/50.6 (NCH_{Bn}), 47.7/46.9 (CH_{Linker}), 29.7 (CH_{Linker}), 28.6/28.0 (CH_{Linker}), 26.4 (3C, Si-^{t}Bu), 23.9 (CH_{Linker}), 18.7 (Cq, Si-^{t}Bu), − 4.60 (Si-CH_3), − 4.80 (Si-CH_3). Due to signal overlap 75 out of 107 carbon atoms were assigned in the ^{13}C spectrum.

^{1}H, ^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 173 Hz, $J_{C1',H1'}$ = 173 Hz, $J_{C1'',H1''}$ = 168 Hz.

HRMS (ESI^+): Calculated for $C_{107}H_{119}N_2O_{22}Si^+[M+NH_4]^+$: 1812.8052; found: 1812.8062.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 39.67 min, λ = 230 nm.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**73**)



Compound **73** was synthesized according a previously described protocol.[367]

A magnetically stirred solution of **71** (180 mg, 100 µmol, 1.0 eq.) in a mixture of CH_2Cl_2/MeOH (v/v = 1:1, 20 ml), *p*-TsOH·H_2O (19.0 mg, 100 µmol, 1.0 eq.) was added. The reaction solution was stirred for 7 h at 50 °C and 12 h at ambient temperature. After complete conversion of the starting material was observed by TLC monitoring, the reaction was neutralized by addition of NEt_3 (200 µl). Organic solvents were removed under reduced pressure and the crude residue was dissolved in CH_2Cl_2 (75 ml) and washed with 1 M HCl (30 ml), sat. aq. $NaHCO_3$ (30 ml) and brine (20 ml) and dried with $MgSO_4$. The solvent was removed under reduced pressure and the crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 2:1) affording **73** (150 mg, 89.2 µmol, 89 %) as a colorless oil.

R_f = 0.22 (^{c}Hex/EtOAc v/v = 2:1)

Optical rotation: $[\alpha]_D^{24}$ = + 15.0 ° (c = 0.3, $CHCl_3$)

^{1}H-NMR (800 MHz, $CDCl_3$): δ [ppm] = 7.95 (d, $J_{CH,CH}$ = 7.7 Hz, 2H, Ar-H), 7.87 (d, $J_{CH,CH}$ = 7.7 Hz, 2H, Ar-H), 7.85 – 7.83 (m, 2H, Ar-H), 7.80 – 7.77 (m, 2H, Ar-H), 7.50 (td, $J_{CH,CH}$ = 7.4 Hz, $J_{CH,CH}$ = 1.4 Hz, 1H, Ar-H), 7.48 – 7.46 (m, 1H, Ar-H), 7.42 – 7.13 (m, 45H, Ar-H), 5.87 (t, $J_{H3'',H4''}$ = $J_{H3'',H2''}$ = 9.7 Hz, 1H, H-3´´), 5.68 (t, $J_{H4'',H3''}$ = $J_{H4'',H5''}$ = 9.8 Hz, 1H, H-4´´), 5.65 – 5.60 (m, 1H, H-2´´), 5.21 – 5.13 (m, 4H, H-1´´, 2 × $CH_{Cbz,}$, CH_{Bn}), 4.87 (d, $J_{H1',H2'}$ = 3.5 Hz, 1H, H-1´), 4.85 (d, $J_{CH,CH}$ = 11.3 Hz, 1H, CH_{Bn}), 4.72 (d, $J_{CH,CH}$ = 11.9 Hz, 1H, CH_{Bn}), 4.62 – 4.57 (m, 3H, H-1, 2 × CH_{Bn}), 4.53 (d, $J_{CH,CH}$ = 12.4 Hz, 1H, CH_{Bn}), 4.46 (d, $J_{CH,CH}$ = 26.7 Hz, 2H, NCH_{Bn}), 4.44 – 4.38 (m, 2H, H-6a´´, CH_{Bn}), 4.32 – 4.28 (m, 2H, H-6b´´, CH_{Bn}), 4.26 – 4.19 (m, 2H, 2 × CH_{Bn}), 4.05 (dd, $J_{H5',H4'}$ = 10.2 Hz, $J_{H5',H6'}$ = 2.4 Hz, 1H, H-5´), 4.03 (dt, $J_{H5'',H4''}$ = 8.9 Hz, $J_{H5'',H6a'}$ = $J_{H5'',H6b''}$ = 4.1 Hz, 1H, H-5´´), 3.97 (t, $J_{H3',H2'}$ = $J_{H3',H2'}$ = 9.2 Hz, 1H, H-3´), 3.95 – 3.89 (m, 2H, H-4, H-4´), 3.86 – 3.81 (m, 1H, H-6a), 3.80 – 3.74 (m, 2H, H-2, H-3), 3.74 – 3.70 (m, 1H, H-5), 3.60 – 3.55 (m, 1H, H-6a´), 3.53 – 3.41 (m, 3H, H-2´, H-6b, CH_{Linker}), 3.39 – 3.28 (m, 2H, H-6b´, CH_{Linker}), 3.22 (t, $J_{CH,CH}$ = 7.7 Hz, 1H, CH_{Linker}), 3.14 (t, $J_{CH,CH}$ = 7.5 Hz, 1H, CH_{Linker}), 1.59 – 1.41 (m, 4H, 4 × CH_{Linker}), 1.34 – 1.11 (m, 2H, 2 × CH_{Linker}).

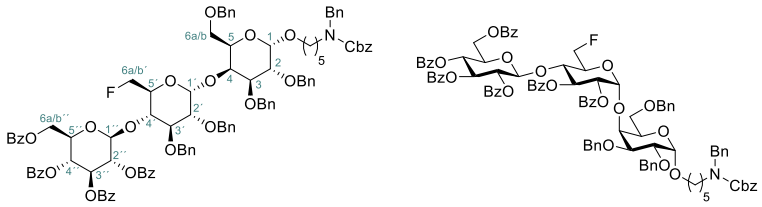
^{13}C-NMR (200 MHz, $CDCl_3$): δ [ppm] = 166.1, 165.9, 165.2, 165.0 (4 × C=O), 156.8/156.3 (C=O-Cbz), 139.4, 138.7, 138.4 (2C), 138.1 (2C,), 137.0/136.9 (7 × Cq), 133.4 (2C), 133.3, 133.0, 129.9 (2C), 129.8 (2C), 129.7, 129.0, 128.9 (2C), 128.6 (2C), 128.5 (3C), 128.4 (4C), 128.3, 128.2, 128.0, 127.9 (2C), 127.7, 127.6 (2C), 127.5, 127.4, 127.3, 127.2, 127.0 (34 × C-Ar), 101.5 (C-1´´), 99.6 (C-1´), 97.7 (C-1), 80.2 (C-3´), 80.0 (C-2´), 78.2 (C-4´), 77.8 (C-4), 77.4 (C-5)*, 75.3 (C-2), 75.1 (CH_{Bn}), 74.1 (CH_{Bn}), 73.5 (CH_{Bn}), 73.4 (C-3´´), 73.0 (CH_{Bn}), 72.6 (CH_{Bn}), 72.5 (C-2´´), 72.3 (C-5´´), 71.1 (C-5´), 69.9 (C-4´´), 69.5 (C-3), 68.4 (C-6), 68.1/ 68.0 (CH_{Linker}), 67.3/67.2

(CH_{Cbz}), 63.2 (C-6´´), 60.5 (C-6´), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2/29.1 (CH_{Linker}), 28.0/27.6 (CH_{Linker}), 23.5 (CH_{Linker}). * Assigned from HSQC-Spectrum due to signal superimposition with solvent peak. Due to signal overlap 76 out of 101 carbon atoms were assigned in the ^{13}C spectrum.

HRMS (ESI^+): Calculated for $C_{101}H_{102}NO_{22}^+$ $[M+H]^+$: 1697.7153; found: 1697.7183.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 20.19 min, λ = 230 nm.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside(**74**)



Compound **74** was synthesized in accordance with a previously reported fluorination protocol.[324, 325]

To a magnetically stirred solution of **73** (150 mg, 89.2 µmol, 1.0 eq.) in dry CH_2Cl_2 (4 ml), 2,4,6-collidine (48.0 µl, 357 µmol, 4.0 eq.) and DAST (24.0 µl, 179 µmol, 2.0 eq.) were added. The reaction was subjected to microwave irradiation (80 °C, 100 W) for 1 h and subsequently poured into MeOH (50 ml). Solvents were removed under reduced pressure, affording a brown oil which was dissolved in CH_2Cl_2 (50 ml). The organic layer was washed with sat. aq. $NaHCO_3$ (20 ml), 1 M HCl (20 ml) and brine (15 ml) and dried with $MgSO_4$. The solvent was removed under reduced pressure and the crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 4:1) affording **74** (113 mg, 67.2 µmol, 75 %) as a colorless oil.

R_f = 0.46 (^{c}Hex/EtOAc v/v = 2:1)

Optical rotation: $[\alpha]_D^{24}$ = + 21.4 ° (c = 0.3, $CHCl_3$)

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.95 (dd, $J_{CH,CH}$ = 8.3 Hz, $J_{CH,CH}$ = 1.4 Hz, 2H, Ar-H), 7.84 (td, $J_{CH,CH}$ = 7.8 Hz, $J_{CH,CH}$ = 7.3 Hz $J_{CH,CH}$ = 1.4 Hz, 4H, Ar-H), 7.81 – 7.76 (m, 2H, Ar-H), 7.54 – 7.11 (m, 47H, Ar-H), 5.85 (t, $J_{H3´´,H4´´}$ = $J_{H3´´,H2´´}$ = 9.7 Hz, 1H, H-3´´), 5.67 (t, $J_{H4´´,H3´´}$ = $J_{H4´´,H5´´}$ = 9.7 Hz, 1H, H-4´´), 5.62 (dd, $J_{H2´´,H3´´}$ = 9.8 Hz, $J_{H2´´,H1´´}$ = 8.0 Hz, 1H, H-2´´), 5.21 – 5.14 (m, 3H, $CH_{Cbz,}$, CH_{Bn}), 5.11 (d, $J_{H1´´,H2´´}$ = 8.0 Hz, 1H, H-1´´), 4.95 (d, $J_{H1´,H2´}$ = 3.5 Hz, 1H, H-1´), 4.90 (d, $J_{CH,CH}$ = 11.5 Hz, 1H, CH_{Bn}), 4.71 (d, $J_{CH,CH}$ = 12.0 Hz, 1H, CH_{Bn}), 4.67 (d, $J_{CH,CH}$ = 12.2 Hz, 1H, CH_{Bn}), 4.64 (bs, 1H, H-1), 4.60 (d, $J_{CH,CH}$ = 11.9 Hz, 1H, CH_{Bn}), 4.55 (d, $J_{CH,CH}$ = 12.3 Hz, 1H, CH_{Bn}), 4.47 (d, $J_{CH,CH}$ = 17.2 Hz, 2H, NCH_{Bn}), 4.44 – 4.27 (m, 4H, H6a/b´, H6a´´, 2 ×CH_{Bn}), 4.26 – 4.20 (m, 3H, H-6b´´, 2 ×CH_{Bn}), 4.19 – 4.09 (m, 1H, H-5´), 4.02 – 3.96 (m, 3H, H-5´´, H-3´, H-4), 3.96 – 3.91 (m, 1H, H-4´), 3.91 – 3.70 (m, 5H, H-6a, H-6a/b´, H-3, H-2, H-5), 3.54 – 3.49 (m, 1H, CH_{Linker}), 3.48 (dd, $J_{H2´,H3´}$ = 9.6 Hz, $J_{H2´,H1´}$ = 3.5 Hz, 1H, H-2´), 3.41 (dd, $J_{H6b,H6a}$ = 9.5 Hz, $J_{H6b,H5}$ = 5.9 Hz, 1H, H-6b), 3.38 – 3.29 (m, 1H, CH_{Linker}), 3.27 – 3.10 (m, 2H, 2 × CH_{Linker}), 1.61 – 1.43 (m, 4H, 4 × CH_{Linker}), 1.33 – 1.15 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 166.1, 165.9, 165.2, 165.1 (4 × C=O), 156.8/156.3 (C=O-Cbz), 139.4, 138.6, 138.4, 138.3, 138.2, 138.1 137.0/136.9 (7 × Cq), 133.4 (2C), 133.3, 133.0, 129.9 (2C), 129.8 (2C), 129.7, 129.1, 129.0, 128.9, 128.6 (2C), 128.5 (2C), 128.4 (2C), 128.3, 128.0 (3C), 127.7 (2C), 127.6, 127.5, 127.4, 127.2, 126.8 (29 × C-Ar), 101.6 (C-1´´), 99.5 (C-1´), 97.7 (C-1), 81.2 (d, $J_{C6´,F}$ = 170.7 Hz, C-6´), 80.2 (C-5´´/C-3´/C-4), 79.8 (C-2´), 77.7 (d, $J_{C4´,F}$ = 5.2 Hz, C-4´), 77.3 (2C, C-5´´/C-3´/C-4, C-3/C-2/C-5), 75.2 (C-3/C-2/C-5), 74.9 (CH_{Bn}), 74.2 (CH_{Bn}), 73.4 (2C, C-3´´, CH_{Bn}), 73.1 (CH_{Bn}), 72.8 (CH_{Bn}), 72.6 (C-2´´), 72.4 (C-5´´/C-3´/C-4), 70.0 (d, $J_{C5´,F}$ = 17.3 Hz, C-5´), 69.8 (C-4´´), 69.3 (C-3/C-2/C-5), 68.1 (CH_{Linker}), 67.9 (C-6), 67.3 (CH_{Cbz}), 63.1 (C-6´´), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.2 (CH_{Linker}), 28.1/27.7 (CH_{Linker}), 23.5 (CH_{Linker}). Due to signal overlap 71 out of 101 carbon atoms were assigned in the ^{13}C spectrum.

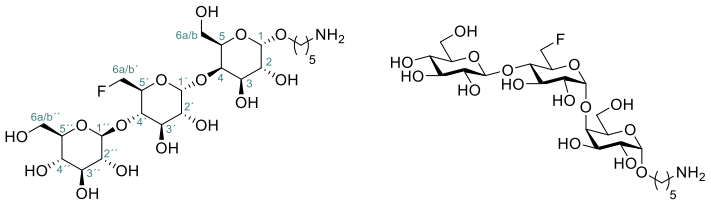
^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 234.9 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 48.1 Hz, $J_{F,H5}$ = 34.3 Hz).

^{1}H, ^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 172 Hz, $J_{C1´H1´}$ = 171 Hz, $J_{C1´´,H1´´}$ = 164 Hz.

HRMS (ESI^+): Calculated for $C_{101}H_{104}O_{21}N_2F^+$ $[M+NH_4]^+$: 1700.7144; found: 1700. 7186.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 21.80 min, λ = 230 nm.

5-Aminopentyl (β-D-glucopyranosyl)-(1→4)-(6-deoxy-6-fluoro-α-D-glucopyranosyl)-(1→4)-α-D-galacto-pyranoside (**5**)



Compound **5** was synthesized according to previously reported protocols.[194, 382]

To a magnetically stirred solution of **74** (70 mg, 41.6 µmol, 1.0 eq.) in MeOH/THF (v/v = 1:1, 8 ml), sodium methoxide (1.25 ml, 0.5 M in MeOH) was added. The solution was stirred for 18 h at ambient temperature, before being neutralized by addition of *Amberlite® IR 120*. The reaction mixture was filtered and solvents were removed under reduced pressure. The crude residue was dried for 17 h under high vacuum and subsequently dissolved in a mixture of MeOH/THF/H_2O/AcOH (v/v = 19:5:4:1, 15 ml). Pd/C (40 mg) was added under an argon atmosphere and the reaction was purged three times with hydrogen. After stirring for 72 h at ambient temperature, the catalyst was removed by filtration through a short plug of *Hyflo®* and solvents were removed under reduced pressure. The crude product was then dissolved in H_2O/MeOH (v/v = 4:1) and subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1), affording **5** (22 mg, 37.4 µmol, 90 % over two steps) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 4.88 – 4.78 (m, 2H, H-1´, H-6a´), 4.64 (d, $J_{H1,H2}$ = 3.7 Hz, 1H, H-1), 4.52 – 4.40 (m, 2H, H-6b´, H-5´), 4.13 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H-1´´), 3.86 (d, $J_{H4,H3}$ = 3.3 Hz, 1H, H-4), 3.75 (dd, $J_{H6a,H6b}$ = 10.5 Hz, $J_{H6a,H5}$ = 8.3 Hz, 1H, H-6a), 3.70 – 3.65 (m, 2H, H-6a´´, H-3), 3.62 (dd, $J_{H5,H6b}$ = 8.3 Hz, $J_{H5,H6a}$ = 5.9 Hz, 1H, H-5), 3.59 – 3.50 (m, 3H, H-2, H-3´, CH_{Linker}), 3.46 – 3.40 (m, 2H, H-6b, H-6b´´), 3.33 – 3.25

(m, 3H, H-2´, H-4´, CH_{Linker}), 3.19 – 3.13 (m, 2H, H-5´´, H-3´´), 3.05 (t, $J_{H4´´,H3´´} = J_{H4´´,H5´´}$ = 9.2 Hz, 1H, H-4´´), 2.99 (t, $J_{H2´´,H1´´} = J_{H2´´,H3´´}$ = 8.5 Hz, 1H, H-2´´), 2.63 (t, $J_{CH,CH}$ = 7.2 Hz, 2H, 2 × CH_{Linker}), 1.56 – 1.43 (m, 4H, 4 × CH_{Linker}), 1.34 (p, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH_{Linker}).

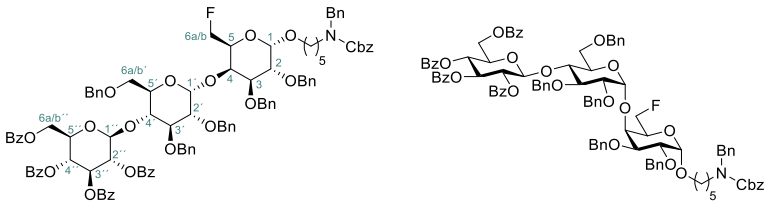
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 103.6 (C-1´´), 99.2 (C-1´), 99.0 (C-1), 81.8 (d, $J_{C6´,F}$ = 168.3 Hz, C-6´), 79.4 (d, $J_{C4´,F}$ = 4.3 Hz, C-4´), 76.9 (C-3´´/C-5´´), 76.5 (C-3´´/C-5´´), 76.4 (C-4), 73.3 (C-2´´), 71.9 (C-2´), 71.3 (C-3´), 71.0 (C-5), 70.1 (C-4´´), 68.5 (C-3), 68.4 (d, $J_{C5´,F}$ = 17.4 Hz, C-5´), 68.3 (C-2), 67.0, (CH_{Linker}), 61.0 (C-6´´), 58.7 (C-6), 40.0 (CH_{Linker}), 29.7(CH_{Linker}), 28.8(CH_{Linker}), 23.0 (CH_{Linker}).

^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 235.0 (td, $J_{F,H6}$ = 47.7 Hz, $J_{F,H5}$ = 33.6 Hz).

^{1}H-^{13}C-coupled HSQC (DMSO-d_6): $J_{C1,H1}$ = 167 Hz, $J_{C1´H1´}$ = 169 Hz, $J_{C1´´,H1´´}$ = 160 Hz.

HRMS (ESI$^+$): Calculated for $C_{23}H_{43}FNO_{15}^+$ [M+H]$^+$: 592.2611; found: 592.2608.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-deoxy-6-fluoro-α-D-galactopyranoside (**72)**



Acceptor **20** (100 mg, 90.6 µmol, 1.0 eq.) and donor **32** (101 mg, 136 µmol, 1.5 eq.) were combined and co-evaporated with toluene (2 × 10 ml). Starting materials were dried for 1 h under high vacuum and subsequently dissolved in dry CH_2Cl_2 (10 ml). Freshly activated 4 Å molecular sieve was added, and the mixture was stirred for 1 h at ambient temperature. The reaction was cooled to 0 °C and TMSOTf (1.60 µl, 9.00 µmol, 0.1 eq.) was added in one portion. After TLC monitoring indicated complete conversion of acceptor **20**, the reaction was neutralized by addition of NEt_3 (100 µl) and filtered through a pad of *Hyflo*®. The filtrate was washed with 1 M HCl (10 ml), sat. aq. $NaHCO_3$ (10 ml) and brine (10 ml) and dried with $MgSO_4$. Solvents were removed under reduced pressure and the crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 4:1) affording **72** (133 mg, 79.0 µmol, 87 %) as a colorless oil.

R_f = 0.23 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3)

Optical rotation: $[\alpha]_D^{22}$ = + 8.4 ° (c = 0.3; $CHCl_3$)

^{1}H-NMR (800 MHz, CD_2Cl_2): δ [ppm] = 7.97 – 7.95 (m, 2H, Ar-H), 7.90 – 7.87 (m, 2H, Ar-H), 7.77 – 7. 74(m, 2H, Ar-H), 7.71 – 7.69 (m, 2H, Ar-H), 7.55 – 7.52 (m, 2H, Ar-H), 7.50 – 7.47 (m, 2H, Ar-H), 7.47 – 7.09 (m, 43H, Ar-H), 5.61 (t, $J_{H3´´,H4´´} = J_{H3´´,H2´´}$ =9.5 Hz, 1H, H-3´´), 5.57 (t, $J_{H4´´,H3´´} = J_{H4´´,H5´´}$ = 9.6 Hz, 1H, H-4´´), 5.51 (dd, $J_{H2´´,H3´´}$ = 9.6 Hz, $J_{H2´´,H1´´}$ =8.1 Hz, 1H, H-2´´), 5.22 (d, $J_{CH,CH}$ = 11.0 Hz, 1H, CH_{Bn}), 5.13 (d, $J_{CH,CH}$ = 20.4 Hz, 2H, CH_{Cbz}), 4.86 (d, $J_{H1´´,H2´´}$ = 8.1 Hz, 1H, H-1´´), 4.83 (d, $J_{H1´,H2´}$ = 3.5 Hz, 1H, H-1´), 4.76 (d, $J_{CH,CH}$ = 11.1 Hz, 1H, CH_{Bn}), 4.70 (d, $J_{CH,CH}$ = 11.3 Hz, 1H, CH_{Bn}), 4.68 – 4.53 (m, 5H, H-1, H-6a, 3 × CH_{Bn}), 4.49 – 4.45 (m, 3H,

CH_{Bn}, 2× NCH_{Bn}), 4.39 (dd, $J_{H6a'',H6b''}$ = 11.9 Hz, $J_{H6a'',H5''}$ =3.3 Hz, 1H, H-6a´´), 4.38 – 4.35 (m, 1H, CH_{Bn}), 4.34 – 4.25 (m, 2H, H-6b, H-6b´´), 4.19 (d, $J_{CH,CH}$ = 11.9 Hz, 1H, CH_{Bn}), 4.15 (d, $J_{CH,CH}$ = 12.0 Hz, 1H, CH_{Bn}), 4.06 (dd, $J_{H4',H5'}$ = 10.3 Hz, $J_{H4',H3'}$ = 8.8 Hz, 1H, H-4´), 4.00 (dt, $J_{H5',H3'}$ = 10.3 Hz, $J_{H5',H6a'}$ = $J_{H5',H6b'}$ = 2.0 Hz, 1H, H-5´), 3.99 – 3.95 (m,1H, H-4), 3.89 – 3.79 (m, 3H, H-5´´, H-3´, H-5), 3.73 – 3.67 (m, 2H, H-2, H-3), 3.56 (dd, $J_{H6a',H6b'}$ = 11.0 Hz, $J_{H6a',H5'}$ = 2.3 Hz, 1H, H-6a´), 3.52 – 3.42 (m, 2H, H-2´, CH_{Linker}), 3.33 – 3.24 (m, 1H, CH_{Linker}), 3.23 – 3.15(m, 2H, 2× CH_{Linker}), 3.01 (dd, $J_{H6b',H6a'}$ = 11.0 Hz, $J_{H6b',H5'}$ = 1.6 Hz, 1H, H-6b´), 1.58 – 1.44 (m, 4H, 4× CH_{Linker}), 1.33 – 1.16 (m, 2H, 2× CH_{Linker}).

^{13}C-NMR (200 MHz, CD_2Cl_2): δ [ppm] = 166.4, 166.0, 165.6, 165.2 (4 × C=O), 157.1/156.5 (C=O-Cbz), 140.2, 139.4, 139.0, 138.9, 138.7, 137.8/137.7 (6 × Cq), 134.0, 133.8, 133.7, 133.5, 130.3, 130.2, 130.1, 130.0, 129.6 (2C), 129.5, 129.3, 129.0 (2C), 128.9 (2C), 128.8 (3C), 128.5, 128.3, 128.2 (2C), 128.1, 128.0, 127.9, 127.7, 127.5 (28 × C-Ar), 100.9 (C-1´´), 100.4 (C-1´), 98.4 (C-1), 81.6 (d, $J_{C6,F}$ = 164.4 Hz, C-6), 80.6 (C-3´), 80.2 (C-2´), 77.9 (C-4´), 77.5 (C-2/C-3), 77.2 (C-4), 75.6 (2C, CH_{Bn}, C-2/C-3), 75.0 (CH_{Bn}), 74.1(CH_{Bn}), 73.8 (2C, CH_{Bn}, C-3´´), 72.8 (2C, CH_{Bn}, C-2´´), 72.4 (C-5´´), 71.1 (C-5´), 70.4 (C-4´´), 69.3 (d, $J_{C5,F}$ = 24.9 Hz, C-5), 68.6 (CH_{Linker}), 67.8 (C-6´), 67.5 (CH_{Cbz}), 63.6 (C-6´´), 51.0/50.6 (NCH_{Bn}), 47.7/46.9 (CH_{Linker}), 29.7 (CH_{Linker}), 28.5/28.0 (CH_{Linker}), 23.9 (CH_{Linker}). Due to signal overlap 69 out of 101 carbon atoms were assigned in the ^{13}C spectrum.

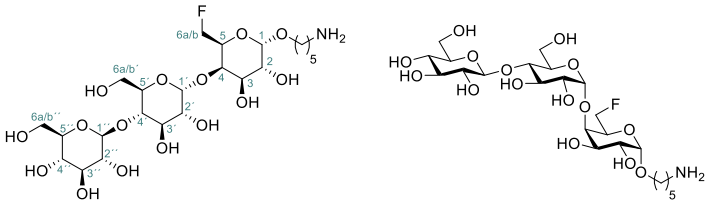
^{19}F-NMR (377 MHz, CD_2Cl_2): δ [ppm] = − 230.8 - − 231.3 (m).

^{1}H-^{13}C-coupled HSQC (CD_2Cl_2): $J_{C1,H1}$ = 171 Hz, $J_{C1´H1´}$ = 171 Hz, $J_{C1´´,H1´´}$ = 166 Hz.

HRMS (ESI$^+$): Calculated for $C_{101}H_{104}FN_2O_{21}^+$ $[M+NH_4]^+$:1700.7144; found: 1700.7185.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 25.48 min, λ = 230 nm.

5-Aminopentyl (β-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→4)-6-deoxy-6-fluoro-α-D-galacto-pyranoside (**6**)



Compound **6** was synthesized in accordance to previously described deprotection protocols.[194, 382]

To a magnetically stirred solution of the fluorinated trisaccharide **72** (100 mg, 59.4 µmol, 1.0 eq.) in a mixture of MeOH/THF (v/v = 1:1, 6 ml), sodium methoxide (1.78 ml, 1.5 M in MeOH) was added. The reaction solution was stirred for 4 h at ambient temperature, before being neutralized by addition of *Amberlite® IR120*. The reaction mixture was filtered and solvents were removed under reduced pressure. The crude residue was dried for 17 h under high vacuum. Subsequently the crude residue was dissolved in a mixture of MeOH/THF/H_2O/AcOH (v/v = 10:5:4:1, 10 ml) and Pd/C (50 mg) was added under an argon atmosphere. The reaction was purged three times with hydrogen and stirred for 27 h at ambient temperature. The catalyst was filtered off (*Hyflo®*) and the solvents were removed under reduced pressure. The crude product was dissolved in H_2O/MeOH (v/v = 4:1) and

subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1), affording **6** (32 mg, 54.5 µmol, 92 % over two steps) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 4.77 – 4.57 (m, 4H, H-1, H-1´, H-6a, H-6b), 4.23 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H-1´´), 4.10 – 4.03 (m, 1H, H-5´/H-5´´), 3.96 – 3.90 (m, 1H, H-5), 3.83 (s, 1H, H-4), 3.71 – 3.64 (m, 3H, H-3, H-6a´, H-6a´´), 3.61 – 3.53 (m, 3H, H-2, CH_{Linker}, H-6b´/H-6b´´), 3.50 (t, $J_{H3´,H2´}$ = $J_{H3´,H4´}$ = 9.2 Hz, 1H, H-3´), 3.42 (dd, $J_{H6b´/H-6b´´,H6a´/H-6a´´}$ = 11.7 Hz, $J_{H6b´/H-6b´´,H5´/H5´´}$ = 6.4 Hz, 1H, H-6b´/H-6b´´), 3.38 – 3.31(m, 2H, H-4´, CH_{Linker}), 3.29 – 3.23 (m, 1H, H-2´), 3.16 (s, 2H, H-3´´, H-5´/H-5´´), 3.06 (t, $J_{H4´´, H3´´}$ = $J_{H4´´, H5´´}$ = 9.3 Hz, 1H, H-4´´), 2.98 (t, $J_{H2´´, H1´´}$ = $J_{H2´´, H3´´}$ = 8.6 Hz, 1H, H-2´´), 2.68 (s, 2H, 2 × CH_{Linker}), 1.56 – 1.47 (m, 4H, 4 × CH_{Linker}), 1.38 – 1.32 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] =103.1 (C-1´´), 100.1 (C-1´), 99.0 (C-1), 82.8 (d, $J_{C6,F}$ = 163.2 Hz, C-6), 79.9 (C-4´), 78.4 (d, $J_{C4,F}$ = 6.4 Hz, C-4), 76.8 (C-3´´, C-5´/C-5´´), 76.5 (C-3´´, C-5´/C-5´´), 73.3 (C-2´´), 72.1 (C-2´), 71.4 (C-3´), 70.4 (C-5´/C-5´´), 70.0 (C-4´´) 69.7 (d, $J_{C5,F}$ = 21.1 Hz, C-5), 68.3 (2C, C-3, C-2), 67.2 (CH_{Linker}), 61.0 (C-6´/C-6´´), 59.7 (C-6´/C-6´´), 39.5 (CH_{Linker})*, 28.6 (CH_{Linker}), 28.5 (CH_{Linker}), 22.9 (CH_{Linker}).* Assigned from HSQC-Spectrum due to signal superimposition with Solvent peak.

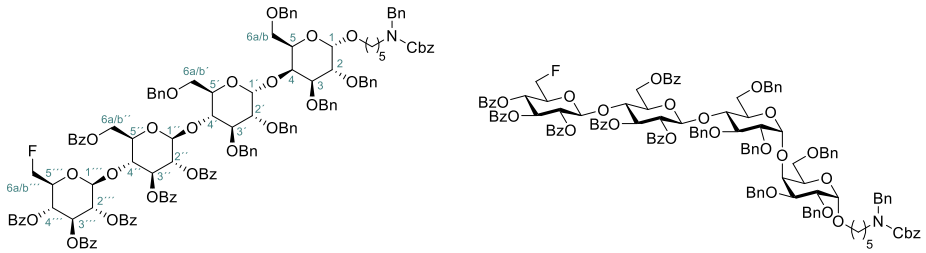
^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 227.4 (td, $J_{F,H6}$ = 47.4 Hz, $J_{F,H5}$ = 14.5 Hz).

^{1}H-^{13}C-coupled HSQC (DMSO-d_6): $J_{C1,H1}$ = 167 Hz, $J_{C1´H1´}$ = 168 Hz, $J_{C1´´,H1´´}$ = 159 Hz.

HRMS (ESI$^+$): Calculated for $C_{23}H_{43}FNO_{15}^+$ [M+H]$^+$: 592.2611; found: 592.2606

Synthesis of tetrasaccharides

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)- 2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**14**)



Donor **26** (200 mg, 180 µmol, 1.5 eq.) and acceptor **18** (143 mg, 120 µmol, 1.0 eq.) were combined, co-evaporated with dry toluene (15 ml) and dried under high-vacuum for 1 h. Subsequently, starting materials were dissolved in dry CH_2Cl_2 (10 ml) and stirred for 1 h over freshly activated 4 Å molecular sieve. The reaction was cooled to 0 °C and TMSOTf (2.19 µl, 12.0 µmol, 0.1 eq.) was added in one portion. The reaction was stirred for 1.5 h at 0 °C before another portion of TMSOTf (3.50 µl, 19.2 µmol, 0.16 eq.) was added. After further stirring for 0.5 h, the reaction was deemed complete and neutralized by addition of NEt_3(100 µl). The mixture was diluted with CH_2Cl_2, and filtered through a pad of *Hyflo*®. The filtrate was washed with 1 M HCl (10 ml), sat. aq. $NaHCO_3$ (10 ml) and

brine (10 ml) and dried over $MgSO_4$. The crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 4:1) affording **14** (225 mg, 105 µmol, 87 %) as a colorless oil.

R_f = 0.26 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3)

Optical rotation: $[\alpha]_D^{24}$ = + 12.6 ° (c = 0.3, $CHCl_3$)

^{1}H-NMR (800 MHz, CD_2Cl_2): δ [ppm] = 8.05 – 7.99 (m, 6H, Ar-H), 7.84 – 7.79 (m, 2H, Ar-H), 7.77 – 7.72 (m, 4H, Ar-H), 7.62 – 7.59 (m, 1H, Ar-H), 7.56 – 7.49 (m, 3H, Ar-H), 7.47 – 7.39 (m, 8H, Ar-H), 7.38 – 7.22 (m, 34H, Ar-H), 7.21 – 7.15 (m, 7H, Ar-H), 7.14 – 7.09 (m, 2H, Ar-H), 7.07 – 7.00 (m, 3H, Ar-H), 5.68 (t, $J_{H3''',H4'''}$ = $J_{H3''',H2'''}$ = 9.6 Hz, 1H, H-3´´´), 5.49 – 5.46 (m, 1H, H-3´´), 5.45 – 5.40 (m 2H, H-2´´´, H-2´´), 5.23 – 5.17 (m, 2H, H-4´´´, CH_{Bn}), 5.14 (d, $J_{CH,CH}$ = 20.3 Hz, 2H, CH_{Cbz}), 4.90 (d, $J_{H1',H2'}$ = 3.7 Hz, 1H, H-1´), 4.81 (d, $J_{H1''',H2'''}$ = 7.9 Hz, 1H, H-1´´´), 4.68 (d, $J_{H1'',H2''}$ = 8.0 Hz, 1H, H-1´´), 4.66 (d, $J_{CH,CH}$ = 11.2 Hz, 1H, CH_{Bn}), 4.63 (d, $J_{CH,CH}$ = 11.3 Hz, 1H, CH_{Bn}), 4.59 (s, 1H, H-1), 4.52 – 4.47 (m, 4H, 4 × CH_{Bn}), 4.46 (s, 2H, NCH_{Bn}), 4.42 (dd, $J_{H6a'',H6b''}$ = 11.8 Hz, $J_{H6a'',H5''}$ = 1.8 Hz, 1H, H-6a´´), 4.31 (d, $J_{CH,CH}$ = 12.5 Hz, 1H, CH_{Bn}), 4.28 (dd, $J_{H6b'',H6a''}$ = 11.7 Hz, $J_{H6b'',H5''}$ = 5.0 Hz, 1H, H-6b´´), 4.25 – 4.20 (m, 3H, 3 × CH_{Bn}), 4.18 (dd, $J_{H4'',H5''}$ = 9.9 Hz, $J_{H4'',H3''}$ = 8.8 Hz, 1H, H-4´´), 4.11 (d, $J_{CH,CH}$ = 11.9 Hz, 1H, CH_{Bn}), 4.06 (dt, $J_{H5',H4'}$ = 10.3 Hz, $J_{H5',H6a'}$ = $J_{H5',H6b'}$ = 2.0 Hz, 1H, H-5´), 4.02 – 3.94 (m, 3H, H-4´, H-4, H-6a´´´), 3.83 – 3.79 (m, 2H, H-3´, H-6a), 3.79 – 3.66 (m, 4H, H-2, H-3, H-5, H-6b´´´), 3.63 (dddd, $J_{H5''',F}$ = 20.5 Hz, $J_{H5''',H4'''}$ = 10.2 Hz, $J_{H5''',H6a'''}$ = 5.3 Hz, $J_{H5''',H6b'''}$ = 2.3 Hz, 1H, H-5´´´), 3.53 (dd, $J_{H6a',H6b'}$ = 10.8 Hz, $J_{H6a',H5'}$ = 2.4 Hz, 1H, H-6a´), 3.52 – 3.44 (m, 1H, CH_{Linker}), 3.43 (ddd, $J_{H5'',H4''}$ = 10.0 Hz, $J_{H5'',H6b''}$ = 4.9 Hz, $J_{H5'',H6b''}$ = 1.8 Hz, 1H, H-5´´), 3.39 – 3.34 (m, 2H, H-2´, H-6b), 3.32 – 3.24 (m, 1H, CH_{Linker}), 3.23 – 3.14 (m, 2H, 2 × CH_{Linker}), 3.01 (dd, $J_{H6b',H6a'}$ = 10.8 Hz, $J_{H6b',H5'}$ = 1.6 Hz, 1H, H-6b´), 1.55 – 1.43 (m, 4H, 4 × CH_{Linker}), 1.33 – 1.15 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (200 MHz, CD_2Cl_2): δ [ppm] = 166.2, 165.9, 165.8, 165.4, 165.3 (2C, 6 × C=O), 157.1/156.5 (C=O-Cbz), 140.2, 139.4, 139.1, 139.0, 138.9, 138.8, 138.6 137.8/137.7 (8 × Cq), 134.1 (2C), 133.8 (2C), 133.7, 133.6, 130.4 (2C), 130.3, 130.2 (2C), 130.1, 129.7, 129.4, 129.2 (3C), 129.1, 129.0 (3C), 128.9 (3C), 128.8 (3C), 128.7 (2C), 128.6, 128.4, 128.3, 128.2 (2C), 128.1, 128.0 (2C), 127.9 (2C), 127.7, 127.6, 127.3 (42 × C-Ar), 101.0 (C-1´´´), 100.7 (C-1´´), 99.9 (C-1´), 98.3 (C-1), 81.3 ($J_{C6''',F}$ = 171.3 Hz, C-6´´´), 80.7 (C-3´), 80.1 (C-2´), 77.9 (C-4´), 77.8 (C-2/C-3), 77.0 (C-4), 76.7 (C-4´´), 75.5 (C-2/C-3), 75.4 (CH_{Bn}), 74.8 (CH_{Bn}), 74.0 (C-3´´), 73.7 (2C, 2 × CH_{Bn}), 73.6 (d, $J_{C5''',F}$ = 19.8 Hz, C-5´´´), 73.4 (2C, C-3´´´, C-5´´), 73.2 (CH_{Bn}), 72.9 (C-2´´), 72.6 (CH_{Bn}), 72.3 (C-2´´´), 70.9 (C-5´), 69.8 (C-5), 68.9 (d, $J_{C4''',F}$ = 7.0 Hz, C-4´´´), 68.4 (C-6), 68.3 (CH_{Linker}), 67.9 (C-6´), 67.4 (CH_{Cbz}), 63.2 (C-6´´), 51.0/50.6 (NCH_{Bn}), 47.7/46.9 (CH_{Linker}), 29.7 (CH_{Linker}), 28.5/28.0 (CH_{Linker}), 23.9 (CH_{Linker}). Due to signal overlap 94 out of 128 carbon atoms were assigned in the ^{13}C spectrum.

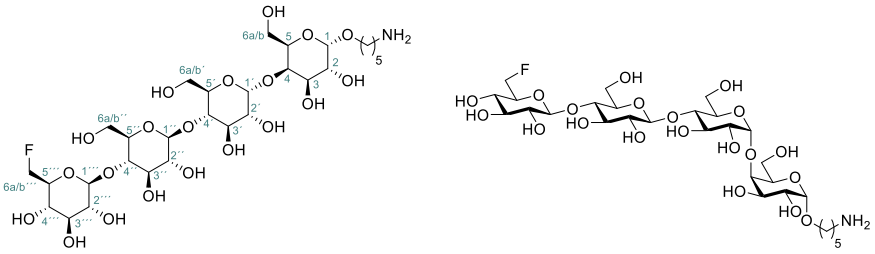
^{19}F-NMR (377 MHz, CD_2Cl_2): δ [ppm] = − 230.0 (td, $J_{F,H6a'''}$ = $J_{F,H6b'''}$ = 46.7 Hz, $J_{F,H5'''}$ = 20.3 Hz).

^{1}H, ^{13}C-coupled HSQC (CD_2Cl_2): $J_{C1,H1}$ = 169 Hz, $J_{C1'H1'}$ = 170 Hz, $J_{C1'',H1''}$ = 165 Hz, $J_{C1''',H1'''}$ = 161 Hz.

MALDI-TOF: Calculated for $C_{129}H_{129}FNO_{29}^+$ [M+H+MeOH]$^+$: 2175.87; found: 2175.90.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 25.62 min, λ = 230 nm.

5-Aminopentyl (6-deoxy-6-fluoro-β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→4)-(α-D-gluco-pyranosyl)-(1→4)-α-D-galactopyranoside (**4**)



Deprotection was conducted according to literature known proceedings.[194, 382]

To a magnetically stirred solution of tetrasaccharide **14** (110 mg, 51.4 µmol, 1.0 eq.) in a mixture of MeOH/THF (v/v = 1:1, 6 ml), sodium methoxide (1.50 ml, 0.5 M in MeOH) was added. The reaction solution was stirred for 5 h at ambient temperature, before being neutralized by addition of *Amberlite® IR 120*. The reaction mixture was filtered and solvents were removed under reduced pressure. The crude residue was dried for 17 h under high vacuum. Subsequently the crude residue was dissolved in a mixture of MeOH/THF/H_2O/AcOH (v/v = 19:5:4:1, 10 ml) and Pd/C (50 mg) was added under an argon atmosphere. The reaction was purged three times with hydrogen and stirred for 40 h at ambient temperature. The catalyst was filtered off (*Hyflo®*) and solvents were removed under reduced pressure. The crude product was dissolved in H_2O/MeOH (v/v = 4:1) and subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1), affording **4** (36 mg, 46.6 µmol, 91% over two steps) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 4.82 (d, $J_{H1´,H2´}$ = 3.7 Hz, 1H, H-1´), 4.65 (d, $J_{H1,H2}$ = 3.6 Hz, 1H, H-1), 4.62 – 4.46 (m, 2H, H-6a´´´, H-6b´´´), 4.36 (d, $J_{H1´´´,H2´´´}$ = 7.9 Hz, 1H, H-1´´´), 4.31 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H-1´´), 4.13 (dt, $J_{H5´,H6b´}$ = $J_{H5´,H4´}$ = 10.3 Hz, $J_{H5´,H6a´}$ = 3.1 Hz, 1H, H-5´), 3.87 – 3.85 (m, 1H, H-4), 3.77 (d, $J_{H6a´´,H6b´´}$ = 11.4 Hz, 1H, H-6a´´), 3.72 (dd, $J_{H6a,\ H6b}$ = 10.6 Hz, $J_{H6a,\ H5}$ = 8.0 Hz, 1H, H-6a), 3.68 (dd, $J_{H6a´,H6b´}$ = 11.9 Hz, $J_{H6a´,H5´}$ = 3.5 Hz, 1H, H-6a´), 3.66 – 3.62 (m, 2H, H-3, H-5), 3.61 – 3.52 (m, 5H, H-3´, H-2, H-6b´, H-6b´´, CH_{Linker}), 3.50 – 3.42 (m, 2H, H-5´´´, H-6b), 3.39 – 3.29 (m, 5H, CH_{Linker}, H-3´´, H-4´, H-4´´, H-5´´), 3.26 (dd, $J_{H2´,H3´}$ = 9.6, $J_{H2´,H1´}$ = 3.7 Hz, 1H, H-2´), 3.20 (t, $J_{H3´´´,H2´´´}$ = $J_{H3´´´,H4´´´}$ = 9.0 Hz, 1H, H-3´´´), 3.10 (t, $J_{H4´´´,H3´´´}$ = $J_{H4´´´,H5´´´}$ = 9.4 Hz, 1H, H-4´´´), 3.06 (t, $J_{H2´´,H1´´}$ = $J_{H2´´,H3´´}$ = 8.1 Hz, 1H, H-2´´), 3.00 (t, $J_{H2´´´,H1´´´}$ = $J_{H2´´´,H3´´´}$ = 8.5 Hz, 1H, H-2´´´), 2.67 (s, 2H, 2 × CH_{Linker}), 1.57 – 1.43 (m, 4H, 4 × CH_{Linker}), 1.35 (p, $J_{CH,CH}$ = 7.6 Hz, 2H, 2 × CH_{Linker}).

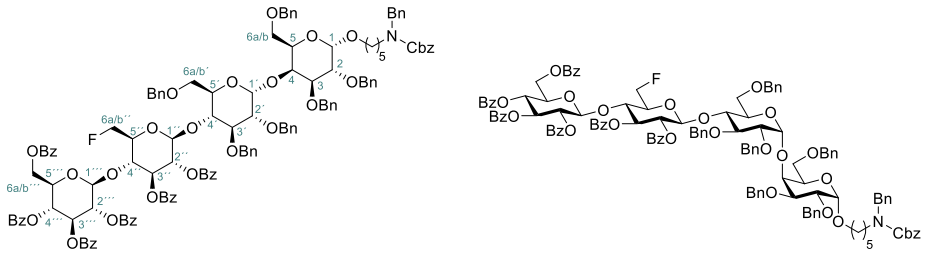
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 102.9 (C-1´´´), 102.7 (C-1´´), 99.4 (C-1´), 99.0 (C-1), 82.7 (d, $J_{C6´´´,F}$ = 169.0 Hz, C-6´´´), 79.9 (C-4´), 79.7 (C-4´´), 77.2 (C-4), 76.2 (C-3´´´), 74.8 (C-3´´/C-5´´), 74.7 (d, $J_{C5´´´,F}$ = 17.1 Hz, C-5´´´), 74.5 (C-3´´/C-5´´), 73.1 (2C, C-2´´, C-2´´´), 72.1 (C-2´), 71.4 (C-3´), 71.0 (C-3), 70.2 (C-5´), 68.8 (C-5), 68.7 ($J_{C4´´´,F}$ = 6.6 Hz, C-4´´´), 68.5 (C-2), 66.9 (CH_{Linker}), 60.2 (C-6´´), 59.7 (C-6´), 59.0 (C-6), 39.4 (CH_{Linker})*, 28.8 (CH_{Linker}), 28.7 (CH_{Linker}), 22.9 (CH_{Linker}). * Assigned from HSQC-Spectrum due to signal superimposition with solvent peak.

^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 232.1 (td, $J_{F,H6a´´´}$ = $J_{F,H6b´´´}$ = 47.8 Hz, $J_{F,H5´´´}$ = 23.4 Hz). [ppm]

^{1}H-^{13}C-coupled HSQC (DMSO-d$_6$): $J_{C1,H1}$ = 167 Hz, $J_{C1´H1´}$ = 169 Hz, $J_{C1´´,H1´´}$ = 158 Hz, $J_{C1´´´,H1´´´}$ = 161 Hz.

HRMS (ESI$^+$): Calculated for $C_{29}H_{53}O_{20}NF^+$ [M+H]$^+$: 754.3139; found: 754.3134.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-6-deoxy-6-fluoro-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**15**)



Donor **27** (210 mg, 188 µmol, 1.5 eq.) and acceptor **18** (150 mg, 126 µmol, 1.0 eq.) were combined, co-evaporated with dry toluene (15 ml) and dried under high-vacuum for 1 h. Subsequently, starting materials were dissolved in dry CH_2Cl_2 (10 ml) and stirred for 1 h over freshly activated 4 Å molecular sieve. The reaction was cooled to 0 °C and TMSOTf (2.30 µl, 12.6 µmol, 0.1 eq.) was added in one portion. The reaction was stirred for 1.5 h at 0 °C before another portion of TMSOTf (2.30 µl, 12.6 µmol, 0.1 eq.) was added. The reaction was slowly warmed to ambient temperature and stirred until complete conversion of the donor was observed *via* TLC monitoring. The reaction was stopped by addition of NEt_3 (100 µl), diluted with CH_2Cl_2, and filtered through *Hyflo*®. The filtrate was washed with 1 M HCl (10 ml), sat. aq. $NaHCO_3$ (10 ml) and brine (10 ml) and dried with $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **15** (225 mg, 104 µmol, 83 %) as a colorless oil.

R$_f$ = 0.27 (^{c}Hex/EtOAc v/v = 2:1 + 1 % NEt_3)

Optical rotation: $[\alpha]_D^{24}$ = + 7.2 ° (c = 0.3, $CHCl_3$)

^{1}H-NMR (800 MHz, $CDCl_3$): δ [ppm] = 8.05 – 8.02 (m, 2H, Ar-H), 7.91 – 7.89 (m, 2H, Ar-H), 7.88 – 7.85 (m, 2H, Ar-H), 7.77 (ddt, $J_{CH,CH}$ = 10.9 Hz, $J_{CH,CH}$ = 6.8 Hz, $J_{CH,CH}$ = 1.4 Hz, 5H, Ar-H), 7.66 – 7.63 (m, 2H, Ar-H), 7.62 – 7.58 (m, 1H, Ar-H), 7.50 – 7.09 (m, 56H, Ar-H), 5.79 (t, $J_{H3´´´,H4´´´}$ = $J_{H3´´´,H2´´´}$ = 9.7 Hz, 1H, H-3´´´), 5.57 (dd, $J_{H2´´´,H3´´´}$ = 9.9 Hz, $J_{H2´´´,H1´´´}$ = 7.9 Hz, 1H, H-2´´´), 5.44 (t, $J_{H4´´´,H3´´´}$ = $J_{H4´´´,H5´´´}$ = 9.6 Hz, 1H, H-4´´´), 5.32 (t, $J_{H3´´,H4´´}$ = $J_{H3´´,H2´´}$ = 9.3 Hz, 1H, H-3´´), 5.29 (dd, $J_{H2´´,H3´´}$ = 9.9 Hz, $J_{H2´´,H1´´}$ = 7.8 Hz, 1H, H-2´´), 5.15 (d, $J_{CH,CH}$ = 18.6 Hz, 2H, CH_{Cbz}), 5.05 (d, $J_{CH,CH}$ = 10.1 Hz, 1H, CH_{Bn}), 4.92 (d, $J_{H1´,H2´}$ = 3.7 Hz, 1H, H-1´), 4.89 (d, $J_{H1´´´,H2´´´}$ = 7.9 Hz, 1H, H-1´´´), 4.77 (d, $J_{CH,CH}$ = 11.9 Hz, 1H, CH_{Bn}), 4.68 (d, $J_{CH,CH}$ = 10.1 Hz, 1H, CH_{Bn}), 4.61 (d, $J_{CH,CH}$ = 11.9 Hz, 1H, CH_{Bn}), 4.59 (d, $J_{CH,CH}$ = 12.1 Hz, 1H, CH_{Bn}), 4.53 (t, $J_{CH,CH}$ = 11.7 Hz, 2H 2 × CH_{Bn}), 4.50 – 4.42 (m, 4H, NCH_{Bn}, H-1, H-6a´´), 4.42 – 4.34 (m, 1H, H-6b´´), 4.31 (d, $J_{H1´´,H2´´}$ = 7.8 Hz, 1H, H-1´´), 4.28 (d, $J_{CH,CH}$ = 12.5 Hz, 1H, CH_{Bn}), 4.18 – 4.10 (m, 4H, H-4´´, 3 × CH_{Bn}), 4.03 (dt, $J_{H5´,H4´}$ = 10.0 Hz, $J_{H5´,H6´}$ = 2.0 Hz, 1H, H-5´), 3.98 (s, 1H, H-4), 3.93 – 3.78 (m, 7H, H-3´, H-4´, H-5´´´, H-6a, H-6a´´´, H-6b´´´, CH_{Bn}), 3.74 – 3.66 (m, 3H, H-2, H-3, H-5), 3.48 – 3.38 (m, 3H, H-2´, H-6a´, CH_{Linker}), 3.38 – 3.33 (m, 1H, H-6b), 3.34 – 3.23 (m, 1H, CH_{Linker}),

3.19 (t, $J_{CH,CH}$ = 7.7 Hz, 1H, CH_{Linker}), 3.11 (t, $J_{CH,CH}$ = 7.6 Hz, 1H, CH_{Linker}), 2.96 – 2.85 (m, 2H, H-5´´, H-6b´), 1.56 – 1.44 (m, 4H, 4 × CH_{Linker}), 1.32 – 1.08 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (200 MHz, $CDCl_3$): δ [ppm] = 165.8 (2C), 165.4, 165.1, 164.8, 164.7 (6 × C=O), 156.8/156.3 (C=O-Cbz), 139.2, 139.0, 138.6, 138.4, 138.3, 138.1, 138.0, 137.7, 137.0/136.9 (9 × Cq), 133.8, 133.5, 133.4, 133.2, 133.1, 129.9, 129.8, 129.7 (2C), 129.6, 129.5, 129.2, 129.1 (2C), 129.0, 128.8 (2C), 128.7 (2C), 128.5, 128.4 (4C), 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4 (2C), 127.3 (34 × C-Ar), 101.2 (C-1´´´), 100.2 (C-1´´), 99.9 (C-1´), 97.9 (C-1), 80.6 (d, $J_{C6´´,F}$ = 173.7 Hz, C-6´´), 80.1 (C-3´), 79.6 (C-2´), 77.5 (C-3/C-5), 77.2 (C-4)*, 77.1 (C-4´)*, 75.7 (CH_{Bn}), 75.4 (d, $J_{C4´´,F}$ = 6.1 Hz, C-4´´), 74.9 (C-2), 74.2 (CH_{Bn}), 73.7 (CH_{Bn}), 73.6 (d, $J_{C5´´,F}$ = 19.3 Hz, C-5´´), 73.5 (CH_{Bn}), 73.2 (CH_{Bn}), 73.1 (C-3´´´/C-3´´), 73.0 (C-3´´´/C-3´´), 72.5 (CH_{Bn}), 72.3 (C-2´´), 72.2 (C-5´´´), 72.0 (C-2´´´), 70.5 (C-5´), 69.5 (C-4´´´), 69.4 (C-3/C-5), 68.1/68.0 (CH_{Linker}), 67.9 (C-6), 67.3/67.2 (CH_{Cbz}), 67.0 (C-6´), 62.7 (C-6´´´), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.2/29.1 (CH_{Linker}), 28.0/27.6 (CH_{Linker}), 23.5 (CH_{Linker}). * Assigned from HSQC spectrum due to signal superimposition with solvent peak. Due to signal overlap 87 out of 128 carbon atoms were assigned in the ^{13}C spectrum.

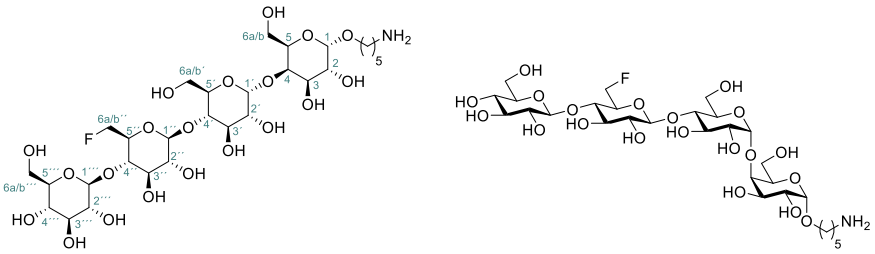
^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 230.5 (td, $J_{F,H6´´}$ = 47.8 Hz, $J_{F,H5´´}$ = 30.8 Hz).

^{1}H, ^{13}C-coupled HSQC (CD_2Cl_2): $J_{C1,H1}$ = 170 Hz, $J_{C1´H1´}$ = 169 Hz, $J_{C1´´,H1´´}$ = 164 Hz, $J_{C1´´´,H1´´´}$ = 162 Hz

MALDI-TOF: Calculated for $C_{128}H_{124}FNO_{28}^{+}$ $[M+NH_4]^+$: 2160.87; found: 2160.95.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 25.80 min, λ = 230 nm.

5-Aminopentyl-(β-D-glucopyranosyl)-(1→4)-(6-deoxy-6-fluoro-β-D-glucopyranosyl)-(1→4)-(α-D-gluco-pyranosyl)-(1→4)-α-D-galactopyranoside (**7**)



Deprotection was conducted according to literature known proceedings.[194, 382]

To a magnetically stirred solution of **15** (100 mg, 46.7 µmol, 1.0 eq.) in MeOH/THF (v/v = 1:1, 10 ml), sodium methoxide (1.40 ml, 0.5 M in MeOH) was added. The reaction solution was stirred for 17 h at ambient temperature, before being neutralized by addition of *Amberlite® IR 120*. The reaction was filtered and solvents were removed under reduced pressure. The crude residue was dried for 17 h under high vacuum. Subsequently the crude residue was dissolved in a mixture of MeOH/THF/H_2O/AcOH (v/v = 19:5:4:1, 40 ml) and Pd/C (50 mg) was added under an argon atmosphere. The reaction was purged three times with hydrogen and stirred for 40 h at ambient temperature. The catalyst was filtered off (*Hyflo®*) and solvents were removed under reduced pressure. The crude product was then dissolved in H_2O/MeOH (v/v = 4:1) and subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1), affording **7** (33 mg, 44.0 µmol, 94 % over two steps) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 4.82 (d, $J_{H1´,H2´}$ = 3.9 Hz, 1H, H-1´), 4.78 – 4.62 (m, 3H, H-6a´´, H-6b´´, H-1), 4.40 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H-1´´), 4.19 (d, $J_{H1´´´,H2´´´}$ = 7.9 Hz, 1H, H-1´´´), 4.16 (dt, $J_{H5´H4´}$ = 10.1 Hz, $J_{H5´H6a´}$ = $J_{H5´H6b´}$ = 3.2 Hz, 1H, H-5´), 3.86 (s, 1H, H-4), 3.75 – 3.62 (m, 6H, H-6a, H-6a´, H-6a´´´,H-3, H-5, H-5´´), 3.61 – 3.52 (m, 4H, CH_{Linker}, H-2, H-3´, H-6b´´´), 3.44 (dd, $J_{H6b,H6a}$ = 10.6 Hz, $J_{H6b,H5}$ = 5.8 Hz, 1H, H-6b), 3.42 – 3.37 (m, 3H, H-6b´, H-4´, H-3´´), 3.35 – 3.29 (m, 2H, H-4´´, CH_{Linker}), 3.26 (dd, $J_{H2´,H3´}$ = 9.7 Hz, $J_{H2´,H1´}$ = 3.6 Hz, 1H, H-2´), 3.21 – 3.12 (m, 2H, H-5´´´, H-3´´´), 3.07 (t, $J_{H2´´,H1´´}$ = $J_{H2´´,H3´´}$ = 8.4 Hz, 1H, H-2´´), 3.04 (t, $J_{H4´´´,H3´´´}$ = $J_{H4´´´,H5´´´}$ = 9.2 Hz, 1H, H-4´´´), 2.99 (t, $J_{H2´´´,H1´´´}$ = $J_{H2´´´,H3´´´}$ = 8.5 Hz, 1H, H-2´´´), 2.67 (s, 2H, 2 × CH_{Linker}), 1.55 – 1.46 (m, 4H, 4 × CH_{Linker}), 1.38 – 1.32 (m, 2H, 2 × CH_{Linker}).

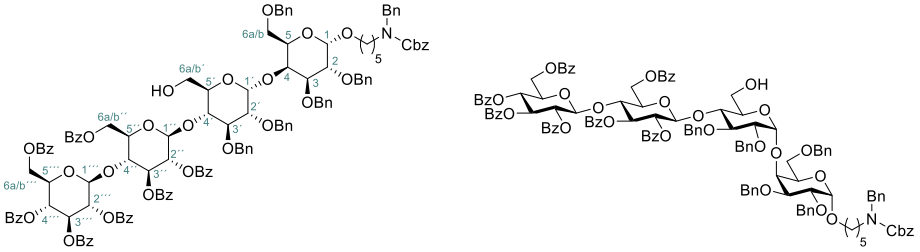
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 103.4 (C-1´´´), 102.6 (C-1´´), 99.4 (C-1´), 99.0 (C-1), 82.1 (d, $J_{C6´´,F}$ = 167.5 Hz, C-6´´), 79.8 (C-4´), 79.1(d, $J_{C4´´,F}$ = 5.5 Hz, C-4´´), 77.2 (C-4), 77.0 (C-3´´´/C-5´´´), 76.4 (C-3´´´/C-5´´´), 74.6 (C-3´´), 73.2 (C-2´´´), 72.9 – 72.8 (2C, C-2´´, C-5´´ [d, $J_{C5´´,F}$ = 16.7 Hz]), 72.2 (C-2´), 71.3 (C-3´), 71.0 (C-3/C-5), 70.1 (C-4´´´), 70.0 (C-5´), 68.8 (C-3/C-5), 68.5 (C-2), 66.9 (CH_{Linker}), 61.1 (C-6´), 59.5 (C-6´´´), 59.0 (C-6), 39.4 (CH_{Linker})*, 28.7 (CH_{Linker}), 28.6 (CH_{Linker}), 22.9 (CH_{Linker}). * Assigned from HSQC spectrum due to signal superimposition with solvent peak.

^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 231.9 (td, $J_{F,H6a´´}$ = $J_{F,H6b´´}$ = 47.5 Hz, $J_{F,H5´´}$ = 23.2 Hz).

^{1}H-^{13}C-coupled HSQC (DMSO-d_6): $J_{C1,H1}$ = 166 Hz, $J_{C1´H1´}$ = 170 Hz, $J_{C1´´,H1´´}$ = 160 Hz, $J_{C1´´´,H1´´´}$ = 161 Hz.

HRMS (ESI^{+}): Calculated for $C_{29}H_{53}O_{20}NF^{+}$ [M+H]$^{+}$: 754.3139; found: 754.3127.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**75**)



TBS cleavage was conducted in accordance with protocol.[367]

Donor **25** (420 mg, 345 µmol, 1.5 eq.) and TBS-protected acceptor **19** (280 mg, 230 µmol, 1.0 eq.) were combined, co-evaporated with dry toluene (5 ml) and dry CH_2Cl_2 (5 ml) and dried under high vacuum for 1 h. Subsequently the starting materials were dissolved in dry CH_2Cl_2 (10 ml) and freshly dried 4 Å molecular sieve was added to the reaction solution. The mixture was stirred for 1 h at ambient temperature and cooled to – 20 °C. Subsequently, TMSOTf (4.00 µl, 23.0 µmol, 0.1 eq.) was added. The reaction was allowed to slowly warm to ambient temperature, before another portion of TMSOTf (4.00 µl, 23.0 µmol, 0.1 eq.) was added after 0.5 h. The reaction was stirred for further 1.5 h before being stopped by addition of NEt_3 (100 µl). The mixture was diluted with CH_2Cl_2 and filtered through a short plug of *Hyflo*®. The organic phase was washed with 1 M HCl (10 ml), sat. aq.

$NaHCO_3$ (10 ml) and brine (10 ml) and dried with $MgSO_4$. The crude product was subjected to column chromatography (^{c}Hex/EtOAc v/v = 4:1) affording the TBS-protected tetrasaccharide intermediate as an inseparable mixture together with a decomposition product of the cellobiose donor **25**.

The thus obtained product was dissolved in CH_2Cl_2/MeOH (20 ml, v/v = 1:1) and *p*-TsOH (44.0 mg, 231 µmol, 1.0 eq.) was added. The reaction was warmed to 50 °C and stirred for 6 h, before being neutralized by addition of NEt_3 (200 µl). Solvents were removed under reduced pressure and the crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **75** (150 µmol, 65 % over two steps) as an amorphous solid.

R_f = 0.30 (^{c}Hex/EtOAc v/v = 2:1).

Optical rotation: $[\alpha]_D^{24}$ = + 30.1 ° (c = 0.5, $CHCl_3$)

^{1}H-NMR (800 MHz, $CDCl_3$): δ [ppm] = 8.00 –7.97 (m, 4H, Ar-H), 7.96 – 7.92 (m, 4H, Ar-H), 7.84 (d, $J_{CH,CH}$ = 7.7 Hz, 2H, Ar-H), 7.74 (t, $J_{CH,CH}$ = 7.6 Hz, 4H, Ar-H), 7.55 – 7.50 (m, 2H, Ar-H), 7.47 (t, $J_{CH,CH}$ = 7.3 Hz, 1H, Ar-H), 7.45 – 7.11 (m, 50H, Ar-H), 6.99 (t, $J_{CH,CH}$ = 7.6 Hz, 2H, Ar-H), 6.90 (t, $J_{CH,CH}$ = 7.3 Hz, 1H, Ar-H), 5.72 (t, $J_{H3'',H4''} = J_{H3'',H2''}$ = 9.4 Hz, 1H, H-3´´), 5.63 (t, $J_{H3''',H4'''} = J_{H3''',H2'''}$ = 9.6 Hz, 1H, H-3´´´), 5.50 (dd, $J_{H2'',H3''}$ = 9.8 Hz, $J_{H2'',H1''}$ = 8.1 Hz, 1H, H-2´´), 5.45 (dd, $J_{H2''',H3'''}$ = 9.6 Hz, $J_{H2''',H1'''}$ = 7.9 Hz, 1H, H-2´´´), 5.29 (t, $J_{H4''',H3'''} = J_{H4''',H5'''}$ = 9.6 Hz, 1H, H-4´´´), 5.16 (d, $J_{CH,CH}$ = 18.7 Hz, 2H, CH_{Cbz}), 5.07 (d, $J_{CH,CH}$ = 11.4 Hz, 1H, CH_{Bn}), 5.01 (d, $J_{H1'',H2''}$ = 8.1 Hz, 1H, H-1´´), 4.82 – 4.77 (m, 3H, H-1´´´, H-1´, CH_{Bn}), 4.63 – 4.55 (m, 3H, H-1, 2 × CH_{Bn}), 4.53 – 4.48 (m, 2H, 2 × CH_{Bn}), 4.48 – 4.41 (m, 3H, NCH_{Bn}, CH_{Bn}), 4.34 – 4.28 (m, 2H, CH_{Bn}, H-6´´/H-6´´´), 4.26 – 4.16 (m, 4H, H-4´´, H-6´´/H-6´´´, 2 × CH_{Bn}), 4.02 – 3.96 (m, 2H, H-5´, H-6´´/H-6´´´), 3.91 (t, $J_{H3',H4'} = J_{H3',H2'}$ = 9.2 Hz, 1H, H-3´), 3.88 (s, 1H, H-4), 3.82 – 3.62 (m, 7H, H-5´´´, H-6a, H-6´´/H-6´´´, H-4´, H-2, H-3, H-5), 3.61 – 3.56 (m, 1H, H-5´´), 3.50 – 3.44 (m, 2H, H-6a´, CH_{Linker}), 3.41 (dd, $J_{H6b,H6a}$ = 9.4 Hz, $J_{H6b,H5}$ = 6.1 Hz, 1H, H-6b), 3.36 (dd, $J_{H2',H3'}$ = 9.8 Hz, $J_{H2',H1'}$ = 3.5 Hz, 1H, H-2´), 3.34 – 3.25 (m, 2H, H-6b´, CH_{Linker}), 3.23 – 3.19 (m, 1H, CH_{Linker}), 3.16 – 3.10 (m, 1H, CH_{Linker}), 1.57 – 1.40 (m, 4H, 4 × CH_{Linker}), 1.26 – 1.11 (m, 2H, 2 × CH_{Linker}).

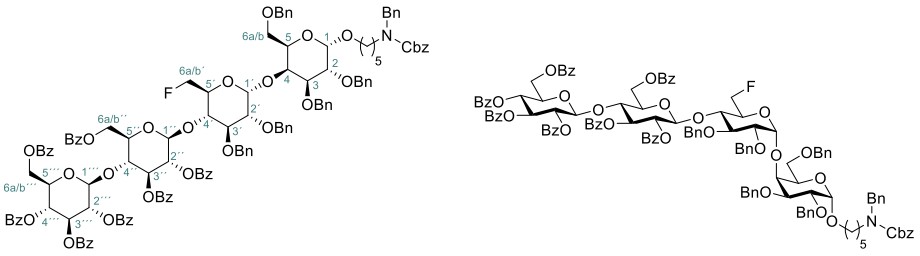
^{13}C-NMR (200 MHz, $CDCl_3$): δ [ppm] = 165.8, 165.7 (2C), 165.5, 165.2, 165.1, 164.7 (7 × C=O), 156.8/156.3 (C=O-Cbz), 139.4, 138.6, 138.5, 138.3, 138.1, 138.0, 137.0/136.9 (7 × Cq), 133.5 (2C), 133.3, 133.2, 130.1, 129.9, 129.8 (3C), 129.7, 129.6, 129.1, 128.8, 128.7, 128.6 (3C), 128.5, 128.4 (2C), 128.3 (2C), 128.1, 128.0, 127.9 (3C), 127.7, 127.6 (2C), 127.5, 127.4, 127.3, 126.9, 126.5 (35 × C-Ar), 101.4 (C-1´´), 100.8 (C-1´´´), 99.5 (C-1´), 97.7 (C-1), 80.4 (C-3´), 79.9 (C-2´), 78.3 (C-4´), 77.8 (C-4), 77.4 (C-5)*, 76.0 (C-4´´), 75.3 (C-2), 74.8 (CH_{Bn}), 74.0 (CH_{Bn}), 73.5 (CH_{Bn}), 73.2 (2C, C-3´´, C-5´´), 73.0 (2C, CH_{Bn}, C-3´´´), 72.6 (2C, CH_{Bn}, C-2´´),72.4 (C-5´´´), 71.9 (C-2´´´), 71.0 (C-5´), 69.6 (C-3), 69.5 (C-4´´´), 68.5 (C-6), 68.1/68.0 (CH_{Linker}), 67.3/67.2 (CH_{Cbz}), 62.7 (C-6´´/C-6´´´), 62.5 (C-6´´/C-6´´´), 60.5 (C-6´), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2/29.1 (CH_{Linker}), 28.0/27.6 (CH_{Linker}), 23.5 (CH_{Linker}). * Assigned from HSQC spectrum due to signal superimposition with solvent peak. Due to signal overlap 86 out of 128 carbon atoms were assigned in the ^{13}C spectrum.

^{1}H-^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 169 Hz, $J_{C1'H1'}$ = 168 Hz, $J_{C1'',H1''}$ = 160 Hz, $J_{C1''',H1'''}$ = 162 Hz.

MALDI-TOF: Calculated for $C_{129}H_{128}FNO_{31}^+$ [M+H+MeOH]$^+$: 2187.85; found: 2188.76.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 22.72 min, λ = 230 nm.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**16**)



Fluorination was conducted in accordance with previously described protocols.[324, 325]

In a microwave vessel equipped with a stirring bar, **75** (140 mg, 69.6 µmol, 1.0 eq.) was dissolved in dry CH_2Cl_2 (2 ml). Subsequently, 2,4,6-collidine (28.0 µl, 0.21 mmol, 3.0 eq.) and DAST (14.0 µl, 0.10 mmol, 1.5 eq.) were added. The reaction solution was heated for 1 h in a microwave oven (80 °C, 100 W). Since the reaction was not deemed complete by TLC monitoring, another portion of 2,4,6-collidine (28.0 µl, 0.21 mmol, 3.0 eq.) and DAST (14.0 µl, 0.10 mmol, 1.5 eq.) were added and microwave heating was continued for another 1 h. Subsequently the reaction was cooled to 0 °C and stopped by addition of MeOH (2 ml). Organic solvents were removed under reduced pressure. The crude residue was dissolved in CH_2Cl_2 (20 ml) and washed with sat. aq. $NaHCO_3$ solution (10 ml) and brine (10 ml) and dried with $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 4:1) affording **16** (112 mg, 51.9 µmol, 75 %) as a colorless foam.

R_f = 0.43 (^{c}Hex/EtOAc v/v = 2:1)

Optical rotation:$[\alpha]_D^{24}$ = + 30.6 ° (c = 0.3, $CHCl_3$)

^{1}H-NMR (800 MHz, $CDCl_3$): δ [ppm] = 7.97 – 7.94 (m, 4H, Ar-H), 7.93 – 7.90 (m, 4H, Ar-H), 7.81 – 7.79 (m, 2H, Ar-H), 7.75 – 7.71 (m, 4H, Ar-H), 7.53 – 7.49 (m, 2H, Ar-H), 7.49 – 7.09 (m, 51H, Ar-H), 6.98 (t, $J_{CH,CH}$ = 7.6 Hz, 2H, Ar-H), 6.87 (t, $J_{CH,CH}$ = 7.3 Hz, 1H, Ar-H), 5.69 (t, $J_{H3'',H4''}$ = $J_{H3'',H2''}$ = 9.5 Hz, 1H, H-3´´), 5.64 – 5.58 (m, 1H, H-3´´´), 5.51 – 5.46 (m, 1H, H-2´´), 5.45 – 5.42 (m, 1H, H-2´´´), 5.28 (t, $J_{H4''',H3'''}$ = $J_{H4''',H5'''}$ = 9.1 Hz, 1H, H-4´´´), 5.15 (d, $J_{CH,CH}$ = 19.1 Hz, 2H, CH_{Cbz}), 5.05 (d, $J_{CH,CH}$ = 11.5 Hz, 1H, CH_{Bn}), 4.95 (d, $J_{H1'',H2''}$ = 8.0 Hz, 1H, H-1´´), 4.88 (d, $J_{H1',H2'}$ = 3.6 Hz, 1H, H-1´), 4.83 (d, $J_{CH,CH}$ = 11.6 Hz, 1H, CH_{Bn}), 4.76 (d, $J_{H1''',H2'''}$ = 7.9 Hz, 1H, H-1´´´), 4.64 (d, $J_{CH,CH}$ = 12.3 Hz, 1H, CH_{Bn}), 4.61 – 4.58 (m, 2H, H-1, CH_{Bn}), 4.53 – 4.49 (m, 2H, 2 × CH_{Bn}), 4.45 (d, $J_{CH,CH}$ = 25.2 Hz, 2H, NCH_{Bn}), 4.41 (d, $J_{CH,CH}$ = 12.0 Hz, 1H, CH_{Bn}), 4.39 – 4.36 (m, 1H, CH_{Bn}), 4.28 – 4.16 (m, 5H, 2 × CH_{Bn}, H-6a´, H-4´´, H-6a´´), 4.13 (dd, $J_{H6b'',H6a''}$ = 12.0 Hz, $J_{H6b'',H5''}$ = 4.0 Hz, 1H, H-6b´´), 4.07 (dd, $J_{H5',F}$ = 34.1 Hz, $J_{H5',H4}$ = 10.3 Hz, 1H, H-5´), 3.97 – 3.94 (m, 2H, H-6a´´´, H-4), 3.90 (t, $J_{H3',H4'}$ = $J_{H3',H2'}$ = 9.3 Hz, 1H, H-3´), 3.85 – 3.68 (m, 6H, H-6b´, H-4´, H-2, H-3, H-5, H-6a), 3.66 – 3.62 (m, 2H, H-5´´´, H-6b´´´), 3.53 (ddd, $J_{H5'',H4''}$ = 9.9, $J_{H5'',H6b''}$ = 3.9, $J_{H5'',H6a''}$ = 1.8 Hz, 1H, H-5´´), 3.50 – 3.41 (m, 1H, CH_{Linker}), 3.41 – 3.35 (m, 2H, H-2´, H-6b), 3.35 – 3.26 (m, 1H, CH_{Linker}), 3.23 – 3.16 (m, 1H, CH_{Linker}), 3.15 – 3.10 (m, 1H, CH_{Linker}), 1.60 – 1.40 (m, 4H, 4 × CH_{Linker}), 1.31 – 1.11 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (200 MHz, $CDCl_3$): δ [ppm] = 165.8 (2C), 165.7, 165.5, 165.3, 165.1, 164.7 (7 × C=O), 156.8/156.3 (C=O-Cbz), 139.3, 138.6, 138.3 (2C), 138.2 (2C), 138.1, 138.03, 137.0/136.9 (9 × Cq), 133.5 (2C), 133.3, 133.2, 130.1, 129.9, 129.8 (2C), 129.7, 129.6, 129.1, 128.8, 128.7 (2C), 128.6 (2C), 128.5, 128.4 (3C), 128.3, 128.1, 128.0 (2C), 127.7 (2C), 127.6, 127.5, 127.4, 127.3, 126.9, 126.2 (32 × C-Ar), 101.6 (C-1´´), 100.9 (C-1´´´), 99.5 (C-1´), 97.7 (C-1), 81.0 (d, $J_{C6´,F}$ = 170.9 Hz, C-6´), 80.4 (C-3´), 79.7 (C-2´), 77.7 (d, $J_{C4´,F}$ = 3.8 Hz, C-4´), 77.2 (C-3*), 77.1 (C-4*), 76.0 (C-4´´), 75.1 (C-2), 74.6 (CH_{Bn}), 74.1 (CH_{Bn}), 73.3 (2C, C-5´´, CH_{Bn}), 73.1 (2C, 2 × CH_{Bn}),73.0 (C-3´´), 72.8 (C-3´´´), 72.6 (C-2´´), 72.4 (C-5´´´), 71.9 (C-2´´´), 69.9 (d, $J_{C5´,F}$ = 16.3 Hz, C-5´), 69.6 (C-4´´´), 69.3 (C-5), 68.1/68.0 (CH_{Linker}), 67.9 (C-6), 67.3 (CH_{Cbz}), 62.7 (C-6´´´), 62.4 (C-6´´), 50.6/50.3 (NCH_{Bn}), 47.3/46.3 (CH_{Linker}), 29.2/29.1 (CH_{Linker}), 28.1/27.6 (CH_{Linker}), 23.5 (CH_{Linker}). * Assigned from HSQC spectrum due to signal superimposition with solvent peak. Due to signal overlap 85 out of 128 carbon atoms were assigned in the ^{13}C spectrum.

^{1}H-NMR (800 MHz, CD_2Cl_2): δ [ppm] = 7.98 (t, $J_{CH,CH}$ = 8.4 Hz, 4H, Ar-H), 7.92 (dd, $J_{CH,CH}$ = 11.6 Hz, $J_{CH,CH}$ = 7.7 Hz, 4H, Ar-H), 7.82 (d, $J_{CH,CH}$ = 7.7 Hz, 2H, Ar-H), 7.75 (d, $J_{CH,CH}$ = 7.8 Hz, 2H, Ar-H), 7.73 (d, $J_{CH,CH}$ = 7.8 Hz, 2H, Ar-H), 7.58 – 7.53 (m. 2H, Ar-H), 7.51 (t, $J_{CH,CH}$ = 7.3 Hz, 1H, Ar-H), 7.49 – 7.09 (m, 50H, Ar-H), 7.03 (t, $J_{CH,CH}$ = 7.5 Hz, 2H, Ar-H), 6.92 (t, $J_{CH,CH}$ = 7.4 Hz, 1H, Ar-H), 5.71 (t, $J_{H3´´,H4´´}$ = $J_{H3´´,H2´´}$ = 9.5 Hz, 1H, H-3´´), 5.67 (t, $J_{H3´´´,H4´´´}$ = $J_{H3´´´,H2´´´}$ = 9.6 Hz, 1H, H-3´´´), 5.48 (dd, $J_{H2´´,H3´´}$ = 9.8 Hz, $J_{H2´´,H1´´}$ = 8.1 Hz, 1H, H-2´´), 5.42 (dd, $J_{H2´´´,H3´´´}$ = 9.7 Hz, $J_{H2´´´,H1´´´}$ = 8.0 Hz, 1H, H-2´´´), 5.36 – 5.31 (m, 1H, H-4´´´), 5.14 (d, $J_{CH,CH}$ = 21.7 Hz, 2H, CH_{Cbz}), 5.10 (d, $J_{CH,CH}$ = 11.7 Hz, 1H, CH_{Bn}), 5.01 (d, $J_{H1´´,H2´´}$ = 8.1 Hz, 1H, H-1´´), 4.92 (d, $J_{H1´,H2´}$ = 3.6 Hz, 1H, H-1´), 4.88 – 4.82 (m, 2H, H-1´´´, CH_{Bn}), 4.72 – 4.67 (m, 1H, H-1), 4.62 – 4.58 (m, 2H, 2 × CH_{Bn}), 4.54 – 4.49 (m, 2H, 2 × CH_{Bn}), 4.46 (s, 2H, NCH_{Bn}), 4.44 – 4.39 (m, 2H, 2 × CH_{Bn}), 4.30 (d, $J_{H6a´´,H6b´´}$ = 11.7 Hz, 1H, H-6a´´), 4.28 – 4.20 (m, 4H, H-4´´, H-6a´, 2 × CH_{Bn}), 4.16 (dd, $J_{H6b´´,H6a´´}$ = 12.1, $J_{H6b´´,H5´´}$ = 4.1 Hz, 1H, H-6b´´), 4.10 (dd, $J_{H5´,F}$ = 34.1 Hz, $J_{H5´,H4´}$ = 10.3 Hz, 1H, H-5´), 4.01 (s, 1H, H-4), 3.96 (dd, $J_{H6a´´´,H6b´´´}$ = 11.6 Hz, $J_{H6a´´´,H5´´´}$ = 2.8 Hz, 1H, H-6a´´´), 3.90 (t, $J_{H3´,H4´}$ = $J_{H3´,H2´}$ = 9.3 Hz, 1H, H-3´), 3.84 – 3.67 (m, 8H, H-6b´, H-3, H-5, H-2, H-4´, H-5´´´, H-6b´´´,H-6a), 3.58 (dt, $J_{H5´´,H4´´}$ = 9.8 Hz, $J_{H5´´,H6a´´}$ = $J_{H5´´,H6b´´}$ = 2.6 Hz, 1H, H-5´´), 3.54 – 3.45 (m, 1H, CH_{Linker}), 3.41 (dd, $J_{H2´,H3´}$ = 9.7 Hz, $J_{H2´,H1´}$ = 3.6 Hz, 1H, H-2´), 3.39 – 3.35 (m, 1H, H-6b), 3.34 – 3.25 (m, 1H, CH_{Linker}), 3.24 – 3.14 (m, 2H, 2 × CH_{Linker}), 1.56 – 1.44 (m, 4H, 4 × CH_{Linker}), 1.34 – 1.21 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (200 MHz, CD_2Cl_2): δ [ppm] = 166.1, 166.0, 165.9 (2C), 165.6, 165.4, 165.3 (7 × C=O), 157.1/156.5 (C=O-Cbz), 139.9, 139.1 (2C), 138.9, 138.8, 138.5, 137.8/137.7 (7 × Cq), 134.0, 133.9, 133.8, 133.7 (2C), 130.4, 130.2, 130.1 (4C), 130.0, 129.6, 129.4, 129.3, 129.2, 129.1, 129.0 (2C), 128.9 (2C), 128.8 (3C), 128.6, 128.5, 128.3, 128.2 (2C), 128.1, 128.0, 127.9 (2C), 127.7, 127.3, 126.7 (36 × C-Ar), 101.9 (C-1´´), 101.3 (C-1´´´), 99.5 (C-1´), 98.0 (C-1), 81.6 (d, $J_{C6´,F}$ = 171.3 Hz, C-6´), 80.7 (C-3´), 80.4 (C-2´), 78.0 (d, $J_{C4´,F}$ = 5.3 Hz, C-4´), 77.5 (C-2/C-3), 77.0 (C-4), 76.6 (C-4´´), 75.9 (C-2/C-3), 75.0 (CH_{Bn}), 74.7 (CH_{Bn}), 73.8 (C-3´´), 73.6 (2C, C-3´´´, C-5´´), 73.5 (CH_{Bn}), 73.4 (CH_{Bn}), 73.1 (C-2´´), 73.0 (CH_{Bn}), 72.8 (C-5/C-5´´´), 72.4 (C-2´´´), 70.2 (d, $J_{C5´,F}$ = 17.3 Hz, C-5´), 69.9 (C-4´´´), 69.6 (C-5/C-5´´´), 68.4 (CH_{Linker}), 68.3 (C-6), 67.5/67.4 (CH_{Cbz}), 63.0 (C-6´´´), 62.6 (C-6´´), 51.0/50.6 (NCH_{Bn}), 47.7/46.9 (CH_{Linker}), 29.7 (CH_{Linker}), 28.5/28.0 (CH_{Linker}), 23.9 (CH_{Linker}). Due to signal overlap 87 out of 128 carbon atoms were assigned in the ^{13}C spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 234.9 - − 235.4 (m).

^{19}F-NMR (377 MHz, CD_2Cl_2): δ [ppm] = − 235.1 (td, $J_{F,H6´}$ = 47.8 Hz, $J_{F,H5´}$ = 35.0 Hz).

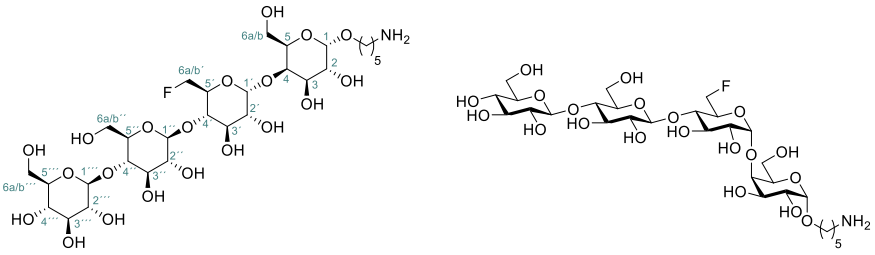
^{1}H, ^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 169 Hz, $J_{C1´H1´}$ = 170 Hz, $J_{C1´´,H1´´}$ = 160 Hz, $J_{C1´´´,H1´´´}$ = 158 Hz.

^{1}H, ^{13}C-coupled HSQC (CD_2Cl_2): $J_{C1,H1}$ = 170 Hz, $J_{C1´,H1´}$ = 172 Hz, $J_{C1´´,H1´´}$ = 162 Hz, $J_{C1´´´,H1´´´}$ = 160 Hz.

MALDI-TOF: Calculated for $C_{128}H_{122}FNO_{29}Na^+$ $[M+Na]^+$: 2179.8; found: 2179.6.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1ml/min): t_R = 24.07 min, λ = 230 nm.

5-Aminopentyl (β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→4)-(6-deoxy-6-fluoro-α-D-gluco-pyranosyl)-(1→4)-α-D-galactopyranoside (**8**)



Deprotection was conducted according to literature known proceedings.[194, 382]

To a magnetically stirred solution of **16** (46 mg, 21.3 µmol, 1.0 eq.) in MeOH/THF (v/v = 1:1, 8 ml), sodium methoxide (640 µl, 0.5 M in MeOH) was added. The reaction solution was stirred for 18 h at ambient temperature, before being neutralized by addition of *Amberlite® IR 120*. The reaction mixture was filtered and solvents were removed under reduced pressure. The crude residue was dried for 17 h under high vacuum. Subsequently it was dissolved in a mixture of MeOH/THF/H_2O/AcOH (v/v = 19:5:4:1, 20 ml) and Pd/C (40 mg) was added under an argon atmosphere. The reaction was purged three times with hydrogen and stirred for 48 h at ambient temperature. The catalyst was filtered off (*Hyflo®*) and solvents were removed under reduced pressure. The crude product was dissolved in H_2O/MeOH (v/v = 4:1) and subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1), affording **8** (15.0 mg, 19.9 µmol, 93 % over two steps) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 4.88 – 4.78 (m, 2H, H-1´, H-6a´), 4.64 (d, $J_{H1,H2}$ = 3.6 Hz, 1H, H-1), 4.52 – 4.40 (m, 2H, H-6b´, H-5´), 4.25 (d, $J_{H1´´´,H2´´´}$ = 7.9 Hz, 1H, H-1´´´), 4.22 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H-1´´), 3.86 (d, $J_{H4,H3}$ = 3.2 Hz, 1H, H-4), 3.78 (d, $J_{H6a´´,H6b´´}$ = 11.3 Hz, 1H, H-6a´´), 3.75 (t, $J_{H6a,H6b}$ = $J_{H6a,H5}$ = 9.4 Hz, 1H, H-6a), 3.70 – 3.64 (m, 2H, H-3, H-6a´´´), 3.62 (t, $J_{H5,H6a}$ = $J_{H5,H6b}$ = 7.1 Hz, 1H, H-5), 3.60 – 3.54 (m, 4H, H-6b´´, CH_{Linker}, H-2, H-3´), 3.46 – 3.39 (m, 2H, H-6b, H-6b´´´), 3.38 – 3.28 (m, 5H, H-3´´, CH_{Linker}, H-4´, H-4´´, H-5´´), 3.27 (dd, $J_{H2´,H3´}$ = 9.6 Hz, $J_{H1´,H2´}$ = 3.6 Hz, 1H, H-2´), 3.19 – 3.14 (m, 2H, H-3´´´, H-5´´´), 3.08 – 3.03 (m, 2H, H-4´´´, H-2´´), 2.98 (t, $J_{H2´´´,H1´´´}$ = $J_{H2´´´,H3´´´}$ = 8.5 Hz, 1H, H-2´´´), 2.62 (s, 2H, 2 × CH_{Linker}), 1.56 – 1.42 (m, 4H, 4 × CH_{Linker}), 1.35 (q, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH_{Linker}).

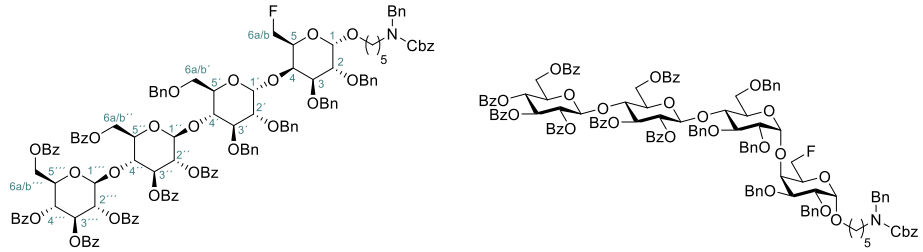
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 103.2 (C-1´´´), 103.1 (C-1´´), 99.1 (C-1´), 99.0 (C-1), 81.8 (d, $J_{C6´,F}$ = 167.7 Hz, C-6´), 79.5 (C-4´´), 79.4 (d, $J_{C4´,F}$ = 5.2 Hz, C-4´), 76.9 (C-5´´´), 76.4 (C-4), 76.3 (C-3´´´), 74.9 (C-3´´/C-5´´), 74.8 (C-3´´/C-5´´), 73.3 (C-2´´´), 73.0 (C-2´´), 71.9 (C-2´), 71.3 (C-3´), 71.0 (C-5), 70.0 (C-4´´´), 68.5 − 68.3 (3C, C-2, C-3, C-5´), 67.0 (CH_{Linker}), 61.0 (C-6´´´), 60.3 (C-6´´), 58.7 (C-6), 40.0 (CH_{Linker}), 29.8/28.8 (CH_{Linker}), 24.1/ 24.0 (CH_{Linker}), 23.0 (CH_{Linker}).

^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 235.0 (td, $J_{F,H6a'} = J_{F,H6b'} = 47.6$ Hz, $J_{F,H5'} = 33.3$ Hz).

^{1}H, ^{13}C-coupled HSQC (DMSO-d_6): $J_{C1,H1} = 167$ Hz, $J_{C1'H1'} = 169$ Hz, $J_{C1'',H1''} = 160$ Hz, $J_{C1''',H1'''} = 161$ Hz.

HRMS (ESI$^+$): Calculated for $C_{29}H_{53}FNO_{20}^+$ [M+H]$^+$: 754.3139; found: 754.3142.

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-deoxy-6-fluoro- α-D-galactopyranoside (**17**)



Donor **25** (165 mg, 136 µmol, 1.5 eq.) and acceptor **20** (100 mg, 96.6 µmol, 1.0 eq.) were combined, co-evaporated with dry toluene (5 ml) and dried under high vacuum for 1 h. Subsequently the starting materials were dissolved in dry CH_2Cl_2 (10 ml) and freshly dried 4 Å molecular sieve was added. The mixture was stirred for 40 min at ambient temperature, cooled to 0 °C and TMSOTf (4.00 µl, 23.0 µmol, 0.1 eq.) was added. The reaction was slowly warmed to room temperature and stirred for 1.5 h. Another portion of TMSOTf (10.0 µl, 57.5 µmol, 0.25 eq.) was added. The reaction was stirred for another 15 minutes before being stopped by addition of NEt_3 (100 µl). The mixture was diluted with CH_2Cl_2 and filtered through a short plug of *Hyflo*®. The filtrate was washed with 1 M HCl (10 ml), sat. aq. $NaHCO_3$ (10 ml) and brine (10 ml) and dried over $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording tetrasaccharide **17** (165 mg, 76.4 µmol, 84 %) as a colorless oil.

R_f = 0.39 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3)

Optical rotation: $[\alpha]_D^{22} = + 30.0$ ° (c = 0.33; $CHCl_3$)

^{1}H-NMR (800 MHz, $CDCl_3$): δ [ppm] = 8.07 – 8.03 (m, 2H, Ar-H), 7.94 – 7.88 (m, 6H, Ar-H), 7.75 – 7.71 (m, 4H, Ar-H), 7.69 – 7.64 (m, 2H, Ar-H), 7.51 (t, $J_{CH,CH} = 7.1$ Hz, 2H, Ar-H), 7.49 – 7.12 (m, 49H, Ar-H), 7.11 (dd, $J_{CH,CH} = 7.2$, $J_{CH,CH} = 2.3$ Hz, 2H, Ar-H), 7.02 – 6.94 (m, 3H, Ar-H), 5.65 (t, $J_{H3''',H4'''} = J_{H3''',H2'''} = 9.6$ Hz, 1H, H-3´´´), 5.53 (dd, $J_{H2''',H3'''} = 9.7$ Hz, $J_{H2''',H1'''} = 7.9$ Hz, 1H, H-2´´´), 5.35 – 5.27 (m, 3H, H-4´´´, H-2´´,H-3´´), 5.15 (d, $J_{CH,CH} = 19.9$ Hz, 2H, CH_{Cbz}), 5.11 (d, $J_{CH,CH} = 11.0$ Hz, 1H, CH_{Bn}), 4.78 (d, $J_{H1',H2'} = 3.6$ Hz, 1H, H-1´), 4.73 – 4.62 (m, 3H, H-1´´´, H-6a, CH_{Bn}), 4.62 – 4.54 (m, 3H, 3 × CH_{Bn}), 4.51 – 4.48 (m, 2H, 2 × CH_{Bn}), 4.48 – 4.43 (m, 3H, NCH_{Bn}, H-1), 4.39 – 4.35 (m, 1H, H-1´´), 4.35 – 4.31 (m, 2H, H-6a´´, CH_{Bn}), 4.32 – 4.18 (m, 2H, H-6b´´, H-6b), 4.10 (d, $J_{CH,CH} = 12.0$ Hz, 1H, CH_{Bn}), 4.06 – 4.01 (m, 1H, H-4´´), 3.95 – 3.89 (m, 4H, H-5´, H-4, H-6a´´´, CH_{Bn}), 3.88 (t, $J_{H4',H3'} = J_{H4',H5'} = 9.5$ Hz, 1H, H-4´), 3.85 – 3.78 (m, 1H, H-5), 3.76 (t, $J_{H3',H4'} = J_{H3',H2'} = 9.3$ Hz, 1H, H-3´), 3.73 – 3.68 (m, 1H, H-3), 3.65 (dd, $J_{H2,H3} = 10.0$ Hz, $J_{H2,H1} = 3.8$ Hz, 1H, H-2), 3.63 – 3.58 (m, 2H, H-5´´´, H-6b´´´), 3.49 – 3.39 (m, 2H, H-6a´, CH_{Linker}), 3.37 (dd, $J_{H2',H3'} = 9.8$ Hz, $J_{H2',H1'} = 3.6$ Hz, 1H, H-2´), 3.34 –

3.24 (m, 1H, CH_{Linker}), 3.23 – 3.18 (m, 2H, H-5´´, CH_{Linker}), 3.15 – 3.11 (m, 1H, CH_{Linker}), 2.88 (dd, $J_{H6b´,H6a´}$ = 10.7 Hz, $J_{H6b´,H5´}$ = 1.9 Hz, 1H, H-6b´), 1.65 – 1.40 (m, 4H, 4 × CH_{Linker}), 1.25 – 1.09 (m, 2H, 2 × CH_{Linker}).

^{13}C-NMR (200 MHz, $CDCl_3$): δ [ppm] = 165.8, 165.7 (2C), 165.4, 165.1, 164.9 (2C, 7 × C=O), 156.8/156.3 (C=O-Cbz), 139.5, 138.9, 138.3, 138.2, 138.1, 138.0, 137.7, 137.0/136.9 (8 × Cq), 133.6, 133.5, 133.3 (2C), 133.1, 129.9 (2C), 129.8 (2C), 129.7, 129.6, 129.2 (2C), 128.9, 128.8, 128.7 (2C), 128.6, 128.5 (3C), 128.4 (3C), 128.0, 127.8, 127.6, 127.5, 127.3 (29 × C-Ar), 100.9 (C-1´´´), 100.3 (C-1´), 100.1 (C-1´´), 98.0 (C-1), 80.8 (d, $J_{C6,F}$ = 160.8 Hz, C-6), 80.2 (C-3´), 78.9 (C-2´), 77.3 (C-4´)*, 77.1 (C-3)*, 77.0 (C-4)*, 76.3 (C-4´´), 75.0 (CH_{Bn}), 74.9 (C-2), 74.3 (CH_{Bn}), 73.8 (CH_{Bn}), 73.6 (CH_{Bn}), 73.3 (C-3´´), 72.9 (C-3´´´), 72.7 (C-5´´), 72.6 (CH_{Bn}), 72.3 (2C, C-5´´´, C-2´´), 71.9 (C-2´´´), 70.6 (C-5´), 69.7 (C-4´´´), 68.7 (d, $J_{C5,F}$ = 25.3 Hz, C-5), 68.2 (CH_{Linker}), 67.3 (CH_{Cbz}), 66.8 (C-6´), 62.8 (C-6´´), 62.7 (C-6´´´), 50.6/50.3 (NCH_{Bn}), 47.2/46.3 (CH_{Linker}), 29.2/29.1 (CH_{Linker}), 28.0/27.6 (CH_{Linker}), 23.5 (CH_{Linker}). * Assigned from HSQC spectrum due to signal superimposition with solvent peak. Due to signal overlap 81 out of 128 carbon atoms were assigned in the ^{13}C spectrum.

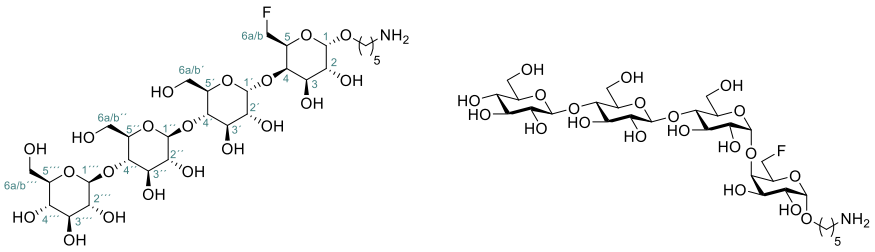
^{1}H, ^{13}C-coupled HSQC ($CDCl_3$): $J_{C1,H1}$ = 170 Hz, $J_{C1´H1´}$ = 168 Hz, $J_{C1´´,H1´´}$ = 163 Hz, $J_{C1´´´,H1´´´}$ = 163 Hz.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 230.6 - − 231.1 (m).

MALDI-TOF: Calculated for $C_{129}H_{129}FNO_{29}^{+}$ $[M+H+MeOH]^+$: 2189.85; found: 2189.95.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 10 min 100 % B, flow: 1 ml/min): t_R = 23.76 min, λ = 230 nm.

5-Aminopentyl (β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→4)-6-deoxy-6-fluoro- α-D-galactopyranoside (**9**)



Deprotection was conducted according to literature known proceedings.[194, 382]

To a magnetically stirred solution of tetrasaccharide **17** (100 mg, 46.4 µmol, 1.0 eq.) in a mixture of MeOH/THF (v/v = 1:1, 10 ml), sodium methoxide (1.40 ml, 0.5 M in MeOH) was added. The reaction solution was stirred for 8 h at ambient temperature, before being neutralized by addition of *Amberlite® IR 120*. The reaction mixture was filtered and solvents were removed under reduced pressure. The crude residue was dried for 17 h under high vacuum. Subsequently the crude residue was dissolved in a mixture of MeOH/THF/H_2O/AcOH (v/v = 19:5:4:1, 20 ml) and Pd/C (50 mg) was added under an argon atmosphere. The reaction was purged three times with hydrogen and stirred for 48 h at ambient temperature. The catalyst was removed by filtration (*Hyflo®*) and solvents were removed under reduced pressure. The crude product was dissolved in H_2O/MeOH (v/v = 4:1) and subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1), affording **9** (30 mg, 39.5 µmol, 85 % over two steps) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 4.78 – 4.57 (m, 4H, H-1, H-1´, H-6a, H-6b), 4.30 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H-1´´), 4.24 (d, $J_{H1´´´,H2´´´}$ = 7.9 Hz, 1H, H-1´´´), 4.10 – 4.07 (m, 1H, H-5´), 3.96 – 3.90 (m, 1H, H-5), 3.83 (bs, 1H, H-4), 3.77 (d, $J_{H-6a´´;H-6b´´}$ = 11.2 Hz, 1H, H-6a´´), 3.71 – 3.65 (m, 3H, H-3, H-6a´,H-6a´´´), 3.61 – 3.54 (m, 4H, H-2, H-6b´, H-6b´´, CH_{Linker}), 3.51 (t, $J_{H3´,H2´}$ = $J_{H3´,H4´}$ = 9.1 Hz, 1H, H-3´), 3.41 (dd, $J_{H6b´´´,H6a´´´}$ = 11.7, $J_{H6b´´´,H5´´´}$ = 6.6 Hz, 1H, H-6b´´´), 3.38 – 3.31 (m, 5H, H-4´, H-3´´, H-4´´, H-5´´, CH_{Linker}), 3.26 (dd, $J_{H2´,H3´}$ = 9.6 Hz, $J_{H2´,H1´}$ = 3.7 Hz, 1H, H-2´), 3.21 – 3.13 (m, 2H, H-3´´´,H-5´´´), 3.07 – 3.03 (m, 2H, H-4´´´, H-2´´), 2.98 (t, $J_{H2´´´,H1´´´}$ = $J_{H2´´´,H3´´´}$ = 8.5 Hz, 1H, H-2´´´), 2.65 (s, 2H, 2 × CH_{Linker}), 1.56 – 1.46 (m, 4H, 4 × CH_{Linker}), 1.42 – 1.29 (m, 2H, 2 × CH_{Linker}).

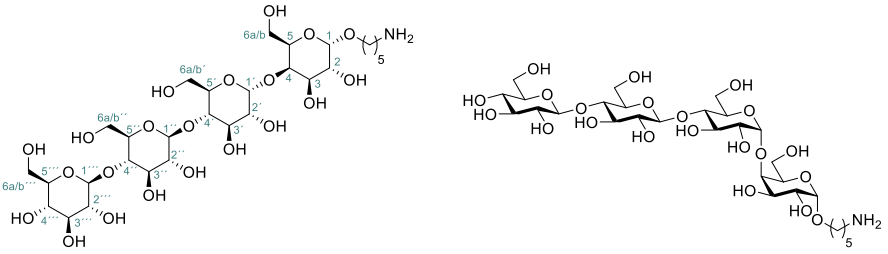
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 103.3 (C-1´´´), 102.7 (C-1´´), 100.1 (C-1´), 99.0 (C-1), 82.8 (d, $J_{C6,F}$ = 163.2Hz, C-6), 80.3 (C-4´´), 79.8 (C-4´), 78.4 (d, $J_{C4,F}$ = 5.7 Hz, C-4), 76.9 (C-5´´´), 76.5 (C-3´´´), 74.8 (2C, C-3´´, C-5´´), 73.3 (C-2´´´), 73.0 (C-4´´´/C-2´´), 72.0 (C-2´), 71.3 (C-3´), 70.4 (C-5´), 70.0 (C-4´´´/C-2´´), 69.7 (d, $J_{C5,F}$ = 21.1 Hz, C-5), 68.3 (2C, C-2, C-3), 67.2 (CH_{Linker}), 61.0 (C-6´´´), 60.3 (C-6´´), 59.6 (C-6´), 39.6 (CH_{Linker})*, 29.2 (CH_{Linker}), 28.6 (CH_{Linker}), 22.9 (CH_{Linker}). * Assigned from HSQC-Spectrum due to signal superimposition with solvent peak.

^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 227.4 (td, $J_{F,H6a}$ = $J_{F,H6b}$ = 47.1 Hz, $J_{F,H5}$ = 14.4 Hz).

^{1}H, ^{13}C-coupled HSQC (DMSO-d_6): $J_{C1,H1}$ = 169 Hz, $J_{C1´H1´}$ = 168 Hz, $J_{C1´´,H1´´}$ = 160 Hz, $J_{C1´´´,H1´´´}$ = 161 Hz.

HRMS (ESI$^+$): Calculated for $C_{29}H_{53}FNO_{20}^+$ [M+H]$^+$: 754.3139; found: 754.3128.

5-Aminopentyl (β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→4)-(α-D-glucopryranosyl)-(1→4)- α-D-galactopyranoside (**10**)



Deprotection was conducted according to literature known proceedings.[194]

To a magnetically stirred solution of **75** (120 mg, 55.6 µmol, 1.0 eq.) in a mixture of MeOH/THF (v/v = 2.5: 3.5, 7 ml), sodium methoxide (1.00 ml, 0.5 M in MeOH) was added. The reaction solution was stirred for 6 h at ambient temperature, before being neutralized by addition of *Amberlite® IR 120*. The reaction mixture was filtered and solvents were removed under reduced pressure. The crude residue was purified by column chromatography (CH_2Cl_2/MeOH = 20:1 → 10:1). The thus obtained product was dissolved in a mixture of CH_2Cl_2/^{t}BuOH/H_2O (v/v = 1:16:8 25 ml) and Pd(OH)/C (100 mg) was added under an argon atmosphere. The reaction was then purged three times with hydrogen and stirred for 24 h at ambient temperature. The catalyst was filtered off (*Hyflo®*) and solvents were removed under reduced pressure. The crude product was then dissolved in H_2O/MeOH (v/v = 4:1) and subjected to RP-phase column chromatography (H_2O/MeOH, v/v = 4:1), affording **10** (29 mg, 38.6 µmol, 69 % over two steps) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 8.14 (s, 3H, NH_3^+*), 5.42 (s, 1H, OH), 5.27 (s, 1H, OH), 5.14 (s, 1H, OH), 5.10 (s, 2H, 2 × OH), 4.83 (bs, 1H, H-1´), 4.77 (s, 1H, OH*), 4.74 – 4.66 (m, 3H, 3 × OH*), 4.65 (bs, 1H, H-1), 4.58 (s, 1H, OH*), 4.47 (s, 1H, OH*), 4.44 (s, 1H, OH*), 4.33 (d, $J_{H1´´,H-2´´}$ = 8.0 Hz, 1H, H-1´´), 4.27 (d, $J_{H1´´´,H-2´´´}$ = 7.9 Hz, 1H, H-1´´´), 4.16 – 4.09 (m, 1H, H-5´), 3.86 (bs, 1H, H-4), 3.76 (d, $J_{H6a´´,H6b´´}$ = 11.1 Hz, 1H, H-6a´´), 3.74 – 3.62 (m, 5H, H-3, H-5, H-6a, H-6a´, H-6a´´´), 3.61 – 3.50 (m, 5H, H-3´, H-2, CH_{Linker}, H-6b´´, H-6b´), 3.46 – 3.29 (m, 7H, H-6b, H-6b´´´, CH_{Linker}, H-3´´, H-4´´, H-5´´, H-4´), 3.28 – 3.24 (m, 1H, H-2´), 3.21 – 3.15 (m, 2H, H-3´´´, H-5´´´), 3.08 – 3.03 (m, 2H, H-4´´´, H-2´´), 2.99 – 2.96 (m, 1H, H-2´´´), 2.78 – 2.67 (m, 2H, 2 × CH_{Linker}), 1.62 – 1.48 (m, 4H, 4 × CH_{Linker}), 1.39 – 1.33 (m, 2H, 2 × CH_{Linker}).

^{1}H-NMR (400 MHz, DMSO-d_6/D_2O*): Anomeric Signals: δ [ppm] = 4.78 (d, $J_{H1´,H-2´}$ = 3.7 Hz, 1H, H-1´), 4.66 (d, $J_{H1,H-2}$ = 3.5 Hz, H-1), 4.30 (d, $J_{H1´´,H-2´´}$ = 7.9 Hz, 1H, H-1´´), 4.23 (d, $J_{H1´´´,H-2´´´}$ = 7.9 Hz, 1H, H-1´´´).

* To verify assignment of OH-groups and NH_2-group, D_2O was added to the NMR tube, resulting in disappearance of the OH and NH_2 signals.

^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 103.2 (C-1´´´), 102.7 (C-1´´), 99.4 (C-1´), 99.0 (C-1), 80.3 (C-4´´), 79.8 (C-4´), 77.2 (C-4), 76.8 (C-5´´´), 76.5 (C-3´´´), 74.8 (2C, C-3´´, C-5´´), 73.4 (C-2´´´), 73.1 (C-2´´), 72.1 (C-2´), 71.4 (C-3´), 70.9 (C-3), 70.2 (C-5´), 70.0 (C-4´´´), 68.8 (C-5), 68.4 (C-2), 66.8 (CH_{Linker}), 60.9 (C-6´´´), 60.2 (C-6´´), 59.5 (C-6´), 58.9 (C-6), 38.7 (CH_{Linker}), 28.6 (CH_{Linker}), 26.7 (CH_{Linker}), 22.8 (CH_{Linker}).

^{1}H, ^{13}C-coupled HSQC (DMSO-d_6): $J_{C1,H1}$ = 166 Hz, $J_{C1´H1´}$ = 169 Hz, $J_{C1´´,H1´´}$ = 160 Hz, $J_{C1´´´,H1´´´}$ = 159 Hz.

HRMS (ESI^+): Calculated for $C_{29}H_{54}O_{21}N^+$ $[M+H]^+$: 752.3183; found: 752.3274.

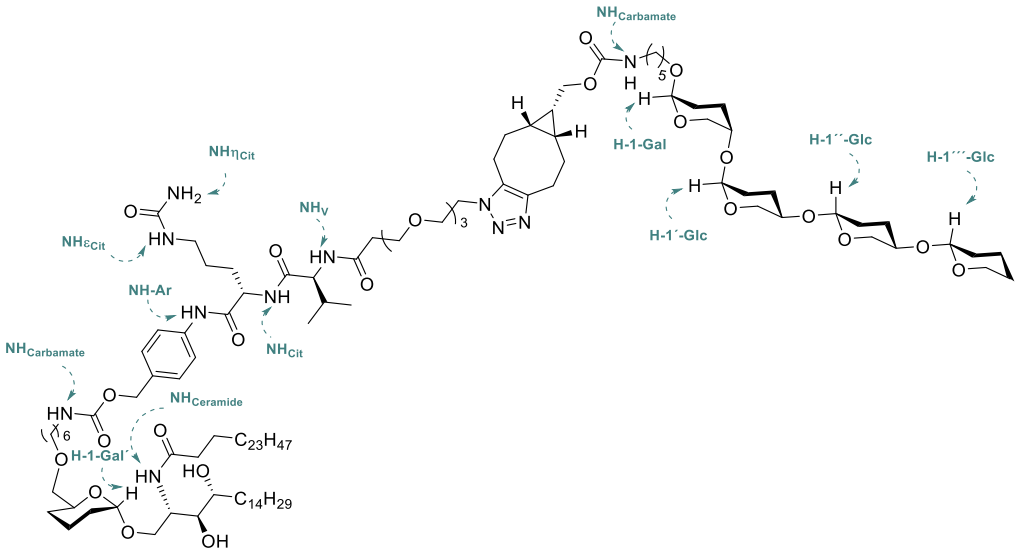
Vaccine candidate assembly

General procedure of SPAAC conjugation

Conjugation reaction was conducted following a previously described protocol.[285, 286]

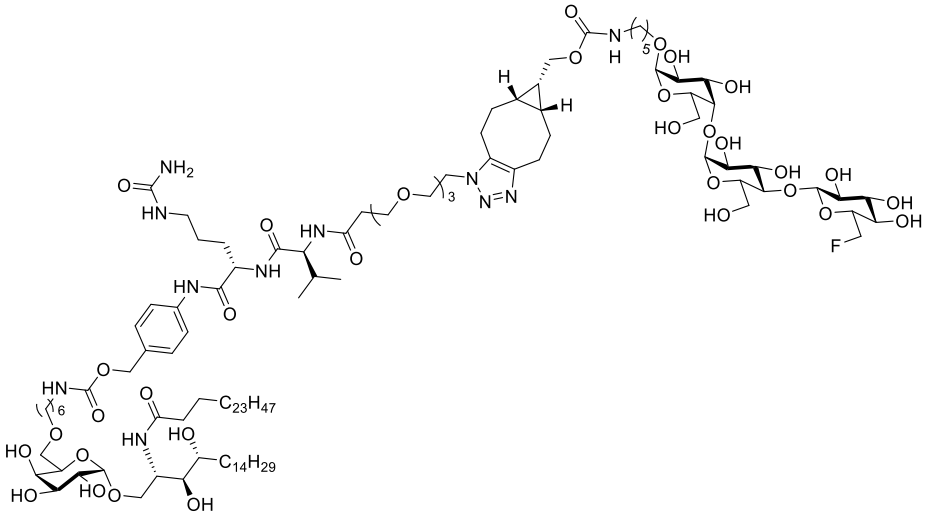
The corresponding oligosaccharide **3**, **4**, **10** (1.0 eq.) was dissolved in dry DMF (300 - 400 µl) and a solution of BCN-NHS (1.3 eq.) in dry DMF (100 µl – 200 µl) was added. Subsequently NEt_3 (2.0 eq.) was added and the reaction was stirred for 24 h at ambient temperature. The reaction was diluted by addition of H_2O (Total Volume: 10 ml) and lyophilized. The crude residue was dissolved in MeOH (0.5 ml -1 ml) and precipitated from cold Et_2O (10 ml). The white precipitate was washed with Et_2O (3 × 10ml) and dried under a gentle flow of nitrogen. The thus obtained products **77** - **79** (4.0 eq.) were dissolved in dry DMSO (200 µl – 300 µl) and added to a solution of **76** (1.0 eq.) in DMSO (300 µl). The reaction was stirred at ambient temperature for 4 d and lyophilized. The crude product was washed thoroughly with water (4 × 5ml) to afford the corresponding SPAAC conjugate was a colourless solid.

Abbreviation used in subsequent assignment of characteristic NMR signals:



Scheme 48: Description of abbreviations used in assignment of characteristic signals of lipo-polysaccharide conjugates **11 -13**. For clarity reasons, hydroxy groups of carbohydrates are not shown.

SPAAC conjugate of C6´´- fluorinated Trisaccharide **11**



According to the general procedure, 6´´- trisaccharide **3** (13.0 mg, 22.0 µmol, 1.0 eq.) was reacted with BCN-NHS ester (8.41 mg, 28.6 µmol, 1.3 eq.) and NEt_3 (6.10 µl, 44.0 µmol, 2.0 eq.) to afford the carbamate intermediate **79**. The thus obtained carbamate (4.0 eq.) was reacted with **76** (8.00 mg, 5.00 µmol, 1.0 eq.) following the general protocol. This afforded the SPAAC conjugate **11** (9.00 mg, 3.81 µmol, 76 %) as colourless solid after lyophilization.

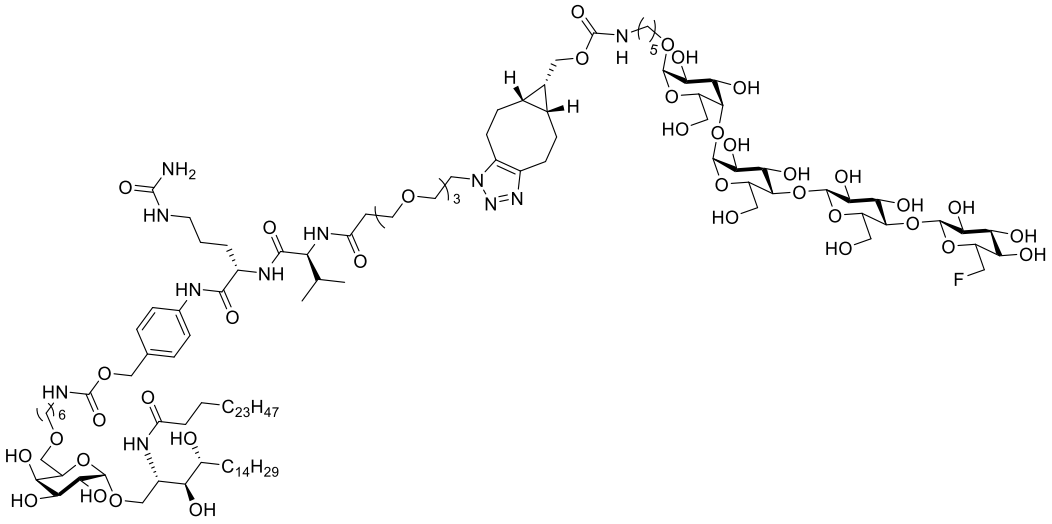
^{1}H-NMR (800 MHz, DMSO-d_6, characteristic signals): δ [ppm] = 9.99 (s, 1H, NH-Ar), 8.13 (d, $J_{NH,H\alpha}$ = 7.5 Hz, 1H, NH_{Cit}), 7.87 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH_V), 7.60 – 7.56 (m, 3H, NH-Ceramide, 2 × Ar-H), 7.26 (d, $J_{CH,CH}$ = 8.2 Hz, 2H, Ar-H), 7.17 (t, $J_{NH,CH}$ = 5.7 Hz, 1H, $NH_{Carbamate}$), 7.10 (t, $J_{NH,CH}$ = 5.7 Hz, 1H, $NH_{Carbamate}$), 5.98 (t, $J_{NH,CH}$ = 6.0 Hz, 1H, $H\varepsilon_{Cit}$), 5.41 (s, 2H, $H\eta_{Cit}$), 4.92 (s, 2H, CH_2-Ar), 4.82 (d, $J_{H1,H2}$ = 3.7 Hz, 1H, H-1-Gal/H-1-Gal´), 4.67 – 4.66 (m, 4H, H-1-Gal/H-1-Gal´, H-1´-Glc, 2 × OH), 4.62 – 4.48 (m, 5H, H6´´-6F-Glc, 3 × OH), 4.32 (d, $J_{H1,H2}$ = 7.9 Hz, 1H, H-1´´-6F-Glc´), 4.27 – 4.20 (m, 3H, $H_{V\alpha}$, 2 × OH).

^{13}C-NMR (200 MHz, DMSO-d_6, characteristic signals): δ [ppm] = 171.6 (C=O-Ceramide), 171.1 (C=O-Val), 170.6 (C=O-Cit), 170.3 (C=O-PEG), 158.9 (Cξ-Cit), 156.4 (C=O-Carbamate-BCN), 156.1 (C=O-Carbamate-dipeptide), 143.2 (C_q-Triazole), 138.6 (C_q-Ar), 133.8 (C_q-Triazole), 131.9 (C_q-Ar), 128.6, 118.9 (4 × C-Ar), 102.9 (C-1´´-F-Glc), 99.4 (C-1´-Glc), 99.3, (C-1-Gal/C-1-Gal´), 98.9 (C-1-Gal/C-1-Gal´), 82.6 (d, $J_{C6´´,F}$ = 169.7 Hz, C-6´´-F-Glc), 57.4 (V_α), 53.1 (Cit_α), 19.2 ($V_{\gamma2}$), 18.1 ($V_{\gamma1}$) 14.0 (CH_3-Ceramide).

^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 232.13 (td, $J_{F,H6a´´}$ = $J_{F,H6b´´}$ = 47.9 Hz, $J_{F,H5´´}$ = 23.7 Hz).

HRMS (ESI^+): Calculated for $C_{118}H_{210}FN_{11}O_{35}^{2+}$ $[M+2H]^{2+}$: 1180.7499; found: 1180.7494.

SPAAC conjugate of C6´´´- fluorinated Tetrasaccharide **12**



According to the general procedure, 6´´´F-tetrasaccharide **4** (20 mg, 26.5 µmol, 1.0 eq.) was reacted with BCN-NHS ester (10.0 mg, 34.5 µmol, 1.3 eq.) and NEt_3 (7.30 µl, 53.0 µmol, 2.0 eq.) to afford the carbamate intermediate **78**. The thus obtained carbamate (4.0 eq.) was reacted with **76** (10.6 mg, 6.64 µmol, 1.0 eq.) following the general protocol. This afforded the SPAAC conjugate **12** (13.0 mg, 5.15 µmol, 78 %) as colorless powder after lyophilization.

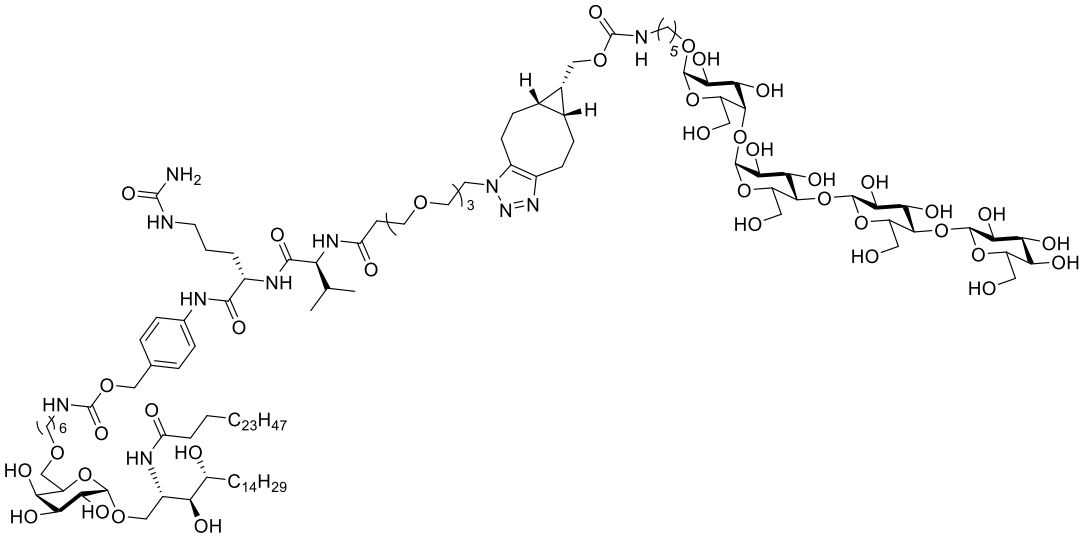
^{1}H-NMR (800 MHz, DMSO-d_6, characteristic signals): δ [ppm] = 9.98 (s, 1H, NH-Ar), 8.12 (d, $J_{NH,H\alpha}$ = 7.4 Hz, 1H, NH_{Cit}), 7.87 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH_V), 7.63 − 7.54 (m, 3H, $NH_{Ceramide}$, 2 × Ar-H), 7.31 – 7.21 (m, 2H, 2 × Ar-H), 7.16 (t, $J_{NH,CH}$ = 5.7 Hz, 1H, $NH_{Carbamate}$), 7.10 (t, $J_{NH,CH}$ = 5.8 Hz, 1H, $NH_{Carbamate}$), 6.00 – 5.95 (m, 1H, $H\varepsilon_{Cit}$), 5.41 (s, 2H, $H\eta_{Cit}$), 4.92 (s, 2H, CH_2-Ar), 4.82 (d, $J_{H1,H2}$ = 3.7 Hz, 1H, H-1-Gal/ H-1-Gal´), 4.69 – 4.62 (m, 4H, H-1-Gal/ H-1-Gal´, H-1´-Glc, 2 × OH), 4.63 – 4.49 (m, 5H, H6´´´-6F-Glc, 3 × OH), 4.37 – 4.35 (m, 2H, H-1´´´-Glc, OH), 4.30 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H1´´-Glc), 4.25 – 4.21 (m, 2H, $H_{V\alpha}$, OH).

^{13}C-NMR (200 MHz, DMSO-d_6, characteristic signals): δ [ppm] = 171.6 (C=O-Ceramide), 171.2 (C=O-Val), 170.6 (C=O-Cit), 170.3 (C=O-PEG), 158.9 (Cξ-Cit), 156.4 (C=O-Carbamate-BCN), 156.1 (C=O-Carbamate-dipeptide), 143.2 (C_q-Triazole), 138.6 (C_q-Ar), 133.8 (C_q-Triazole), 131.7 (C_q-Ar), 128.6, 118.9 (4 × C-Ar), 102.9 (C-1´´´- Glc), 102.7 (C-1´´- Glc), 99.4 (C-1-Gal´/ C-1-Gal), 99.3 (C-1-Gal´/ C-1-Gal), 98.9 (C-1´-Glc), 82.6 (d, $J_{C6´´´,F}$ = 168.4 Hz, Cc-6´´´-F-Glc), 57.4 (V_α), 53.1 (Cit_α), 19.2 ($V_{\gamma2}$), 18.1 ($V_{\gamma1}$), 14.0 (CH_3-Ceramide), 13.9 (CH_3-Ceramide).

^{19}F-NMR (377 MHz, DMSO-d_6): δ [ppm] = − 232.13 (td, $J_{F,H6a´´´}$ = $J_{F,H6b´´´}$ = 47.8 Hz, $J_{F,H5´´´}$ = 23.5 Hz).

MALDI-TOF: Calculated for $C_{124}H_{218}FN_{11}O_{40}Na^+$ $[M+Na]^+$: 2543.52; found: 2542.31.

SPAAC conjugate of tetrasaccharide **13**



According to the general procedure, tetrasaccharide **10** (19 mg, 25.3 µmol, 1.0 eq.) was reacted with BCN-NHS ester (9.6 mg, 32.9 µmol, 1.3 eq.) and NEt_3 (7.00 µl, 50.6 µmol, 2.0 eq.) to afford the carbamate intermediate **77**. The thus obtained carbamate (4.0 eq.) was reacted with **76** (10.1 mg, 6.33 µmol, 1.0 eq.) following the general protocol. This afforded the SPAAC conjugate **13** (13.0 mg, 5.15 µmol, 82 %) as colorless solid after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6, characteristic signals): δ [ppm] = 9.98 (s, 1H, NH-Ar), 8.12 (d, $J_{NH,H\alpha}$ = 7.4 Hz, 1H, NH_{Cit}), 7.87 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH_V), 7.60 – 7.56 (m, 3H, $NH_{Ceramide}$, 2 × Ar-H), 7.29 – 7.24 (m, 2H, 2 × Ar-H), 7.16 (t, $J_{NH,CH}$ = 5.7 Hz, 1H, $NH_{Carbamate}$), 7.10 (t, $J_{NH,CH}$ = 5.8 Hz, 1H, $NH_{Carbamate}$), 5.98 (t, $J_{NH,CH}$ = 5.9 Hz, 1H, $NH_{\varepsilon Cit}$), 5.43 – 5.40 (m, 3H, $H\eta_{Cit}$, OH), 4.92 (s, 2H, CH_2-Ar), 4.82 (d, $J_{H1,H2}$ = 3.7 Hz, 1H, H-1-Gal/ H-1-Gal´), 4.68 – 4.63 (m, 4H, H-1-Gal´/ H1-Gal, H-1´-Glc, 2 × OH), 4.30 (d, $J_{H1´´,H2´´}$ = 7.9 Hz, 1H, H-1´´-Glc), 4.26 – 4.21 (m, 3H, H-1´´´-Glc, $H_{V\alpha}$, OH).

^{13}C-NMR (200 MHz, DMSO-d_6, characteristic signals): δ [ppm] = 171.6 (C=O-Ceramide), 171.2 (C=O-Val), 170.6 (C=O-Cit), 170.3 (C=O-PEG), 158.9 (Cξ-Cit), 156.4 (C=O-Carbamate-BCN), 156.1 (C=O-Carbamate-dipeptide), 143.2 (C_q-Triazole), 138.6 (Cq-Ar), 133.8 (C_q-Triazole), 131.7 (C_q-Ar), 128.6, 118.9 (4 × C-Ar), 103.3 (C-1´´´- Glc), 102.7(C-1´´- Glc), 99.4 (C-1´-Glc), 99.3 (C-1-Gal/ C-1-Gal´), 98.9 (C-1-Gal/ C-1-Gal´), 57.4 (V_α), 53.1 (Cit_α), 19.2 ($V_{\gamma 2}$), 18.1 ($V_{\gamma 1}$), 14.0 (CH_3-Ceramide), 13.9 (CH_3-Ceramide).

MALDI-TOF: Calculated for $C_{124}H_{223}N_{12}O_{41}^+$ $[M+NH_4]^+$: 2536.57; found: 2535.50.

Part II:

A set of rhamnosylation specific antibodies enables the detection of novel protein glycosylations in bacteria

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II.1 Introduction

It is assumed that native proteomes exceed the complexity of predictions from genomic data analysis by three orders of magnitude.[400] This in large parts is attributed to further processing steps occurring either directly on the nascent polypeptide chain or on fully folded proteins.[401] These additional modification steps are generally referred to as post-translational modifications (PTMs) and can either occur from spontaneous reactions, or from the action of specialized enzymes.[400, 401] Further, PTMs can be divided into two categories. The first category subsumes cleavage steps within the primary peptide sequence, either by action of proteases or to a lesser extend *via* autocatalytic cleavage. Category two summarizes (enzymatic) derivatisation of existing amino acid side-chains *via* attachment of molecular moieties. The chemical nature of such covalent modifications can be highly diverse and ranges from those of small molecular weight (e.g. methyl groups or phosphates) to highly complex modifications such as oligosaccharides or small proteins (e.g. bacterial ubiquitin-like protein (Pup)).[402, 403] In principle, 15 of the 20 proteogenic amino acids provide suitable side-chain functionalities for post-translational modifications.[400, 401] However, thiols (Cys), hydroxyls (Ser, Thr) and amides (Asn) are examples for most commonly modified functional groups.[402] It is estimated that in higher eucaryotes, up to 5 % of the genome is dedicated to enzymes involved in post-translational protein modifications.[400, 401] Although bacteria may appear as simpler organisms, their proteome also comprises various PTMs from which are the most abundant are phosphorylation, acetylation and glycosylation.[401, 404] PTMs are involved in most of the major cellular processes (e.g. signal transduction, transcription regulation and pathogenesis) and their often dynamic, substoichiometric and reversible nature allows living cells to adapt without the need for protein degradation or *de-novo* protein biosynthesis.[402, 405-407]

Protein glycosylation is arguably the most complex PTM and although the understanding of bacterial glycosylation systems has significantly expanded over last decades it is still assumed that this knowledge covers only the "*tip of an iceberg*".[408] Recent discoveries of unusual glycosylation mechanism (e.g. sequential Asn glycosylation in *H. influenzae*) or glycosylation patterns (e.g. *N*-rhamnosylation/GlcNAcylation of arginine) might support this assumption and indicate a larger diversity in the bacterial glycoproteome that is yet to be discovered. In view to the development of novel antiinfectives, research dedicated towards identifying novel glycosylation patterns and sites might in turn offer new perspectives for combating bacterial infectious diseases.[409]

II.1.2 Protein glycosylation in bacteria

Protein glycosylation generally refers to covalent attachment of mono- or polysaccharide residues onto amino acid side chains. Here in particular, Ser/Thr (*O*-linked) or Asn (*N*-linked) are frequent sites for enzymatic glycan attachment.[410] Less frequently, bacterial glycosylations are for instance also found on cysteine (*S*-linked) or *N*-linked to arginine or *O*-linked to tyrosine.[411-417] In contrast to glycosylation of proteins in higher organisms, which has been accepted and studied by the scientific community since the late 1930´s, protein glycosylation in procaryotic organisms has long been overlooked. This slowly changed in 1976 with first descriptions of *O*-glycosylated *S*-layer glycoproteins of *Helobacterium salinarium*, *Clostridium thermosaccharolyticum* and *Clostridium thermohydrosulfuricum*.[418-420] More than two decades later, the first bacterial *N*-glycosylation pathway was described in *Campylobacter jejuni*, and subsequently became one of the most thoroughly studied bacterial glycosylation systems so far.[421-424] Compared to their eukaryotic counterparts, bacterial glycoproteins are more

diverse in terms of glycan composition (e.g. pseudaminic acid (Pse) or *N,N´*-Diacetylbacillosamine (diNAcBac)) and linkages.[425-428] In bacteria, glycoproteins are most frequently found on cellular surfaces or appendages (flagella or pili), although exceptions such as the glutathione synthetase in *Citrobacter rodentium* or the translation elongation factor P (EF-P) – both cytosolic proteins – have been recently discovered (*vide infra*).[402, 410, 411, 415, 424, 425, 429]

Bacterial glycosylation can be generally classified according to the biosynthetic mechanism from which the glycan-protein linkage is derived.[425] Thus, saccharides can either be transferred *en-bloc* onto the proteinogenic acceptor *via* a lipid carrier or in a sequentially utilizing soluble glycosyltransferases. For instance, protein *O*-glycosylation *via en-bloc* transfer has been described for the glycosylation of the major subunit of type IV pili (PilE) in *Neisseria meningitidis* (see Figure 14A).[430-434] This glycosylation begins with the attachment of 2,4-diacetamido-2,4,6-trideoxyhexose (DATDH) or glyceramido acetamido trideoxyhexose (GATDH) onto a lipid carrier (undecaprenyl phosphate (UndP)) catalysed by the bifunctional enzymes PglB or PglB2.[425, 432, 435] Subsequent elongation of this UndPP-monosaccharide precursor by the PglA and PglE glycosyltransferases furnishes a membrane-bound trisaccharide, which is translocated into the periplasm (flippase PglF) and subsequently transferred *en-bloc* onto a serine residue of PilE (oligosaccharyltransferase (OST): PglL).[436-440]

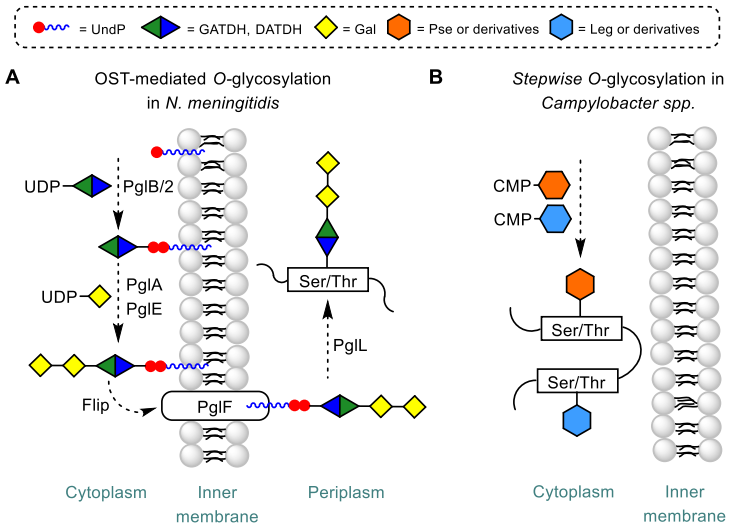


Figure 14: Examples for *en-bloc* or stepwise *O*-glycosylations in bacteria (according to *Li et al*[425]). **A**) OST-mediated *O*-glycosylation of PilE in *N. meningitidis*. The bifunctional enzymes PglB and PglB2 catalyse conversion of UDP-GlNAc to UDP-DATDH (PglB) or UDP-GATDH (PglB) and subsequent transfer onto the lipid carrier moiety. Attachment of galactose residues by the action of the PglA and PglE glycosyltransferases furnishes a membrane bound trisaccharide which is translocated into the periplasm (PglF) and transferred *en-bloc* (PglL) onto Ser/Thr of the target protein. **B**) Stepwise *O*-glycosylation of flagellin in *Campylobacter spp*. Here several surface-accessible Ser/Thr residues are *O*-glycosylation with sialic acid-like sugars.

By contrast, *Campylobacter spp.* employs a stepwise glycosylation mechanism for *O*-glycosylation of their flagellin proteins. Here, several Ser and Thr residues are modified with sialic acid-like sugars (e.g. pseudaminic acid (Pse), legionaminic acid (Leg) or derivatives thereof), which seem essential for motility and flagellin assembly (see Figure 14 B).[424, 427, 441-444] Notably, glycan attachment appears to be mainly driven by surface accessibility of the requisite Ser/Thr residues rather than by a distinct consensus sequence.[427] A related transfer mechanism was reported for the assembly of a rhamnose-linked heterogenous oligosaccharide on Ser or Thr of type a flagellin in *Pseudomonas aeruginosa*.[445] Interestingly, a truncated version of this glycan was found on flagellin of the *P. aeruginosa* JJ962 strain, consisting of a single *O*-linked rhamnose moiety.

With regard to bacterial *en-bloc N*-glycosylation systems, the prototypic oligosaccharide transferase-mediated *N*-glycosylation system of *Campylobacter jejuni* ranks amongst the best described bacterial glycosylation systems.[421, 422, 424, 446] Thus far, more than 65 proteins of *C. jejuni* have been identified to be *N*-glycosylated with more than 150 additional proteins being predicted as potential targets of this particular glycosylation system.[424, 447, 448]

C. jejuni thereby utilizes *en-bloc* transfer for the attachment of a preassembled heptasaccharide onto the amide function of asparagine located within a Asp/Glu-X_1-Asn-X_2-Ser/Thr (with X ≠ Pro) consensus sequence (see Figure 15 A).[425, 449] Cytosolic assembly of the heptasaccharide precursor starts with PglC mediated transfer of diNAcBac (derived from UDP-diNAcBac) onto a membrane bound lipid carrier (UndP), providing diNAcBac-α1-PP-Und as anchoring point for subsequent *O*-glycosylation steps.[450, 451] Further elongation *via* an orchestrated action of three distinct glycosyltransferases (PglA, PgLJ and PglH) generates a linear hexasaccharide intermediate.[424, 452-454] In a last cytosolic glycosylation step, PglI introduces a branching Glucose (Glc) residue at the central *N*-Acetylgalactosamine (GalNAc) unit to furnish the fully assembled, membrane-bound heptasaccharide.[451] Upon completion, the preassembled heptasaccharide is translocated into the periplasm by the flippase PglK, where the OST PglB catalyses the *en-bloc* transfer onto target proteins.[455-457]

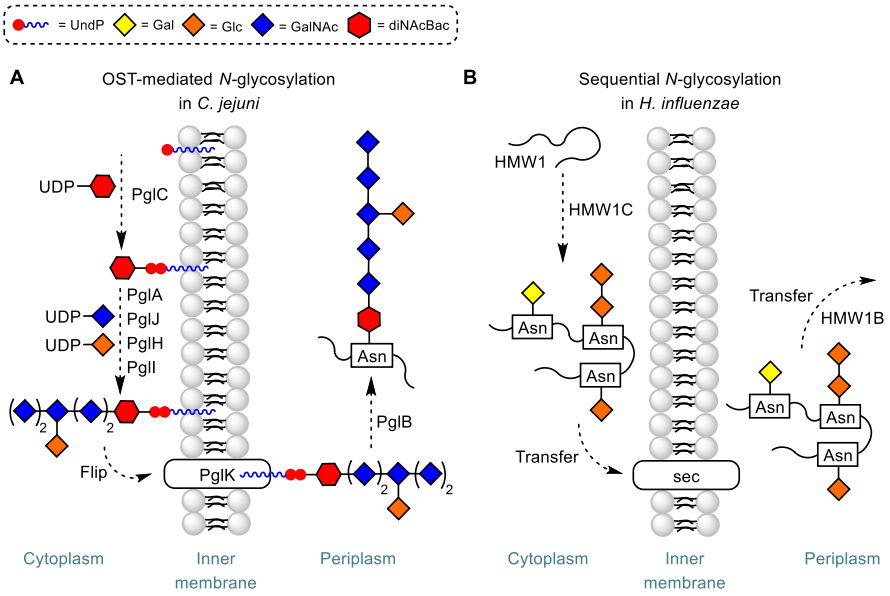


Figure 15: Overview of bacterial *N*-glycosylation pathways (according to *Lassak et al.*[401] and *Li et al.*[425]). **A**) OST mediated protein *N*-glycosylation of *C. jejuni*. Here a membrane bound heptasaccharide precursor is assembled *via* the orchestrated action of five cytosolic glycosyltransferases (PglC, PglA, PglJ, PglHa and PglI). Upon completion, the assembled heptasaccharide is translocated into periplasm (PglK), where it is transferred *en-bloc* onto a Asn residue of the target protein. **B**) Sequential *N*-glycosylation of the HMW1 protein of *H. influenzae*. Using nucleotide sugars (UDP-glucose, UDP-galactose) as donor substrates, cytosolic glycosyltransferase HMW1C mediates asparagine glycosylation of HMW1 in a sequential manner. *N*-glycosylation motifs found on HMW1 of *H. influenzae* include mono- and disaccharide residues. The latter however demands for initial attachment of a glucose residue onto Asn which subsequently is further elongated by HMW1C to form the respective disaccharide moiety. Subsequently, glycosylated HMW1 is transferred into the periplasm (sec) and further to the cell-surface *via* HMW1B.

A markedly different *N*-glycosylation pathway utilizing sequential glycan transfer has been reported for instance in *H. influenzae* (see Figure 15 B).[458, 459] Here, one of the high molecular-weight adhesin proteins (HMW1) is glycosylated with glucose (Glc) or galactose (Gal) residues at various Asn side-chains to prevent premature degradation and support bacterial-cell surface tethering.[458, 460] The corresponding glycosyltransferase HMW1C utilizes nucleotide sugars (UDP-Gal, UDP-Glc) for the attachment of mono- or disaccharide residues onto Asn, mostly in the context of a Asn-X-Ser/Thr consensus sequence. However, formation of disaccharide structures seem to require a Glc residue at the reducing end and suggests a biosynthetic pathway which starts with Glc attachment

to the Asn acceptor and subsequent hexose-hexose bond formation.[459] HMW1C thereby mediates both biosynthetic steps (Asn glycosylation and *O*-glycoside assembly) in a multifunctional fashion.[459] Glycosylated HMW1 is subsequently transferred from the cytosol into the periplasm *via* the general secretory pathway (sec) and finally to the cell-surface *via* the outer membrane translocator HMW1B.[401, 461, 462]

In addition, the *N*-glycosidic attachments of monosaccharides to the guanidine side-chain of arginine has been reported recently.[411, 414-416, 463, 464] For instance, the groups of *Hartland* and *Shao* independently reported enzymatic arginine glycosylation of host proteins by entero pathogenic *Escherichia coli.* [414, 416] Here glycosyltransferase NleB catalyses the transfer of a single *N*-Acetylglucosamine (GlcNAc) residue onto arginine, thereby blocking receptor-induced apoptosis of infected cells. Interestingly, upon *N*-GlcNAcylation of Arg^{256}, NleB also enhances activity of an intra-bacterial glutathione synthetase of *C. rodentium* thereby fulfilling an important role in regulating bacterial physiology.[415] Similarly, *Lassak et al*. first discovered the activation of EF-P *via* attachment of a rather uncommon glycosidic motif consisting of a single L-rhamnose moiety bound to arginine.[411] In the following section, this particular bacterial glycosylation will be discussed in more detail, as it encouraged our research on yet undiscovered protein rhamnosylations in bacteria (*vide infra*).

II.1.3 Arginine rhamnosylation of bacterial elongation factor P

Specialized elongation factors have evolved in bacteria, eukaryotes and archaea to alleviate ribosomal stalling and foster rapid translation of polyproline motifs.[465-473] In bacteria, elongation factor P (EF-P) resembles in its size and shape those of *t*RNA and aids proline-proline peptide bond formation *via* optimal positioning and orienting P-Site *t*RNA.[465, 474-476] Activation of EF-P frequently (recent exceptions see reference[477]) demands for PTM of positively charged and highly conserved amino acid residues located in its KOW-like *N*-domain.[9, 411, 466, 477, 478] In contrast to eukaryotes and archaea, which merely rely on (deoxy) hypusination for activation of their ortholog (initiation factor e/aIF5A), EF-P activation strategies are chemically more diverse in bacteria (see Figure 16).[476, 478] For instance, approximately 26 % of bacteria utilize an attachment of (R)-β-lysine to a conserved lysine residue for activation of EF-P.[479-482] It is worth mentioning that γ-proteobacteria (e.g. *Escherichia coli*) can further hydroxylate the modified lysine residue to eventually form (R)-β-lysylated hydroxy lysine.[411, 483] Apparat from that, 5-aminopentanolylation of lysine was described as a further activation strategy and has been observed for instance in *Bacillus subtilis* and *Staphylococcus aureus*.[484, 485] While (R)-β-lysylation and 5-aminopentanolylation are similar regarding their chemical structure, a markedly different activation strategy has been discovered in 9 % of bacteria including clinically relevant pathogens such as *Pseudomonas aeruginosa*, *Bordetella pertussis* and *Neisseria meningitidis*.[411, 463, 464] This particular activation strategy utilizes post-translationally attachment of single L-rhamnose moiety onto a highly conserved arginine residue on EF-P.

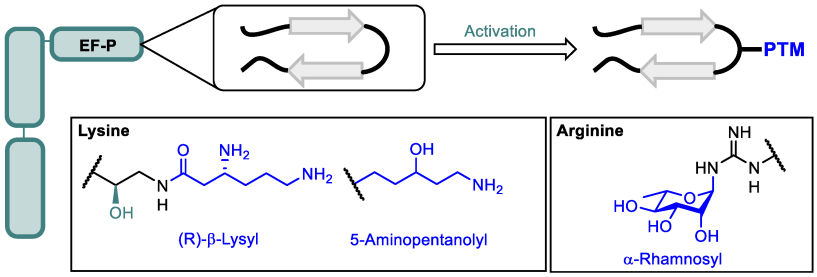


Figure 16: Post-translational modification for activation of the bacterial elongation factor P (EF-P). Known modification include attachment of (R)-β-lysine or 5-aminopentanol to a highly conserved lysine residue located in the in KOW-like domain of EF-P or α-rhamnosylation of a highly conserved arginine residue equivalently positioned in the KOW-like domain.

The inverting glycosyltransferase EarP was identified as the modifying enzyme and utilizes dTDP-β-L-rhamnose for the formation of the α-rhamnosidic linkage on arginine (see Figure 17 A).[411, 486, 487] Crystallographic and biochemical investigations on EarP from *Pseudomonas putida*, *N. meningitidis* and *P. aeruginosa* showed that EarP belongs to the GT-B superfamily with a clamp-like structure build of opposing Rossmann-like domains (see Figure 18 B).[9, 488, 489] The dTDP-β-L-rhamnose donor binding site consists of several conserved residues and was assigned either at the C-domain of EarP (*P. putida*) or within the cleft between the C-terminal and N-terminal domain (*N. meningitidis, P. aeruginosa*) respectively. Based on crystal structure analysis of EarP from *P. Putida, Krafczyk et al.* proposed a three-step catalytic cycle consisting of an initial binding of dTDP-β-L-rhamnose to the ground state of EarP with the L-rhamnose moiety adopting its common $^1C_4$ conformer.[9] Upon subsequent binding of EF-P to the *N*-terminal domain, the Arg^{32} acceptor residue on EF-P becomes glycosylated in accordance to a S_N2-like mechanism. This in turn results in a decrease of EarP/EF-P binding affinity and hence to product release from the active site. Further, *Krafczyk et al.* identified two negatively charged residues (Asp^{13}, Asp^{17}) as promising candidates for forming the catalytic active dyad for EarP-mediated rhamnosylation of EF-P.

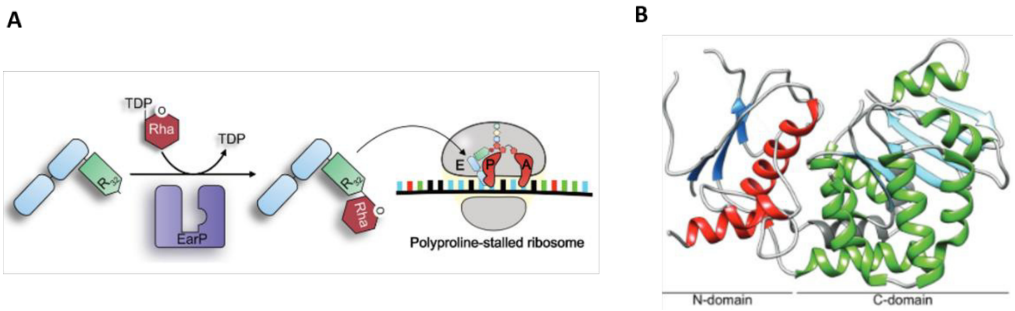


Figure 17: : Rhamnosylation of bacterial elongation factor P. **A**) Activation of EF-P *via* EarP mediated arginine rhamnosylation (Krafczyk *et al.*[9]) **B**) Ribbon representation of crystal structure of EarP from *P. putida* (Krafczyk *et al.*[9]).

A refined model for EarP mediated arginine rhamnosylation in *Neisseria meningitidis* was proposed by *Yanagisawa* and co-workers and confirmed a S_N2-like rhamnosylation mechanism with an Asp residue (Asp^{20} which corresponds to Asp^{17} of EarP from *P. putida*) serving as the general base.[488] In the binary complex (dTDP-β-L-rhamnose/EarP) the rhamnose moiety adopted its common $^1C_4$ conformation, with an equatorial alignment of the anomeric dTDP leaving group. This conformational alignment however opposes a S_N2-like nucleophilic attack (either from η-nitrogen of Arg or non-related nucleophiles) due to steric shielding of the anomeric centre. Hence, *Yanagisawa* and co-workers proposed that subsequent EF-P-binding induces rotational movement of the *N*-terminal domain (EarP) and conformational rearrangement of a conserved donor binding loop. This acceptor-binding-induced structural reorientation in turn disrupts former hydrogen bonds between EarP and the rhamnose

moiety and sterically induces a conformational distortion of the rhamnose moiety (e.g. $^{1}C_4 \rightarrow {}^{5}S_1$-non chair conformation, Figure 18). The axial positioning of the dTDP-leaving group within this newly formed non-chair conformer, facilitates a S_N2-like nucleophilic attack of the $Arg^{EF\text{-}P}$-η-nitrogen and hence formation of the observed α-*N*-rhamnosidic linkage.

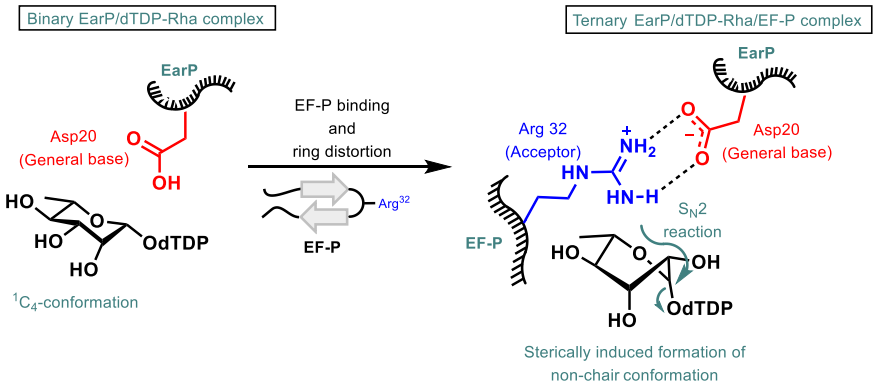


Figure 18: Simplified depiction of steric-induced conformational distortion at the rhamnose moiety as proposed by *Yanagisawa* and co-workers.[488] Upon binding of EF-P, rotational movement of the *N*-terminal domain (EarP) and conformational rearrangement of a conserved donor binding loop induces conformational distortion of the rhamnose moiety (e.g. $^{1}C_4 \rightarrow {}^{5}S_1$-non chair conformation) and thus facilitates glycosylation of arginine in a S_N2-like fashion.

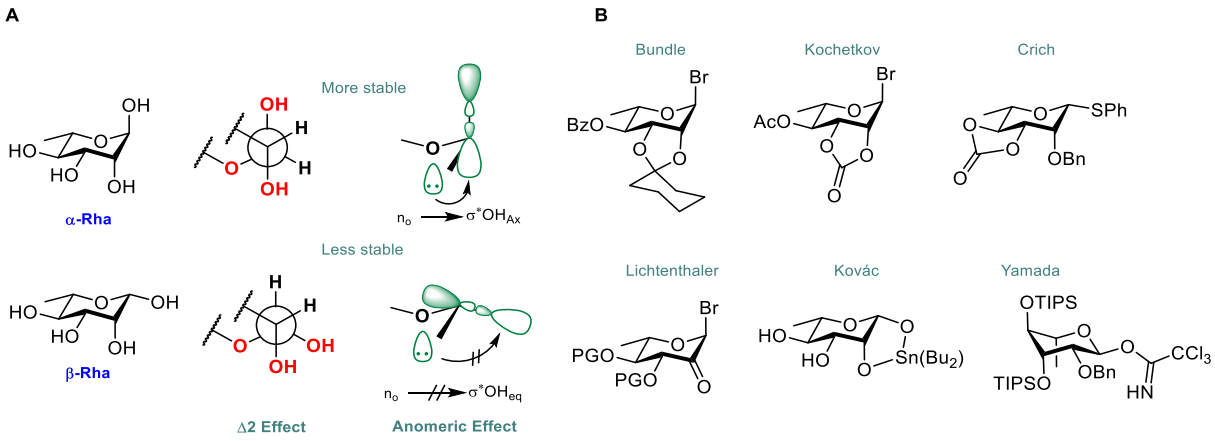
Recently, a enzymatic mechanism that comprises a stepwise structural change of EarP was suggested for EF-P rhamnosylation in *P. aeruginosa*.[489] In contrast to *N. meningitidis*, EarP mediated rhamnosylation in *P. aeruginosa* involves a more pronounced conformational rearrangement step upon donor substrate binding, which facilitates EF-P binding *via* "donor-binding-induced fit". Further conformational reorientations during formation of the ternary (EF-P/dTDP-Rha/EF-P) complex ensure proper spatial arrangement of catalytic active side chains (e.g. Asp^{16} as general base to activate the arginine acceptor) and $Arg^{EF\text{-}P}$-acceptor activation. Similarly, to *N. meningitidis*, steric constraints in the tertiary complex enforces conformational distortion of the rhamnose ring and thus aid *N*- glycosidic bond formation *via* a S_N2-like mechanism.

In terms of acceptor specificity, EF-P is the only known substrate of the EarP glycosyltransferase and recognition of a specific molecular shape, rather than a certain sequon, is held accountable for this sharp specificity.[488, 490, 491] The uncommon glycosylation motif of a single L-rhamnose moiety and its regulatory role in activating EF-P in various bacterial pathogens, stimulated further research dedicated to the question about a putatively more diverse bacterial rhamnoproteome. In regards to probing protein rhamnosylation in bacteria, chemical synthesis can provide access to defined glycan entities for the development of suitable biochemical tools (e.g. rhamnosylation-specific antibodies[9, 487]) to detect hitherto unknown rhamnoproteins in different bacterial phyla.

II.1.4 Chemical β-rhamnosylations

L-Rhamnose is a deoxy sugar frequently found in bacterial glycans (e.g. CPS, lipopolysaccharides) or occasionally in glycopeptides or -proteins.[411, 445, 463, 464, 492-501] In bacteria and archaea, L-rhamnosidic linkages are generally assembled from dTDP-β-L-rhamnose as common precursor and respective biosynthetic genes can be found in 42 % of bacterial- and 21 % of archaeal-genomes.[502] By contrast, these genes seem to be completely absent in humans and consequently biosynthetic enzymes from the rhamnose-pathway as well as rhamnosyl transferases (*vide supra*) became attractive targets for the development of novel antibacterial therapeutics.[9, 411, 503]

Furthermore, the abundance of *O*-rhamnosidic linkages in bacterial surface glycans, mark them particularly interesting targets for vaccine development and spurred the search for chemical approaches towards stereocontrolled assembly of *O*-rhamnosidic bonds.[18] Here in particular 1,2-*cis* rhamnosides (β-rhamnose) emerged as a formidable challenge, due to a lack of neighbouring group participation and unfavourable stereoelectronic effects (Δ2 effect, anomeric effect, see Scheme 49 A) that impede their controlled formation.[18, 21, 504-506] In addition, axial orientation of C-2 substituents further hampers β-glycosylation by steric hindrance.[21] In contrast to the closely related mannose system, the absence of a C-6-hydroxy group in rhamnose renders it more reactive and thus makes stereoselectivity harder to achieve. This is further reinforced by the inapplicability of 4,6-*O*-benzyliden acetal protection to gather conformational disarming as commonly applied in 1,2-*cis* mannosylation reactions.[21, 507, 508]

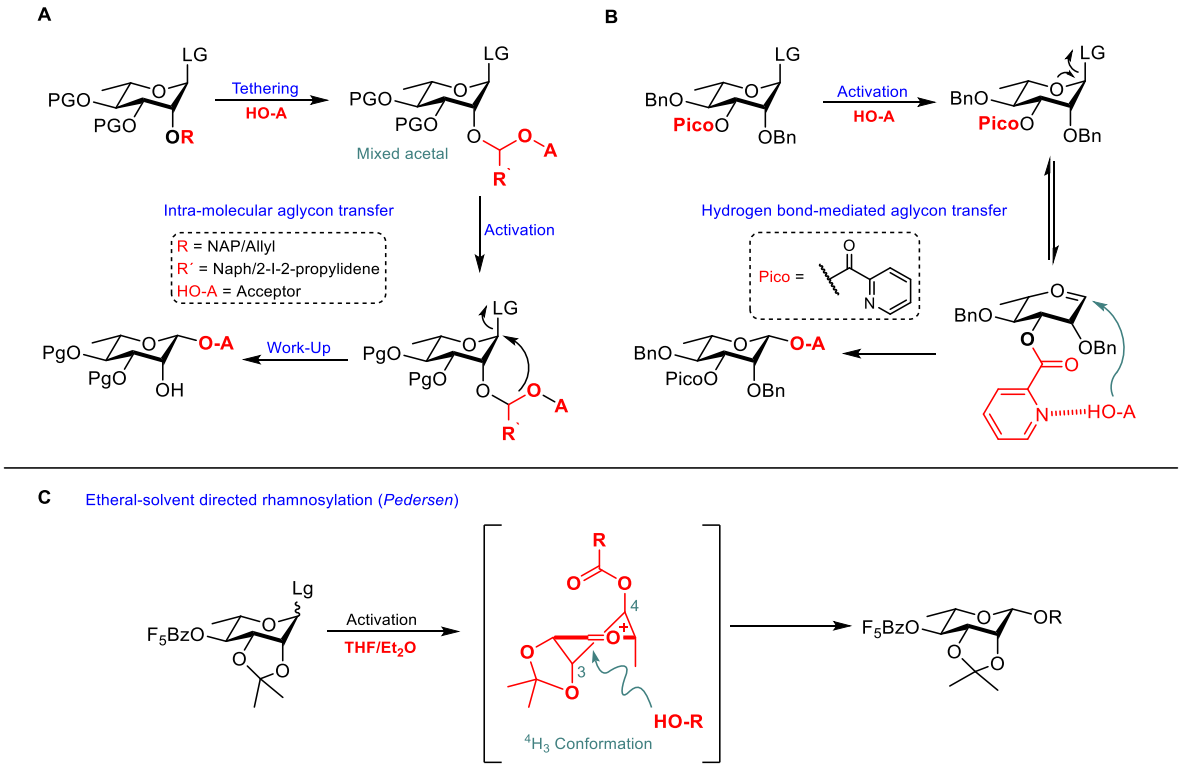


Scheme 49: **A**) Illustration of stereoelectronic effects favouring α-rhamnoside formation and impede stereoselective β-rhamnosylations: Δ2 and anomeric effect. **B**) Early examples of rhamnosyl donor building blocks facilitating the formation of β-rhamnosidic linkages. Common approaches targeted towards destabilising or supressing formation of the oxocarbenium ion intermediate and hence facilitate a S_N2-like reaction mechanism. Further approaches for β-rhamnosylations included 1,2-*cis* tethering *via* stannylen acetal formation (*Kovác*) or steric induced ring-flipping (*Yamada*).

In 1980 *Bundle* and co-workers reported a first synthetic approach towards stereoselective β-rhamnoside formation, by utilizing a rhamnosyl bromide donor equipped with a 2,3-*O*-cyclohexylidene protecting group (see Scheme 49 B).[509] Glycosylation under Koenigs-Knorr conditions gave high to moderate yields and selectivity. Soon after, *Kochetkov* introduced the 2,3-*O*-carbonate protecting group to facilitate β-selectivity in rhamnosylation reactions under heterogenous Koenigs-Knorr conditions.[510] This approach was later expanded to 3,4-*O*-carbonates assuming that their pronounced electron withdrawing character supports 1,2-*cis* rhamnoside formation (*Crich* and co-worker).[511] Following the same line of thought, *Lichtenthaler* and co-workers introduced an ulosyl approach, which exploits an electron withdrawing C-2-carbonyl to suppress oxocarbenium ion intermediate formation and facilitate a S_N2-type glycosylation.[512-514] Also circumventing the formation of oxocarbenium ion intermediates, but in a different way, *Kováč* utilized a 1,2-*cis* stannylene-acetal- tethered rhamnose nucleophile in S_N2-type displacement reactions of secondary and primary triflates installed at the glycosyl acceptor moiety.[515] In contrast, *Yamada* (and later *Bols*) introduced ring-flipped, super-armed rhamnosyl donors − comprising sterically demanding silyl ether protecting groups − for β-selective rhamnosylation reactions.[516, 517]

More recently, intra-molecular aglycon transfer came to the fore as approach towards β-rhamnoside formation (see Scheme 50 A).[508, 518, 519] Here the respective glycosyl acceptor is tethered to the C-2 protecting group (e.g. allylic ether or naphthylmethyl ether) to provide a pre-formed spatial arrangement of acceptor and donor. Upon activation, this arrangement facilitates delivery of the acceptor from the β-side and formation of 1,2-*cis* linked rhamnosides.[520]

In a related manner, β-selectivity can also be achieved *via* hydrogen bond-mediated aglycon delivery (see Scheme 50 B).[18, 521] Hence, a picoloyl protecting group installed at the C3-position serves a platform for hydrogen bond formation and promotes nucleophilic attack in a *syn*-fashion. This for example was recently applied in the synthesis of a hexasaccharide from *S. pneumoniae* serotype 2 as well as during preparation of a trisaccharidic motif from *P. fluorescens* BIM B-582.[522, 523]



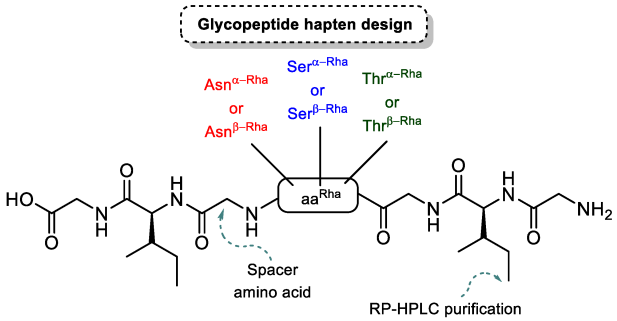
Scheme 50: More recent synthetic studies for stereoselective formation of β-*O*-rhamnosidic linkages. **A**) Intra-molecular aglycon transfer based on covalent acceptor-tethering to a C-2-protecting group. **B**) Hydrogen bond mediated aglycon transfer using picoloyl-mediated hydrogen bonding to ensure proper spatial arrangement of the donor-acceptor pair. **C**) Conceivable mechanism of β-rhamnosylation as proposed by *Pedersen* and co-workers. In ethereal solvents the oxocarbenium ion favours a $^4H_3$ half chair which promotes nucleophilic attack from the β-site.

A systematic study about influence of reaction parameters (e.g. promotor, protecting groups and solvent) on the stereochemical outcome of rhamnosylation reactions was provided by *Pedersen* and co-workers.[21] Here a combination of a disarming fluorobenzoyl ester group and ethereal solvents was found to facilitate β-rhamnosylation of simple alcohols. It was rationalized, that in ethereal solvents the oxocarbenium ion favours a $^4H_3$ half chair conformation which promotes the observed selectivity (see Scheme 50 C).

II.2 Objective and synthetic considerations of Part II

The discovery of the regulatory role of arginine mono-rhamnosylation (Arg^{Rha}) in EF-P-activation of various clinically relevant pathogens[411, 463, 464], attracted the idea of mono-rhamnosylation being a more common, but thus far widely unappreciated, protein glycosylation in bacteria. Assuming that such a rhamnoproteome may also include linkages beyond that of Arg^{Rha}, we aimed at expanding the limited biochemical toolbox of *anti*-Arg^{Rha} antibodies[9, 487] by a set of antibodies covering the most prominent bacterial *O*- and *N*-glycosylation sites (namely Ser^{Rha}, Thr^{Rha} and Asn^{Rha}). This in turn should enable a facile detection of novel bacterial rhamnoproteins *via* western-blot analysis and thus provide a promising starting point for probing novel bacterial rhamnosylation sites and deciphering their role in bacterial physiology.

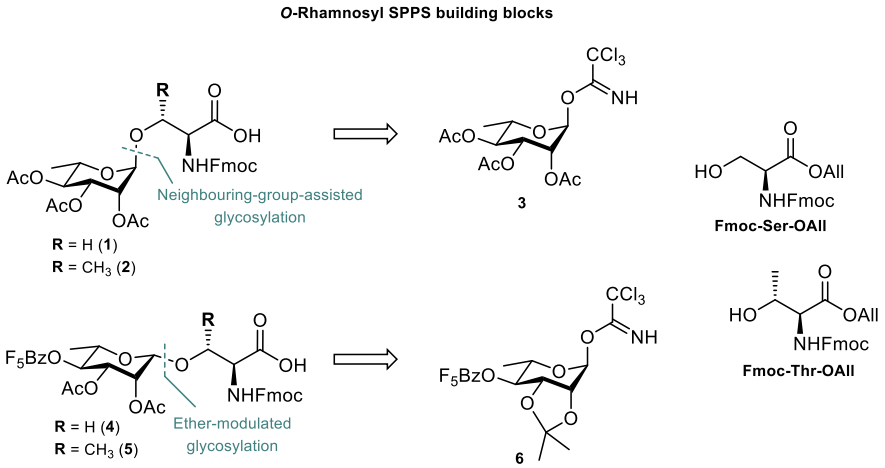
Providing this intended set of polyclonal antibodies first requires a careful selection of suitable haptens/immunogens for elicitation of a robust immune response against the modification of interest. Commonly used approaches in this regard rely on native proteins, or synthetic peptidic fragments thereof, comprising the PTM of interest. [8, 9, 11, 487, 524-526] The lack of information about respective target proteins naturally associated to our aim of chasing a thus far mostly putative glycosidic protein modification, however suggested an approach based on an artificial peptide sequence that comprises the respective rhamnosyl amino acid epitope (see Scheme 51). Inspired by recent works of *Hu* and co-workers[8, 487] we opted for an artificial glycopeptide comprising two glycine residues flanking the respective rhamnosylated amino acid. These glycine residues were supposed to function as spacer amino acids to avoid interactions between antibodies and the surrounding amino acid side chains. Our design also provided for two additional isoleucine residues to ease RP-HPLC purification. In addition, we aimed at a set of immunogens, in which each hapten comprises only a single rhamnosidic anomer (e.g., $Ser^{\alpha\text{-}Rha}$ and $Ser^{\beta\text{-}Rha}$). This additional requirement was included as it may ensure subsequent immunization with defined ratios of both anomeric glycoforms and thus support antibody cross-reactivity against both naturally occurring configurations of *O*-rhamnosidic linkages.[18]



Scheme 51: Design considerations and molecular components of targeted artificial glycopeptide haptens for the generation of specific *anti*-Ser^{Rha}, *anti*-Thr^{Rha} and *anti*-Asn^{Rha} antibodies. Glycine residues flanking the rhamnosylated amino acid epitope serve as spacer amino acids to prevent interactions between antibody and adjacent side-chain residues. Additional isoleucine residues were included to facilitate RP-HPLC purification.

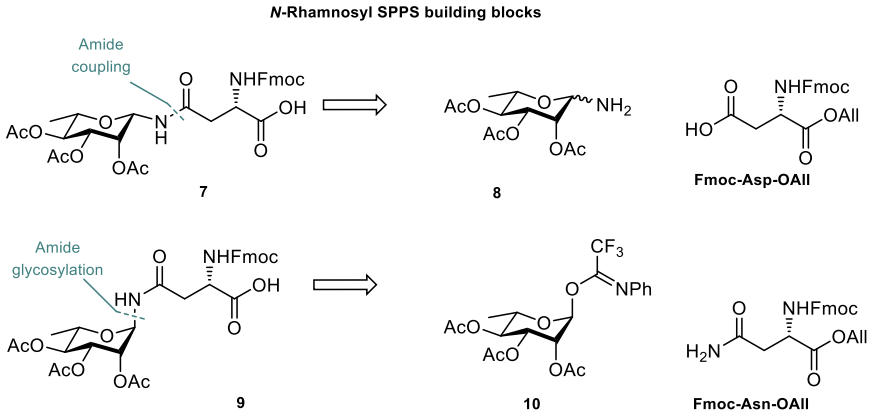
For glycopeptide hapten-assembly, we decided to use a cassette-approach relying on pre-fabricated rhamnosyl amino acid building blocks. By following this strategy, the desired anomeric configuration of the rhamnose moiety could readily be installed and unambiguously assigned prior to its incorporation *via* SPPS. Consequently, six rhamnosyl amino acid building blocks (**1**, **2**, **4**, **5**, **7** and **9**) were identified as key intermediates *en route* to our targeted collection of rhamnosylated glycopeptide haptens.

The α-*O*-rhamnosylated serine and threonine building blocks **1** and **2** should be accessible from neighbouring group assisted glycosylation of Fmoc-Ser/Thr-OAll using rhamnosyl trichloroacetimidate donor **3** (see Scheme 52). For assembly of the chemically more challenging β-glycosidic linkages of **4** and **5** on the other hand, we opted for an ether-modulated glycosylation, based on *Pedersen´s* methodology.[21] This particular approach was chosen as it utilizes a protecting group pattern compatible with common conditions in glycosyl amino acid assembly and subsequent glycopeptide synthesis *via* Fmoc-SPPS.



Scheme 52: Chemical structures of *O*-rhamnosylated serine and threonine SPPS building blocks **1**, **2**, **4**, **5** as well as key intermediates for stereoselective glycosidic bond formation.

For the assembly of the β-rhamnosylated asparagine building block **7** we intended a synthetic strategy based on PyBOP-mediated amid coupling of rhamnosyl amine **8** to Fmoc-Asp-OAll (see Scheme 53). The tendency of rhamnosyl amine **8** to form predominantly β-linked products[486] on the other hand though diminished the applicability of this approach for assembly of α-linked rhamnosyl asparagine derivative **9**. Consequently, a direct amide-glycosylation approach using PTFA donor **10** was supposed as most effective strategy for α-selective *N*-glycosidic bond formation.



Scheme 53: Chemical structures of *N*-rhamnosylated asparagine SPPS building blocks **7** and **9** as well as key intermediates for stereoselective glycosidic bond formation.

II.3 Manuscript

A set of rhamnosylation-specific antibodies enables detection of novel protein glycosylations in bacteria

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Daniel Gast‡, Franziska Koller‡, Ralph Krafczyk, Lukas Bauer, Swetlana Wunder, Jürgen Lassak* and Anja Hoffmann-Röder*, *Org. Biomol. Chem*. **2020**, 18, 6823 – 6828

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‡ Authors contributed equally

Declaration of contributions:

Franziska Koller, Ralph Krafczyk and Jürgen Lassak performed determination of BSA-Loading, antibody characterisation, bacterial growth, detection of rhamnosylated proteins and wrote the corresponding parts of supplementary information. Lukas Bauer helped with the organic synthesis. Swetlana Wunder contributed in the synthesis of *O*-rhamnosylated glycopeptides. Anja Hoffmann-Röder and Jürgen Lassak designed the study and provided scientific supervision. Daniel Gast conducted the chemical synthesis and characterisation of rhamnosyl amino acid building blocks and glycopeptides. In addition, Daniel Gast prepared the BSA-conjugates and wrote the chemical part of the supporting information. Anja Hoffmann-Röder, Jürgen Lassak, Daniel Gast, Franziska Koller and Ralph Krafczyk wrote and edited the final manuscript.

Franziska Koller **Daniel Gast**

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A set of rhamnosylation-specific antibodies enables detection of novel protein glycosylations in bacteria†

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Despite its potential importance for bacterial virulence, protein rhamnosylation has not yet been sufficiently studied. Specific *anti*-Ser^{Rha}, *anti*-Thr^{Rha} and *anti*-Asn^{Rha} antibodies allowed the identification of previously unknown monorhamnosylated proteins in cytosol and membrane fractions of bacterial cell lysates. Mapping of the complete rhamnoproteome in pathogens should facilitate development of targeted therapies against bacterial infections.

Introduction

To perform their specific biological function, peptides and proteins are usually altered post-translationally by attachment of specific groups. Glycosylation, *i.e.* the enzymatic attachment of sugar residues to the side chains of amino acids, is among the most common and diverse post-translational modifications (PTMs). Such protein glycosylations occur in all domains of life and regulate important cellular processes, including differentiation, signal transduction and the immune response.[1] In addition, bacterial protein glycosylation often plays an important role in the pathogenicity of bacteria.[2] Typical prokaryotic glycosylation patterns include *O*-linked glycans, preferably bound to serine (Ser), threonine (Thr), and less frequently to tyrosine (Tyr), as well as *N*-bound sugars.[3,4] The latter includes the extensively studied glycosylation pattern of *Campylobacter jejuni*,[5] in which a pre-assembled heptasaccharide is enzymatically transferred *en-bloc* onto asparagine (Asn), as well as the attachment of monosaccharides.[6–8] For instance, the glycosyltransferase HMW1C from *Haemophilus influenzae* attaches various single glucose and galactose units to its target protein, the adhesin HMW1, with the help of the sugar nucleotides UDP-glucose and UDP-galactose as activated glycosyl donors.[9,10] Similarly NleB1, initially reported to attach *N*-acetylglucosamine to arginine (Arg) side chains of host proteins, also regulates bacterial physiology by promoting glycosylation of an specific Arg residue of the bacterial glutathione synthetase (GshB) in *Citrobacter rodentium*.[11–13] Another example for this rather unusual Arg glycosylation was recently described for translation elongation factor P (EF-P), which is estimated to be glycosylated in approximately 10% of all bacteria,[14] including clinically important germs like *Pseudomonas aeruginosa*[14,15] and *Neisseria meningitidis*.[16] Here, a single rhamnose (Rha) moiety is linked to a key Arg unit of EF-P by the glycosyltransferase EarP.[14,17] Rhamnosylation of EF-P is essential for preventing ribosome stalling when translating polyproline motifs. It also represents the first example for a protein regulation by *N*-monoglycosylation in bacteria.[14,18] However, the widespread existence of synthesis pathways for activated rhamnose dinucleotide precursors (dT/UDP-β-L-rhamnose, GDP-α-D-rhamnose) in bacteria, archaea and eukaryotes raises the question whether such monorhamnosylations are indeed only curiosities or whether they rather represent a general mechanism for functional protein regulation in prokaryotes.[6] More importantly, and in view of the close link between protein glycosylation and bacterial pathogenicity, research in this direction might also offer new therapeutic avenues to combat bacterial infectious diseases,[19] which has become even more urgent with the spread of infections caused by multiresistant germs. An important prerequisite for the development of new antiinfectives or therapeutic antibacterial approaches is the identification of (new) glycoprotein targets and their specific biological functions. Despite recent technological advances, profiling of bacterial glycosylation sites is still a difficult task and usually requires specific enrichment strategies due to the dynamics of glycosylation and its substoichiometry.[20] Here, immunoblotting using modification-specific antibodies is particularly useful, although multiple analyses with different (complementary) antibodies are often

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†Electronic supplementary information (ESI) available: Details on chemical procedures and characterizations of new compounds; details on antibody evaluation and detection of rhamnoproteins. See DOI: 10.1039/d0ob01289k
‡These authors contributed equally to this work.

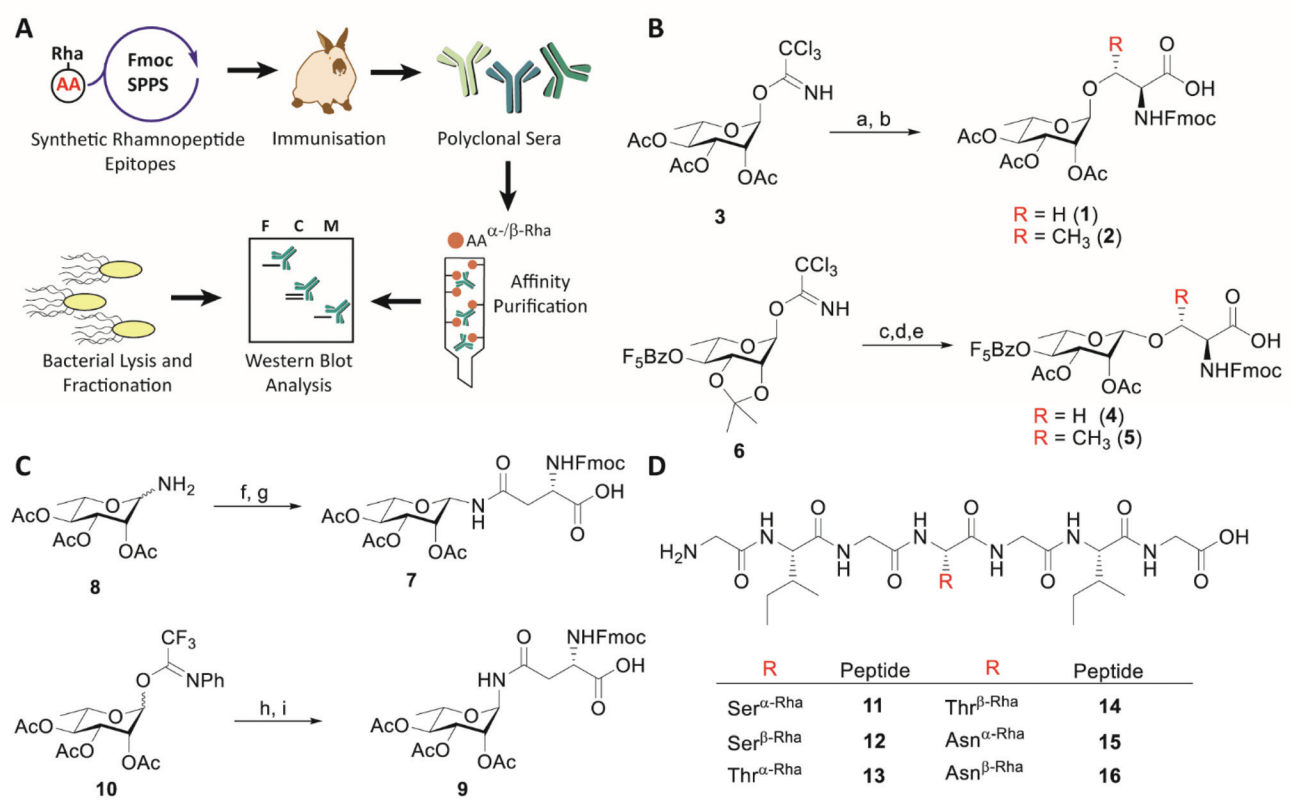

Fig. 1 (A) Generation of polyclonal *anti*-Ser^{Rha}, *anti*-Thr^{Rha} and *anti*-Asn^{Rha} from synthetic rhamnopeptide epitopes and their use in profiling of the bacterial rhamnosyl proteome (F = flagella, C = cytosol fraction, M = membrane fraction). (B) Synthesis of α-*O*-rhamnosyl serine and threonine building blocks: reagents and conditions: (a) serine: Fmoc-L-Ser-OAll, TMSOTf, CH_2Cl_2, 74%; threonine: Fmoc-L-Thr-OAll. TMSOTf, CH_2Cl_2, 75%; (b) $PhSiH_3$, [$Pd(PPh_3)_4$], CH_2Cl_2, 97% (**1**), 85% (**2**). Synthesis of β-*O*-rhamnosyl serine and threonine building blocks: reagents and conditions: (c) serine: Fmoc-L-Ser-OAll, $HNTf_2$, Et_2O/CH_2Cl_2 76%; threonine: Fmoc-L-Thr-OAll, $HNTf_2$, Et_2O/CH_2Cl_2 85%; (d) (1) TFA, H_2O, (2) Ac_2O, pyridine, serine: 87% threonine: 74% over 2 steps; (e) [$Pd(PPh_3)_4$], *N*-methyl aniline, THF, 98% (**4**), 94% (**5**). (C) Synthesis of β-*N*-rhamnosyl asparagine building block: reagents and conditions: (f) Fmoc-L-Asp-OAll, PyBOP, DIPEA, DMF, 79%; (g) $PhSiH_3$, [$Pd(PPh_3)_4$], CH_2Cl_2, 81%. Synthesis of α-*N*-rhamnosyl asparagine building block: reagents and conditions: (h) Fmoc-L-Asn-OAll, TMSOTf, $MeNO_2/CH_2Cl_2$, 63%; (i) $PhSiH_3$, [$Pd(PPh_3)_4$], CH_2Cl_2, 76%. (D) Schematic depiction of *O*- and *N*-rhamnosylated glycopeptides **11–16** for antibody generation.

needed.[21] So far, only *anti*-Arg^{Rha} antibodies have been described for the detection of protein monorhamnosylation in cell lysates.[17,22] However, to be able to detect further potentially important rhamnose *N*- and *O*-linked glycosylation patterns, we are now expanding the existing biochemical toolbox with this study. Novel, and specific *anti*-Asn^{Rha}, *anti*-Ser^{Rha} and *anti*-Thr^{Rha} polyclonal antibodies are presented, which should allow for the detection of previously unknown monorhamnosylated bacterial proteins (Fig. 1A).

Results and discussion

Based on previous work of Hu and co-workers, who generated first *anti*-Arg^{Rha} antibodies with the aid of synthetic glycopeptides,[22,23] we also used an artificial glycopeptide scaffold of the sequence Gly-Ile-Gly-AA^{Rha}-Gly-Ile-Gly for production of our extended set of specific *anti*-AA^{Rha} antibodies. Here, AA^{Rha} stands either for Asn$^{α/β-Rha}$, Ser$^{α/β-Rha}$ or Thr$^{α/β-Rha}$, which represent chemically pre-formed Rha-building blocks of Asn, Ser, and Thr. In general, these pre-assembled rhamnosyl amino acids can be incorporated into any given peptide sequence by solid-phase peptide synthesis (SPPS) according to the Fmoc-protocol. Chemical approaches towards such building blocks have been reported earlier,[24,25] with exception of *N*-linked Asn^{Rha} and the synthetically challenging β-*O*-rhamnosyl Ser/Thr building blocks. Detailed information on the preparation of all of these (novel) building blocks are provided in the ESI.† In brief, *O*-linked α-rhamnosylated Ser and Thr SPPS building blocks **1** and **2** were assembled from Fmoc-L-Ser-OAll[26] and Fmoc-L-Thr-OAll[26] *via* TMSOTf-catalysed glycosylation with trichloroacetimidate donor **3**[27] (Fig. 1B). Subsequent reductive deallylation furnished the desired products stereochemically pure and in 72% and 64% overall yield, respectively. Similarly, the corresponding β-rhamnosylated Ser-*O*- and Thr-*O*-building blocks **4** and **5** were obtained from Fmoc-L-Ser-OAll[26] and Fmoc-L-Thr-OAll[26] using rhamnosyl donor **6**[28] and Tf_2NH as synthesis promotor. Acidic cleavage of the isopropylidene acetal protecting group, followed by reacetylation and deallylation, then provided the corresponding β-*O*-rhamnosy-

lated Ser and Thr derivatives **4** and **5**, in 65% and 59% overall yield, respectively. *N*-Linked β-rhamnosylated Asn SPPS building block **7** was obtained in 64% yield by PyBOP-mediated coupling of rhamnosylamine precursor **8**[29] to Fmoc-L-Asp-OAll[30] and Pd-catalysed deallylation (Fig. 1C). In contrast, α-*N*-linked rhamnosyl Asn derivative **9** was not accessible *via* this route due to the high racemization tendency of the intermediate rhamnosylamine **8**.[29] However, TMSOTf-catalyzed glycosylation of Fmoc-L-Asn-OAll with *N*-phenyl trifluoroacetimidate donor **10**[31,32] and subsequent ester cleavage furnished the desired product in 48% yield. The stereochemical assignments of all six *N*- and *O*-rhamnosyl amino acid building blocks were proven by ^{1}H–^{13}C-coupled HSQC NMR experiments (see ESI†).[29,33] With the pre-formed amino acid building blocks **1**, **2**, **4**, **5**, **7** and **9** at hands, a set of *O*- and *N*-rhamnosylated glycopeptides (**11–16**) comprising either α- or β-rhamnosylated Ser, Thr or Asn residues was synthesized on 2-chlorotrityl resin using standard Fmoc-SPPS procedures (see ESI†). Additional glycine units were introduced into the sequence to act as separators, while the corresponding isoleucine residue should facilitate HPLC purification (Fig. 1D). Polyclonal *anti*-Asn^{Rha}, *anti*-Ser^{Rha}, and *anti*-Thr^{Rha} antibodies were raised commercially by Eurogentec GmbH, Germany, according to their Rabbit Speedy 28-day (AS superantigen) program. For more details please refer to the ESI.† To ensure cross reactivity of the antibodies to both anomeric rhamnosyl linkages, equimolar mixtures of α- and β-configured glycopeptides were used for immunization of rabbits. The specificities of the obtained *anti*-Ser^{Rha}, *anti*-Thr^{Rha} and *anti*-Asn^{Rha} polyclonal antibodies were investigated with semi-synthetic glycopeptide conjugates (BSA-Ser$^{α-/β-Rha}$, BSA-Thr$^{α-/β-Rha}$, BSA-Asn$^{α-/β-Rha}$) derived from bovine serum albumin (BSA) and the corresponding α- or β-rhamnosylated glycopeptides (Fig. 2). In addition, the BSA-conjugates of the naked, non-glycosylated peptide sequences were prepared as negative controls (BSA-Ser^{NP}, BSA-Thr^{NP}, BSA-Asn^{NP}). All three polyclonal antibodies strongly bound their cognate glycopeptide hapten up to a concentration range of only 1–2 ng of BSA conjugate (Fig. 2D and ESI Fig. 6B and 7B†). By contrast, the antisera neither recognized the corresponding naked BSA conjugates lacking the sugar moiety, nor synthetic EF-P variants (EF-P^{Ser-NP}, EF-P^{Thr-NP}, EF-P^{Asn-NP}) in which the internal loop region carrying the natural rhamnosylation site was substituted by the corresponding naked peptide (Fig. 2A, ESI Fig. 6A and 7A†).[14] This demonstrates that the rhamnosylated glycosylamino acids are key structural elements of the antibodies' cognate haptens. This finding was confirmed by competition assays, in which the antibodies were pre-incubated with either L-rhamnose (up to 15 mM) or the respective amino acid (L-Ser, L-Thr, L-Asn, up to 15 mM) prior to immunodetection. Here, no impairment in recognition of the corresponding rhamnosylated BSA conjugates was observed (Fig. 2C, ESI Fig. 6A and 7A†). Moreover, cross-reactivity of the antibodies to non-cognate glycopeptide antigens was tested. While *anti*-Thr^{Rha} could detect β-*O*-rhamnosylated Ser (Fig. 2B) and *vice versa* (ESI, Fig. 6C†), almost no binding to *N*-rhamnosylated Asn was observed. *Anti*-Asn^{Rha}, on the other hand, did not recognize *N*-Arg rhamnosylation but showed some cross-reactivity with *O*-rhamnosylated Ser and

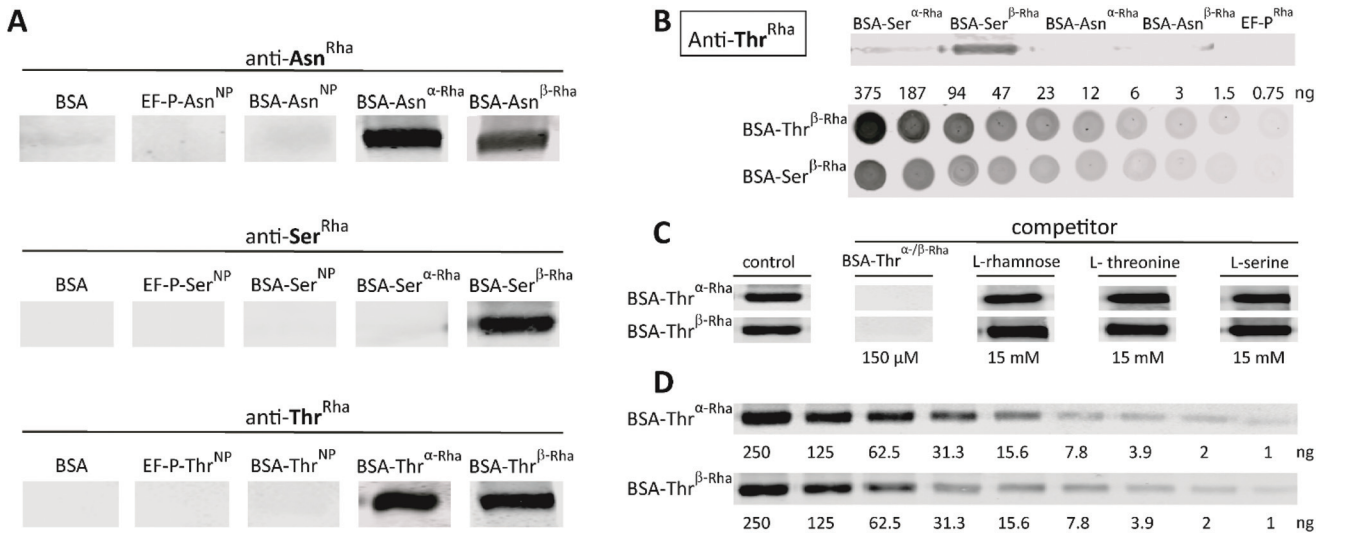

Fig. 2 Sensitivity and specificity analysis of *anti*-aa^{Rha} specific antibodies. (A) Specificity analysis: 0.5 µg of the respective sample were subjected to SDS-PAGE and subsequent immunodetection analysis with 0.2 mg ml^{-1} of *anti*-aa^{Rha}. BSA, synthetic EF-P variants comprising the naked peptide sequence in their loop region (EF-P-aa^{NP}) and BSA coupled to the naked peptide (BSA-aa^{NP}) served as negative controls. α- and β-rhamnosylated peptides coupled to BSA (BSA-aa$^{α-/β-Rha}$) served as positive controls. (B) *Anti*-Thr^{Rha} cross-reactivity analysis: 0.5 µg of the respective sample were subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg ml^{-1} of *anti*-Thr^{Rha}. Upper part: Immunodetection of BSA-Ser$^{α-/β-Rha}$, BSA-Asn$^{α-/β-Rha}$ and EF-P^{Rha}. Lower part: Immunodetection of varying concentrations of BSA-Thr$^{β-Rha}$ and BSA-Ser$^{β-Rha}$. (C) Cross-reactivity analysis of *anti*-Thr^{Rha} against L-rhamnose, L-Thr and L-Ser. *Anti*-Thr^{Rha} was preincubated prior to immunodetection with BSA-Thr$^{α-/β-Rha}$ (150 µM), L-rhamnose, L-Thr and L-Ser (15 mM). (D) *Anti*-Thr^{Rha} sensitivity analysis: immunodetection of varying concentrations of BSA-Thr$^{α-/β-Rha}$. Antibody concentrations were kept constant at 0.2 mg ml^{-1}.

Thr (ESI Fig. 7C†). It should be noted however, that the cognate antigen/antibody pairing was always superior (Fig. 2B lower part, and ESI Fig. 6 and 7†). Interestingly, whereas the *anti*-Ser^{Rha} antibody recognized almost exclusively the β-*O*-rhamnosylated glycopeptide BSA conjugate (Fig. 2A and ESI Fig. 6A†), no such anomeric preference was observed for the other antibodies. Finally, none of the antibodies was found cross-reactive with naturally occurring oligosaccharides carrying L-rhamnose at the reducing end linked to Asn or Ser/Thr. Thus, immunoblotting experiments with the *S*-layer protein from *Geobacillus stearothermophilus*, characterized by a *N*-rhamnosylated trisaccharide repeating unit,[34] as well as with the *O*-rhamnosylated flagellar filament protein FliC from *P. aeruginosa*[35] turned out negative and clearly demonstrate the desired specificity of the antibodies for monorhamnosylation (ESI Fig. 9†). With the rhamnosylation-specific antibodies *anti*-Ser^{Rha}, *anti*-Thr^{Rha} and *anti*-Asn^{Rha} at hands, we aimed at detection of rhamnosylated endogenous proteins from distinct phyla (Fig. 3A). Since thus far monorhamnosylation of proteins has only been reported for bacteria,[8,14–16] we have restricted our selection to prokaryotes and in particular to those microorganisms known to synthesize nucleoside diphosphate (NDP)–rhamnose (*e.g.* as donor substrate for EF-P-Arg rhamnosylation[14]). The only exceptions in this regard are *Micrococcus luteus* and *Staphylococcus aureus* which lack the corresponding NDP–rhamnose pathways and served as negative controls. To cover all potential rhamnosylation motifs, the already known *anti*-Arg^{Rha} antibody[17] was also included in the study. Sensitivity analysis based on 10^9 cells per western blot allowed estimation of the lower detection limit to be in the range of 60–150 rhamnosylation events per cell. This in turn should reveal most of the putative rhamnoproteome. To enhance sensitivity, the flagella of swimming bacteria were isolated, and cells were further separated into cytosol and membrane fractions prior to immunoblotting. Cellular fractionation does not only allow for conclusions on the localization of detected gly-

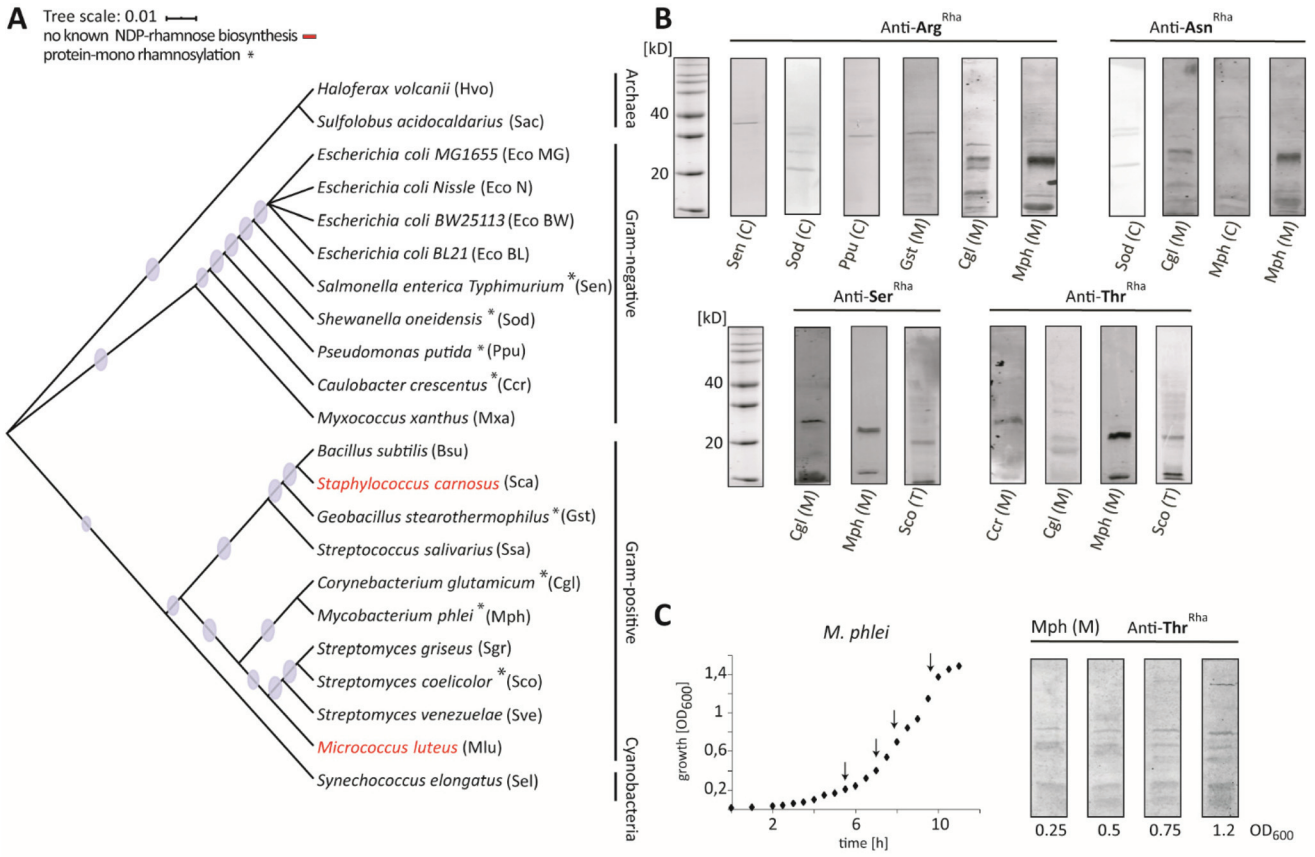

Fig. 3 (A) Phylogenetic distribution of microorganisms selected for rhamnoproteome analysis. Depicted is a rooted phylogenetic tree based on 16s rDNA sequences. The tree was calculated using ClustalX and visualized using iTOL. Bootstrap values (0–1000) are given as transparent circles. The circle size indicates the probability of correct branching with the largest circles having a bootstrap value of 1000 and the smallest circle with a bootstrap value of 533. A star marks those bacteria in which monorhamnosylation was detected. Black font indicates for a known NDP–rhamnose biosynthesis pathway, whereas a red font marks those bacteria where the respective genes are absent. (B) Immunodetection of rhamnosylated proteins using *anti*-Arg^{Rha}, *anti*-Asn^{Rha}, *anti*-Ser^{Rha} and *anti*-Thr^{Rha}. Prokaryotic samples were separated into cytosolic (C) and membrane (M) fractions and subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg ml^{-1} of the corresponding antibody. (C) Immunodetection of *M. phlei* rhamnoproteins from different growth phases. Arrows indicate the different time points of sample collection. Immunodetection is exemplified for *anti*-Thr^{Rha} (0.2 mg ml^{-1}).

coproteins but also accumulates those rhamnopeptides present in the membrane or being part of the flagellum. This enrichment is important since many prokaryotic protein glycosylation events occur on cellular appendages or membrane proteins.[8,36–39] While protein monorhamnosylation was not detected in the selected archaea, bands indicative of rhamnoproteins were readily identified in immunoblotting experiments with different bacterial phyla (Fig. 3B). Specifically, *N*-rhamnosylation was detected in both Gram-negative and Gram-positive species, whereas *O*-rhamnosylation was mainly observed in the latter. Rhamnoproteins appeared to be both, membrane-bound and cytosolic, while flagellin rhamnosylation was not detected (Fig. 3B and ESI Fig. 11†). In addition, significant differences in the relative abundance of monorhamnosylated proteins were revealed for various bacteria. While EF-P seems to be the only rhamnoprotein in *P. putida*, multiple rhamnoproteins were detected in *Corynebacterium glutamicum* and *Mycobacterium phlei.* It is noteworthy, that for the latter the dTDP-β-L-rhamnose biosynthesis pathway is essential.[40] Interestingly, the number of detected monorhamnosylated proteins varied over the time of growth with an additionally band appearing exclusively in the stationary phase of *M. phlei* (Fig. 3C and ESI Fig. 10†).

Conclusion

In summary, new polyclonal antibodies for the specific recognition of protein monorhamnosylation in bacteria were developed from novel synthetic designer *N*- and *O*-glycopeptide antigens. These *anti*-Ser^{Rha}, *anti*-Thr^{Rha} and *anti*-Asn^{Rha} antisera can be applied together with already known *anti*-Arg^{Rha} antibodies as useful tools for biochemical and proteomic studies, *e.g.* identifying potential *N*- and *O*-rhamnosylation sites. Thus, we have successfully used the complete set of antibodies in immunoblotting experiments to detect novel monorhamnosylated proteins in various bacteria. The existence of rhamnose-modified proteins in several bacterial species (8 out of 22 species tested) supports the assumption that the already known regulatory *N*-monorhamnosylation of translation elongation factor EF-P is not an exception. Rather, protein monorhamnosylation seems to be a more common PTM occurring across bacterial family boundaries. Ongoing research on the rhamnoproteome, for which the present study is the starting point, will require determination and (functional) characterization of the specific protein glycosylation sites. This will not only expand our knowledge of the bacterial glycoproteome but might potentially open a new way for combating infectious and antibiotic-resistant bacterial diseases.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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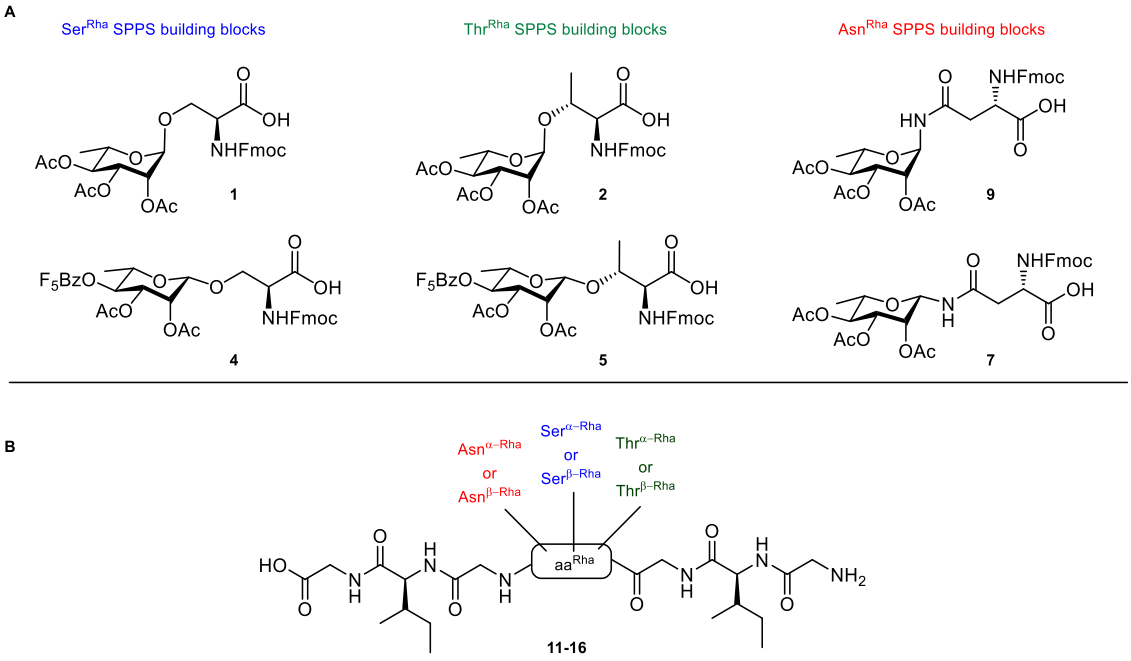
II.4 Conclusion and outlook

Post-translational modification (PTM) is a common strategy of living cells to expand the chemical repertoire provided by the proteinogenic amino acids and to adapt without the need of protein degradation or *de-novo* biosynthesis. Protein glycosylation is arguably the most complex PTM and was recently assumed as an emerging key player in bacterial physiology.[402] Further, recent discoveries of unusual glycosylation motif such as EarP mediated mono-rhamnosylation of arginine[411], suggest a possibly larger diversity in the bacterial glycoproteome than known so far.

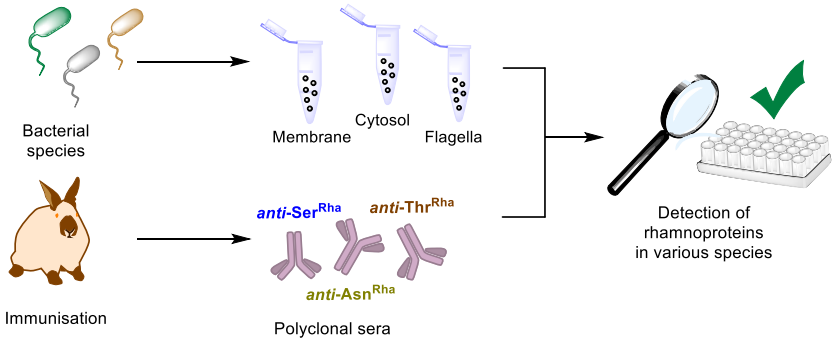
Within this chapter, we aimed at the generation of rhamnosylation-specific antibodies (*anti*-Asn^{Rha}, *anti*-Ser^{Rha} and *anti*-Thr^{Rha}) to expand the limited biochemical toolbox for detecting protein rhamnosylation in bacterial lysates. In a first step, we developed synthetic approaches for (novel) pre-assembled *O*- and *N*-rhamnosylated amino acid building blocks (see Scheme 54 A) for incorporation into any given peptide sequence *via* Fmoc-SPPS. On the basis of an artificial peptide sequence, we further used this building blocks to assemble a set of stereochemically pure glycopeptide haptens for antibody generation (see Scheme 54 B). Polyclonal sera were raised commercially by *Eurogentec* GmbH in accordance to their rabbit speedy 28-day program.



Scheme 54: **A**) *O*- and *N*-rhamnosyl amino acid building blocks for SPPS. **B**) Artificial haptens for generation of *anti*-Asn^{Rha}, *anti*-Ser^{Rha} and *anti*-Thr^{Rha} antibodies.

Antibody-characterization *via* western blot analysis and competition assays unveiled the rhamnosylated amino acids as the crucial recognition element. Despite a certain degree antibody cross-reactivity for non-cognate glycopeptides was observed (e.g. *anti*-Thr^{Rha} antibody also recognises Ser$^{\beta\text{-}Rha}$), the corresponding antibody-binding to the parent hapten was found superior in all cases. Further, the antibodies´ specificity for protein monorhamnosylation was verified using natural occurring rhamnose containing polysaccharides. By making use of this set of novel antibodies in combination with an *anti*-Arg^{Rha} antibody (previously generated commercially from a glycopeptide synthesized in our group[9]), we detected rhamnoproteins in 8 of 22 bacterial species (see Scheme 55). This provided first indications for protein-rhamnosylation as a more common post-translational

modification in bacteria and sets a promising starting point for further investigations on hitherto unknown rhamnoproteins in bacteria.



Scheme 55: Detection of rhamnosylated proteins *via* western blot analysis.

Accordingly, future work in this direction will include the generation of monoclonal − modification specific − antibodies to facilitate antibody-based affinity enrichment and further analysis of bacterial rhamnoproteins *via* tandem mass spectrometry.[11, 527, 528] Here either semisynthetic protein-conjugates (see supporting information part II) or fully synthetic two-component immunogens (in accordance to the design described in part I) could be used for monoclonal antibody generation.

II.5 Experimental data part II

A set of rhamnosylation-specific antibodies enables detection of novel protein glycosylations in bacteria

II.5.1 Supporting Information

A set of rhamnosylation-specific antibodies enables detection of novel protein glycosylations in bacteria

Reproduced from

Daniel Gast[‡], Franziska Koller[‡], Ralph Krafczyk, Lukas Bauer, Swetlana Wunder, Jürgen Lassak* and Anja Hoffmann-Röder*, *Org. Biomol. Chem*. **2020**, 18, 6823 – 6828

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Authors Contributions to this Supporting Information can be found above in the

Declaration of contributions Section.

Note: Numbering of Compounds and Figures/Tables in the Supporting information section corresponds to the Numbering/cross-references used in the Manuscript (*vide supra*). The only exception in this regard are reference numbers, which has been adapted to the referencing order of this thesis to facilitate orientation of the reader.

Methods and reagents:

If not otherwise noted, all reactions were magnetically stirred and conducted in oven-dried glassware. Reactions were performed in an argon atmosphere using standard Schlenk techniques. Solvents for moisture sensitive reactions (Diethyl ether, Dichloromethane, Tetrahydrofuran) were dried according to standard procedures and distilled prior to use or purchased from *Acros Organics* as "*extra dry*" reagents stored over molecular sieve and under inert gas atmosphere (*N,N*-Dimethylformamide (DMF)). Commercially available reagents were purchased from Sigma-Aldrich or TCI-Chemicals and were used without further purification. Analytical thin-layer chromatography (TLC) was used for monitoring of reactions. TLC was performed on pre-coated silica gel 60 F254 aluminium plates (*Merck KGaA*, Darmstadt) and visualized by exposure to ultraviolet light (UV, 254 nm) and/or staining with either a 1:1 mixture of 1 M H_2SO_4 in EtOH and 3 % 4-methoxyphenol solution in EtOH (Carbohydrates) or Seebach reagent (Cerium phosphomolybdic acid (5.0 g) concentrated sulfuric acid (16 ml), water (200 ml) and Cerium(IV) sulphate (2.0 g)). If not otherwise stated, purification of substances was achieved by standard flash column chromatography on silica (35-70 µm particle size) from Acros organics.

NMR spectroscopy:

Proton nuclear magnetic resonance (^{1}H-NMR) were recorded in deuterated solvents ($CDCl_3$, DMSO-d_6) on a *Bruker Avance III HD* 400 MHz spectrometer equipped with a *CyroProbe*™, a *Varian VXR 400 S* spectrometer, a *Bruker AMX600* spectrometer or a *Bruker Avance III HD* 800 MHz spectrometer equipped with a *Cyro Probe*™. Chemical Shifts (δ scale) were reported in parts per million (ppm) and calibration was carried out with residual protic solvents as internal reference. For indication of multiplicities the following abbreviations were used: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) as well as combinations thereof. Carbon nuclear magnetic resonance spectra (^{13}C-NMR) were recorded on the aforementioned spectrometers with 100 MHz, 150 MHz and 200 MHz and chemical shifts (δ) were also given in ppm referenced to the central carbon signal of the solvents. For further assignment of the ^{1}H and ^{13}C-NMR signals, 2D-NMR experiments (COSY, HSQC, HMBC) were used. If necessary, anomeric configuration of rhamnose derivatives were proved by using proton coupled HSQC experiments. Numbering of proton and carbon atoms does not necessarily correspond to the IUPAC and are further described in the respective section.

High-resolution mass spectrometry (HRMS):

High resolution (HR-ESI) mass spectra were recorded on a *Thermo Finnigan LTQ FT* spectrometer either in positive or negative ionization mode.

High performance liquid chromatography (HPLC):

Analytical RP-HPLC was conducted at a *JASCO* system (PU-2080 Plus, LG-2080-02-S, DG-2080-53 and MD-2010 Plus) on *Phenomenex Aeris Peptide* column (C18, 5 µm, 250 mm × 4.6 mm; later referred to as "*Aeris*") or *Phenomenex Luna* column (C18, 5 µm, 250 mm × 4.6 mm; later referred to as "*Luna*"). A gradient of water (A)/acetonitrile (B) containing 0.1 % TFA with a flow-rate of 1 ml/min was used as eluent. Semi-preparative RP-HPLC was performed at a *JASCO* system (PU-2087 Plus, LG-2080-02-S and UV-2075 Plus) on a *Phenomenex Aeris Peptide* column (C18, 250 × 21.2 mm), with a flow of 16 ml/min. Exact composition of gradients are presented in Table 1 and Table 2 below.

Table 1: Description of gradient A for HPLC-purification

Time [min]	**0**	**40**	**60**
Water [%]	95	20	0
MeCN [%]	5	80	100
TFA [%]	0.1	0.1	0.1
Flow [ml/min]	15	15	15

Table 2: Description of gradient B for HPLC-purification

Time [min]	0	40	60
Water [%]	95	40	0
MeCN [%]	5	60	100
TFA [%]	0.1	0.1	0.1
Flow [ml/min]	15	15	15

Optical rotation:

Optical rotations were measured on a *Perkin-Elmer polarimeter 241* at the Sodium-D-line (589 nm) at the given temperature in °C. Concentration c is given in g/100 ml in the solvent stated in brackets ($CHCl_3$).

Microwave assisted peptide synthesis (for unglycosylated peptides (naked peptides NP); 0.1 mmol scale)

A pre-loaded Gly-Wang resin (*Rapp Polymere*) with a loading of 0.66 mmol/g was swelled 0.5 h in 5 ml DMF prior to use. DIC/OXYMA (0.5 M in DMF) and DIPEA (0.1 M in DMF) were used as coupling reagents. Fmoc-protected amino acids (Fmoc-Asn(Trt)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Thr(*t*-Bu)-OH, Fmoc-Gly-OH and Fmoc-Ile-OH from *Orpegen Pharma* or *Sigma Aldrich*) as well as the Fmoc-TEG-CO_2H-Spacer were coupled with a double coupling protocol (2 × coupling protocol: 75 °C, 170 W, 15 sec → 90 °C, 30 W, 120 sec, 5.0 eq. of Fmoc-AA-OH per coupling). Deprotection of the Fmoc-protecting group was achieved by treatment with 20 % piperidine in DMF (2 × deprotection protocol: 75 °C, 155 W, 15 sec → 90 °C, 30 W, 50 sec). Upon completion of the solid-phase peptide synthesis, the resin-bound peptide was cleaved from the resin by treatment with TFA/H_2O/Tri-*iso*-propylsilane (98:1:1) for 1 h. After filtration, the crude product was precipitated from 40 ml of cold diethyl ether. The crude peptide was collected by centrifugation and the supernatant was discarded. The peptide was washed with cold diethyl ether and dried under a flow of nitrogen. The crude residue was dissolved in water and lyophilized prior to purification by RP-HPLC.

Conventional peptide synthesis (rhamnosylated glycopeptides)

a) Resin swelling:

H-Gly-2-ClTrt resin (Loading: 1.1 mmol/g) in a 2 ml SPPS vessel was added 2 ml DMF and was shaken for 30 min at room temperature.

b) Coupling of non-glycosylated amino acid building blocks:

Synthesis on a 0.1 mmol scale:

To a solution of Fmoc aa-OH (4.0 eq.) in 455 µl DMF was added a 1 M PyBOP solution in DMF (400 µl, 4.0 eq.), a 1 M HOBT·H_2O solution in DMF (400 µl, 4.0 eq.) and a 2 M DIPEA solution in DMF (400 µl, 8.0 eq.). The solution was shaken for 1 minute before being added to the resin. The mixture was agitated at room temperature (1 h per coupling for Gly, 1.5 h per coupling for Ile). For Gly a double coupling protocol was applied, Ile was coupled *via* a single coupling protocol. After each coupling step, the resin was filtered and washed with DMF (5 × 2 ml).

Synthesis on a 0.05 mmol scale:

To a solution of Fmoc aa-OH (8.0 eq.) in 455 µl DMF was added a 1 M PyBOP solution in DMF (400 µl, 8.0 eq.), a 1 M HOBT· H_2O solution in DMF (400 µl, 8.0 eq.) and a 2 M DIPEA solution in DMF (400 µl, 16.0 eq.). The solution was shaken for 1 minute before being added to the resin. The mixture was agitated at room temperature (1 h per coupling for Gly, 1.5 h per coupling for Ile). For Gly a double coupling protocol was applied, Ile was coupled *via* a single coupling protocol. Subsequently, the resin was filtered and washed with DMF (5 × 2 ml).

c) Coupling of rhamnosyl amino acid building blocks (1, 2, 4, 5, 7 and 9) and Fmoc-TEG-CO_2H Spacer:

For the incorporation of the rhamnosylated amino acid blocks, as well as the Fmoc-TEG-CO_2H Spacer a double coupling protocol was used. Conditions for both coupling steps can be found in the corresponding section below.

d) Deprotection of rhamnosylated glycopeptides[8] and cleavage from resin

Removal of the acetyl protecting groups was accomplished by addition of 2 ml of a 5 % N_2H_2 solution in DMF to the resin-bound peptide. The mixture was agitated for 17 h at room temperature. Subsequently, the resin was filtered and washed with DMF (5 × 2 ml), MeOH (5 × 2 ml) and CH_2Cl_2 (5 × 2 ml). Cleavage from resin was conducted by treatment with 1 ml of a mixture of TFA/H_2O/Tri-*iso*-propylsilane (98:1:1). The solution was concentrated under a flow of Nitrogen before being added dropwise to chilled Et_2O for precipitation of the crude peptide. The mixture was centrifuged and the supernatant was discarded. The crude peptide was dissolved in water and lyophilized prior to purification by RP-HPLC.

Assignment of peptide NMR signals:

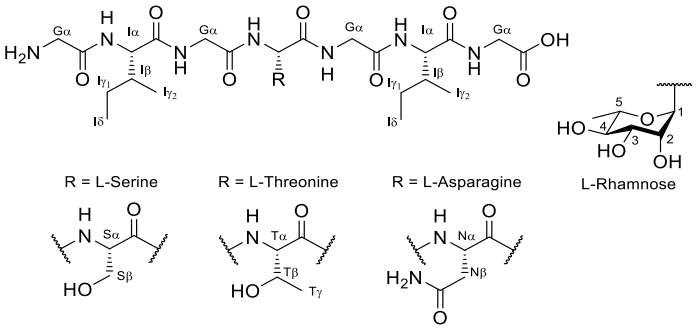


Figure 1: Assignment of peptide NMR-signals.

Structure and abbreviation of TEG-spacer for BSA conjugation:

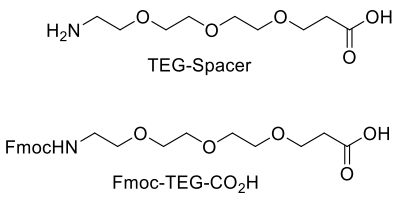


Figure 2: Chemical structures of TEG-spacer derivatives and abbreviations used in the following.

Antibody generation and purification

The polyclonal antibodies (*anti*-Asn^{Rha}, *anti*-Ser^{Rha}, *anti*-Thr^{Rha}) were commercially produced by *Eurogentec* using the *Rabbit Speedy 28-day* (AS superantigen) program. Two rabbits were immunized per antibody production by multi-site subcutaneous injections (4 injections: day 0, 7, 10, 18) with mono-rhamnosylated peptides coupled to Keyhole limpet hemocyanin (KLH).

Per injection 500 µl Freund´s adjuvant with mycobacterial particles were administered together with 500 µl antigen with a concentration of 400 µg/ml (equimolar mixtures of the corresponding α- and β-rhamnosylated glycopeptide, 200 µg in total per rabbit). The final bleed was taken after 28 days and provided 70 ml serum. The polyclonal antibodies were extracted from the serum by affinity chromatography (AS-PURI-MED). Therefore, the corresponding glycopeptide was coupled to a matrix (AF-Amino TOYO). The serum was loaded, and unrelated antibodies were washed off. Antibodies recognizing the rhamnosylated peptides were eluted and the affinity of antibodies was analysed by indirect Enzyme-linked Immunosorbent Assay (ELISA). The plate was coated with the corresponding glycopeptide (100 ng/well) and carrier protein KLH as control. Different dilutions of the serum, flow through, purified antibody and pre-immune sera were tested. The optical density of the chromogenic substrate (secondary HRP-conjugated antibody, chromogenic substrate *o*-phenylenediamine) was measured at 492 nm (OD492). For all purified antibodies, sigmoidal ELISA curves were observed for the glycopeptide. Final analysis with BIOANALYSER (*Agilent*) revealed 89.4 %, 68.3 % and 84.6 % antibody purity for *anti*-Asn^{Rha}, *anti*-Ser^{Rha} and *anti*-Thr^{Rha}, respectively.

SDS-PAGE and Western Blotting

Electrophoretic separation of proteins was carried out using SDS-PAGE as described by *Lämmli*.[529] Separated proteins were visualized in gel with 0.5 % (Vol/Vol) 2-2-2-trichloroethanol[530] and transferred to a nitrocellulose membrane by vertical Western Blotting. Antigens were detected using 0.2 µg/ml *anti*-Arg^{Rha}, 0.2 µg/ml *anti*-Asn^{Rha}, 0.2 µg/ml *anti*-Ser^{Rha} and 0.2 µg/ml *anti*-Thr^{Rha}. Primary antibodies (rabbit) were targeted with 0.1 µg/ml anti-rabbit IgG (IRDye® 680RD) (donkey) antibodies (abcam). Target proteins were visualized *via Odyssey*® CLx Imaging System (LI-COR, Inc).

Construction of plasmids for sensitivity and specificity analysis

In order to obtain EF-P variants comprising the peptide sequence of the naked (unmodified) peptides (NP) (Gly-Ile-Gly-Asn-Gly-Ile-Gly (Asn^{NP}), Gly-Ile-Gly-Ser-Gly-Ile-Gly (Ser^{NP}) and Gly-Ile-Gly-Thr-Gly-Ile-Gly (Thr^{NP})) at its loop region (EF-P$^{Asn\text{-}NP}$, EF-P$^{Ser\text{-}NP}$, EF-P$^{Thr\text{-}NP}$) the corresponding peptide sequence were introduced into His$_6$-tagged *efp*$_{P.pu}$ (pBAD24-*efp*$_{P.pu}$)[491] by overlap extension PCR.[531] Oligonucleotides used in this study are listed in table 3. Kits were used according to the manufacturers' directions. Plasmid DNA was isolated using a Hi Yield plasmid minikit (*Süd-Laborbedarf* GmbH), DNA fragments were purified by employing a Hi Yield PCR clean-up (Süd-Laborbedarf). Sequence amplifications by PCR were performed utilizing the Q5 high-fidelity DNA polymerase (NEB). All constructs were analysed by Sanger sequencing (LMU Sequencing Service). Standard methods were performed according to the instructions of in the literature.[532]

Table 3: Oligonucleotides for plasmid construction

Oligonucleotide	Sequence (5'-3')
Seq33_fw	GGC GTC ACA CTT TGC TAT GC
pBAD-HisA_rev	CAG TTC CCT ACT CTC GCA TG
EF-P(Ppu)synL_Asn_OL_Fw	GGC ATT GGC AAC GGC ATT GGC ATC ATG AAG ACC AAG CTG AAG AAC C
EF-P(Ppu)synL_Asn_OL_Rev	GCC AAT GCC GTT GCC AAT GCC GGT GAA CTC AGC TTT TTG AAC CAG
EF-P(Ppu)synL_Thr_OL_Fw	GGC ATT GGC ACC GGC ATT GGC ATC ATG AAG ACC AAG CTG AAG AAC C
EF-P(Ppu)synL_Thr_OL_Rev	GCC AAT GCC GGT GCC AAT GCC GGT GAA CTC AGC TTT TTG AAC CAG
EF-P(Ppu)synL_Ser_OL_Fw	GGC ATT GGC AGC GGC ATT GGC ATC ATG AAG ACC AAG CTG AAG AAC C
EF-P(Ppu)synL_Ser_OL_Rev	GCC AAT GCC GCT GCC AAT GCC GGT GAA CTC AGC TTT TTG AAC CAG

Protein production and purification

The His_6-tagged EF-$P_{P.pu}$ containing the peptide sequence of the naked peptides was transformed in chemically competent *E. coli* LMG194 as strain for protein production. After promotor induction with arabinose (0.2 % (wt/vol)), cells were incubated at 37 °C for 3 h and lysed afterwards by sonication. The His_6-tagged protein was purified using Ni-nitrilotriacetic acid (Ni-NTA; Qiagen) according to the manufacturer's instructions.

Synthesis and characterization of rhamnosylated amino acid building blocks:

Synthesis of *O*-rhamnosyl serine and threonine SPPS building blocks:

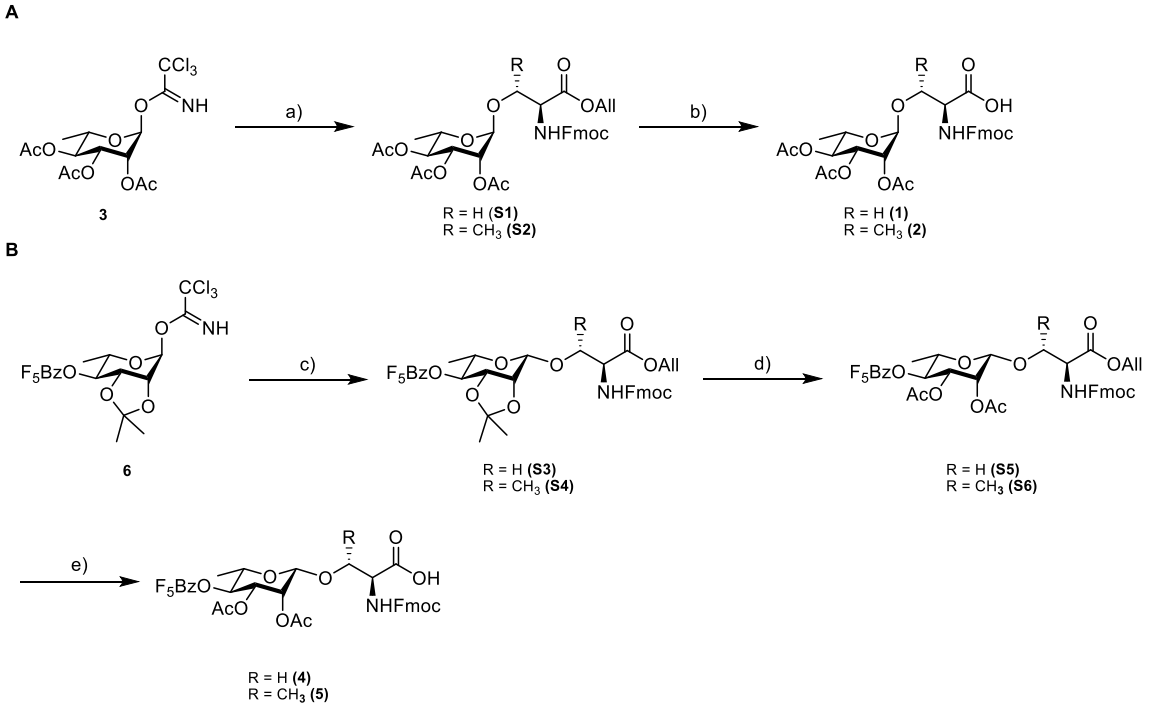
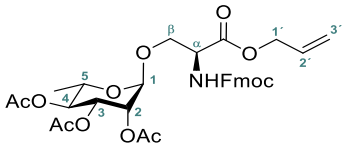


Figure 3: General overview of the chemical synthesis of *O*- rhamnosylated serine and threonine SPPS building blocks. **A**) Synthesis of α-*O*-rhamnosyl serine and threonine building blocks: Reagents and conditions: a) Serine: Fmoc-Ser-OAll, TMSOTf, CH_2Cl_2, 74%; Threonine: Fmoc-Thr-OAll, TMSOTf, CH_2Cl_2, 75 %; b) $PhSiH_3$, [$Pd(PPh_3)_4$], CH_2Cl_2, 97 % (**1**), 85 % (**2**). **B**) Synthesis of β-*O*-rhamnosyl serine and threonine building blocks: Reagents and Conditions: Serine: Fmoc-Ser-OAll, $HNTf_2$, Et_2O/CH_2Cl_2 76%; threonine: Fmoc-Thr-OAll, $HNTf_2$, Et_2O/CH_2Cl_2 85 %; d) 1) TFA, H_2O, Ac_2O, pyridine 87 % (**S5**), 74 % (**S6**) over two steps; e) [$Pd(PPh_3)_4$], *N*-methylaniline, THF, 98 % (**4**), 94 % (**5**).

N^{α}-Fluorenylmethoxycarbonyl-*O*-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-L-serine allyl ester (**S1**)



2,3,4-Tri-*O*-acetyl-L-rhamnopyranosyl trichloroacetimidate **3**[533] (2.01 g, 4.63 mmol, 1.7 eq.) and Fmoc-Ser-OAll[534] (1.00 g, 2.72 mmol, 1.0 eq.) were combined and co-evaporated with toluene (2 × 10 ml). The mixture was dried 1 h under high vacuum before being dissolved in dry CH_2Cl_2 (35 ml). Freshly activated 4 Å molecular sieve was added and the reaction mixture was stirred for 1 h at room temperature before being chilled to 0 °C. Subsequently, TMSOTf (54.0 µl, 0.30 mmol, 0.1 eq.) was added in one portion and the stirring was continued at 0 °C until complete conversion of the acceptor was detected by TLC monitoring. The reaction was stopped by addition of NEt_3 (200 µl) filtered through a pad of *Hyflo* and concentrated to dryness under reduced pressure. The crude product was directly subjected to flash chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **S1** (1.29 g, 2.02 mmol, 74 %) as a colourless foam.

R*f* = 0.52 (^{c}Hex/EtOAc v/v = 1:1).

Optical rotation $[\alpha]_D^{22} = -18.0°$ (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.79 – 7.73 (m, 2H, 2 × CH-Fmoc), 7.65 (d, $J_{CH,CH}$ = 6.4 Hz, 2H, 2 × CH-Fmoc), 7.42 – 7.38 (m, 2H, 2 × CH-Fmoc), 7.37 – 7.30 (m, 2H, 2 × CH-Fmoc), 5.97 (ddt, $J_{H2´,H3trans}$ = 16.6 Hz, $J_{H2´,H3cis}$ = 10.3 Hz, $J_{H2´,H1}$ = 6.0 Hz, 1H, H-2´), 5.71 (d, $J_{NH,H\alpha}$ = 8.6 Hz, 1H, NH-Fmoc), 5.39 (d, $J_{H3´trans,\ H2´}$ = 17.1 Hz, 1H, H-3´$_{trans}$), 5.32 – 5.27 (m, 2H, H-2, H-3´$_{cis}$), 5.17 (dd, $J_{H3,H4}$ = 10.1 Hz, $J_{H3,H2}$ = 3.5 Hz, 1H, H-3), 5.06 (t, $J_{H4,H3} = J_{H4,H5}$ = 10.0 Hz, 1H, H-4), 4.75 (s, 1H, H-1), 4.71 (d, $J_{H1´,H2´}$ = 6.0 Hz, 2H, H-1´), 4.64 (dt, $J_{H\alpha,NH}$ = 8.8 Hz, $J_{H\alpha,H\beta}$ = 3.1 Hz, 1H, H-α), 4.45 – 4.37 (m, 2H, CH_2-Fmoc), 4.27 (t, $J_{CH,CH}$ = 7.3 Hz, 1H, CH-Fmoc), 4.19 (dd, $J_{H\beta,H\beta´}$ = 9.9 Hz, $J_{H\beta,H\alpha}$ = 3.1 Hz, 1H, H-β), 3.76 (m, 1H, H-5), 3.70 (dd, $J_{H\beta´,H\beta}$ = 9.9 Hz, $J_{H\beta´,H\alpha}$ = 3.1 Hz, 1H, H-β´), 2.15 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.21 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3).

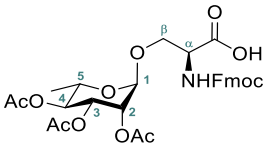
^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 170.2 (C=O), 170.0 (2C, 2 × C=O), 169.4 (C=O), 156.1 (Fmoc-C=O), 144.0 (Cq-Fmoc), 143.9 (Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 131.4 (C-2´), 127.9 (2C, 2 × CH-Fmoc), 127.3 (2C, 2 × CH-Fmoc), 125.4 (2C, 2 × CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 119.8 (C-3´), 97.7 (C-1), 71.0 (C-4), 69.6 (C-2), 69.0 (C-3), 68.1 (C-β), 67.6 (CH_2-Fmoc), 66.9 (C-5), 66.7 (C-1´), 54.3 (C-α), 47.3 (CH-Fmoc), 21.0 (OAc), 20.9 (OAc), 20.8 (OAc), 17.5 (rha-CH_3).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 175 Hz.

HRMS (ESI+): Calculated for $C_{33}H_{41}O_{12}N_2$ $[M+NH_4]^+$: 657.2654; found: 657.2670.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 18.55 min, λ = 230 nm.

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-L-serine (**1**)



Cleavage of the allylic ester was conducted according to a slightly modified procedure.[486]

To a magnetically stirred solution of rhamnosyl-serine derivative **S1** (1.00 g, 1.56 mmol, 1.0 eq.) in dry CH_2Cl_2 (25 ml), Phenylsilane (427 µl, 3.43 mmol, 2.2 eq.) and $[Pd(PPh_3)_4]$ (36.0 mg, 31.3 µmol, 0.02 eq.) were added. The reaction was stirred at ambient temperature until complete conversion of the starting material was observed by TLC monitoring. The reaction was quenched by addition of water (1.50 ml) and concentrated to dryness under reduced pressure. The crude residue was co-evaporated with toluene (2 × 15 ml) and directly subjected to flash chromatography (^{c}Hex/EtOAc v/v = 2:1 + 1 % AcOH → 1:1 + AcOH) affording **1** (911 mg, 1.52 mmol, 97 %) as a colourless foam.

R$_f$ = 0.22 (^{c}Hex/EtOAc v/v = 1:1 + 1 % AcOH).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ [ppm] = 7.76 (dd, $J_{CH,CH}$ = 7.6 Hz, $J_{CH,CH}$ = 2.5 Hz, 2H, 2 × CH-Fmoc), 7.66 – 7.60 (m, 2H, 2 × CH-Fmoc), 7.39 (t, $J_{CH,CH}$ = 7.6 Hz, 1H, 2 × CH-Fmoc), 7.32 (td, $J_{CH,CH}$ = 7.6 Hz, $J_{CH,CH}$ = 2.9 Hz, 2H, 2 × CH-Fmoc), 5.97 (d, $J_{NH,H\alpha}$ = 8.5 Hz, 1H, NH-Fmoc), 5.32 (dd, $J_{H2,H3}$ = 3.5 Hz, $J_{H2,H1}$ = 1.7 Hz, 1H, H-2), 5.21 (dd, $J_{H3,H4}$ = 10.2 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 5.05 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.9 Hz, 1H, H-4), 4.78 (s, 1H, H-1), 4.69 (dt, $J_{H\alpha,NH}$ = 8.6 Hz, $J_{H\alpha,H\beta}$ = 3.3 Hz, 1H, H-α), 4.45 – 4.36 (m, 2H, CH_2-Fmoc), 4.28 – 4.17 (m, 2H, CH-Fmoc, H-β), 3.88 – 3.81 (m, 1H, H-5), 3.75 (dd, $J_{H\beta',H\beta}$ = 10.2 Hz, $J_{H\beta',H\alpha}$ = 3.3 Hz, 1H, H-β´), 2.16 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.21 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3).

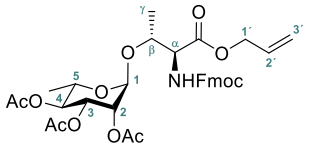
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 172.2 (C=O), 170.5 (C=O), 170.4 (C=O), 170.2 (C=O), 156.3 (Fmoc-C=O), 143.9 (Cq-Fmoc), 143.8 (Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 127.9 (2C, 2 × CH-Fmoc), 127.3 (2C, 2 × CH-Fmoc), 125.4 (2C, 2 × CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 97.8 (C-1), 71.0 (C-4), 69.7 (C-2), 69.3 (C-3), 67.9 (C-β), 67.7 (CH_2-Fmoc), 67.0 (C-5), 54.0 (C-α), 47.2 (CH-Fmoc), 21.1 (OAc), 20.9 (2C, 2 × OAc), 17.4 (rha-CH_3).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 175 Hz.

HRMS (ESI+): Calculated for $C_{30}H_{37}O_{12}N_2$ $[M+NH_4]^+$: 617.2341; found: 617.2347.

For further analytical data see reference.[535, 536]

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*O*-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-L-threonine allyl ester (**S2**)



2,3,4-tri-*O*-acetyl-L-rhamnopyranosyl trichloroacetimidate **3**[533] (1.94 g, 4.46 mmol, 1.7 eq) and Fmoc-Thr-OAll[534] (1.00 g, 2.62 mmol, 1.0 eq.) were combined and co-evaporated with toluene (2 × 10 ml). The mixture was dried 1 h under high vacuum before being dissolved in dry CH_2Cl_2 (35 ml). Freshly activated 4 Å molecular sieve was added and the reaction mixture was stirred for 1 h at room temperature, before being chilled to 0 °C. Subsequently, TMSOTf (54.0 µl, 0.30 mmol, 0.1 eq.) was added in one portion and the stirring was continued at 0 °C until complete conversion of the acceptor was detected by TLC monitoring. The reaction was stopped by addition of NEt_3 (200 µl) filtered through a pad of *Hyflo* and concentrated to dryness under reduced pressure. The crude product was directly subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **S2** (1.29 g, 1.97 mmol, 75 %) as a colourless oil.

R$_f$ = 0.17 (^{c}Hex/EtOAc v/v = 3:1).

Optical rotation: $[\alpha]_D^{24} = -47.4°$ (c = 0.33, $CHCl_3$).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ [ppm] = 7.77 (d, $J_{CH,CH}$ = 7.8 Hz, 2H, 2 × CH-Fmoc), 7.69 – 7.62 (m, 2H, 2 × CH-Fmoc), 7.41 (td, $J_{CH,CH}$ = 7.2 Hz, $J_{CH,CH}$ = 2.4 Hz, 2H, 2 × CH-Fmoc), 7.39 – 7.29 (m, 2H, 2 × CH-Fmoc), 5.96 (ddt, $J_{H2´,H3´trans}$ = 16.5 Hz, $J_{H2´,H3´cis}$ = 10.4 Hz, $J_{H2´,H1´}$ = 6.0 Hz, 1H, H-2´), 5.56 (d, $J_{NH,Hα}$ = 9.8 Hz, 1H, NH-Fmoc), 5.37 (dt, $J_{H3´trans,\ H2´}$ = 17.2 Hz, $J_{H3´trans,\ H1´}$ = 1.5 Hz, 1H, H-3$_{´trans}$), 5.31 – 5.26 (m, 1H, H-3$_{´cis}$), 5.22 (dd, $J_{H2,H3}$ = 3.5 Hz, $J_{H2,H1}$ = 1.7 Hz, 1H, H-2), 5.16 (dd, $J_{H3,H4}$ = 10.1 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 5.05 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.8 Hz, 1H, H-4), 4.84 (d, $J_{H1,H2}$ = 1.8 Hz, 1H, H-1), 4.74 (ddt, $J_{CH,CH}$ = 13.0 Hz, $J_{H1´,H2´}$ = 5.9 Hz, $J_{H1´,H3cis´}$ = $J_{H1´,H3´trans}$ = 1.3 Hz, 1H, H-1´), 4.63 (ddt, $J_{CH,CH}$ = 12.9 Hz, $J_{H1´,H2´}$ = 6.0 Hz, $J_{H1´,H3cis´}$ = $J_{H1´,H3´trans}$ = 1.3 Hz, 1H, H-1´), 4.53 (dd, $J_{Hα,NH}$ = 9.8 Hz, $J_{Hα,Hβ}$ = 2.4 Hz, 1H, H-α), 4.49 – 4.35 (m, 3H, H-β, CH_2-Fmoc), 4.28 (t, $J_{CH,CH}$ = 7.2 Hz, 1H, CH-Fmoc), 3.73 (dq, $J_{H5,H4}$ = 10.0 Hz, $J_{H5,CH3}$ = 6.3 Hz, 1H, H-5), 2.16 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.26 (d, $J_{Hγ,Hβ}$ = 6.1 Hz, 3H, H-γ), 1.20 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3).

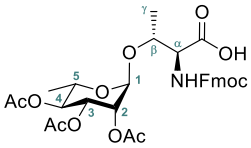
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 170.3 (C=O), 170.1 (C=O), 170.0 (2C, 2 × C=O), 156.8 (Fmoc-C=O), 144.0 (Cq-Fmoc), 143.8 (Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 131.4 (C-2´), 127.9 (2C, 2 × CH-Fmoc), 127.3 (2C, 2 × CH-Fmoc), 125.4 (2C, 2 × CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 119.6 (C-3´), 94.2 (C-1), 71.9 (C-β), 70.9 (C-4), 70.1 (C-2), 69.1 (C-3), 67.7 (CH_2-Fmoc), 67.1 (C-5), 66.6 (C-1´), 58.7 (C-α), 47.3 (CH-Fmoc), 21.0 (OAc), 20.9 (2C, 2 × OAc), 17.5 (rha-CH_3), 15.1 (Cγ).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 172 Hz.

HRMS (ESI+): Calculated for $C_{34}H_{39}O_{12}NK$ $[M+K]^+$: 692.2104; found: 692.2103.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 16.55 min, λ = 230 nm.

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*O*-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-L-threonine (**2**)



Cleavage of the allylic ester was conducted according to a slightly modified procedure.[486]

Rhamnosyl amino acid **S2** (1.15 g, 1.76 mmol, 1.0 eq.) was dissolved in dry CH_2Cl_2 (30 ml), before Phenylsilane (482 µl, 3.87 mmol, 2.2 eq.) and $[Pd(PPh_3)_4]$ (41.0 mg, 35.2 µmol, 0.02 eq.) were added. The reaction was stirred at ambient temperature until complete conversion of the starting material was observed by TLC monitoring. The reaction was stopped by addition of water (0.5 ml) and concentrated to dryness. The crude residue was co-evaporated with toluene (2 × 25 ml) and directly subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 2:1 + 1 % AcOH → 1:1 + 1 % AcOH) affording **2** (920 mg, 1.50 mmol, 85 %) as a colourless foam.

R$_f$ = 0.29 (^{c}Hex/EtOAc v/v = 1:1 + 1 % AcOH).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ [ppm] = 7.77 (d, $J_{CH,CH}$ = 7.9 Hz, 2H, 2 × CH-Fmoc), 7.64 (t, $J_{CH,CH}$ = 6.5 Hz 2H, 2 × CH-Fmoc), 7.40 (td, $J_{CH,CH}$ = 7.2 Hz, $J_{CH,CH}$ = 2.7 Hz, 2H, 2 × CH-Fmoc), 7.37 – 7.30 (m, 2H, 2 × CH-Fmoc), 5.67 (d, $J_{NH,H\alpha}$ = 9.7 Hz, 1H, NH-Fmoc), 5.23 (dd, $J_{H2,H3}$ = 3.5 Hz, $J_{H2,H1}$ =1.7 Hz, 1H, H-2), 5.18 (dd, $J_{H3,H4}$ = 10.0 Hz, $J_{H3,H2}$ = 3.5 Hz, 1H, H-3), 5.04 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.9 Hz, 1H, H-4), 4.85 (d, $J_{H1,H2}$ = 1.8 Hz, 1H, H-1), 4.55 (dd, $J_{H\alpha,NH}$ = 9.7 Hz, $J_{H\alpha,H\beta}$ = 2.4 Hz, 1H, H-α), 4.53 – 4.36 (m, 3H, CH_2-Fmoc, H-β), 4.26 (t, $J_{CH,CH}$ = 7.2 Hz, 1H, CH-Fmoc), 3.89 – 3.76 (m, 1H, H-5), 2.16 (s, 3H, OAc), 1.99 (s, 6H, 2 × OAc), 1.26 (d, $J_{H\gamma,H\beta}$ = 6.2 Hz, 3H, H-γ), 1.18 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3).

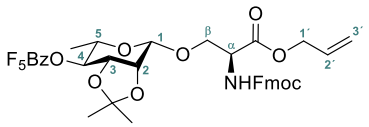
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 173.5 (C=O), 170.4 (2C, C=O), 170.2 (C=O), 156.9 (Fmoc-C=O), 143.9 (Cq-Fmoc), 143.8 (Cq-Fmoc), 141.4 (2C, 2 ×Cq-Fmoc), 127.9 (2C, 2 × CH-Fmoc), 127.3 (2C, 2 × CH-Fmoc), 125.4 (2C, 2 × CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 94.4 (C-1), 71.9 (C-β), 70.8 (C-4), 70.2 (C-2), 69.3 (C-3), 67.8 (CH_2-Fmoc), 67.3 (C-5), 58.3 (C-α), 47.2 (CH-Fmoc), 21.1 (OAc), 20.9 (2C, 2 × OAc), 17.3 (rha-CH_3), 15.2 (C-γ).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 172 Hz.

HRMS (ESI+): Calculated for $C_{31}H_{35}O_{12}NK$ $[M+K]^+$: 652.1791; found: 652.1794.

Further analytical data see reference.[535]

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*O*-(2,3-*O*-Isopropylidene-4-*O*-pentafluorobenzoyl-β-L-rhamnopyranosyl)-L-serine allyl ester (**S3**)



2,3-*O*-Isopropylidene-4-*O*-pentafluorobenzoyl-α-L-rhamnosyl trichloroacetimidate **6**[21] (724 mg, 1.30 mmol, 1.0 eq.) and Fmoc-Ser-OAll[534] (588 mg, 1.60 mmol, 1.2 eq.) were combined, co-evaporated with toluene (20 ml) and dried under high-vacuum for 2 h. The starting materials were dissolved in Et_2O/CH_2Cl_2 (45 ml, v/v = 2:1) and freshly activated 4 Å molecular sieve was added. The mixture was stirred for 1 h before being cooled to − 78 °C. Subsequently the reaction was started by addition $HNTf_2$ (37.0 mg, 0.13 mmol, 0.1 eq.). After complete consumption of the rhamnosyl donor was observed by TLC monitoring, the reaction was neutralized by addition of NEt_3 (200 µl) diluted with CH_2Cl_2 (150 ml) and filtered through a short plug of *Hyflo*. Organic solvents were removed under reduced pressure and the crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **S3** (740 mg, 1.99 mmol, 76 %) as a colourless oil.

R$_f$ = 0.19 (^{c}Hex/EtOAc v/v = 3:1).

Optical rotation: $[\alpha]_D^{24}$ =+ 26.4° (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.76 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.65 – 7.60 (m, 2H, 2 × CH-Fmoc), 7.40 (t, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.32 (t, $J_{CH,CH}$ = 7.4 Hz, 2H, 2 × CH-Fmoc), 6.01 – 5.86 (m, 2H, H-2´, NH-Fmoc), 5.36 (d, $J_{H3´trans,\ H2´}$ = 17.2 Hz, 1H, H-3´$_{trans}$), 5.28 – 5.20 (m, 2H, H-3´$_{cis}$, H-4), 4.75 (s, 1H, H-1), 4.70 (d, $J_{H1´,H2´}$ = 5.7 Hz, 2H, H-1´), 4.59 (dd, $J_{Hα,NH}$ = 8.3 Hz, $J_{Hα,Hβ}$ = 3.9 Hz, 1H, H-α), 4.48 – 4.36 (m, 2H, CH_2-Fmoc), 4.28 – 4.22 (m, 3H, CH-Fmoc, H-2, H-3), 4.22 – 4.16 (m, 2H, H-β), 3.57 (dq, $J_{H5,H4}$ = 12.4 Hz, $J_{H5,CH3}$ = 6.4 Hz, 1H, H-5), 1.62 (s, 3H, CH_3, Isopropylidene), 1.38 (s, 3H, CH_3, Isopropylidene), 1.31 (d, $J_{CH3,H5}$ = 6.3 Hz, 3H, rha-CH_3).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 169.9 (C=O), 158.2 (C=O), 156.3 (Fmoc-C=O), 146.6 − 146.4 (m, CF), 144.9 − 143.9 (m, CF), 144.5 − 144.3 (m, CF),144.0 (Cq-Fmoc), 143.9 (Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 138.8 −138.6 (m, CF), 137.1−136.9 (m, CF), 131.7 (C-2´), 127.9 (2C, 2 × CH-Fmoc), 127.2 (2C, 2 × CH-Fmoc), 125.3 (2C, 2 × CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 118.8 (C-3´), 111.7 (C_q), 99.1 (C-1), 76.8 (C-3), 76.7 (C-4), 74.3 (C-2), 70.5 (C-β), 70.0 (C-5), 67.4 (CH_2-Fmoc), 66.4 (C-1´), 54.6 (C-α), 47.3 (CH-Fmoc), 27.5 (CH_3, Isopropylidene), 26.3 (CH_3, Isopropylidene), 17.9 (rha-CH_3). Note: Due to low signal intensity, the quaternary C-atom of the pentafluorobenzoyl protecting group could not be assigned in the ^{13}C-NMR spectrum.

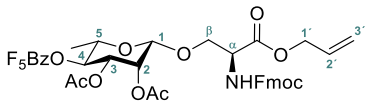
^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 160 Hz.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = −137.5 (dp, *J* = 17.0 Hz, *J* = 5.8 Hz), −147.5 (tt, *J* = 21.0 Hz, *J* = 4.9 Hz), −159.8 – −160.0 (m).

HRMS (ESI$^+$): Calculated for $C_{37}H_{38}F_5O_{10}N_2$ $[M+NH_4]^+$: 765.2441; found: 765.2446.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 22.23 min, λ = 230 nm.

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*O*-(2,3-*O*-Acetyl-4-*O*-pentafluorobenzoyl-β-L-rhamno-pyranosyl)-L-serine allyl ester (**S5**)



Compound **S3** (980 mg**,** 1.31 mmol, 1.0 eq.) dissolved in 90 % aqueous TFA (10 ml) and stirred for 1 h at ambient temperature. After complete conversion of the starting material was observed by TLC monitoring, the reaction was diluted with toluene (20 ml) and concentrated under reduced pressure. The crude residue was co-evaporated with toluene (2 × 15 ml) and dried in high-vacuum for 1 h. The crude diol was dissolved in acetic acid anhydride (15 ml) and pyridine (1 ml) was added carefully. The reaction was stirred at ambient temperature until TLC monitoring indicated complete disappearance of the starting material. The reaction was diluted with EtOAc (35 ml) and washed with 1 M HCl (15 ml), sat. aq. $NaHCO_3$ (3 × 30 ml) and brine (15 ml) and dried with $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **S5** (904 mg, 1.14 mmol, 87 % over two steps) as a colourless oil.

R*f* = 0.41 (^{c}Hex/EtOAc v/v = 2:1).

Optical rotation: $[\alpha]_D^{22}$ = + 46.8° (c = 0.33, $CHCl_3$).

^{1}H-NMR: (800 MHz, $CDCl_3$): δ [ppm] = 7.77 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.65 – 7.57 (m, 2H, 2 × CH-Fmoc), 7.40 (t, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.32 (td, $J_{CH,CH}$ = 7.4 Hz, $J_{CH,CH}$ = 4.2 Hz, 2H, 2 × CH-Fmoc), 5.94 – 5.87 (m, 1H, H-2´), 5.86 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH-Fmoc), 5.46 (d, $J_{H2,H3}$ = 3.3 Hz, 1H, H-2), 5.35 – 5.29 (m, 2H, H-4, H-3´$_{trans}$), 5.29 – 5.25 (m, 1H, H-3´$_{cis}$), 5.07 (dd, $J_{H3,H4}$ = 10.1 Hz, $J_{H3,H2}$ = 3.3 Hz, 1H, H-3), 4.70 – 4.61 (m, 3H, H-1, H-1´), 4.52 (dd, $J_{H\alpha,NH}$ = 8.8 Hz, $J_{H\alpha,H\beta}$ = 3.0 Hz, 1H, H-α), 4.47 (dd, J_{CH2} = 10.8 Hz, $J_{CH,CH}$ = 7.0 Hz, 1H, CH_2-Fmoc), 4.38 (dd, $J_{CH,CH}$ = 10.6 Hz, $J_{CH,CH}$ = 7.2 Hz, 1H, CH_2-Fmoc), 4.25 (t, $J_{CH,CH}$ = 7.1 Hz, 1H, CH-Fmoc), 4.19 (dd, $J_{H\beta,H\beta'}$ = 10.4 Hz, $J_{H\beta,H\alpha}$ = 3.1 Hz, 1H, H-β), 4.06 (dd, $J_{H\beta',H\beta}$ = 10.5 Hz, $J_{H\beta',H\alpha}$ = 2.8 Hz, 1H, H-β´), 3.61 (dq, $J_{H5,H4}$ = 12.2 Hz, $J_{H5,CH3}$ = 6.2 Hz, 1H, H-5), 2.19 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.34 (d, $J_{CH3,H5}$ = 6.1 Hz, 3H, rha-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 170.4 (C=O), 170.1 (C=O), 169.4 (C=O), 158.2 (C=O), 156.2 (Fmoc-C=O), 146.4 – 146.2 (m, CF), 144.1 – 143.6 (m, 3C, CF, 2 × Cq-Fmoc), 142.4 – 142.1 (m, CF), 141.5 (Cq-Fmoc), 141.4 (Cq-Fmoc), 139.3 – 138.9 (m, CF), 136.8 –136.4 (m, CF), 131.6 (C-2´), 127.9 (2C, 2 × CH-Fmoc), 127.2 (2C, 2 × CH-Fmoc), 125.3 (CH-Fmoc), 125.2 (CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc) 119.2 (C-3´), 98.5 (C-1), 73.0 (C-4), 70.6 (2C, C-3, C-5), 70.4 (C-β), 68.8 (C-2), 67.2 (CH_2-Fmoc), 66.5 (C-1´), 54.5 (C-α), 47.3 (CH-Fmoc), 20.9 (OAc), 20.6 (OAc), 17.4 (rha-CH_3). Note: Quaternary C-atom of the pentafluorobenzoyl protecting group could not be assigned in the ^{13}C-NMR spectrum.

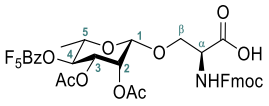
^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 159 Hz.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 139.0 (dp, *J* = 16.4 Hz, *J* = 5.8 Hz), −147.7 (tt, *J* = 20.8 Hz, *J* = 4.4 Hz), −159.3 – −159.6 (m).

HRMS (ESI^{+}): Calculated for $C_{38}H_{38}F_5O_{12}N_2$ $[M+NH_4]^+$: 809.2339; found: 809.2350.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 21.68 min, λ = 230 nm.

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*O*-(2,3-*O*-Acetyl-4-*O*-pentafluorobenzoyl-β-L-rhamno-pyranosyl) -L-serine (**4**)



Cleavage of the allylic ester was conducted according to a literature known protocol.[537]

To a magnetically stirred solution of allylic ester **S5** (700 mg, 0.88 mmol, 1.0 eq.) in dry THF (20 ml), [$Pd(PPh_3)_4$] (104 mg, 0.09 mmol, 0.1 eq.) and *N*-methylaniline (953 µl, 8.80 mmol, 10 eq.) was added. The reaction was stirred at ambient temperature until TLC monitoring indicated complete conversion of the starting material. Subsequently, the reaction was concentrated to dryness under reduced pressure and the crude residue was co-evaporated with toluene (2 × 10 ml). The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1 + 1 % AcOH → 1:1 + 1 % AcOH) affording **4** (650 mg, 0.86 mmol, 98 %) as a colourless foam.

R$_f$ = 0.12 (^{c}Hex/EtOAc v/v = 1:1 + 1 % AcOH).

Optical rotation: $[\alpha]_D^{24}$ = + 63.0° (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.77 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.61 (t, $J_{CH,CH}$ = 8.4 Hz 2H, 2 × CH-Fmoc), 7.40 (t, $J_{CH,CH}$ = 7.4 Hz, 2H, 2 × CH-Fmoc), 7.34 – 7.30 (m, 2H, 2 × CH-Fmoc), 5.94 (d, $J_{NH,H\alpha}$ = 8.4 Hz, 1H, NH-Fmoc), 5.49 (d, $J_{H2,H3}$ = 3.3 Hz, 1H, H-2), 5.31 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.8 Hz, 1H, H-4), 5.12 (dd, $J_{H3,H4}$ = 10.2 Hz, $J_{H3,H2}$ = 3.3 Hz, 1H, H-3), 4.72 (s, 1H, H-1), 4.55 (d, $J_{H\alpha,NH}$ = 8.2 Hz, 1H, H-α), 4.48 (dd, $J_{CH,CH}$ = 10.6 Hz, $J_{CH,CH}$ = 7.0 Hz, 1H, CH_2-Fmoc), 4.40 (dd, $J_{CH,CH}$ = 10.6 Hz, $J_{CH,CH}$ = 7.1 Hz, 1H, CH_2-Fmoc), 4.27 – 4.16 (m, 2H, CH-Fmoc, H-β), 4.11 (d, $J_{H\beta',H\beta}$ = 10.3 Hz, 1H, H-β´), 3.69 – 3.61 (m, 1H, H-5), 2.19 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.34 (d, $J_{CH3,H5}$ = 6.1 Hz, 3H, rha-CH_3).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 173.2 (C=O), 171.1 (C=O), 170.3 (C=O), 158.2 (C=O), 156.6 (Fmoc-C=O), 146.0 – 145.8 (m, CF), 144.0 – 143.6 (m, 3C, CF, 2 × Cq-Fmoc), 141.5 (Cq-Fmoc) 141.4 (Cq-Fmoc), 138.8 – 138.6 (m, CF), 137.1 – 136.9 (m, CF), 127.9 (2C, 2 × CH-Fmoc), 127.2 (2C, 2 × CH-Fmoc), 125.2 (2C, 2 × CH-Fmoc), 120.2 (2C, 2 × CH-Fmoc), 98.4 (C-1), 73.1 (C-4), 70.7 (C-3), 70.4 (C-5), 69.9 (C-β), 69.2 (C-2), 67.5 (CH_2-Fmoc), 54.2 (C-α), 47.2 (CH-Fmoc), 20.9 (OAc), 20.6 (OAc), 17.4 (rha-CH_3). Note: Due to low signal intensity of C-atoms belonging to the pentafluorobenzoyl protecting group only 33 out of 35 carbon atoms were assigned from the ^{13}C-NMR spectrum.

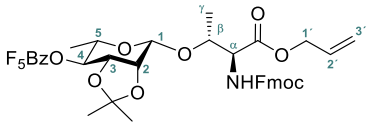
^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 159 Hz.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = −139.0 (dp, *J* = 16.5 Hz, *J* = 5.6 Hz), −147.6 (tt, *J* = 21.0 Hz, *J* = 4.4 Hz), −159.3 – −159.5 (m).

HRMS (ESI^-): Calculated for $C_{35}H_{29}O_{12}NF_5$ [M-H-]: 750.1615; found: 750.1640.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 19.27 min, λ = 230 nm.

N^{α}-Fluorenylmethoxycarbonyl-*O*-(2,3-*O*-Isopropylidene-4-*O*-pentafluorobenzoyl-β-L-rhamno-pyranosyl)-L-threonine allyl ester (**S4**)



2,3-*O*-Isopropylidene-4-*O*-pentafluorobenzoyl-α-L-rhamnosyl trichloroacetimidate **6**[21] (724 mg, 1.33 mmol, 1.0 eq.) and Fmoc-Thr-OAll[534] (610 mg, 1.60 mmol, 1.2 eq.) were combined, co-evaporated with toluene (20 ml) and CH_2Cl_2 (20 ml) and dried under high-vacuum for 2 h. Subsequently the starting materials were dissolved in Et_2O/CH_2Cl_2 (45 ml, v/v = 2:1) and freshly activated 4 Å molecular sieve was added. The mixture was stirred for 1 h before being cooled to − 78 °C. Subsequently the reaction was started by addition $HNTf_2$ (32.0 mg, 0.11 mmol, 0.1 eq.). After complete consumption of the rhamnosyl donor was observed by TLC monitoring, the reaction was neutralized by addition of NEt_3 (200 µl), diluted with CH_2Cl_2 and filtered through a short plug of *Hyflo*. Organic solvents were removed under reduced pressure and the crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 5:1) affording **S4** (865 mg, 1.14 mmol, 85 %) as a colourless foam.

R$_f$ = 0.33 (^{c}Hex/EtOAc v/v = 3:1 + 1 % NEt_3).

Optical rotation: $[\alpha]_D^{24}$ = + 21.6° (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.80 – 7.74 (m, 2H, 2 × CH-Fmoc), 7.67 – 7.60 (m, 2H, 2 × CH-Fmoc), 7.44 – 7.38 (m, 2H, 2 × CH-Fmoc), 7.32 (td, $J_{CH,CH}$ = 7.2 Hz, $J_{CH,CH}$ = 3.1 Hz, 2H, 2 × CH-Fmoc), 5.93 (ddt, $J_{H2´,H3´trans}$ = 16.4 Hz, $J_{H2´,H3´cis}$ = 10.5 Hz, $J_{H2´,H1´}$ = 5.8 Hz, 1H, H-2´), 5.73 (d, $J_{NH,Hα}$ = 9.6 Hz, 1H, NH-Fmoc), 5.37 (dd, $J_{H3´trans, H2´}$ = 17.2 Hz, $J_{H3´trans, H1´}$ = 1.5 Hz, 1H, H-3´$_{trans}$), 5.27 (dd, $J_{H3´cis, H2´}$ = 10.4 Hz, $J_{H3´cis, H1´}$ = 1.3 Hz, 1H, H-3´$_{cis}$), 5.19 (dd, $J_{H4,H5}$ = 9.7 Hz, $J_{H4,H3}$ = 7.4 Hz, 1H, H-4), 4.77 (d, $J_{H1,H2}$ = 2.1 Hz, 1H, H-1), 4.67 (dt, $J_{H1´,H2´}$ =5.9 Hz, $J_{H1´,H3´trans}$ = $J_{H1´,H3´cis}$ = 1.4 Hz, 2H, H-1´), 4.49 (qd, $J_{Hβ,Hγ}$ = 6.4 Hz, $J_{Hβ,Hα}$ = 2.5 Hz, 1H, H-β), 4.45 (dd, $J_{Hα,NH}$ = 9.6 Hz, $J_{Hα,Hβ}$ = 2.5 Hz, 1H, H-α), 4.43 (d, $J_{CH,CH}$ = 7.2 Hz, 2H, CH_2-Fmoc), 4.26 (t, $J_{CH,CH}$ = 7.6 Hz, 1H, CH-Fmoc), 4.19 (dd, $J_{H3,H4}$ = 7.4 Hz, $J_{H3,H2}$ = 5.5 Hz, 1H, H-3), 4.14 (dd, $J_{H2,H3}$ = 5.7 Hz,$J_{H2,H1}$ = 2.2 Hz, 1H, H-2), 3.53 (dq, $J_{H5,H4}$ = 9.8 Hz, $J_{H5,CH3}$ = 6.2 Hz, 1H, H-5), 1.63 (s, 3H, CH_3, Isopropylidene), 1.41 (d, $J_{Hγ,Hβ}$ = 6.5 Hz, 3H, H-γ), 1.38 (s, 3H, CH_3, Isopropylidene), 1.32 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 170.3 (C=O), 158.3 (C=O), 156.9 (Fmoc-C=O), 146.6 − 146.4 (m, CF-Ar), 144.9 − 144.7 (m, CF-Ar), 144.0 (Cq-Fmoc), 143.8 (Cq-Fmoc), 142.8 − 142.6 (m, CF-Ar), 141.5 (Cq-Fmoc), 141.4 (Cq-Fmoc), 138.8 − 138.6 (m, CF-Ar), 137.1 − 136.9 (m, CF-Ar), 131.6 (C-2´), 127.9 (2C, 2 × CH-Fmoc), 127.2 (2C, 2 × CH-Fmoc), 125.3 (2C, 2 × CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 119.3 (C-3´), 111.7 (C_q), 99.5 (C-1), 77.0 (C-β), 76.9 (2C,C-3, C-4), 74.6 (C-2), 69.8 (C-5), 67.4 (CH_2-Fmoc), 66.3 (C-1´), 58.8 (C-α), 47.3 (CH-Fmoc), 27.7 (CH_3, Isopropylidene), 26.3 (CH_3, Isopropylidene), 19.0 (Cγ), 17.9 (rha-CH_3). Note: Signal of C-β was obscured by the solvent signal and was therefore assigned by 2D-HSQC analysis. Quaternary C-atom of the pentafluorobenzoyl protecting group could not be assigned in the ^{13}C-NMR spectrum.

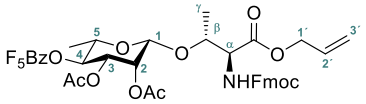
^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = − 137.5 (dp, *J* = 16.9 Hz, *J* = 5.8 Hz), −147.7 (tt, *J* = 20.8 Hz, *J* = 4.9 Hz), −159.9 – −160.1 (m).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 159 Hz.

HRMS (ESI^+): Calculated for $C_{38}H_{40}F_5O_{10}N_2$ $[M+NH_4]^+$: 779.2598; found: 779.2603.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 23.57 min, λ = 230 nm.

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*O*-(2,3-O-Acetyl-4-*O*-pentafluorobenzoyl-β-L-rhamno-pyranosyl)-L-threonine allyl ester (**S6**)



Rhamnosyl amino acid **S4** (590 mg, 0.78 mmol, 1.0 eq.) was dissolved in 90 % aqueous TFA (30 ml) and stirred for 1 h at ambient temperature. After TLC monitoring indicated complete conversion of the starting material, TFA was removed under reduced pressure and the crude residue was co-evaporated with toluene (20 ml) and dried 1 h under high vacuum. The crude diol was then dissolved in acetic anhydride (15 ml) and pyridine (1 ml) was added. The reaction was left to stir 1.5 h at room temperature, before being poured onto water. The aqueous mixture carefully neutralized with Na_2CO_3 and subsequently extracted with CH_2Cl_2 (2 × 20 ml). The combined organic layers were washed with brine (25 ml) and dried with $MgSO_4$. The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1) affording **S6** (463 mg, 0.58 mmol, 74 % over two steps) as a colourless foam.

R$_f$ = 0.53 (^{c}Hex/EtOAc v/v = 2:1).

Optical rotation: $[\alpha]_D^{24}$ = + 21.0° (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.77 (d, $J_{CH,CH}$ = 7.6 Hz, 2H, 2 × CH-Fmoc), 7.67 – 7.61 (m, 2H, 2 × CH-Fmoc), 7.40 (t, $J_{CH,CH}$ = 7.2 Hz, 2H, 2 × CH-Fmoc), 7.32 (t $J_{CH,CH}$ = 7.2 Hz, 2H, 2 × CH-Fmoc), 5.94 (ddt, $J_{H2',H3'trans}$ = 16.5 Hz, $J_{H2',H3'cis}$ = 10.4 Hz, $J_{H2',H1'}$ = 5.9 Hz, 1H, H-2´), 5.60 (d, $J_{NH,H\alpha}$ = 9.7 Hz, 1H, NH-Fmoc), 5.41 – 5.35 (m, 2H, H-3´$_{trans}$, H-2), 5.33 – 5.27 (m, 2H, H-3´$_{cis}$, H-4), 5.05 (dd, $J_{H3,H4}$ = 10.2 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 4.71 – 4.62 (m, 3H, H-1´, H-1), 4.49 – 4.35 (m, 4H, CH_2-Fmoc, H-β, H-α), 4.26 (t, $J_{CH,CH}$ = = 7.3 Hz, 1H, CH-Fmoc), 3.62 (dq, $J_{H5,H4}$ = 9.7 Hz, $J_{H5,CH3}$ = 6.3 Hz, 1H, H-5), 2.23 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.39 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3), 1.33 (d, $J_{H\gamma,H\beta}$ = 6.4 Hz, 3H, H-γ).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 170.6 (C=O), 170.2 (C=O), 170.0 (C=O), 158.2 (C=O), 156.8 (Fmoc-C=O), 146.0 – 145.8 (m, CF), 144.4 – 144.2 (m, CF), 144.1 (Cq-Fmoc), 143.8 (Cq-Fmoc), 142.8 – 142.5 (m, CF), 141.5 (Cq-Fmoc), 141.4 (Cq-Fmoc), 138.8 – 138.6 (m, CF), 137.1 – 136.9 (m, CF), 131.5 (C-2´), 127.9 (CH-Fmoc), 127.8 (CH-Fmoc) 127.2 (2C, 2 × CH-Fmoc), 125.4 (CH-Fmoc), 125.3 (CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 119.8 (C-3´), 98.9 (C-1), 77.7 (C-β), 73.1 (C-4), 70.6 (C-3), 70.3 (C-5), 69.5 (C-2), 67.4 (CH_2-Fmoc), 66.6 (C-1´), 58.7 (C-α), 47.3 (CH-Fmoc), 21.1 (OAc), 20.5 (OAc), 18.3 (C-γ), 17.5 (CH_3-rha). Note: Quaternary C-atom of the pentafluorobenzoyl protecting group could not be assigned in the ^{13}C-NMR spectrum.

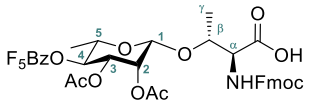
^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = −138.9 – −139.1 (m), −147.7 (tt, J = 20.8 Hz, J = 4.4 Hz), −159.3 – −159.6 (m)

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 161 Hz.

HRMS (ESI^+): Calculated for $C_{39}H_{40}F_5O_{10}N_2$ $[M+NH_4]^+$: 823.2496; found: 823.2498.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 25.88 min, λ = 230 nm.

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*O*-(2,3-*O*-Acetyl-4-*O*-pentafluorobenzoyl-β-L-rhamno-pyranosyl)-L-threonine (**5**)



Cleavage of the allylic ester was conducted according to a literature known protocol.[537]

To a magnetically stirred solution of rhamnosyl threonine derivative **S6** (1.15 g, 1.43 mmol, 1.0 eq.) in dry THF (25 ml), $[Pd(PPh_3)_4]$ (162 mg, 0.14 mmol, 0.1 eq.) and *N*-methylaniline (1.55 ml, 14.3 mmol, 10 eq.) was added. The reaction was stirred at ambient temperature until TLC monitoring indicated complete conversion of the starting material. Subsequently, the reaction was concentrated to dryness under reduced pressure and the crude residue was co-evaporated with toluene (2 × 10 ml). The crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 3:1 + 1 % AcOH → 2:1 + 1 % AcOH) affording **5** (1.03 g, 1.35 mmol, 94 %) as a colourless foam.

R$_f$ = 0.33 (^{c}Hex/EtOAc v/v = 1:1 + 1 % AcOH).

Optical rotation: $[\alpha]_D^{24}$ = + 37.8 (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.76 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.64 – 7.58 (m, 2H, 2 × CH-Fmoc), 7.42 – 7.37 (m, 2H, 2 × CH-Fmoc), 7.30 (t, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 5.78 (d, $J_{NH,H\alpha}$ = 9.4 Hz, 1H, NH-Fmoc), 5.45 (d, $J_{H2,H3}$ = 3.4 Hz, 1H, H-2), 5.31 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.9 Hz, 1H, H-4), 5.17 (dd, $J_{H3,H4}$ = 10.2 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 4.85 (s, 1H, H-1), 4.52 – 4.45 (m, 2H, H-β, H-α), 4.43 – 4.35 (m, 2H, CH_2-Fmoc), 4.22 (t, $J_{CH,CH}$ = 7.3 Hz, 1H, CH-Fmoc), 3.73 – 3.66 (m, 1H, H-5), 2.24 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.39 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3), 1.34 (d, $J_{H\gamma,H\beta}$ = 6.4 Hz, 3H, H-γ).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 172.9 (C=O), 171.7 (C=O), 170.4 (C=O), 158.2 (C=O), 157.1 (Fmoc-C=O), 145.9 − 145.8 (m, CF-Ar), 144.0 (Cq-Fmoc), 143.7 (Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 138.8 − 138.5 (m, CF-Ar), 137.1 − 136.9 (m, CF-Ar), 127.9 (2C, 2 × CH-Fmoc), 127.2 (2C, 2 × CH-Fmoc), 125.4 (CH-Fmoc), 125.3 (CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 98.8 (C-1), 77.6 (C-β), 73.2 (C-4), 70.7 (C-3), 70.2 (C-5), 69.9 (C-2), 67.6 (CH_2-Fmoc), 58.4 (C-α), 47.2 (CH-Fmoc), 21.1 (OAc), 20.6 (OAc), 18.3 (Cγ), 17.5 (rha-CH_3). Note: Due to low signal intensity of the carbon atoms belonging to the pentafluorobenzoyl protecting group only 33 out of 36 carbon atoms were assigned in the ^{13}C-NMR spectrum.

^{19}F-NMR (377 MHz, $CDCl_3$): δ [ppm] = −139.1 (dp, *J* = 16.6 Hz, *J* = 5.7 Hz), −147.8 (tt, *J* = 20.7 Hz, *J* = 4.3 Hz), −159.5 – −159.7 (m).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 159 Hz.

HRMS (ESI^+): Calculated for $C_{36}H_{36}F_5O_{12}N_2$ $[M+NH_4]^+$: 783. 2183; found: 783.2178.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 21.03 min, λ = 230 nm.

Synthesis of *N*-rhamnosyl asparagine SPPS building blocks:

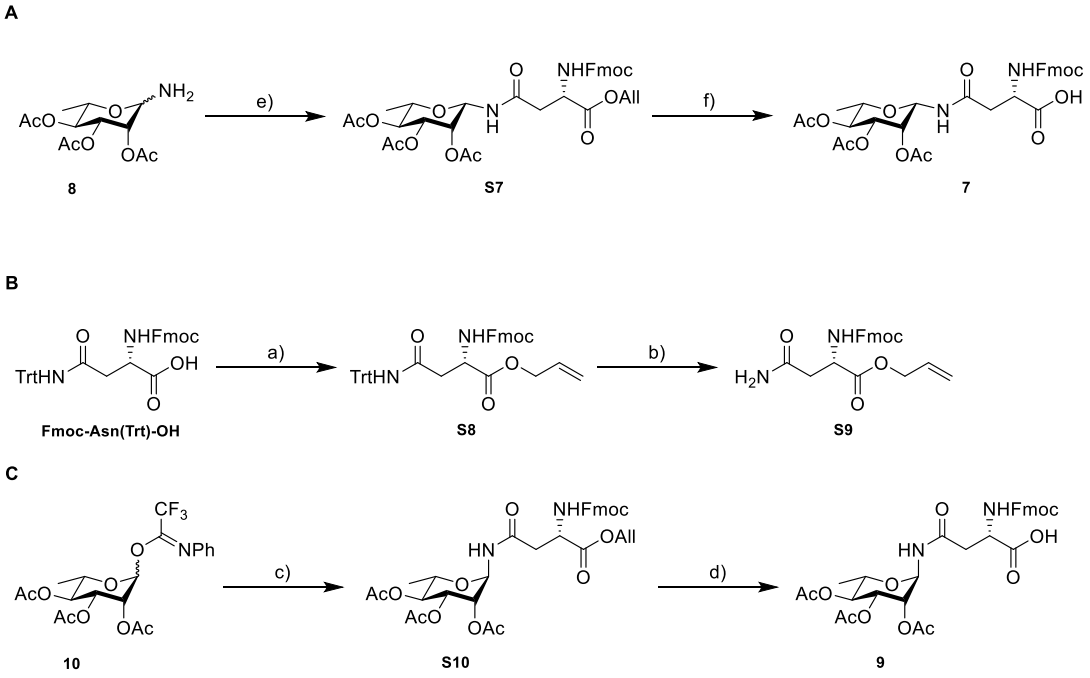
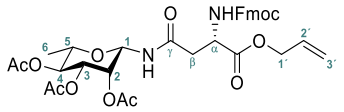


Figure 4: General overview of the chemical synthesis of *N*-rhamnosylated asparagine SPPS building blocks. **A**) Synthesis of β-*N*-rhamnosyl asparagine building block; Reagents and conditions: e) Fmoc-Asp-OAll, PyBOP, DIPEA, DMF, 79 %; f) $PhSiH_3$, $[Pd(PPh_3)_4]$, CH_2Cl_2, 81 %; **B**) Synthesis of Fmoc-Asn-OAll acceptor **S10**: Reagents and conditions: a) $NaHCO_3$, Allyl bromide, DMF, 85 %, b) TFA, H_2O, CH_2Cl_2, 89 %. **C**) Synthesis of α-*N*-rhamnosyl asparagine building block: Reagents and conditions: c) Fmoc-Asn-OAll (**S9**), TMSOTf, $MeNO_2/CH_2Cl_2$, 63 %; d) $PhSiH_3$, $[Pd(PPh_3)_4]$, CH_2Cl_2, 76 %.

N^{α}-Fluorenylmethoxycarbonyl-N^{γ}-(2,3,4-Tri-*O*-acetyl-β-L-rhamnosyl)-L-asparagine allyl ester (**S7**)



To a magnetically stirred solution of Fmoc-Asp-OAll[538] (2.40 g, 6.07 mmol, 1.7 eq.) in DMF (20 ml), PyBOP (4.43 g, 8.50 mmol, 2.4 eq.) were added at 0 °C. The solution was adjusted to pH = 9 by careful addition of DIPEA and stirred for 3 min. Subsequently a solution of rhamnosyl amine **8**[486] (1.04 g, 3.59 mmol, 1.0 eq.) in DMF (20 ml) was added slowly. After complete addition of the amine, the pH was again adjusted to pH = 9 and the reaction was stirred for 2 h at room temperature. Subsequently the organic solvents were removed under reduced pressure and the crude product was dissolved in CH_2Cl_2 (150 ml). The organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 50 ml), 1 M HCl (50 ml), H_2O (25 ml) and brine (25 ml). The organic layer was dried over $MgSO_4$ and all organic solvent were removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH v/v = 120:1) furnishing **S7** (1.89 g, 2.84 mmol, 79 %).

R_f = 0.16 (CH_2Cl_2/MeOH v/v = 120:1).

Optical rotation: $[\alpha]_D^{18}$ = + 13.8° (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.76 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.60 (t, $J_{CH,CH}$ = 6.6 Hz, 2H, 2 × CH-Fmoc), 7.40 (t, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.31 (tt, $J_{CH,CH}$ = 7.4 Hz, $J_{CH,CH}$ = 1.4 Hz, 2H, 2 × CH-Fmoc), 6.49 (d, $J_{NH,H1}$ = 9.2 Hz, 1H, NHγ), 5.90 (m,2H, NH-Fmoc ,H-2´), 5.47 (d, $J_{H1,NH}$ = 9.0 Hz, 1H, H-1), 5.36 – 5.27 (m, 2H, H-2, H-3´trans), 5.24 (d, 1H, $J_{H3´cis,\ H2´}$ = 10.4 Hz, H-3´cis), 5.07 – 4.97 (m, 2H, H-3, H-4), 4.67 (d, $J_{H1´;H2´}$ = 5.8 Hz, H-1´), 4.62 (dt, $J_{Hα,NH}$ = 8.9 Hz, $J_{Hα,Hβ}$ = 4.7 Hz, 1H, H-α), 4.42 – 4.31 (m, 2H, CH_2-Fmoc), 4.23 (t, $J_{CH,CH}$ = 7.0 Hz, 1H, CH-Fmoc), 3.62 (dq, $J_{H5,H4}$ = 8.9 Hz, $J_{H5,CH3}$ = 6.2 Hz, H-5), 2.96 (dd, $J_{Hβ,Hβ´}$ =16.4 Hz, $J_{Hβ,Hα}$ = 4.8 Hz, 1H, H-β), 2.84 (dd, $J_{Hβ´,Hβ}$ = 16.4 Hz, $J_{Hβ´,Hα}$ = 4.5 Hz, 1H, H-β´), 2.21 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.23 (d, $J_{CH3,H5}$ = 6.1 Hz, 3H, rha-CH_3).

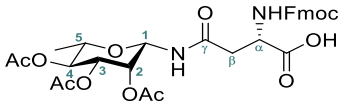
^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 170.6 (C=O), 170.5 (C=O) 170.1 (2C, 2 × C=O), 169.5 (C=O), 156.3 (C=O-Fmoc), 143.9 (2C, 2 × Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 131.6 (C-2´), 127.9 (2C, 2 × CH-Fmoc), 127.2 (2C, 2 × CH-Fmoc), 125.3 (2C, 2 × CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 119.2 (C-3´), 75.8 (C-1), 72.5 (C-5), 71.6 (C3/C4), 70.2 (2C, C3/C4, C-2), 67.5 (CH_2-Fmoc), 66.7 (C-1´), 50.6 (C-α), 47.2 (CH-Fmoc), 38.3 (C-β), 21.1 (OAc), 20.9 (OAc), 20.7 (OAc), 17.6 (rha-CH_3).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 154 Hz.

HRMS (ESI^+): Calculated for $C_{34}H_{39}O_{12}N_2$ $[M+H]^+$: 667.2498; found: 667.2498.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 12.09 min, λ = 230 nm.

N$^{\alpha}$-Fluorenylmethoxycarbonyl-*N*$^{\gamma}$-(2,3,4-Tri-*O*-acetyl-β-L-rhamnosyl)-L-asparagine (**7**)



Cleavage of the allylic ester was conducted according to a slightly modified procedure.[486]

A magnetically stirred solution of **S7** (1.00 g, 1.50 mmol, 1.0 eq.) in dry CH_2Cl_2 (25 ml) were added Phenylsilane (0.41 ml, 3.30 mmol, 2.2 eq.) and [$Pd(PPh_3)_4$] (35.0 mg, 30.0 µmol, 0.02 eq.). Upon completion, H_2O (20 ml) was added and the reaction was stirred for another 20 min at room temperature. Subsequently, solvents were removed under reduced pressure and the crude residue was co-evaporated with toluene to furnish a black amorphous solid. The crude product was subjected to flash column chromatography (ʿHex/EtOAc v/v = 1:2 + 1 % AcOH) affording **7** (758 mg, 1.21 mmol, 81 %) as a beige foam.

R$_f$ = 0.06 (ʿHex/EtOAc v/v = 1:2 + 1 % AcOH).

Optical rotation: $[\alpha]_D^{18}$ = + 22.6° (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.74 (d, $J_{CH,CH}$ = 7.6 Hz, 2H, 2 × CH-Fmoc), 7.61 – 7.57 (m, 2H, 2 × CH-Fmoc), 7.38 (t, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.29 (td, $J_{CH,CH}$ = 7.4 Hz, $J_{CH,CH}$ = 3.3 Hz, 2H, 2 × CH-Fmoc), 7.06 (d, $J_{NH,H1}$ = 9.0 Hz, 1H, NHγ), 6.13 (d, $J_{NH,H\alpha}$ = 7.6 Hz, 1H, NH-Fmoc), 5.49 (dd, $J_{H1,NH}$ = 9.1 Hz, $J_{H1,H2}$ = 1.4 Hz, 1H, H-1), 5.38 (dd, $J_{H2,H3}$ = 3.2 Hz, $J_{H2,H1}$ = 1.2 Hz, 1H, H-2), 5.08 (dd, $J_{H3,H4}$ = 10.2 Hz, $J_{H3,H2}$ = 3.3 Hz, 1H, H-3), 5.01 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.8 Hz, 1H, H-4), 4.62 – 4.56 (m, 1H, H-α), 4.44-4.29 (m, 2H, CH_2-Fmoc), 4.21 (t, $J_{CH,CH}$ = 7.2 Hz, 1H, CH-Fmoc), 3.67 (dq, $J_{H5,H4}$ = 9.5 Hz, $J_{H6,CH3}$ = 6.1 Hz, 1H, H-5), 3.00 – 2.93 (m, 1H, H-β), 2.82 (dd, $J_{H\beta',H\beta}$ = 16.4 Hz, $J_{H\beta',H\alpha}$ = 5.6 Hz, 1H, H-β´), 2.17 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.22 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3).

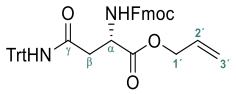
^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 173.2 (C=O) , 170.8 (C=O), 170.2 (2C, 2 × C=O), 156.6 (C=O-Fmoc), 143.8 (2C, 2 × Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 127.9 (2C, 2 × CH-Fmoc), 127.3 (2C, 2 × CH-Fmoc), 125.3 (2C, 2 × CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 76.0 (C-1), 72.6 (C-5), 71.6 (C-3), 70.2 (C-4), 70.1 (C-2), 67.7 (CH_2-Fmoc), 50.3 (C-α), 47.1 (CH-Fmoc), 37.9 (C-β), 21.0 (OAc), 20.9 (OAc), 20.7 (OAc), 17.6 (rha-CH_3). Note: Due to signal overlap only 30 out of 31 carbon atoms are assigned in the ^{13}C-spectra.

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 157 Hz.

HRMS (ESI^+): Calculated for $C_{31}H_{38}O_{12}N_3$ $[M+NH_4]^+$: 644.2450; found: 644.2447.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 18.27 min, λ = 230 nm.

N^{α}-Fluorenylmethoxycarbonyl-N^{γ}-trityl-L-asparagine allyl ester (**S8**)



Compound **S8** was synthesized according to a slightly modified procedure.[539]

To a magnetically stirred mixture of Fmoc-Asn(Trt)-OH (3.00 g, 5.03 mmol, 1.0 eq.) and $NaHCO_3$ (1.06 g, 12.6 mmol, 2.5 eq.) in DMF (50 ml), Allyl bromide (1.30 ml, 15.1 mmol, 3.0 eq.) was added slowly. The reaction mixture was stirred for 48 h at room temperature. Subsequently, solvents were removed under reduced pressure and the crude residue was dissolved in CH_2Cl_2 (150 ml), washed with water (20 ml) and brine (20 ml) and dried with $MgSO_4$. Solvent was again removed under reduced pressure and the crude residue was crystallized from ^{c}Hex/EtOAc (v/v = 10:1) to furnish **S8** (2.73 g, 4.29 mmol, 85 %) as a colourless solid.

R$_f$ = 0.30 (3:1 ^{c}Hex/EtOAc).

Optical rotation: $[\alpha]_D^{22}$ = + 16.0° (c = 1.0; $CHCl_3$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.76 (dd, $J_{CH,CH}$ = 7.6 Hz, $J_{CH,CH}$ = 4.0 Hz, 2H, 2 × CH-Fmoc), 7.59 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.40 (t, $J_{CH,CH}$ = 7.6 Hz, 2H, 2 × CH-Fmoc), 7.34 – 7.27 (m, 10H, 2 × CH-Fmoc, Ar-H), 7.20 – 7.14 (m, 7H, Ar-H), 6.68 (s, 1H, NH_{γ}), 6.12 (d, $J_{NH,H\alpha}$ = 8.9 Hz, 1H, NH-Fmoc), 5.83 (ddt, $J_{H2´,H3´trans}$ = 16.4 Hz, $J_{H2´,H3´cis}$ = 10.9 Hz, $J_{H2´,H1´}$ = 5.7 Hz, 1H, H-2´), 5.31 – 5.24 (m, 1H, H-3´$_{trans}$), 5.19 (dd, $J_{H3´cis,\ H2´}$ = 10.4 Hz, $J_{H3´cis,\ H3´trans/H1´}$ = 1.4 Hz, 1H, H-3´$_{cis}$), 4.64 (dt, $J_{H\alpha,NH}$ = 8.8 Hz, $J_{H\alpha,H\beta}$ = 4.2 Hz, 1H, H-α), 4.59 (d, $J_{H1´,H2´}$ = 5.7 Hz, 2H, H-1´), 4.42 (dd, $J_{CH,CH}$ = 10.3 Hz, $J_{CH,CH}$ = 7.0 Hz, 1H, CH_2-Fmoc), 4.28 (dd, $J_{CH,CH}$ = 10.3 Hz, $J_{CH,CH}$ = 7.5 Hz, 1H, CH_2-Fmoc), 4.21 (t, $J_{CH,CH}$ = 7.1 Hz, 1H, CH-Fmoc), 3.14 (dd, $J_{H\beta,H\beta´}$ = 15.9 Hz, $J_{H\beta,H\alpha}$ = 4.5 Hz, 1H, H-β), 2.83 (dd, $J_{H\beta´,H\beta}$ = 15.9 Hz, $J_{H\beta´,H\alpha}$ = 4.2 Hz, 1H, H-β´).

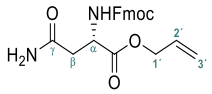
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 170.8 (C=O), 169.4 (C=O), 156.4 (Fmoc-C=O), 144.4 (C-Ar), 144.0 (Cq-Fmoc), 143.9 (Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 131.7 (C-2´), 128.8 (C-Ar), 128.2 (C-Ar), 127.8 (2C, 2 × CH-Fmoc), 127.4 (C-Ar), 127.2 (2C, 2 × CH-Fmoc), 125.4 (CH-Fmoc), 125.3 (CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 118.7 (C-3´), 71.1 (Cq), 67.4 (CH_2-Fmoc), 66.5 (C-1´), 51.1 (C-α), 47.2 (CH-Fmoc), 38.7 (C-β).

Note: Due to signal overlap only 27 out of 41 carbon atoms are assigned from the ^{13}C-spectrum.

HRMS (ESI^+): Calculated for $C_{41}H_{37}O_5N_2$ $[M+H]^+$: 637.2697; found: 637.2695.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 25.08 min, λ = 230 nm.

N^{α}-Fluorenylmethoxycarbonyl-L-asparagine allyl ester (**S9**)



Compound **S9** was synthesized according to a modified procedure from reference.[540]

To a magnetically stirred solution of asparagine derivative **S8** (2.64 g, 4.15 mmol, 1.0 eq.) in CH_2Cl_2 (20 ml), water (2 ml) and trifluoroacetic acid (20 ml) were added. The reaction mixture was stirred at room temperature until complete conversion of the starting material was observed by TLC monitoring. Subsequently, all organic solvents were removed under reduced pressure and the crude residue was co-evaporated with toluene (2 × 25 ml) and dried under high vacuum. The crude product was then crystallized from cold ^{c}Hex, to furnish **S9** (1.45 g, 3.68 mmol, 89 %) as a colourless solid.

R$_f$ = 0.03 (2:1 ^{c}Hex/EtOAc + 1 % NEt_3).

Optical rotation: $[\alpha]_D^{23}$ = + 22.0° (c = 0.5; $CHCl_3$).

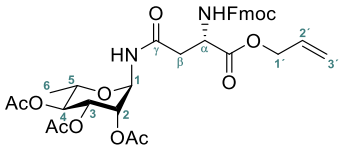
^{1}H-NMR (400 MHz, DMSO-d_6): δ [ppm] = 7.89 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.75 (d, $J_{NH,H\alpha}$ = 8.2 Hz, 1H, NH-Fmoc), 7.70 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.42 (t, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.33 (tt, $J_{CH,CH}$ = 7.4 Hz, $J_{CH,CH}$ = 1.5 Hz, 2H, 2 × CH-Fmoc), 5.87 (ddt, $J_{H2´,H3´trans}$ = 17.3 Hz, $J_{H2´,H3´cis}$ = 10.4 Hz, $J_{H2´,H1´}$ = 5.2 Hz, 1H, H-2´), 5.30 (dq, $J_{H3´trans,H2´}$ = 17.3 Hz, $J_{H3´trans,H1´}$ = $J_{H3´trans,H3´cis}$ = 1.8 Hz, 1H, H-3´trans), 5.18 (dq, $J_{H3´cis,H2´}$ = 10.6 Hz, $J_{H3´cis,H1´}$ = $J_{H3´cis,H3´trans}$ = 1.5 Hz, 1H, H-3´cis), 4.58 – 4.53 (m, 2H, H-1´), 4.51 – 4.41 (m, 1H, H-α), 4.34 – 4.17 (m, 3H, CH-Fmoc, CH_2-Fmoc), 2.60 (dd, $J_{H\beta,H\beta}$ = 15.6 Hz, $J_{H\beta,H\alpha}$ = 5.7 Hz, 1H, H-β), 2.54 – 2.45 (m, 1H, H-β´).

^{13}C-NMR (100 MHz, DMSO-d_6): δ [ppm] = 171.4 (C=O), 170.8 (C=O), 155.9 (Fmoc-C=O), 143.8 (2C, 2 × Cq-Fmoc), 140.7 (Cq-Fmoc), 132.4 (C-2´), 127.7 (2C, 2 × CH-Fmoc), 127.1 (2C, 2 × CH-Fmoc), 125.3 (2C, 2 × CH-Fmoc), 120.2 (2C, 2 × CH-Fmoc), 117.5 (C-3´), 65.8 (CH_2-Fmoc), 64.9 (C-1´), 50.7 (C-α), 46.6 (CH-Fmoc), 36.7 (C-β). Note: Due to Signal overlapping, only 3 out of the 4 quaternary carbon atoms belonging to the Fmoc-Protecting group can be assigned from the ^{13}C NMR spectrum.

HRMS (ESI$^+$): Calculated for $C_{22}H_{23}O_5N_2$ [M+H]$^+$: 395.1601; found: 395.1601.

RP-HPLC (Luna, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 8.67 min, λ = 230 nm.

N^{α}-Fluorenylmethoxycarbonyl-N^{γ}-(2,3,4-Tri-*O*-acetyl-α-L-rhamnosyl)-L-asparagine allyl ester (**S10**)



Fmoc-Asn-OAll **S9** (1.03 g, 2.60 mmol, 1.0 eq.) and Trifluoroacetimidate **10**[343] (2.40 g, 5.20 mmol, 2.0 eq.) were co-evaporated with dry toluene (2 × 20 ml) and dried in high vacuum for 1 h. The reactants were solved in a mixture of $MeNO_2/CH_2Cl_2$ (v/v = 3:2, 50 ml). Subsequently, freshly activated 4 Å molecular sieve was added and the reaction solution was stirred for 0.5 h at room temperature. After cooling the reaction mixture to 0 °C, TMSOTf (94.0 µl, 0.52 mmol, 0.2 eq.) was added. The reaction was allowed to warm to ambient temperature and was stirred until it was deemed complete. The reaction was stopped by addition of Triethylamine (2 ml) and filtered through a short pad of *Hyflo*®. The filter cake was washed with CH_2Cl_2 (150 ml). The combined organic layers were washed with 1 M HCl (75 ml), saturated aqueous $NaHCO_3$ (75 ml) and brine (50 ml) and dried over $MgSO_4$. Organic solvents were removed under reduced pressure and the crude product was subjected to flash column chromatography (^{c}Hex/EtOAc v/v = 2:1 + 1 % AcOH) affording **S10** (1.10 g, 1.64 mmol, 63 %) as a colourless foam.

R$_f$ = 0.23 (^{c}Hex/EtOAc v/v = 2:1).

Optical rotation: $[\alpha]_D^{24} = -19.8°$ (c = 0.33, $CHCl_3$).

^{1}H-NMR: (600 MHz, $CDCl_3$): δ [ppm] = 7.76 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.60 (dd, $J_{CH,CH}$ = 7.5 Hz, $J_{CH,CH}$ = 4.8 Hz, 2H, 2 × CH-Fmoc), 7.40 (t, $J_{CH,CH}$ = 7.4 Hz, 2H, 2 × CH-Fmoc), 7.31 (t, $J_{CH,CH}$ = 7.4 Hz, 2H, 2 × CH-Fmoc), 6.93 (d, $J_{NH,H1}$ = 8.6 Hz, 1H, NHγ), 6.11 (d, $J_{NH,H\alpha}$ = 8.6 Hz, 1H, NH-Fmoc), 5.89 (ddt, $J_{H2´,H3´trans}$ = 16.4 Hz, $J_{H2´,H3´cis}$ = 10.9 Hz, $J_{H2´,H1´}$ = 5.5 Hz, 1H, H-2´), 5.61 (dd, $J_{H1,NH}$ = 8.6 Hz, $J_{H1,H2}$ = 4.1 Hz, 1H, H-1), 5.32 (d, $J_{H3´trans,\ H2´}$ = 17.1 Hz, 1H, H-3´$_{trans}$), 5.27 – 5.18 (m, 3H, H-3´$_{cis}$, H-2, H-3), 4.97 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 7.2 Hz, 1H, H-4), 4.73 – 4.61 (m, 3H, H-α, H-1´), 4.45 (dd, $J_{CH,CH}$ = 10.3 Hz, $J_{CH,CH}$ = 7.0 Hz, 1H, CH_2-Fmoc), 4.30 (dd, $J_{CH,CH}$ = 10.4 Hz, $J_{CH,CH}$ = 7.6 Hz, 1H, CH_2-Fmoc), 4.24 (t, $J_{CH,CH}$ = 7.0 Hz, 1H, CH-Fmoc), 3.86 – 3.79 (m, 1H, H-5), 2.99 (dd, $J_{Hβ,Hβ´}$ = 16.5 Hz, $J_{Hβ,Hα}$ = 5.2 Hz, 1H, H-β), 2.83 (dd, $J_{Hβ´,Hβ}$ = 16.5 Hz, $J_{Hβ´,Hα}$ = 4.3 Hz, 1H, H-β´), 2.12 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.27 (d, $J_{CH3,H5}$ = 6.6 Hz, 3H, rha-CH_3).

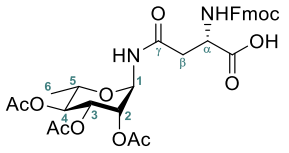
^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 170.7 (C=O), 170.5 (C=O), 170.3 (C=O), 170.2 (C=O), 169.8(C=O), 156.5 (Fmoc-C=O), 144.0 (Cq-Fmoc), 143.7 (Cq-Fmoc), 141.4 (2C, 2 × Cq-Fmoc), 131.5 (C-2´), 127.9 (2C, 2 × CH-Fmoc), 127.3 (2C, 2 × CH-Fmoc), 125.4 (CH-Fmoc), 125.3 (CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 119.0 (C-3´), 74.4 (C-1), 71.1 (C-4), 69.7 (C-5), 69.1 (C-2/C-3), 68.7 (C-2/C-3), 67.7 (CH_2-Fmoc), 66.6 (C-1´), 50.8 (C-α), 47.2 (CH-Fmoc), 38.3 (C-β), 20.9 (2C, 2 × OAc), 20.8 (OAc), 17.0 (rha-CH_3).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 169 Hz.

HRMS (ESI^+): Calculated for $C_{34}H_{38}O_{12}N_2Na^+$ $[M+Na]^+$: 689.2317; found: 689.2313.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 12.17 min, λ = 230 nm.

N^{α}-Fluorenylmethoxycarbonyl-N^{γ}-(2,3,4-Tri-*O*-acetyl-α-L-rhamnosyl)-L-asparagine (**9**)



Cleavage of the allylic ester was conducted according to a slightly modified procedure.[486]

To a magnetically stirred solution of **S10** (800 mg, 1.35 mmol, 1.0 eq.) in dry CH_2Cl_2 (15 ml), Phenylsilane (328 µl, 2.97 mmol, 2.2 eq.) and [$Pd(PPh_3)_4$] (32.0 mg, 27.7 µmol, 0.02 eq.) were added. The reaction was stirred at ambient temperature until complete conversion of the starting material was observed *via* TLC monitoring. Subsequently, the reaction was stopped by addition of 1 ml water and solvents were removed under reduced pressure. The crude product was co-evaporated with toluene (2 × 20 ml) and subjected to flash column chromatography (ᶜHex/EtOAc v/v = 1:1 + 1 % AcOH). α-Rhamnosyl asparagine building block **9** (640 mg, 1.02 mmol, 76 %) was obtained as a colourless foam.

R*f* = 0.10 (ᶜHex/EtOAc v/v = 1:2 + 1 % AcOH).

Optical rotation $[\alpha]_D^{22} = -10.2°$ (c = 0.33, $CHCl_3$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.98 (d, $J_{NH,H1}$ = 8.5 Hz, 1H, NHγ), 7.72 (d, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 7.57 (dd, $J_{CH,CH}$ = 7.6 Hz, $J_{CH,CH}$ = 4.4 Hz, 2H, 2 × CH-Fmoc), 7.36 (t, $J_{CH,CH}$ = 7.4 Hz, 2H, 2 × CH-Fmoc), 7.27 (t, $J_{CH,CH}$ = 7.5 Hz, 2H, 2 × CH-Fmoc), 6.48 (d, $J_{NH,H\alpha}$ = 8.1 Hz, 1H, NH-Fmoc), 5.63 (dd, $J_{H1,NH}$ = 8.6, $J_{H1,H2}$ = 2.6 Hz, 1H, H-1), 5.33 (dd, $J_{H3,H4}$ = 9.3 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 5.26 (t, $J_{H2,H3}$ = $J_{H2,H1}$ = 3.1 Hz, 1H, H-2), 5.03 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.0 Hz, 1H, H-4), 4.69 (q, $J_{H\alpha,NH}$ = $J_{H\alpha,H\beta}$ = 6.0 Hz, 1H, H-α), 4.36 (dd, $J_{CH,CH}$ = 10.5 Hz, $J_{CH,CH}$ = 7.4 Hz, 1H, CH_2-Fmoc), 4.31 (dd, $J_{CH,CH}$ = 10.5 Hz, $J_{CH,CH}$ = 7.4 Hz, 1H, CH_2-Fmoc), 4.18 (t, *J* = 7.4 Hz, 1H, CH-Fmoc), 3.84 – 3.77 (m, 1H, H-5), 3.04 (dd, $J_{H\beta,H\beta'}$ = 16.3 Hz, $J_{H\beta,H\alpha}$ = 5.6 Hz, 1H, H-β), 2.85 (dd, $J_{H\beta',H\beta}$ = 16.2 Hz, $J_{H\beta',H\alpha}$ = 5.0 Hz, 1H, H-β′), 2.13 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.20 (d, $J_{CH3,H5}$ = 6.3 Hz, 3H, rha-CH_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 173.8 (C=O), 171.5 (C=O), 171.3 (C=O), 170.6 (C=O), 169.9 (C=O), 156.8 (Fmoc-C=O), 143.9 (Cq-Fmoc), 143.7 (Cq-Fmoc), 141.4 (Cq-Fmoc), 141.3 (Cq-Fmoc), 127.9 (2C, 2 × CH-Fmoc), 127.2 (2C, 2 × CH-Fmoc), 125.4 (CH-Fmoc), 125.3 (CH-Fmoc), 120.1 (2C, 2 × CH-Fmoc), 75.7 (C-1), 71.0 (C-4), 69.5 (C-3), 69.3 (C-2), 68.4 (C-5), 67.8 (CH_2-Fmoc), 50.7 (C-α), 47.0 (CH-Fmoc), 37.5 (C-β), 21.0 (2C, 2 × OAc), 20.8 (OAc), 17.4 (rha-CH_3).

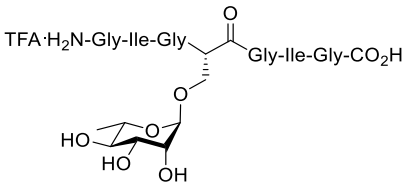
^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 168 Hz.

HRMS (ESI^+): Calculated for $C_{31}H_{35}O_{12}N_2$ $[M+H]^+$: 627.2185, found: 627.2182.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 50 % B → 30 min 100 % B, flow: 1 ml/min): t_R = 7.28 min, λ = 230 nm.

Synthesis and characterization of rhamnosylated glyco-peptide haptens for immunization

Ser$^{\alpha\text{-Rha}}$-Peptide (**11**)



Glycopeptide was synthesized according to general procedure for 0.1 mmol scale peptide synthesis. α-rhamnosyl serine building block **1** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 2 h per coupling. Cleavage of the *O*-acetyl protecting groups was performed according to the general procedure for on-resin deprotection. Cleavage from resin according to the previously described procedure furnished the crude glycopeptide. Purification *via* RP-HPLC (Gradient: B; λ = 212 nm) yielded **11** (52 mg, 63.4 µmol, 63 %) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d$_6$): δ [ppm] = 8.45 (d, $J_{NH,H\alpha}$ = 8.8 Hz, 1H, NH$_I$), 8.35 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH$_G$), 8.33 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH$_G$), 8.24 (t, $J_{NH,H\alpha}$ = 5.7 Hz, 1H, NH$_G$), 8.12 (d, $J_{NH,H\alpha}$ = 8.1 Hz, 1H, NH$_S$), 7.97 (bs, 3H, NH$_3^+$), 7.88 (d, $J_{NH,H\alpha}$ = 8.9 Hz, 1H, NH$_I$), 4.54 – 4.50 (m, 2H, H-1, H$_{S\alpha}$), 4.31 (dd, $J_{H\alpha,NH}$ = 8.9 Hz, $J_{H\alpha,H\beta}$ = 7.2 Hz, 1H, H$_{I\alpha}$), 4.21 (dd, $J_{H\alpha,NH}$ = 8.9 Hz, $J_{H\alpha,H\beta}$ = 7.1 Hz, 1H, H$_{I\alpha}$), 3.86 – 3.67 (m, 7H, 6 × H$_{G\alpha}$, H$_{S\beta}$), 3.63 – 3.59 (m, 3H, H$_{G\alpha}$, H-2), 3.49 (dd, $J_{H\beta,H\beta}$ = 10.2, $J_{H\beta,H\alpha}$ = 4.6 Hz, 1H, H$_{S\beta}$), 3.40 – 3.33 (m, 2H, H-5, H-3), 3.16 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.4 Hz, 1H, H-4), 1.75 – 1.69 (m, 2H, 2 × H$_{I\beta}$), 1.49 – 1.39 (m, 2H, H$_{I\gamma1}$), 1.11 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH$_3$), 1.11 – 1.02 (m, 2H, H$_{I\gamma1}$), 0.86 (d, $J_{H\gamma,H\beta}$ = 6.8 Hz, 3H, H$_{I\gamma2}$), 0.85 (d, $J_{H\gamma,H\beta}$ = 6.8 Hz, 3H, H$_{I\gamma2}$), 0.84 – 0.79 (m, 6H, 2 × H$_{I\delta}$).

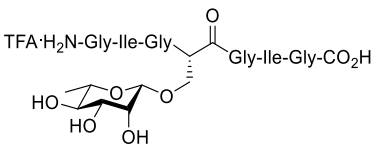
^{13}C-NMR (200 MHz, DMSO-d$_6$): δ [ppm] = 171.3 (C=O), 171.1 (C=O), 170.8 (C=O), 169.4 (C=O), 168.6 (C=O), 168.3 (C=O), 165.8 (C=O), 99.6 (C-1), 71.9 (C-4), 70.6 (C-5/C-3), 70.2 (C-2), 68.5 (C-5/C-3), 66.4 (S$_\beta$), 56.9 (I$_\alpha$), 56.7 (I$_\alpha$), 52.4 (S$_\alpha$), 42.0 (G$_\alpha$), 41.7 (G$_\alpha$), 40.6 (G$_\alpha$), 40.2 (G$_\alpha$), 37.0 (I$_\beta$), 36.9 (I$_\beta$), 24.2 (2C, 2 × I$_{\gamma1}$), 17.9 (CH$_3$-rha), 15.3 (2C, 2 × I$_{\gamma2}$), 11.2 (I$_\delta$), 11.1 (I$_\delta$).

^{1}H-^{13}C-HSQC (DMSO-d$_6$): $J_{H1,C1}$ = 169 Hz.

HRMS (ESI$^+$): Calculated for $C_{29}H_{52}N_7O_{13}^+$ [M+H]$^+$: 706.3618; found: 706.3611.

RP-HPLC: (Aeris, 0.1 % TFA; 0 min 5 % B → 40 min 80 % B, flow: 1ml/min): t_R = 10.96 min, λ = 230 nm.

Ser$^{\beta\text{-Rha}}$-Peptide (**12**)



Glycopeptide was synthesized according to general procedure for 0.05 mmol scale peptide synthesis. β-Rhamnosyl serine building block (**4**) was coupled following a double coupling protocol with 3-fold excess for each coupling

step and a coupling time of 1.5 h per coupling. Cleavage of the *O*-ester protecting groups was performed according to the general procedure for on resin-deprotection. Cleavage from resin according to the previously described procedure furnished the crude glycopeptide. Purification *via* RP-HPLC (Gradient: A; λ = 205 nm) yielded **12** (22 mg, 26.8 µmol, 54 %) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 8.48 (d, $J_{NH,H\alpha}$ = 8.8 Hz, 1H, NH_I), 8.37 (t, $J_{NH,H\alpha}$ = 5.7 Hz, 1H, NH_G), 8.32 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.23 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.10 (d, $J_{NH,H\alpha}$ = 7.7 Hz, 1H, NH_S), 7.81 (d, $J_{NH,H\alpha}$ = 9.0 Hz, 1H, NH_I), 4.44 – 4.40 (m, 2H, $H_{S\alpha}$, H-1), 4.30 (t, $J_{H\alpha,H\beta}$ = $J_{H\alpha,NH}$ = 8.0 Hz, 1H, $H_{I\alpha}$), 4.22 (dd, $J_{H\alpha,NH}$ = 9.0 Hz, $J_{H\alpha,H\beta}$ = 7.1 Hz, 1H, $H_{I\alpha}$), 3.86 – 3.64 (m, 9H, 6 × $H_{G\alpha}$, 2 ×$H_{S\beta}$, H-2), 3.60 (d, $J_{H\alpha,NH}$ = 4.0 Hz, 2H, $H_{G\alpha}$), 3.20 (dd, $J_{H3,H4}$ = 8.8 Hz, $J_{H3,H2}$ = 3.3 Hz, 1H, H-3), 3.13 – 3.04 (m, 2H, H-4, H-5), 1.75 – 1.68 (m, 2H, 2 × $H_{I\beta}$), 1.48 – 1.40 (m, 2H, $H_{I\gamma1}$), 1.17 (d, $J_{CH3,H5}$ = 5.7 Hz, 3H, rha-CH_3), 1.12 – 1.03 (m, 2H, $H_{I\gamma1}$), 0.87 – 0.79 (m, 12H, 2 × $H_{I\gamma2}$, 2 × $H_{I\delta}$).

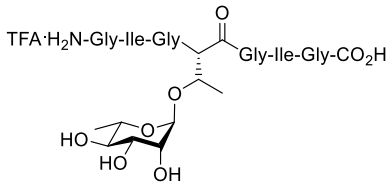
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 171.3 (C=O), 171.1 (C=O), 170.9 (C=O), 169.7 (C=O), 168.7 (C=O), 168.4 (C=O), 165.9 (C=O), 100.2 (C-1), 73.2 (C-3), 72.2 (C-4/C-5), 72.0 (C-4/C-5), 70.4 (C-2), 68.1 (S_β), 56.9 (I_α), 56.6 (I_α), 53.0 (S_α), 42.0 (G_α), 41.7 (G_α), 40.7 (G_α), 40.2 (G_α), 36.9 (2C, 2 × I_β), 24.2 ($I_{\gamma1}$), 24.1 ($I_{\gamma1}$), 17.9 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$), 11.1 (2C, 2 × I_δ).

^{1}H-^{13}C-HSQC (DMSO-d_6): $J_{H1,C1}$ = 156 Hz.

HRMS (ESI$^-$): Calculated for $C_{29}H_{50}N_7O_{13}^-$ [M–H]$^-$: 704.3472; found: 704.34718.

RP-HPLC (Aeris, 0.1% TFA; 0 min 5 % B → 40 min 80 % B, flow: 1ml/min): t_R = 11.15 min, λ = 230 nm.

Thr$^{\alpha\text{-Rha}}$-Peptide (**13**)



Glycopeptide **13** was synthesized according to general procedure for 0.1 mmol scale peptide synthesis. α-Rhamnosyl threonine building block **2** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 1.5 h per coupling. Cleavage of the *O*-acetyl protecting groups were performed according to the general procedure for *on-resin* deprotection. Cleavage from resin according to the general procedure and subsequent purification *via* RP-HPLC purification (Gradient: B; λ = 212 nm) yielded **13** (37 mg, 44.4 µmol, 44 %) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 8.46 (d, $J_{NH,H\alpha}$ = 8.9 Hz, 1H, NH_I), 8.38 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.32 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.07 (t, $J_{NH,H\alpha}$ = 5.6 Hz, 1H, NH_G), 8.00 – 7.93 (m, 4H, NH_I, NH_3^+), 7.79 (d, $J_{NH,H\alpha}$ = 9.0 Hz, 1H, NH_T), 4.59 (d, $J_{H1,H2}$ = 1.6 Hz, 1H, H-1), 4.44 (dd, $J_{H\alpha,NH}$ = 9.1 Hz, $J_{H\alpha,H\beta}$ = 3.7 Hz, 1H, $H_{T\alpha}$),

4.31 (dd, $J_{H\alpha,NH}$ = 8.9 Hz, $J_{H\alpha,H\beta}$ = 7.3 Hz, 1H, $H_{I\alpha}$), 4.21 (dd, $J_{H\alpha,NH}$ = 8.9, $J_{H\alpha,H\beta}$ = 7.0 Hz, 1H, $H_{I\alpha}$), 4.06 (qd, $J_{H\beta,H\gamma}$ = 6.3 Hz, $J_{H\beta,H\alpha}$ = 3.6 Hz, 1H, $H_{T\beta}$), 3.88 – 3.75 (m, 5H, 5× $H_{G\alpha}$), 3.69 (dd, $J_{H\alpha,H\alpha}$ = 17.4 Hz, $J_{H\alpha,NH}$ = 5.8 Hz, $H_{G\alpha}$) 3.63 – 3.58 (m, 2H, 2× $H_{G\alpha}$), 3.56 (dd, $J_{H2,H3}$ = 3.4 Hz, $J_{H2,H1}$ =1.6 Hz, 1H, H-2), 3.40 (dd, $J_{H3,H4}$ = 9.4 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 3.33 (dq, $J_{H5,H4}$ = 9.4 Hz, $J_{H5,CH3}$ = 6.2 Hz, 1H, H-5), 3.15 (t, $J_{H4,H3}$ = $J_{H4,H3}$ = 9.4 Hz, 1H, H-4), 1.75 – 1.69 (m, 2H, 2 × $H_{I\beta}$), 1.49 – 1.40 (m, 2H, $H_{I\gamma1}$), 1.12 – 1.05 (m, 5H, rha-CH_3, $H_{I\gamma1}$), 1.02 (d, $J_{H\gamma,H\beta}$ = 6.2 Hz, 3H, $H_{T\gamma}$), 0.86 (d, $J_{H\gamma,H\beta}$ = 6.8 Hz, 3H, $H_{I\gamma2}$), 0.85 (d, $J_{H\gamma,H\beta}$ = 6.8 Hz, 3H, $H_{I\gamma2}$), 0.82 (t, $J_{H\delta,H\gamma}$ = 7.4 Hz, 3H, $H_{I\delta}$), 0.80 (t, $J_{H\delta,H\gamma}$ = 7.4 Hz, 3H, $H_{I\delta}$).

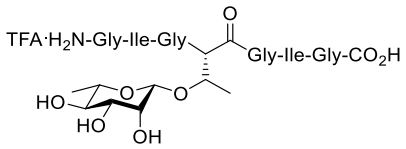
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 171.3 (C=O), 171.1 (C=O), 170.8 (C=O), 169.5 (C=O), 168.9 (C=O), 168.3 (C=O), 165.8 (C=O), 96.0 (C-1), 71.9 (C-4), 70.7 (C-2), 70.5 (C-3), 69.7 (T_β), 68.6 (C-5), 56.9 (I_α), 56.7 (I_α), 56.6 (T_α), 41.9 (G_α), 40.6 (G_α), 40.2 (G_α), 40.0 (G_α), 37.0 (I_β), 36.8 (I_β), 24.2 (2C, 2 × $I_{\gamma1}$), 17.8 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$), 14.5 (T_γ), 11.2 (I_δ), 11.1 (I_δ).

^{1}H-^{13}C-HSQC (DMSO-d_6): $J_{H1,C1}$ = 169 Hz.

HRMS (ESI$^+$): Calculated for $C_{30}H_{54}N_7O_{13}^+$ [M+H]$^+$: 720.3774; found: 720.3776.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 40 min 80 % B, flow: 1ml/min): t_R = 11.10 min, λ = 230 nm.

Thr$^{\beta\text{-Rha}}$-Peptide (**14**)



Glycopeptide was synthesized according to general procedure for 0.05 mmol scale peptide synthesis. β-Rhamnosyl threonine building block **5** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 1.5 h per coupling. Cleavage of the *O*-ester protecting groups was performed according to the general procedure for on-resin deprotection. Cleavage from resin according to the previously described procedure furnished the crude glycopeptide. RP-HPLC purification (Gradient: A; λ = 205 nm) yielded **14** (18 mg, 21.6 µmol, 43 %) after lyophilization.

^{1}H-NMR: (800 MHz, DMSO-d_6): δ [ppm] = 8.46 (d, $J_{NH,H\alpha}$ = 8.8 Hz, 1H, NH_I), 8.38 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.33 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.17 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 7.95 (t, $J_{NH,H\alpha}$ = 5.6 Hz, 3H, NH_3^+), 7.92 (d, $J_{NH,H\alpha}$ = 9.0 Hz, 1H, NH_I), 7.82 (d, $J_{NH,H\alpha}$ = 8.8 Hz, 1H, NH_T), 4.45 (s, 1H, H-1), 4.35 (dd, $J_{H\alpha,NH}$ = 8.9 Hz, $J_{H\alpha,H\beta}$ = 3.6 Hz, 1H, $H_{T\alpha}$), 4.31 (dd, $J_{H\alpha,NH}$ = 8.9 Hz, $J_{H\alpha,H\beta}$ = 7.2 Hz, 1H, $H_{I\alpha}$), 4.23 (dd, $J_{H\alpha,NH}$ = 9.0 Hz, $J_{H\alpha,H\beta}$ = 7.0 Hz, 1H, $H_{I\alpha}$), 4.10 (qd, $J_{H\beta,H\gamma}$ = 6.4 Hz, $J_{H\beta,H\alpha}$ = 3.7 Hz, 1H, $H_{T\beta}$), 3.88 – 3.68 (m, 6H, 6 × $H_{G\alpha}$), 3.63 (d, $J_{H2,H3}$ = 3.4 Hz, 1H, H-2), 3.62 – 3.58 (m, 2H, $H_{G\alpha}$), 3.17 (dd, $J_{H3,H4}$ = 9.0 Hz, $J_{H3,H2}$ = 3.3 Hz, 1H, H-3), 3.10 – 3.01 (m, 2H, H-4, H-5), 1.76 – 1.69 (m, 2H, 2 × $H_{I\beta}$), 1.50 – 1.39 (m, 2H, $H_{I\gamma1}$), 1.15 (d, $J_{CH3,H5}$ = 5.9 Hz, 3H, rha-CH_3), 1.11 (d, $J_{H\gamma,\ H\beta}$ = 6.3 Hz, 3H, $H_{T\gamma}$), 1.10 – 1.03 (m, 2H, $H_{I\gamma1}$), 0.88 – 0.79 (m, 12H, 2 × $H_{I\gamma2}$, 2 × $H_{I\delta}$).

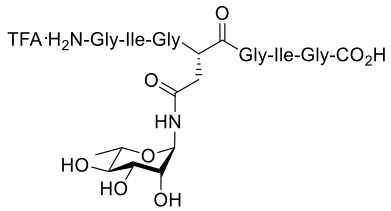
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 171.3 (C=O), 171.1 (C=O), 170.8 (C=O), 169.8 (C=O), 168.8 (C=O), 168.4 (C=O), 165.8 (C=O), 100.8 (C-1), 75.3 (T_β), 73.1 (C-3), 72.0 (C-4/C-5), 71.9 (C-4/C-5), 70.6 (C-2), 56.9 (I_α), 56.7 (I_α), 56.6 (T_α), 41.8 (2C, 2 × G_α), 40.6 (G_α), 40.2 (G_α), 36.9 (2C, 2 × I_β), 24.2 ($I_{\gamma1}$), 24.1 ($I_{\gamma1}$), 18.8 (T_γ), 18.0 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$), 11.2 (I_δ), 11.1 (I_δ).

^{1}H-^{13}C-HSQC (DMSO-d_6): $J_{H1,C1}$ = 156 Hz.

HRMS (ESI$^-$): Calculated for $C_{30}H_{52}N_7O_{13}^-$ [M–H]$^-$: 718.3629; found: 718.3630.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 40 min 80 % B, flow: 1ml/min): t_R = 11.63 min, λ = 230 nm.

Asn$^{\alpha\text{-Rha}}$-Peptide (**15**)



The synthesis of α-rhamnosyl asparagine glycopeptide **15** was performed according to the general procedure for peptide synthesis on a 0.1 mmol scale. α-Rhamnosyl asparagine building block **9** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 2 h per coupling. Deprotection of the acetyl protecting groups was performed according to the general procedure for on-resin deprotection. Cleavage from resin according to the general procedure furnished the crude glycopeptide which was subjected to RP-HPLC purification (Gradient: A; λ = 205 nm) to afford **15** (52 mg, 61.4 µmol, 61 %) after lyophilization.

^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 8.55 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH_I), 8.51 (d, $J_{NH,H1}$ = 8.8 Hz, 1H, $NH_{N\gamma}$), 8.39 (t, $J_{NH,H\alpha}$ = 5.6 Hz, 1H, -NH_G), 8.22 (t, $J_{NH,h\alpha}$ = 5.8 Hz, 1H, NH_G), 8.15 (d, $J_{NH,N\alpha}$ = 7.8 Hz, 1H, NH_N), 8.09 (t, $J_{NH,G\alpha}$ = 5.8 Hz, 1H, NH_G), 7.78 (d, $J_{NH,I\alpha}$ = 9.0 Hz, 1H, NH_I), 5.22 (dd, $J_{H1,NH}$ = 8.8 Hz, $J_{H1,H2}$ = 2.0 Hz, 1H, H-1), 4.55 (td, $J_{N\alpha,NH}$ = 7.8 Hz, $J_{N\alpha,N\beta}$ =5.3 Hz, 1H, $H_{N\alpha}$), 4.29 (dd, $J_{I\alpha,NH}$ = 8.7 Hz, $J_{I\alpha,I\beta}$ = 7.3 Hz, 1H, $H_{I\alpha}$), 4.20 (dd, $J_{I\alpha,NH}$ = 9.0, $J_{I\alpha,I\beta}$ = 7.3 Hz, 1H, $H_{I\alpha}$), 3.83 (dd, $J_{H\alpha,H\alpha}$ = 16.6 Hz, $J_{H\alpha,NH}$ = 6.0 Hz, 1H, $H_{G\alpha}$), 3.77 – 3.67 (m, 5H, $H_{G\alpha}$), 3.64 – 3.60 (m, 3H, $H_{G\alpha}$, H-3), 3.51 (m, 1H, H-2), 3.39 – 3.36 (m, 1H, H-5), 3.20 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.0 Hz, 1H, H-4), 2.60 – 2.55 (m, 2H, 2 × $H_{N\beta}$), 1.76-1.70 (m, 2H, 2 × $H_{I\beta}$), 1.47-1.38 (m, 2H, $H_{I\gamma1}$), 1.11 – 1.04 (m, 5H, rha-CH_3, $H_{I\gamma1}$), 0.86 – 0.78 (m, 12H, 2 × $H_{I\gamma2}$, 2 × $H_{I\delta}$).

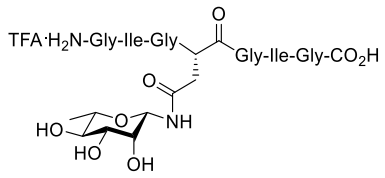
^{13}C-NMR (150 MHz, DMSO-d_6): δ [ppm] = 171.2 (3C, 3 × C=O), 170.9 (C=O), 169.6 (C=O), 168.6 (C=O), 168.4 (C=O), 166.0 (C=O), 77.8 (C-1), 72.1 (C-4), 70.6 (C-2), 70.2 (C-3), 69.5 (C-5), 57.0 (I_α), 56.7 (I_α), 49.6 (N_α), 42.1 (G_α), 41.8(G_α), 40.8 (G_α),40.2 (G_α) 37.3 (N_β), 36.9 (I_β), 36.6 (I_β), 24.2 (2C, 2 × $I_{\gamma1}$), 18.0 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$),11.1 (I_δ), 11.0 (I_δ).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 165 Hz.

HRMS (ESI$^+$): Calculated for $C_{30}H_{53}O_{13}N_8$ [M+H]$^+$: 733.3727; found: 733.3720.

RP-HPLC (Aeris, 0.1 % TFA, 0 min: 8 % B → 10 min: 8 % B → 60 min: 50 % B → 70 min: 100 % B; flow: 1 ml/ min): t_R = 22.45 min, λ = 230 nm.

Asn$^{\beta\text{-Rha}}$-Peptide (**16**)



The synthesis of β-rhamnosyl asparagine glycopeptide **16** was performed according to the general procedure for peptide synthesis on a 0.1 mmol scale. β-Rhamnosyl asparagine building block **7** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 2 h per coupling. Deprotection of the acetyl protecting groups was performed according to the general procedure for on-resin deprotection. Cleavage from resin according to the general procedure furnished the crude glycopeptide which was subjected to RP-HPLC purification (Gradient: A; λ = 205 nm) to afford **16** (54 mg, 63.8 µmol, 64 %) after lyophilization.

^{1}H-NMR (600 MHz, DMSO-d$_6$) δ [ppm] = 8.53 (d, $J_{NH,H\alpha}$ = 8.6 Hz, 1H, NH_I), 8.37 (t, $J_{NH,H\alpha}$ = 5.7 Hz, 1H, NH_G), 8.26 – 8.19 (m, 2H, $NH_{N\gamma}$, NH_G), 8.14 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.11 (d, $J_{NH,H\alpha}$ = 8.0 Hz, 1H, NH_N), 7.80 (d, $J_{NH,\,I\alpha}$ = 8.9 Hz, 1H, NH_I), 4.98 (dd, $J_{H1,NH}$ = 9.1 Hz, $J_{H1,H2}$ = 1.3 Hz, 1H, H-1), 4.59 – 4.54 (m, 1H, $H_{N\alpha}$), 4.29 (dd, $J_{H\alpha,NH}$ = 8.7 Hz, $J_{H\alpha,H\beta}$ = 7.3 Hz, 1H, $H_{I\alpha}$), 4.20 (dd, $J_{H\alpha,NH}$ = 9.0 Hz, $J_{H\alpha,H\beta}$ = 7.4 Hz, 1H, $H_{I\alpha}$), 3.83 (dd, $J_{H\alpha1,H\alpha2}$ = 16.6 Hz, $J_{H\alpha,NH}$ = 5.9 Hz, 1H, $H_{G\alpha}$), 3.77 (dd, $J_{H\alpha,H\alpha}$ = 17.4 Hz, $J_{H\alpha,NH}$ = 5.9 Hz, 1H, $H_{G\alpha}$), 3.74 – 3.67 (m, 4H, 4 × $H_{G\alpha}$), 3.61 (d, 2H, 2 × $H_{G\alpha}$), 3.56 (d, $J_{H2,H3}$ = 3.2 Hz, 1H, H-2), 3.28 (dd, $J_{H3,H4}$ = 8.6 Hz, $J_{H3,H2}$ = 3.1 Hz, 1H, H-3), 3.13-3.06 (m, 2H, H-4, H-5), 2.65 (dd, $J_{H\beta,H\beta}$ = 15.8 Hz, $J_{H\beta,H\alpha}$ = 5.7 Hz, 1H, $H_{N\beta}$), 2.56-2.51 (m, 1H, $H_{N\beta}$), 1.78 – 1.69 (m, 2H, 2 × $H_{I\beta2}$), 1.49 – 1.39 (m, 2H, $H_{I\gamma1}$), 1.12 – 1.05 (m, 5H, rha-CH_3, $H_{I\gamma1}$), 0.86 – 0.79 (m, 12H, 2 × $I_{\gamma2}$, 2 × I_δ).

^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 171.2 (2C, 2 × C=O), 171.1 (C=O), 170.8 (C=O), 169.3 (C=O), 168.5 (2C, 2 × C=O), 165.9 (C=O), 77.4 (C-1), 73.7 (C-3), 73.6 (C-4/C-5), 71.6 (C-4/C-5), 70.7 (C-2), 57.0 (I_α), 56.7 (I_α), 49.5 (N_α), 42.2 (G_α), 41.7 (G_α), 40.6 (G_α), 40.2 (G_α), 37.5 (N_β), 36.9 (I_β), 36.5 (I_β), 24.2 (2C, 2 × $I_{\gamma1}$) , 18.0 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$), 11.1 (I_δ), 11.0 (I_δ).

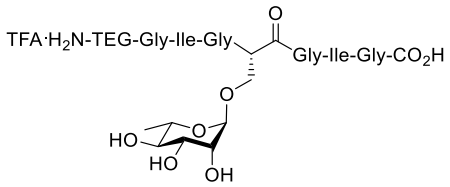
^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 154 Hz.

HRMS (ESI$^+$): Calculated for $C_{30}H_{53}O_{13}N_8$ $[M+H]^+$: 733.3727; found: 733.3717.

RP-HPLC (Aeris, 0.1 % TFA, 0 min: 5 % B → 40 min: 80 % B → 60 min: 100 % B, flow: 1 ml/ min): t_R = 10.73 min, λ = 230 nm.

Synthesis of BSA-conjugates for Western Blot analysis

Ser$^{\alpha\text{-Rha}}$-Peptide with TEG-Spacer (**S11**)



Glycopeptide was synthesized according to general procedure for 0.1 mmol scale peptide synthesis. α-Rhamnosyl serine building block **1** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 1.5 h per coupling. Fmoc-TEG-CO_2H Spacer was coupled with a 4-fold excess and a coupling time of 1 h per coupling. Cleavage of the *O*-acetyl protecting groups was performed according to the general procedure for on-resin deprotection. Cleavage from resin according to the previously described procedure furnished the crude glycopeptide. RP-HPLC purification (Gradient: A; $\lambda = 205$ nm) yielded **S11** (58 mg, 56.7 µmol, 57 %) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 8.33 (t, $J_{NH,H\alpha} = 5.9$ Hz, 1H, NH_G), 8.24 (t, $J_{NH,H\alpha} = 5.8$ Hz, 1H, NH_G), 8.22 (t, $J_{NH,H\alpha} = 5.7$ Hz, 1H, NH_G), 8.11 – 8.08 (m, 2H, NH_G, NH_S), 7.86 (d, $J_{NH,H\alpha} = 8.9$ Hz, 1H, NH_I), 7.85 (d, $J_{NH,H\alpha} = 8.7$ Hz, 1H, NH_I), 7.77 (s, 3H, NH_3^+), 4.53 (d, $J_{H1,\,H2} = 1.6$ Hz, 1H, H-1), 4.51 (ddd, $J_{H\alpha,NH} = 8.1$ Hz, $J_{H\alpha,H\beta} = 6.2$ Hz, $J_{H\alpha,H\beta} = 4.8$ Hz, 1H, $H_{S\alpha}$), 4.24 – 4.17 (m, 2H, 2 × $H_{I\alpha}$), 3.81 – 3.73 (m, 7H, 7 × $H_{G\alpha}$), 3.73 – 3.67 (m, 2H, $H_{G\alpha}$, $H_{S\beta}$), 3.62 – 3.47 (m, 14H, 12 × CH_2-O-Spacer, H-2, $H_{S\beta}$), 3.39 – 3.34 (m, 2H, H-3, H-5), 3.16 (t, $J_{H4,H3} = J_{H4,H5} = 9.4$ Hz, 1H, H-4), 3.01 – 2.95 (m, 2H, CH_2-O-Spacer), 2.38 (t, $J_{CH,CH} = 6.5$ Hz, 2H, CH_2-O-Spacer), 1.76 – 1.67 (m, 2H, 2 × $H_{I\beta}$), 1.46 – 1.39 (m, 2H, $H_{I\gamma1}$), 1.11 (d, $J_{CH3,H5} = 6.2$ Hz, 3H, rha-CH_3), 1.10 – 1.03 (m, 2H, $H_{I\gamma1}$), 0.85 (d, 3H, $J_{H\gamma,H\beta} = 6.8$ Hz, $H_{I\gamma2}$), 0.83 (d, 3H, $J_{H\gamma,H\beta} = 6.8$ Hz, $H_{I\gamma2}$) 0.81 (td, $J_{H\delta,H\gamma} = 7.4$ Hz, $J_{H\delta,H\beta} = 2.7$ Hz, 6H, 2 × $H_{I\delta}$).

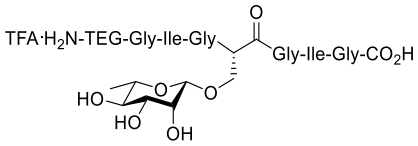
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 171.3 (2C, 2 × C=O), 171.1 (C=O), 170.5 (C=O), 169.4 (C=O), 168.8 (2C, 2 × C=O), 168.3 (C=O), 99.6 (C-1), 71.9 (C-4), 70.5 (C-3), 70.2 (C-2), 69.7 (2C, CH_2-Spacer), 69.6 (CH_2-Spacer), 69.5 (CH_2-Spacer), 68.5 (C-5), 66.7 (2C, CH_2-Spacer), 66.3 (S_β), 56.8 (I_α), 56.7 (I_α), 52.4 (S_α), 42.0 (G_α), 41.9 (2C, 2 × G_α), 40.6 (G_α), 38.7 (CH_2-Spacer), 36.8 (I_β), 36.7 (I_β), 35.8 (CH_2-Spacer), 24.3 ($I_{\gamma1}$), 24.2 ($I_{\gamma1}$), 17.9 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$), 11.2 (2C, 2 × I_δ).

^{1}H-^{13}C-HSQC (DMSO-d_6): $J_{H1,C1} = 171$ Hz.

HRMS (ESI^+): Calculated for $C_{38}H_{69}N_8O_{17}^+$ $[M+H]^+$: 909.4775; found: 909.4777.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 80 % B, flow: 1ml/min)**:** $t_R = 11.07$ min, $\lambda = 230$ nm.

Ser$^{\beta\text{-Rha}}$-Peptide with TEG-Spacer (**S12**)



Glycopeptide was synthesized according to general procedure for 0.05 mmol scale peptide synthesis. α-Rhamnosyl serine building block **4** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 1.5 h per coupling. Fmoc-TEG-CO_2H building block was coupled with a 4-fold excess and a coupling time of 1 h per coupling. Cleavage of the *O*-ester protecting groups was performed according to the general procedure for on resin-deprotection. Cleavage from resin according to the previously described procedure furnished the crude glycopeptide. RP-HPLC purification (Gradient: A; λ = 205 nm) yielded **S12** (20 mg, 19.6 µmol, 39 %) after lyophilization.

^{1}H-NMR: (600 MHz, DMSO-d_6): δ [ppm] = 8.35 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.24 (t, $J_{NH,H\alpha}$ = 5.7 Hz, 1H, NH_G), 8.21 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.09 (t, $J_{NH,H\alpha}$ = 5.7 Hz, 1H, NH_G), 8.06 (d, $J_{NH,H\alpha}$ = 7.8 Hz, 1H, NH_S), 7.84 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH_I), 7.80 (d, $J_{NH,H\alpha}$ = 9.0 Hz, 1H, NH_I), 7.74 (bs, 3H, NH_3^+), 4.45 – 4.40 (m, 2H, $H_{S\alpha}$, H-1), 4.26 – 4.18 (m, 2H, 2 × $H_{I\alpha}$), 3.86 – 3.65 (m, 11H, 8 × $H_{G\alpha}$, $H_{S\beta}$, H-2), 3.63 – 3.47 (m, 12H, 12 × CH_2-O-Spacer), 3.21 (dd, $J_{H3,H4}$ = 8.8 Hz, $J_{H3,H2}$ = 3.2 Hz, 1H, H-3), 3.13 – 3.07 (m, 2H, H-4, H-5), 3.01 – 2.98 (m, 2H, CH_2-O-Spacer), 2.38 (t, $J_{CH,CH}$ = 6.5 Hz, 2H, CH_2-O-Spacer), 1.74 – 1.68 (m, 2H, 2 × $H_{I\beta}$), 1.45 – 1.40 (m, 2H, $H_{I\gamma1}$), 1.17 (d, $J_{CH3,H5}$ = 5.5 Hz, 3H, rha-CH_3), 1.10 – 1.01 (m, 2H, $H_{I\gamma1}$), 0.84 (t, $J_{H\gamma,H\beta}$ = 6.8 Hz, 6H, 2 × $H_{I\gamma2}$), 0.83 – 0.79 (m, 6H, 2 × $H_{I\delta}$).

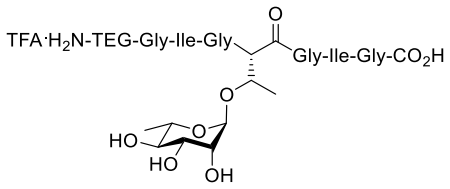
^{13}C-NMR (150 MHz, DMSO-d_6): δ [ppm] = 171.3 (2C, 2 × C=O), 171.0 (C=O), 170.5 (C=O), 169.7 (C=O), 168.8 (C=O), 168.7 (C=O), 168.4 (C=O), 100.1 (C-1), 73.2 (C-3), 72.1 (C-4/C-5), 72.0 (C-4/C-5), 70.4 (C-2), 69.7 (2C, CH_2-Spacer), 69.6 (CH_2-Spacer), 69.5 (CH_2-Spacer), 68.1 (S_β), 66.7 (2C, CH_2-Spacer), 56.8 (I_α), 56.6 (I_α), 53.0 (S_α), 42.0 (G_α), 41.9 (G_α), 41.8 (G_α), 40.6 (G_α), 38.7 (CH_2-Spacer), 36.9 (I_β), 36.7 (I_β), 35.8 (CH_2-Spacer), 24.2 ($I_{\gamma1}$), 24.1 ($I_{\gamma1}$), 17.9 (CH_3-rha), 15.3 (2C, 2 × $I_{\gamma2}$), 11.1 (2C, 2 × I_δ).

^{1}H-^{13}C-HSQC (DMSO-d_6): $J_{H1,C1}$ = 159 Hz.

HRMS (ESI$^+$): Calculated for $C_{38}H_{69}N_8O_{17}^+$ $[M+H]^+$: 909.4775; found: 909.4782

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 80 % B, flow: 1ml/min): t_R = 12.16 min, λ = 230 nm.

Thr$^{\alpha\text{-Rha}}$-Peptide with TEG-Spacer (**S13)**



Glycopeptide was synthesized according to general procedure for 0.1 mmol scale peptide synthesis. α-Rhamnosyl threonine building block **2** was coupled following a double coupling protocol with 3-fold excess for each coupling step and coupling time of 1.5 h per coupling. Fmoc-TEG-CO_2H building block was coupled following a double coupling protocol with 4-fold excess for each coupling step and a coupling time of 1 h per coupling. Cleavage of the *O*- acetyl protecting groups was performed according to the general procedure for on-resin deprotection. Cleavage from resin according to the previously described procedure furnished the crude glycopeptide. RP-HPLC purification (Gradient: A; λ = 205 nm) yielded **S13** (52 mg, 50.1 µmol, 50 %) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 8.31 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.28 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.10 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.06 (t, $J_{NH,H\alpha}$ = 5.5 Hz, 1H, NH_G), 7.95 (d, $J_{NH,H\alpha}$ = 8.8 Hz, 1H, NH_I), 7.85 (d, $J_{NH,H\alpha}$ = 8.8 Hz, 1H, NH_I), 7.76 (s, 3H, NH_3^+), 7.74 (d, $J_{NH,H\alpha}$ = 9.0 Hz, 1H, NH_T), 4.59 (d, $J_{H1,\,H2}$ = 1.6 Hz, 1H, H-1), 4.43 (dd, $J_{H\alpha,NH}$ = 9.0 Hz, $J_{H\alpha,H\beta}$ = 3.6 Hz, 1H, $H_{T\alpha}$), 4.21 (dd, $J_{NH,H\alpha}$ = 8.9 Hz, $J_{H\alpha,H\beta}$ = 7.1 Hz, 2H, 2 × $H_{I\alpha}$), 4.10 – 4.04 (m, 1H, $H_{T\beta}$), 3.85 – 3.74 (m, 7H, 7 × $H_{G\alpha}$), 3.69 (dd, $J_{H\alpha,H\alpha}$ = 17.4 Hz, $J_{H\alpha,NH}$ = 5.8 Hz, 1H, $H_{G\alpha}$), 3.61 – 3.47 (m, 13H, 12 × CH_2-O-Spacer, H-2), 3.41 (dd, $J_{H3,H4}$ = 9.4 Hz, $J_{H3,H2}$ = 3.4 Hz, 1H, H-3), 3.33 (dq, $J_{H5,H4}$ = 9.4 Hz, $J_{H5,CH3}$ = 6.2 Hz, 1H, H-5), 3.14 (t, $J_{H4,H3}$ = $J_{H4,H5}$ =9.4 Hz, 1H, H-4), 2.98 (q, $J_{CH,CH}$ = 5.5 Hz, 2H, CH_2-O-Spacer), 2.38 (t, $J_{CH,CH}$ = 6.5 Hz, 2H, CH_2-O-Spacer), 1.76 – 1.67 (m, 2H, 2 × $H_{I\beta}$), 1.46 – 1.40 (m, 2H, $H_{I\gamma1}$), 1.11 – 1.03 (m, 5H, rha-CH_3, $H_{I\gamma1}$), 1.02 (d, $J_{H\gamma,H\beta}$ = 6.2 Hz, 3H, $H_{T\gamma}$), 0.84 (t, $J_{H\gamma,H\beta}$ = 7.3 Hz, 6H, 2 × $H_{I\gamma2}$), 0.80 (td, $J_{H\delta,H\gamma}$ = 7.4 Hz, $J_{H\delta,H\beta}$ = 3.4 Hz, 6H, 2 × $H_{I\delta}$).

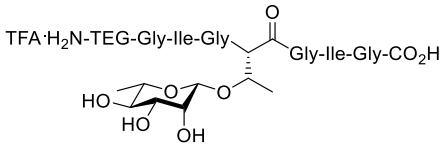
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 171.3 (2C, 2 × C=O), 171.1 (C=O), 170.5 (C=O), 169.5 (C=O), 169.0 (C=O), 168.7 (C=O), 168.4 (C=O), 96.0 (C-1), 71.9 (C-4), 70.7 (C-2), 70.5 (C-3), 69.7 (2C, CH_2-Spacer), 69.6 (2C, CH_2-Spacer, T_β), 69.5 (CH_2-Spacer), 68.6 (C-5), 66.7 (2C, CH_2-Spacer), 56.8 (I_α), 56.7 (T_α), 56.6 (I_α), 42.0 (2C, 2 × G_α), 41.9 (G_α), 40.6 (G_α), 38.7 (CH_2-Spacer), 36.8 (2C, 2 × I_β), 35.9 (CH_2-Spacer), 24.3 ($I_{\gamma1}$), 24.2 ($I_{\gamma1}$), 17.8 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$), 14.5 (T_γ), 11.2 (I_δ), 11.1 (I_δ).

^{1}H-^{13}C-HSQC (DMSO-d_6): $J_{H1,C1}$ = 170 Hz.

HRMS (ESI$^+$): Calculated for $C_{39}H_{71}N_8O_{17}^+$ $[M+H]^+$: 923.4932; found: 923.4944.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 80 % B, flow: 1ml/min)**:** t_R = 12.07 min, λ = 230 nm.

Thr$^{\beta\text{-Rha}}$-Peptide with TEG-Spacer (**S14)**



Glycopeptide was synthesized according to general procedure for 0.05 mmol scale peptide synthesis. β-Rhamnosyl threonine building block **5** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 1.5 h per coupling. Fmoc-TEG-CO_2H building block was coupled with a 4-fold excess for each coupling and a coupling time of 1 h per coupling. Cleavage of the *O*-ester protecting groups was performed according to the general procedure for on-resin deprotection. Cleavage from resin according to the previously described procedure furnished the crude glycopeptide. RP-HPLC purification (Gradient: A; λ = 205 nm) yielded **S14** (25 mg, 24.1 µmol, 48%) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 8.32 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.29 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.16 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.06 (t, $J_{NH,H\alpha}$ = 5.7 Hz, 1H, NH_G), 7.91 (d, $J_{NH,H\alpha}$ = 8.9 Hz, 1H, NH_I), 7.88 (d, $J_{NH,H\alpha}$ = 8.7 Hz, 1H, NH_I), 7.78 (d, $J_{NH,H\alpha}$ = 8.8 Hz, 1H, NH_T), 7.75 (bs, 3H, NH_3^+), 4.44 (s, 1H H-1), 4.34 (dd, $J_{H\alpha,NH}$ = 8.9 Hz, $J_{H\alpha,H\beta}$ = 3.6 Hz, 1H, $H_{T\alpha}$), 4.23 (dd, $J_{H\alpha,NH}$ = 9.0 Hz, $J_{H\alpha,H\beta}$ = 7.0 Hz, 1H, NH_I), 4.20 (dd, $J_{H\alpha,NH}$ = 8.7 Hz, $J_{H\alpha,H\beta}$ = 7.2 Hz, 1H, NH_I), 4.10 (qd, $J_{H\beta,H\gamma}$ = 6.4 Hz, $J_{H\beta,H\alpha}$ = 3.5 Hz, 1H, $H_{T\beta}$), 3.87 – 3.66 (m, 8H, 8 × $H_{G\alpha}$), 3.64 (d, $J_{H2,H3}$ = 3.3 Hz, 1H, H-2), 3.62 – 3.47 (m, 12H, 12 × CH_2-O-Spacer), 3.18 (dd, $J_{H3,H4}$ = 9.0 Hz, $J_{H3,H2}$ = 3.3 Hz, 1H, H-3), 3.08 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.1 Hz, 1H, H-4), 3.04 (dq, $J_{H5,H4}$ = 9.1 Hz, $J_{H5,CH3}$ = 5.9 Hz, 1H, H-5), 3.00 – 2.95 (m, 2H, CH_2-O-Spacer), 2.38 (t, $J_{CH,CH}$ = 6.5 Hz, 2H, CH_2-O-Spacer), 1.75 – 1.68 (m, 2H, 2 × $H_{I\beta}$), 1.46 – 1.39 (m, 2H, $H_{I\gamma1}$), 1.15 (d, $J_{CH3,H5}$ = 5.9 Hz, 3H, rha-CH_3), 1.10 (d, $J_{H\beta,H\gamma}$ = 6.4 Hz, 3H, -$H_{T\gamma}$), 1.09 – 1.03 (m, 2H, $H_{I\gamma1}$), 0.85 (d, $J_{H\gamma,H\beta}$ = 6.9 Hz, 3H, $H_{I\gamma2}$), 0.84 (d, $J_{H\gamma,H\beta}$ = 6.8 Hz, 3H, $H_{I\gamma2}$), 0.82 – 0.79 (m, 6H, 2 × $H_{I\delta}$).

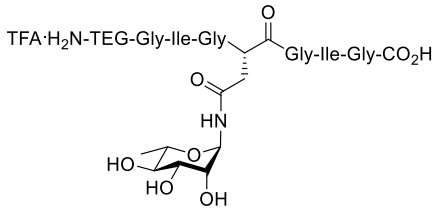
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 171.3 (2C, 2 × C=O), 171.1 (C=O), 170.5 (C=O), 169.8 (C=O), 168.9 (2C, 2 × C=O), 168.4 (C=O), 100.7 (C-1), 75.2 (T_β), 73.1 (C-3), 72.0 (C-4/C-5), 71.9 (C-4/C-5), 70.6 (C-2), 69.7 (2C, CH_2-Spacer), 69.6 (CH_2-Spacer), 69.5 (CH_2-Spacer), 66.7 (2C, CH_2-Spacer), 56.8 (I_α), 56.7 (2C, I_α, T_α), 41.9 (2C, 2 × G_α), 41.8 (G_α), 40.6 (G_α), 38.7 (CH_2-Spacer), 36.9 (I_β), 36.7 (I_β), 35.9 (CH_2-Spacer), 24.2 ($I_{\gamma1}$), 24.1 ($I_{\gamma1}$), 18.8 (T_γ), 18.0 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$), 11.2 (I_δ), 11.1 (I_δ).

^{1}H-^{13}C-HSQC (DMSO-d_6): $J_{H1,C1}$ = 156 Hz.

HRMS (ESI$^+$): Calculated for $C_{39}H_{71}N_8O_{17}^+$ $[M+H]^+$: 923.4932; found: 923.4935.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 80 % B, flow: 1ml/min)**:** t_R = 12.52 min, λ = 230 nm.

Asn$^{\alpha\text{-Rha}}$-Peptide with TEG-Spacer (**S15**)



The synthesis of α-rhamnosyl asparagine glycopeptide **S15** was performed according to the general procedure for peptide synthesis on a 0.1 mmol scale. α-Rhamnosyl asparagine building block **9** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 2 h per coupling. Fmoc-TEG-CO_2H-Spacer was coupled following a double coupling protocol with 4-fold excess for each coupling and a coupling time of 1 h per coupling. Deprotection of the acetyl protecting groups was performed according to the general procedure for on-resin deprotection. Cleavage from resin according to the general procedure furnished the crude glycopeptide. RP-HPLC purification (Gradient: A; λ = 205 nm) afforded **S15** (48 mg, 45.7 µmol, 46 %) after lyophilization.

^{1}H-NMR (800 MHz, DMSO-d_6): δ [ppm] = 8.50 (d, $J_{NH,\ H1}$ = 8.8 Hz, 1H, $NH_{N\gamma}$), 8.29 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.24 (t, $J_{NH,H\alpha}$ = 5.7 Hz, 1H, NH_G), 8.12 (d, $J_{NH,H\alpha}$ = 7.9 Hz, 1H, NH_N), 8.10 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.06 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 7.87 (d, $J_{NH,H\alpha}$ = 8.5 Hz, 1H, NH_I), 7.77 – 7.72 (m, 4H, NH_I, NH_3^+), 5.22 (dd, $J_{H1,NH}$ = 8.8 Hz, $J_{H1,H2}$ = 2.0 Hz, 1H, H-1), 4.55 (td, $J_{N\alpha,NH}$ = 7.8 Hz, $J_{H\alpha,H\beta}$ = 5.4 Hz, 1H, $H_{N\alpha}$), 4.22 – 4.18 (m, 2H, 2 × $H_{I\alpha}$), 3.80 – 3.68 (m, 8H, 8 × $H_{G\alpha}$), 3.63 – 3.48 (m, 14H, 6 × CH_2-O-Spacer, H-2, H-3), 3.39 – 3.35 (m, 1H, H-5), 3.20 (t, $J_{H4,H3}$ = $J_{H4,H5}$ = 9.0 Hz, 1H, H-4), 3.00 − 2.96 (m, 2H, CH_2-O-Spacer), 2.63 – 2.61 (m, 1H, $H_{N\beta}$), 2.54 – 2.52 (m, 1H, $H_{N\beta}$), 2.38 (t, $J_{CH,CH}$ = 6.5 Hz, 2H, CH_2-O-Spacer), 1.76 – 1.69 (m, 2H, 2 × $H_{I\beta}$), 1.44 – 1.40 (m, 2H, $H_{I\gamma1}$), 1.09 (d, $J_{CH3,H5}$ = 6.2 Hz, 3H, rha-CH_3), 1.07 – 1.05 (m, 2H, $H_{I\gamma1}$), 0.85 – 0.79 (m, 12H, 2 × $I_{\gamma2}$, 2 × I_δ).

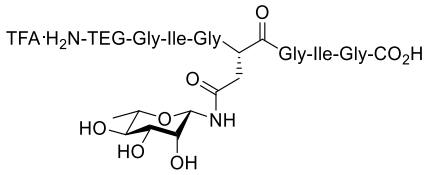
^{13}C-NMR (200 MHz, DMSO-d_6): δ [ppm] = 171.4 (C=O), 171.3 (C=O), 171.1 (2C, 2 × C=O), 170.5 (C=O), 169.6 (C=O), 168.9 (C=O), 168.7 (C=O), 168.4 (C=O), 77.8 (C-1), 72.1 (C-4), 70.6 (C-2/C-3), 70.2 (C-2/C-3), 69.7 (2C, CH_2-O-Spacer), 69.6 (CH_2-O-Spacer), 69.5 (2C, C-5, CH_2-O-Spacer), 66.7 (2C, CH_2-O-Spacer), 56.9 (I_α), 56.6 (I_α), 49.7 (N_α), 42.1 (G_α), 41.9 (G_α), 41.8 (G_α), 40.6 (G_α), 38.7 (CH_2-O-Spacer), 37.3 (N_β), 36.7 (I_β), 36.6 (I_β), 35.8 (CH_2-O-Spacer), 24.3 ($I_{\gamma1}$), 24.2 ($I_{\gamma1}$), 18.0 (rha-CH_3), 15.3 (2C, 2 × $I_{\gamma2}$), 11.1 (2C, 2 × I_δ).

^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 162 Hz.

HRMS (ESI$^+$): Calculated for $C_{39}H_{70}N_9O_{17}$ $[M+H]^+$: 936.4884; found: 936.4884.

RP-HPLC (Aeris, 0.1 % TFA, 0 min: 5 % B → 40 min: 80% B → 60 min: 100 % B, flow: 1 ml/ min): t_R = 11.41 min, λ = 230 nm.

Asn$^{\beta\text{-Rha}}$-Peptide with TEG-Spacer (**S16**)



The synthesis of β-rhamnosyl asparagine glycopeptide **S16** was performed according to the general procedure for peptide synthesis on a 0.1 mmol scale. β-Rhamnosyl asparagine building block **7** was coupled following a double coupling protocol with 3-fold excess for each coupling step and a coupling time of 2 h per coupling. Fmoc-TEG-CO_2H-Spacer was coupled following a double coupling protocol with 5-fold excess for each coupling and a coupling time of 1 h per coupling. Deprotection of the acetyl protecting groups was performed according to the general procedure for deprotection. Cleavage from resin according to the general procedure furnished the crude glycopeptide. RP-HPLC purification (Gradient: A; λ = 205 nm) afforded **S16** (58 mg, 55.3 µmol, 55 %) after lyophilization.

^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 8.26 – 8.17 (m, 3H, $NH_{N\gamma}$, 2 × NH_G), 8.14 – 8.08 (m, 3H, NH_N, 2 × NH_G), 7.86 (d, $J_{NH,I\alpha}$ = 8.6 Hz, 1H, NH_I), 7.78 (d, $J_{NH,I\alpha}$ = 8.9 Hz, 1H, NH_I),7.75 (bs, 3H, NH_3^+), 4.98 (d, $J_{H1,NH}$ = 9.3 Hz, 1H, H-1), 4.56 (q, $J_{N\alpha,NH}$ = $J_{N\alpha,N\beta}$ = 7.1 Hz, 1H, $H_{N\alpha}$), 4.23 – 4.16 (m, 2H, 2 × $H_{I\alpha}$), 3.81 – 3.68 (m, 8H, 8 × $H_{G\alpha}$), 3.62 – 3.47 (m, 13H, H-2, 6 × CH_2-O-Spacer), 3.28 (dd, $J_{H3,H4}$ = 8.7 Hz, $J_{H3,H2}$ = 3.3 Hz, 1H, H-3), 3.14 – 3.06 (m, 2H, H-4, H-5), 3.02 − 2.94 (m, 2H, CH_2-O-Spacer), 2.66 (dd, $J_{H\beta,H\beta}$ = 15.9 Hz, $J_{H\beta,H\alpha}$ = 5.9 Hz, 1H, $H_{N\beta}$), 2.55 – 2.51 (m, 1H, $H_{N\beta}$), 2.38 (t, $J_{CH,CH}$ = 6.5 Hz, 2H, CH_2-O-Spacer), 1.81-1.66 (m, 2H, 2 × $H_{I\beta}$), 1.48 – 1.38 (m, 2H, $H_{I\gamma1}$), 1.11 (d, $J_{CH3,H5}$ = 5.5 Hz, 3H, rha-CH_3), 1.11 – 1.03 (m, 2H, $H_{I\gamma1}$), 0.85 – 0.76 (m, 12H, 2 × $I_{\gamma2}$, 2 × I_δ).

^{13}C-NMR (150 MHz, DMSO-d_6): δ [ppm] = 171.3 (C=O), 171.2 (2C, 2 × C=O), 171.0 (C=O), 170.5 (C=O), 169.4 (C=O), 168.9 (C=O), 168.6 (C=O), 168.5 (C=O), 77.4 (C-1), 73.7 (C-3), 73.6 (C-4/C-5), 71.6 (C-4/C-5), 70.7 (C-2), 69.7 (2C, CH_2-O-Spacer), 69.6 (CH_2-O-Spacer), 69.5 (CH_2-O-Spacer), 66.7 (2C, CH_2-O-Spacer), 56.9 (I_α), 56.7 (I_α), 49.5 (N_α), 42.2 (G_α), 41.9 (G_α), 41.8 (G_α), 40.6 (G_α), 38.7 (CH_2-O-Spacer), 37.4 (N_β), 36.7 (I_β), 36.5 (I_β), 35.8 (CH_2-O-Spacer), 24.3 ($I_{\gamma1}$), 24.2 ($I_{\gamma1}$), 17.9 (rha-CH_3), 15.3 ($I_{\gamma2}$), 15.2 ($I_{\gamma2}$), 11.1 (I_δ), 11.0 (I_δ).

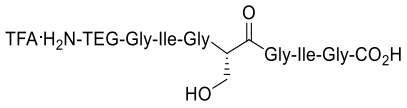
^{1}H-^{13}C-HSQC ($CDCl_3$): $J_{H1,C1}$ = 154 Hz

HRMS (ESI$^+$): Calculated for $C_{39}H_{70}N_9O_{17}$ $[M+H]^+$: 936.4884; found 936.4873.

RP-HPLC (Aeris, 0.1 % TFA, 0 min: 8 % B → 10 min: 8 % B → 60 min: 50% B → 70 min: 100 % B; flow: 1 ml/ min): t_R = 11.79 min, λ = 230 nm.

Synthesis of non-glycosylated Peptides (Naked Peptides (NP))

Naked Ser-Peptide (**S17, Ser^{NP}**)



Naked Ser-Peptide **S17** was synthesized according to the general procedures for microwave assisted peptide synthesis. Cleavage from resin was conducted as described in the general procedure. Purification *via* RP-HPLC (Gradient: A; λ = 205 nm) furnished **S17** (32 mg, 36.5 µmol, 37 %) after lyophilization.

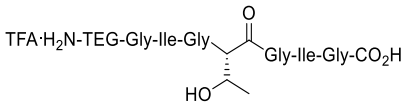
^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 8.33 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.28 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.18 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, -NH_G), 8.11 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 7.95 (d, $J_{NH,H\alpha}$ = 7.6 Hz, 1H, NH_S), 7.91 (d, $J_{NH,H\alpha}$ = 8.4 Hz, 1H, NH_I), 7.81 (s, 3H, NH_3^+), 7.77 (d, $J_{NH,H\alpha}$ = 9.0 Hz, 1H, NH_I), 4.25 (dt, $J_{H\alpha,NH}$ = 7.4 Hz, $J_{H\alpha,H\beta}$ = 5.5 Hz, 1H, $H_{S\alpha}$), 4.21 (dd, $J_{H\alpha,NH}$ = 8.9 Hz, $J_{H\alpha,H\beta}$ = 7.2 Hz, 1H, $H_{I\alpha}$), 4.17 (dd, $J_{H\alpha,NH}$ = 8.4 Hz, $J_{H\alpha,H\beta}$ = 7.2 Hz, 1H, $H_{I\alpha}$), 3.83 – 3.67 (m, 8H, 8 × $H_{G\alpha}$), 3.65 – 3.46 (m, 14H, 2 × $H_{S\beta}$, 12 × CH_2-O-Spacer), 3.01 – 2.93 (m, 2H, CH_2-O-Spacer), 2.38 (t, $J_{CH,CH}$ = 6.5 Hz, 2H, CH_2-O-Spacer), 1.76 – 1.66 (m, 2H, 2 × $H_{I\beta}$), 1.47 – 1.38 (m, 2H, $H_{I\gamma1}$), 1.11 – 1.01 (m, 2H, $H_{I\gamma1}$), 0.88 – 0.75 (m, 12H, 2 × $H_{I\gamma2}$, 2 × $H_{I\delta}$).

^{13}C-NMR (150 MHz, DMSO-d_6): δ [ppm] = 171.3 (C=O), 171.2 (C=O), 171.0 (C=O), 170.5 (C=O), 170.4 (C=O), 169.0 (C=O), 168.8 (C=O), 168.5 (C=O), 69.7 (2C, CH_2-Spacer), 69.6 (CH_2-Spacer), 69.5 (CH_2-Spacer), 66.7 (2C, CH_2-Spacer), 61.8 (S_β), 57.1 (I_α), 56.6 (I_α), 55.3 (S_α), 42.1 (G_α), 41.9 (G_α), 41.8 (G_α), 40.6 (G_α), 38.6 (CH_2-Spacer), 36.8 (I_β), 36.6 (I_β), 35.8 (CH_2-Spacer), 24.3 ($I_{\gamma1}$), 24.1 ($I_{\gamma1}$), 15.3 ($I_{\gamma2}$), 15.2 ($I_{\gamma2}$), 11.1 (2C, 2 × I_δ).

HRMS (ESI$^+$): Calculated for $C_{32}H_{59}N_8O_{13}^+$ $[M+H]^+$: 763.4196; found: 763.4197.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 40 min 80 % B, flow: 1ml/min): t_R = 12.09 min, λ = 230 nm.

Naked Thr-Peptide (**S18, Thr^{NP}**)



Naked Thr-Peptide **S18** was synthesized according to the general procedures for microwave assisted peptide synthesis. Cleavage from resin was conducted as described in the general procedure. Purification *via* RP-HPLC (Gradient: A; λ = 205 nm) yielded **S18** (42 mg, 47.2 µmol, 47 %) after lyophilization.

^{1}H-NMR (800 MHz, $CDCl_3$): δ = 8.32 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.25 (bs, 1H, NH_G), 8.15 – 8.10 (m, 2H, 2 × NH_G), 7.99 (d, $J_{NH,H\alpha}$ = 6.4 Hz, 1H, NH_T), 7.81 (d, $J_{NH,H\alpha}$ = 9.0 Hz, 1H, NH_I), 7.78 (d, $J_{NH,H\alpha}$ = 8.2 Hz, 1H, NH_I), 4.21 (dd, $J_{H\alpha,NH}$ = 9.0 Hz, $J_{H\alpha,H\beta}$ = 7.3 Hz, 1H, $H_{I\alpha}$), 4.19 – 4.16 (m, 2H, $H_{I\alpha}$, $H_{T\alpha}$), 4.02 – 3.98 (m, 1H, $H_{T\beta}$), 3.82 – 3.67 (m, 8H, 8 × $H_{G\alpha}$), 3.62 – 3.46 (m, 12H, 12 × CH_2-O-Spacer), 2.98 (t, $J_{CH,CH}$ = 5.2 Hz, 2H, CH_2-O-

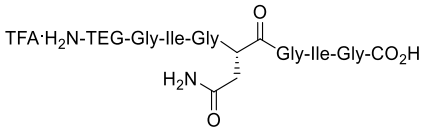
Spacer), 2.38 (t, $J_{CH,CH}$ = 6.5 Hz, 2H, CH_2-O-Spacer), 1.75 – 1.69 (m, 2H, 2 × $H_{I\beta}$), 1.47 – 1.39 (m, 2H, $H_{I\gamma1}$), 1.11 – 1.01 (m, 5H, $H_{T\gamma}$, $H_{I\gamma1}$), 0.85 – 0.82 (m, 6H, 2 × $H_{I\gamma2}$), 0.81 – 0.78 (m, 6H, 2 × $H_{I\delta}$). [ppm]

^{13}C-NMR (200 MHz, $CDCl_3$): δ= 171.3 (C=O), 171.2 (C=O), 171.1 (C=O), 170.5 (2C, 2 × C=O), 169.0 (2C, 2 × C=O), 168.6 (C=O), 69.7 (2C, CH_2-Spacer), 69.6 (CH_2-Spacer), 69.5 (CH_2-Spacer), 66.7 (3C, CH_2-Spacer, T_β), 58.5 (T_α), 57.0 (I_α), 56.7 (I_α), 42.1 (2C, 2 × G_α), 41.9 (G_α), 40.8 (G_α), 38.7 (CH_2-Spacer), 36.7 (I_β), 36.6 (I_β), 35.8 (CH_2-Spacer), 24.3 ($I_{\gamma1}$), 24.1 ($I_{\gamma1}$), 19.5 (T_γ), 15.3 (2C, 2 × $I_{\gamma2}$), 11.1 (2C, 2 × I_δ). [ppm]

HRMS (ESI$^+$): Calculated for $C_{33}H_{61}O_{13}N_8^+$ $[M+H]^+$: 777.4353; found: 777.4341.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 40 min 80 % B, flow: 1ml/min): t_R = 12.09 min, λ = 230 nm.

Naked Asn-Peptide (**S19, Asn^{NP}**)



Naked Asn-Peptide **S19** was synthesized according to the general procedures for microwave assisted peptide synthesis. Cleavage from resin was conducted as described in the general procedure for cleavage from resin. Subsequent purification *via* RP-HPLC (Gradient: A; λ = 205) yielded **S1**9 (35 mg, 38.7 µmol, 39 %) after lyophilization.

^{1}H-NMR: (600 MHz, DMSO-d_6): δ [ppm] = 8.28 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.25 (t, $J_{NH,H\alpha}$ = 5.9 Hz, 1H, NH_G), 8.13 (t, $J_{NH,H\alpha}$ = 5.8 Hz, 1H, NH_G), 8.11 – 8.07 (m, 2H, NH_G, NH_N), 7.93 (d, $J_{NH,H\alpha}$ = 8.4 Hz, 1H, NH_I), 7.76 (d, $J_{NH,H\alpha}$ = 9.0 Hz, 1H, NH_I), 7.39 (s, 1H, NH_N), 6.91 (s, 1H, NH_N), 4.52 (td, $J_{H\alpha,H\beta}$ = 7.5 Hz, $J_{H\alpha,NH}$ = 5.7 Hz, 1H, $H_{N\alpha}$), 4.21 – 4.15 (m, 2H, 2 × $H_{I\alpha}$), 3.81 – 3.66 (m, 8H, 8 × $H_{G\alpha}$), 3.62 – 3.47 (m, 12H, CH_2-O-Spacer), 2.98 (t, $J_{CH,CH}$ = 5.2 Hz, 2H, CH_2-O-Spacer), 2.56 – 2.52 (m, 1H, $H_{N\beta}$), 2.45 (dd, $J_{H\beta,H\beta}$ = 15.6 Hz, $J_{H\beta,H\alpha}$ = 7.2 Hz, 1H, $H_{N\beta}$), 2.38 (t, $J_{CH,CH}$ = 6.5 Hz, 2H, CH_2-O-Spacer), 1.80 – 1.67 (m, 2H, 2 × $H_{I\beta}$), 1.49-1.38 (m, 2H, $H_{I\gamma1}$), 1.13-1.01 (m, 2H, $H_{I\gamma1}$), 0.90 – 0.76 (m, 12H, 2 × $H_{I\gamma2}$, 2 × $H_{I\delta}$).

^{13}C-NMR (150 MHz, DMSO-d_6): δ [ppm] = 171.6 (C=O), 171.4 (C=O), 171.2 (C=O), 171.1 (C=O), 170.5 (C=O), 168.9 (C=O), 168.8 (C=O), 168.5 (C=O), 69.7 (2C, CH_2-Spacer), 69.6 (CH_2-Spacer), 69.5 (CH_2-Spacer), 66.7 (2C, CH_2-Spacer), 57.0 (I_α), 56.8 (I_α), 49.7 (N_α), 42.2 (G_α), 42.0 (G_α), 41.9 (G_α), 40.7 (G_α), 38.7 (CH_2-Spacer), 37.1 (N_β), 36.6 (2C, I_β), 35.8 (CH_2-Spacer), 24.3 ($I_{\gamma1}$), 24.2 ($I_{\gamma1}$), 15.3 (2C, 2 × $I_{\gamma2}$), 11.1 (I_δ), 11.0 (I_δ). Note: Due to signal overlap C-terminal carbonyl could not be assigned in the ^{13}C-NMR.

HRMS (ESI$^+$): Calculated for $C_{33}H_{60}N_9O_{13}$ $[M+H]^+$: 790.4305; found: 790.4308.

RP-HPLC (Aeris, 0.1 % TFA; 0 min 5 % B → 40 min 80 % B, flow: 1ml/min): t_R = 12.09 min, λ = 230 nm.

General Procedure for BSA Conjugation

Synthesis of squarate monoamides was conducted according to a slightly modified procedure.[541]

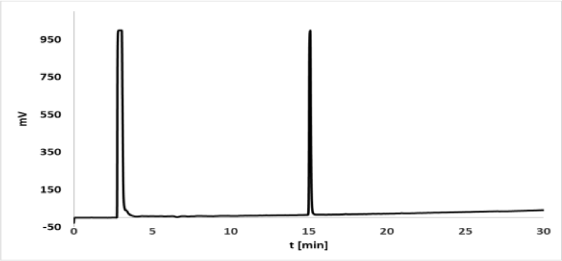
To a magnetically stirred solution of peptide (1.0 eq.) in a mixture of H_2O/EtOH (v/v = 1:1), 3,4-diethoxy-3-cyclobutene-1,2-dione (1.3 eq.) was added. The pH-value was carefully adjusted to pH = 8 by addition of a saturated Na_2CO_3 solution. The reaction was stirred until complete conversion of the starting material was observed by RP-HPLC monitoring. Subsequently the reaction was neutralized by careful addition of 1 M AcOH and lyophilized. The crude product was subjected to RP-HPLC (Gradient: A) and lyophilized, to furnish the desired squarate monoamide as colourless lyophilizate.

Table 4: Experimental data of glycopeptide mono amides for BSA conjugation

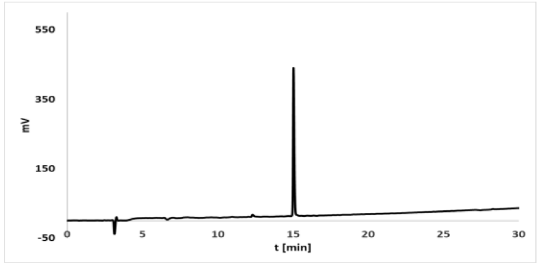
Peptide	Monoamide	ESI-MS [m/z]	RP-HPLC*
Ser$^{\alpha\text{-Rha}}$-GP (**S11**) (34.0 mg, 37.4 µmol)	**S20** 16mg (15.7 µmol, 42 %)	Calc.: $[M+NH_4]^+$: 1050.5201; found: 1050.5227.	t_R = 15.07 min λ = 230 nm
Ser$^{\beta\text{-}}$Rha-GP (**S12**) (15.0 mg, 16.5 µmol)	**S21** 8 mg (7.74 µmol, 47 %)	Calc.: $[M+NH_4]^+$: 1050.5201; found: 1050.5217	t_R = 15.13 min λ = 230 nm
Thr$^{\alpha\text{-Rha}}$-GP (**S13**) (37.0 mg, 40.1 µmol)	**S22** 21 mg (20.1 µmol, 50 %)	Calc.: $[M+NH_4]^+$: 1064.5358; found: 1064.5374.	t_R = 15.11 min λ = 230 nm
Thr$^{\beta\text{-Rha}}$-GP (**S14**) (15.0 mg, 16.3 µmol)	**S23** 6 mg (5.73 µmol, 35 %)	Calc.: $[M+NH_4]^+$: 1064.5358; found: 1064.5377.	t_R = 15.44 min λ = 230 nm
Asn$^{\alpha\text{-Rha}}$-GP (**S15**) (24.0 mg, 22.9 µmol)	**S24** 11 mg (10.4 µmol, 45 %)	Calc.: $[M+Na]^+$: 1082.4864; found: 1082.4864	t_R = 14.63 min λ = 230 nm
Asn$^{\beta\text{-}}$Rha-GP (**S16**) (40.0 mg, 38.0 µmol)	**S25** 22 mg (20.8 µmol, 55 %)	Calc.: $[M+Na]^+$: 1082.4864; found: 1082.4874	t_R = 14.84 min λ = 230 nm
Ser^{NP} (**S17**) (30.0 mg, 39.2 µmol)	**S26** 18 mg (20.3 µmol, 52 %)	Calc.: $[M+NH_4]^+$: 904.4622; found: 904.4631.	t_R = 15.61 min λ = 230 nm
Thr^{NP} (**S18**) (35.0 mg, 45.0 µmol)	**S27** 15 mg (16.7 µmol, 37 %)	Calc.: $[M+NH_4]^+$: 918.4779; found: 918.4789.	t_R = 15.90 min λ = 230 nm
Asn^{NP} (**S19**) (61.0 mg, 69.6 µmol)	**S28** 26 mg (28.4 µmol, 41 %)	Calc.: $[M+Na]^+$: 936. 4285; found: 936.4275	t_R = 15.49 min λ = 230 nm

* Gradient: 0.1 % TFA; 0 min 5 % B → 40 min 80% B, flow: 1ml/min; Column: Phenomenex Aeris Peptide column (C18, 5 µm, 250 mm × 4.6 mm).

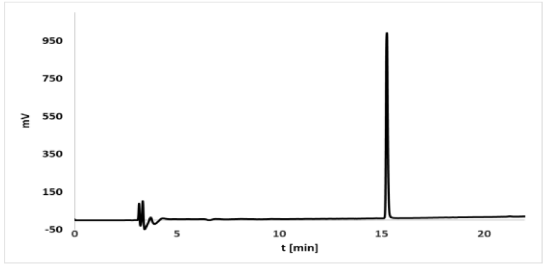
HPLC-Traces of purified squarate monoamides:



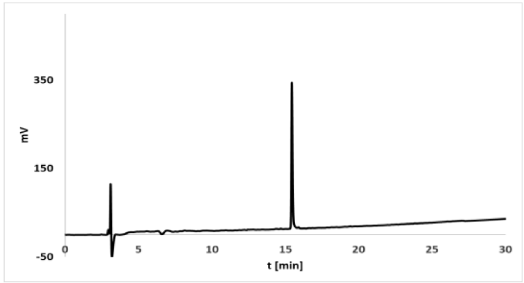
HPLC Trace of $Ser^{\alpha\text{-Rha}}$ peptide Squarate monoamide (**S20**); t_R = 2.81 min = DMSO



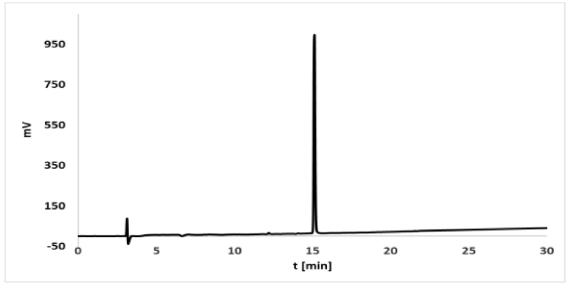
HPLC-Trace of $Ser^{\beta\text{-Rha}}$ Peptide squarate monoamide (**S21**)



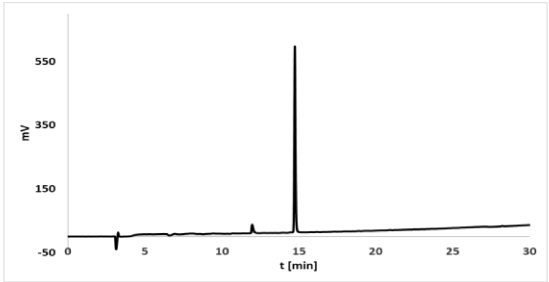
HPLC-Trace of $Thr^{\alpha\text{-Rha}}$ Peptide squarate monoamide (**S22**)



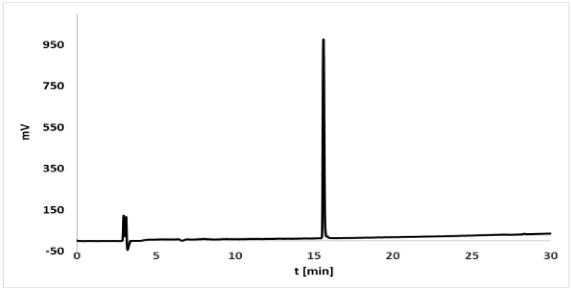
HPLC-Trace of $Thr^{\beta\text{-Rha}}$ Peptide squarate monoamide (**S23**)



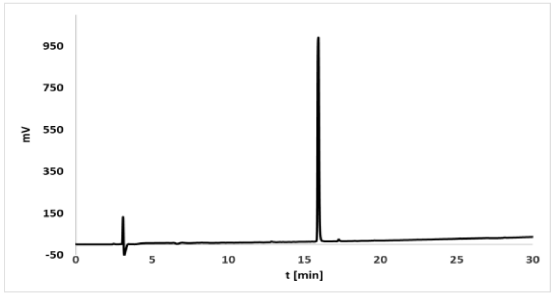
HPLC-Trace of $Asn^{\alpha\text{-Rha}}$ peptide squarate monoamide (**S24**)



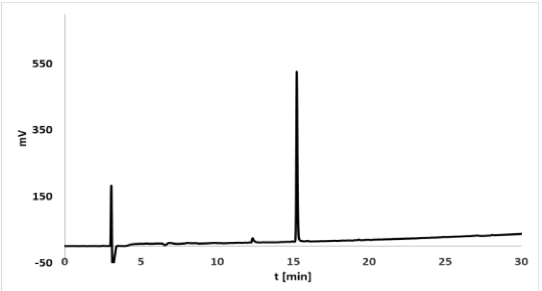
HPLC-Trace of$Asn^{\beta\text{-Rha}}$ peptide squarate monoamide (**S25**)



HPLC-Trace of Ser^{NP} squarate monoamide (**S26**)



HPLC-Trace of Thr^{NP} squarate monoamide (**S27**)



HPLC-Trace of Asn^{NP} squarate monoamide (**S28**)

Conjugation protocol:

BSA conjugation was conducted following a previously reported procedure[541]

For the synthesis of BSA conjugates, the corresponding peptide squaric acid monoamide (2.0 µmol, 25 eq.) was dissolved in 1 ml of disodium phosphate buffer (65 mg Na_2HPO_4 per 1 ml of H_2O, pH = 9.5) and added to a solution of BSA (5 mg, 0.08 µmol, 1.0 eq.) in 1 ml of the same buffer. The mixture was agitated 3 d at 26 °C, before being filtered over an *Amicon Ultra-15* centrifugal filter using a 10 kDa membrane and washed with water until the filtrate was neutral. The residue was lyophilized yielding the corresponding BSA conjugate.

Table 5: Experimental data for BSA conjugation

Monoamide	Conjugate	Yield	Loading [peptide/BSA]
S20 ($Ser^{\alpha\text{-}Rha}$)	**BSA-$Ser^{\alpha\text{-}Rha}$**	6 mg	14
S21 ($Ser^{\beta\text{-}Rha}$)	**BSA-$Ser^{\beta\text{-}Rha}$**	6 mg	18
S22 ($Thr^{\alpha\text{-}Rha}$)	**BSA-$Thr^{\alpha\text{-}Rha}$**	6 mg	17
S23 ($Thr^{\beta\text{-}Rha}$)	**BSA-$Thr^{\beta\text{-}Rha}$**	6 mg	17
S24 ($Asn^{\alpha\text{-}Rha}$)	**BSA-$Asn^{\alpha\text{-}Rha}$**	6 mg	22
S25 ($Asn^{\beta\text{-}Rha}$)	**BSA-$Asn^{\beta\text{-}Rha}$**	6 mg	20
S26 (Ser^{NP})	**BSA-Ser^{NP}**	6 mg	14
S27 (Thr^{NP})	**BSA-Thr^{NP}**	6 mg	17
S28 (Asn^{NP})	**BSA-Asn^{NP}**	6 mg	18

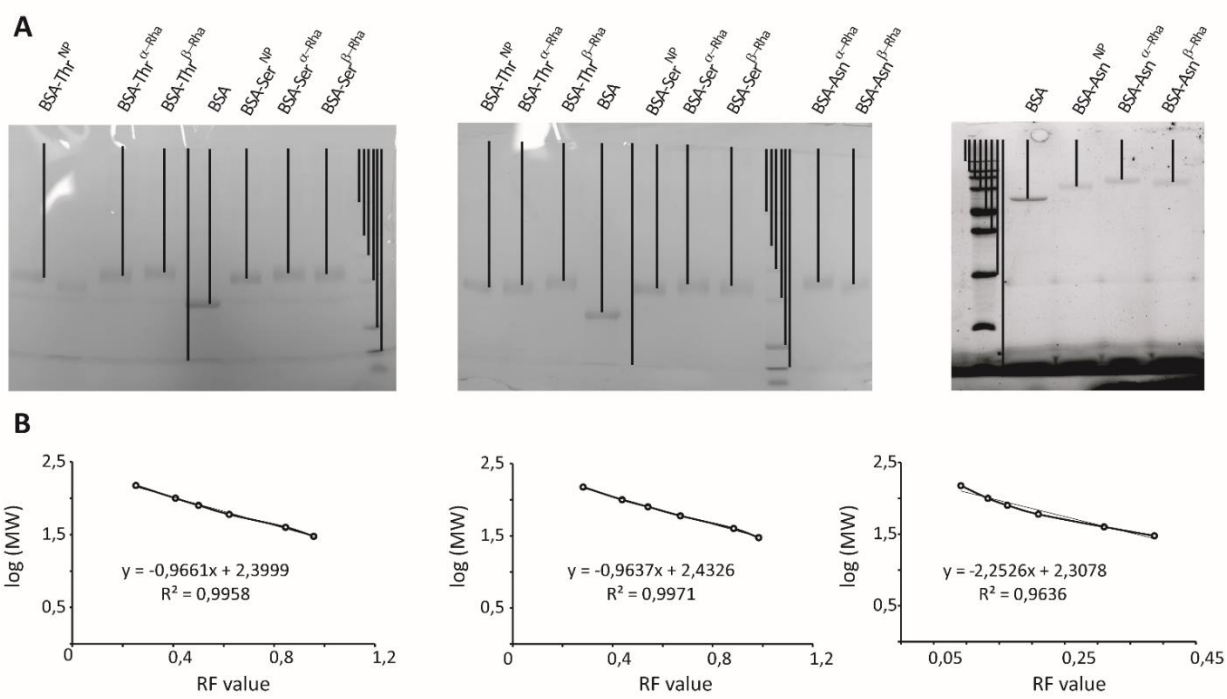


Figure 5: A) SDS-PAGE of aa^{Rha} conjugates. 0.5 µg of BSA, BSA coupled to naked peptide (BSA-aa^{NP}) and BSA conjugates of the corresponding rhamnosylated glycopeptides (BSA-$aa^{\alpha-/\beta-Rha}$) were subjected to SDS-PAGE and subsequent staining with InstantBlue™ (Expedeon Ltd.). **B**) Graphics of linear equation for determining the R_F values. The linear equation was calculated from the protein ladder from the corresponding SDS-PAGE. The mean value of rhamnosylated peptide per BSA molecule was calculated using the linear equation.

Experimental procedures for sensitivity and specificity evaluation of antibodies

Specificity of *anti*-Asn^{Rha}, *anti*-Ser^{Rha} and *anti*-Thr^{Rha}

The specificity of the antibodies was determined by a Western Blot. For this purpose, 0.5 µg of each BSA, BSA-aa^{NP}, EF-P-aa^{Rha} and BSA-$aa^{\alpha-/\beta-Rha}$ were subjected to SDS-PAGE and Western Blot analysis with the corresponding antibody (0.2 µg/ml).

Antigen detection limits were determined by subjecting decreasing amounts of BSA-$aa^{\alpha-/\beta-Rha}$ (500 ng to 0.5 ng) to SDS-PAGE and Western Blot analysis as described above and detection with 0.2 µg/ml of the corresponding antibody.

To investigate cross-reactivity of the antibodies against free L-arginine, L-asparagine, L-serine, L-threonine or L-rhamnose, varying concentrations of BSA-$aa^{\alpha-/\beta-Rha}$ (0.2 µM to 150 µM) and putative competitors (5 mM, 15 mM) were preincubated with the corresponding antibody prior to BSA-$aa^{\alpha-/\beta-Rha}$ (0.5 µg) detection.

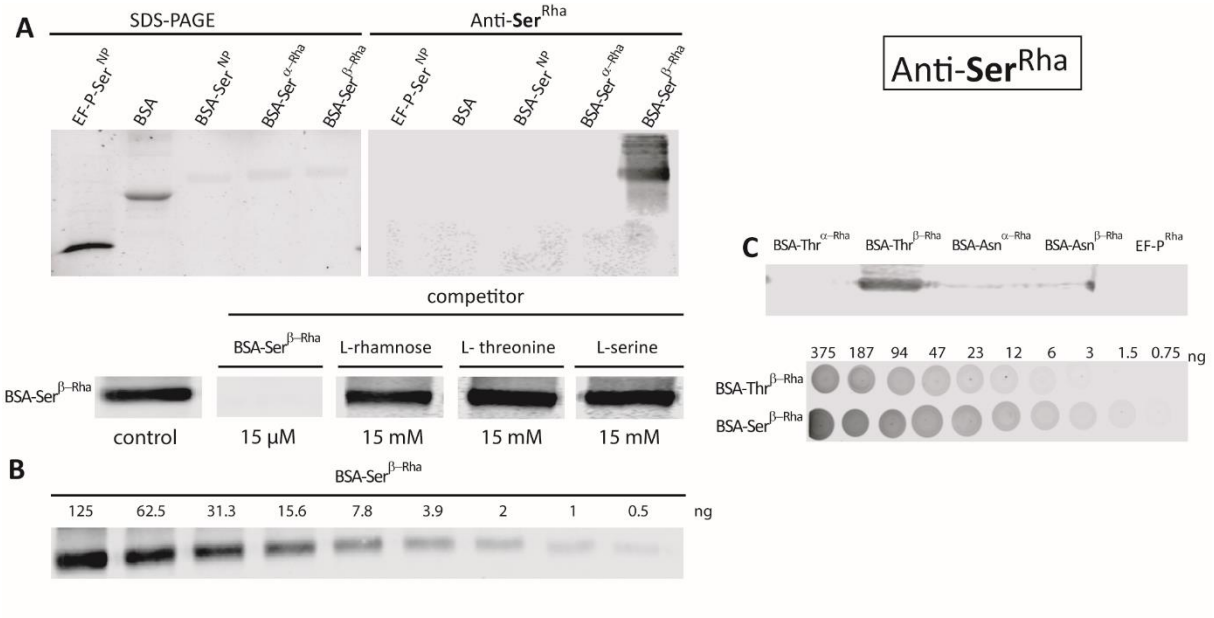


Figure 6: A) *Anti*-Ser^{Rha} specificity analysis: 0.5 µg of the respective sample were subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg/ml of *anti*-Ser^{Rha}. Upper part: BSA, EF-P-Ser^{Rha} and BSA coupled to the naked peptide (BSA-Ser^{NP}). BSA-Ser^{NP} served as negative controls. α- and β- rhamnosylated peptides coupled to BSA (BSA-Ser$^{α-/β-Rha}$) served as positive controls. Lower part: Cross-reactivity analysis of *anti*-Ser^{Rha} against L-rhamnose, L-threonine and L-serine. *Anti*-Ser^{Rha} was preincubated prior to immunodetection with α-/β-Ser^{Rha} (15 µM), L-rhamnose, L-threonine and L-serine (15 mM). **B**) *Anti*-Ser^{Rha} sensitivity analysis of varying concentrations BSA-Ser$^{α-/β-Rha}$. Antibody concentrations were kept constant at 0.2 mg/ml. **C**) Cross-reactivity analysis of *anti*-Ser^{Rha} against BSA-Thr$^{α-/β-Rha}$, BSA-Asn$^{α-/β-Rha}$ and EF-P^{Rha}. 0.5 µg of the respective sample were subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg/ml of *anti*-Ser^{Rha}.

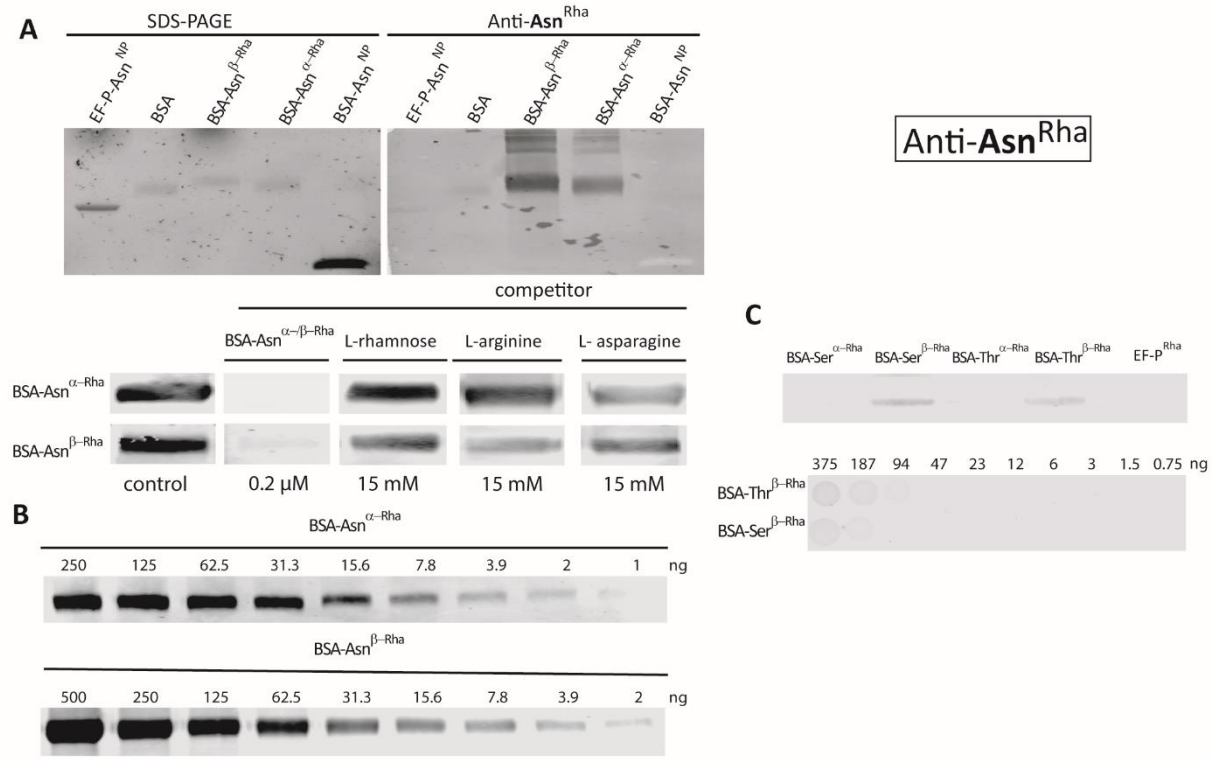


Figure 7: A) *Anti*-Asn^{Rha} specificity analysis: 0.5 µg of the respective sample were subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg/ml of *anti*-Asn^{Rha}. Upper part: BSA, EF-P-Asn^{Rha} and BSA coupled to the naked peptide (BSA-Asn^{NP}). BSA-Asn^{NP} served

as negative controls. α- and β- rhamnosylated peptides coupled to BSA (BSA-Asn$^{\alpha-/\beta-Rha}$) served as positive controls. Lower part: Cross-reactivity analysis of *anti*-Asn^{Rha} against L-rhamnose, L-asparagine and L-arginine. *Anti*-Asn^{Rha} was preincubated prior to immunodetection with BSA-Asn$^{\alpha-/\beta-Rha}$ (0.2 µM), L-rhamnose, L-asparagine and L-arginine (15 mM). **B**) *Anti*-Asn^{Rha} sensitivity analysis of varying concentrations of BSA-Asn$^{\alpha-/\beta-Rha}$. Antibody concentrations were kept constant at 0.2 mg/ml. **C**) Cross-reactivity analysis of *anti*-Asn^{Rha} against BSA-Ser$^{\alpha-/\beta-Rha}$, BSA-Thr$^{\alpha-/\beta-Rha}$ and EF-P^{Rha}. 0.5 µg of the respective sample were subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg/ml of *anti*-Asn^{Rha}.

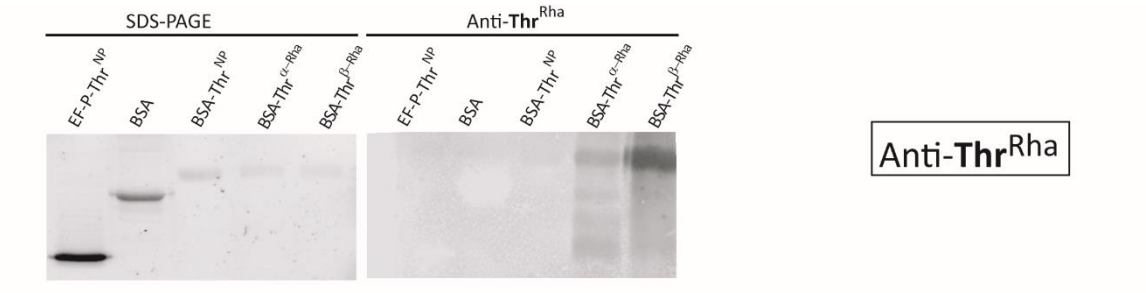


Figure 8: Anti-Thr^{Rha} specificity analysis: 0.5 µg of the respective sample were subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg/ml of anti-Thr^{Rha}. Upper part: BSA, EF-P-Thr^{Rha} and BSA coupled to the naked peptide BSA-Thr^{NP} served as negative controls. α- and β- rhamnosylated peptides coupled to BSA (BSA-Thr$^{\alpha-/\beta-Rha}$) served as positive controls.

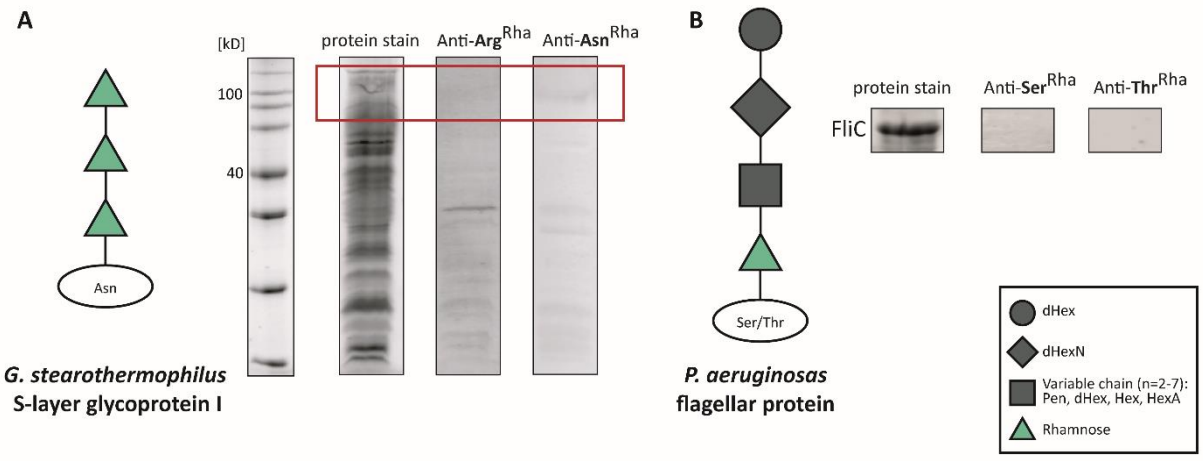


Figure 9: A) Immunodetection of *Geobacillus stearothermophilus* S-layer glycoprotein I. Left: Illustration of the glycanstructure of S-layer glycoprotein I. The S-layer glycoprotein I is poly-rhamnosylated (trisaccharide repeat of rhamnose) at an asparagine residue.[542] Right: The membrane fraction of *G. stearothermophilus,* which contains the poly-rhamnosylated S-layer glycoprotein, was subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg/ml of *anti*-Arg^{Rha} and *anti*-Asn^{Rha}. The S-layer glycoprotein revealed four bands of apparent molecular masses 93, 120, 147 and 170 kDa (area indicated by the red box).[542] **B**) Immunodetection of *Pseudomonas aeruginosa* flagellar protein. Left: Illustration of flagellar glycan structure. The protein is glycosylated at a serine and threonine residue (S260 and T189). A glycan comprising up to 11 monosaccharides units is *O* linked through a rhamnose residue to the protein.[445] Right: The flagellar protein was subjected to SDS-PAGE and subsequent Western Blot analysis with 0.2 mg/ml of *anti*-Ser^{Rha} and *anti*-Thr^{Rha}.

Detection of rhamnosylated proteins in cell lysates

Detection of rhamnosylated proteins in different organisms was carried out using total cell, cytosolic and membrane fractions. These were subjected to SDS-PAGE (total cell fraction: 10 µl, cytosolic fraction 10 µl, membrane fraction: 5 µl (OD600 of 25) and Western Blot analysis as described above.

Bacterial strains and growth conditions

Cells were grown in liquid media according to the growth conditions listed in table 6. When required, media were solidified by using 1.5 % (w/v) agar. The optical density at 600 nm (OD600) of cultures was monitored and cells were harvested at OD600.

Table 6: Bacterial strains and growth conditions

Taxonomy	Strain	Culture condition/Feature	Source
Actinobacteria	*Corynebacterium glutamicum*	BHI medium (DSMZ 215)[543], 30 °C	DSM20300[543]
Actinobacteria	*Mycobacterium phlei*	Lysogeny broth (LB)[544], 37 °C	DSM43239[543]
Actinobacteria	*Micrococcus luteus*	LB, 30 °C	DSM20030[543]
Actinobacteria	*Streptomyces coelicolor*	LB + glycin (0.5 %), 30 °C	DSM40233[543]
Actinobacteria	*Streptomyces griseus*	LB + glycin (0.5 %), 30 °C	DSM40395[543]
Actinobacteria	*Streptomyces venezuelae*	LB + glycin (0.5 %), 30 °C	DSM40230[543]
Alphaproteobacteria	*Caulobacter crescentus*	Caulobacter medium (DSMZ 595)[543], 30 °C	DSM25117[543]
Firmicutes	*Bacillus subtilis*	LB, 30 °C	DSM10[543]
Firmicutes	*Geobacillus stearothermophilus*	LB, 55 °C	DSM22[543]
Firmicutes	*Staphylococcus carnosus*	HD + glycin (0.5 %), 37 °C	DSM20501[543]
Firmicutes	*Streptococcus salivarius*	HD + glycin (0.5 %), 37 °C	DSM20618[543]
Gammaproteobacteria	*Escherichia coli* BL21	LB, 37 °C	[545]
Gammaproteobacteria	*Escherichia coli* BW25113	LB, 37 °C	[546]
Gammaproteobacteria	*Escherichia coli* DH5αλpir	LB, 37 °C / recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 ΔlacZYA-	[547]

		argF U169 ϕ80dlacZΔM15 λpir	
Gammaproteobacteria	*Escherichia coli* LMG194	LB, 37 °C / F- ΔlacX74 galE galK thi rpsL ΔphoA (PvuII) Δara714 leu Tn10	[548]
Gammaproteobacteria	*Escherichia coli* MG1655	LB, 37 °C	DSM18039[543]
Gammaproteobacteria	*Escherichia coli* Nissle 1917	LB, 37 °C	DSM6601[543]
Gammaproteobacteria	*Salmonella enterica* Typhimurium	LB, 37 °C	[549]
Gammaproteobacteria	*Shewanella oneidensis*	LB, 30 °C	ATCC® 700550™[550]
Gammaproteobacteria	*Pseudomonas aeruginosa*	LB, 37 °C	DSM22644[543]
Gammaproteobacteria	*Pseudomonas putida*	LB, 37 °C	DSM100120[543]
Deltaproteobacteria	*Myxococcus xanthus*	CTTYE medium [551], 32 °C	DSM16526[543]
Euryarchaeota	*Haloferax volcanii*	YPC [552], 45 °C	[553]
Sulfolobaceae	*Sulfolobus acidocaldarius*	Brock media [554], 76 °C	DSM639[543]
Cyanobacteria	*Synechococcus elongatus* PCC_6803	Cyanobacteria medium BG11 (DSMZ 1592)[543], 30 °C	[555]

Isolation of cytosolic and membrane fraction

Cell pellets were resuspended to a final O.D. of 25. Cell disruption was achieved for gram negative bacteria by Constant Systems Ltd. continuous-flow cabinet at 1.35 kb. Gram positive bacteria were treated with lysozyme (10 mg/ml) and further lysed by sonication. Cell debris were removed by centrifugation, cytosolic and membrane fraction were separated by ultracentrifugation.

Flagella isolation

Bacterial strains were grown overnight on agar plates (media and growth temperature according to table x). Cells from two plates were resuspended in 2 ml of phosphate-buffered saline (PBS, pH 7.4). The homogenate was passaged 20 times through a needle (*Sterican*® 0.45x12 mm) and centrifuged at 12.000 g at 4 °C for 20 min. The flagellar proteins were precipitated from the supernatant using ammonium sulphate (30 %) overnight at room temperature followed by centrifugation at 12.000 g at 4 °C for 20 min. The protein pellet was resuspended in 100 µl Laemmli-buffer[529] and subjected to SDS-PAGE (20 µl sample).

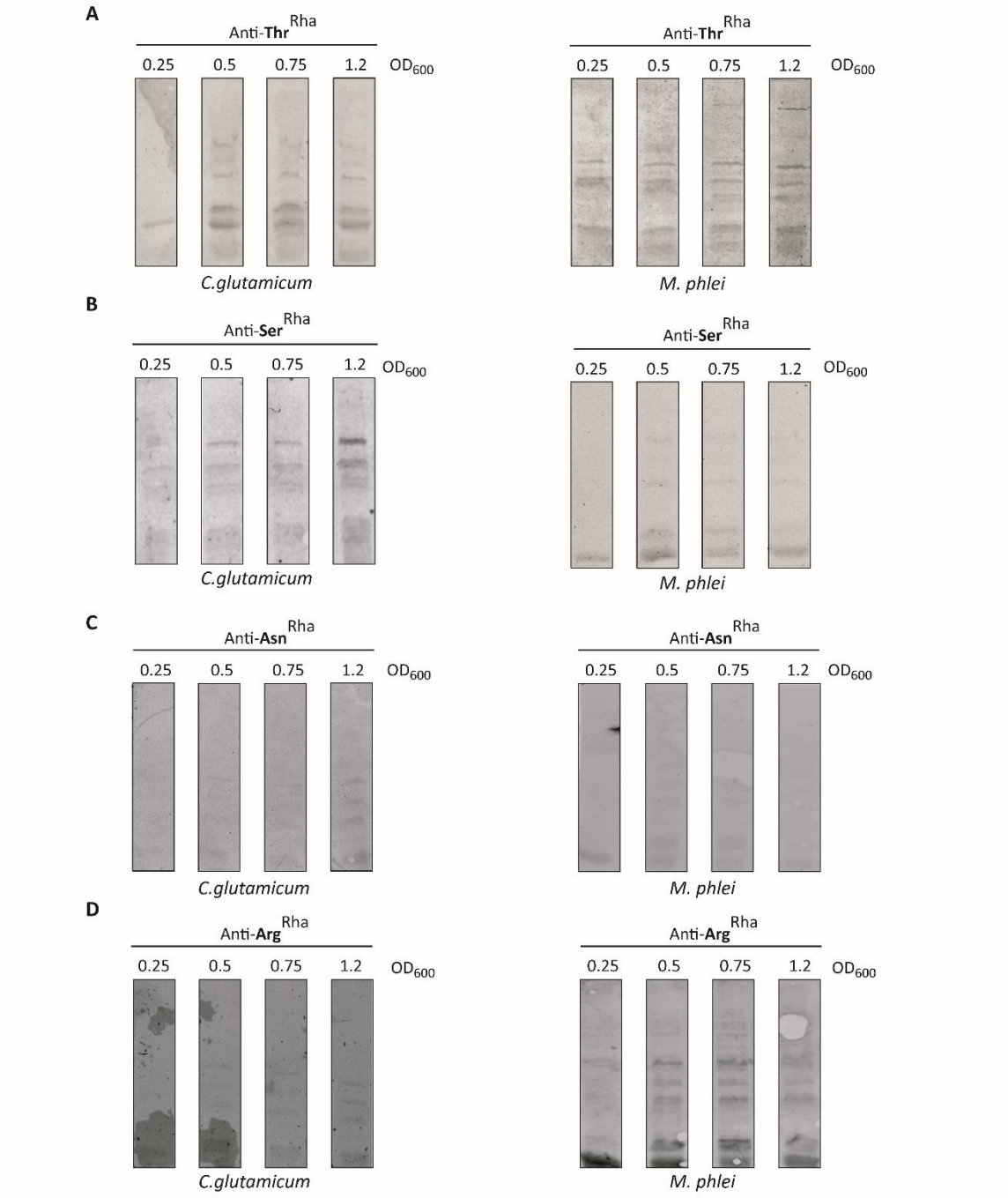


Figure 10: Immunodetection of *Corynebacterium glutamicum* (left) and *Mycobacterium phlei* rhamnoproteins (right) in membrane fractions from different growth phases. Sample were collected at 0.25, 0.5, 0.75 and 1.2 O.D. (600 nm) and subjected to SDS-PAGE with subsequent Western Blot analysis using 0.2 mg/ml of the corresponding antibody: **A**) *anti*-Thr^{Rha}, **B**) *anti*-Ser^{Rha}, **C**) *anti*-Asn^{Rha} and **D**) *anti*-Arg^{Rha}.

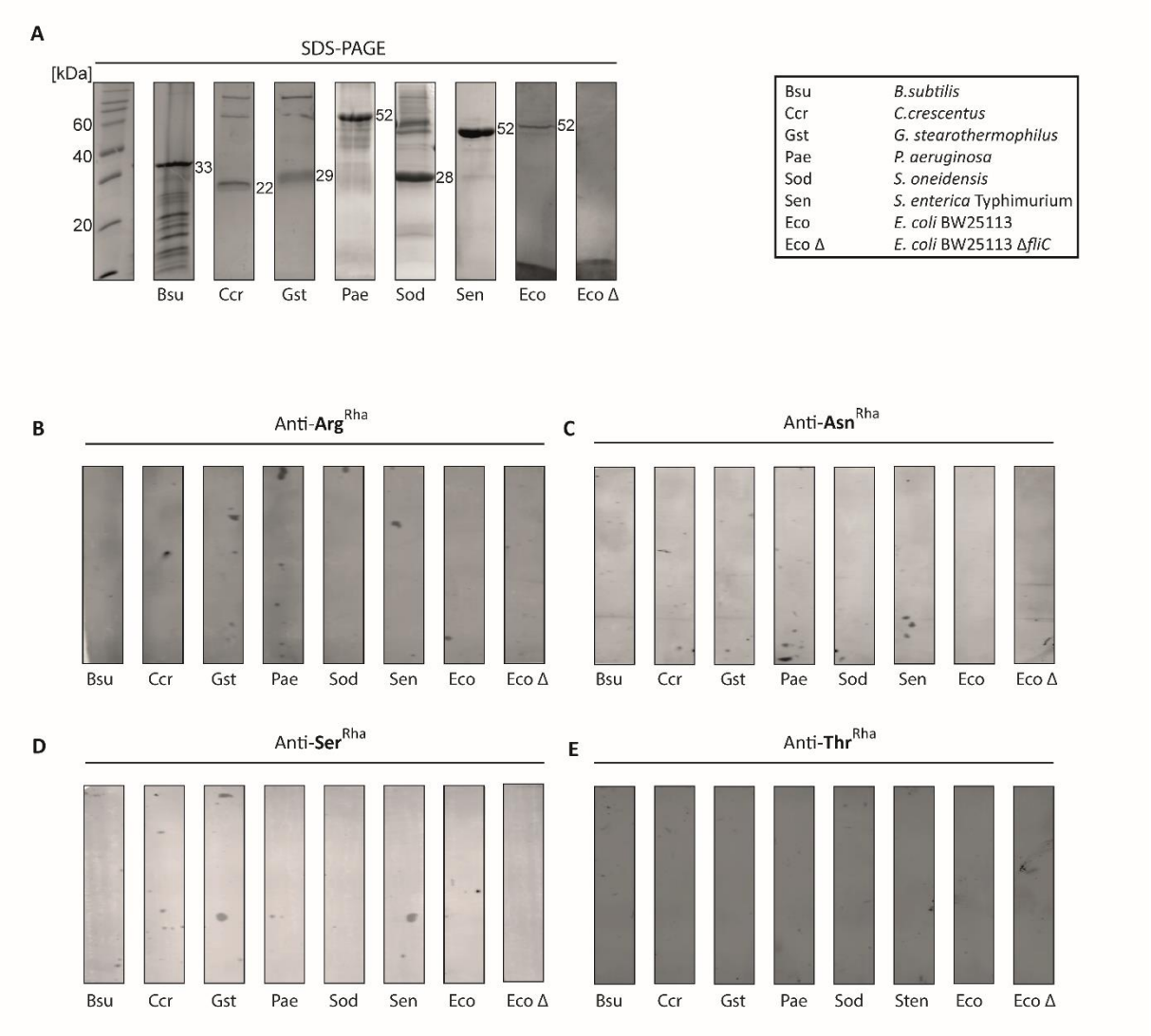
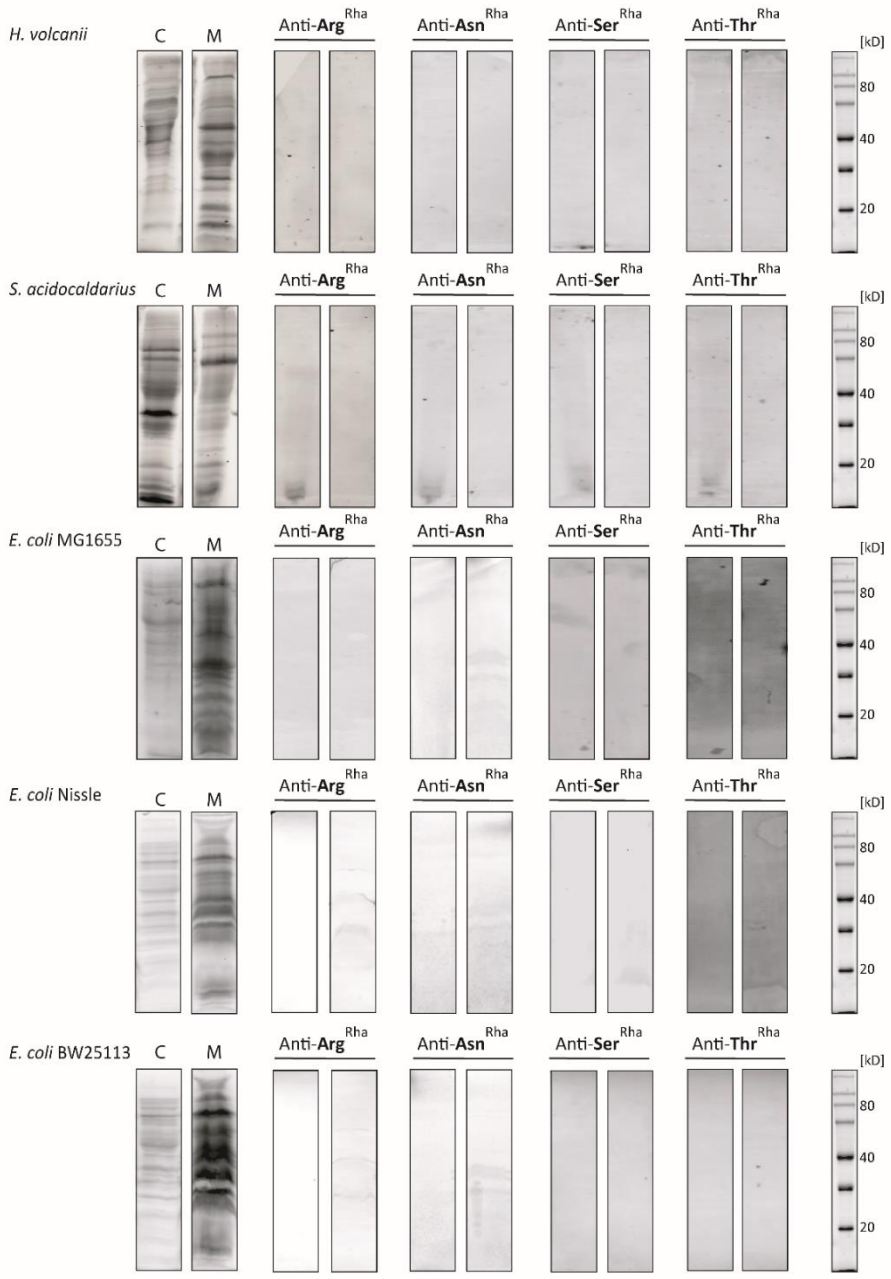
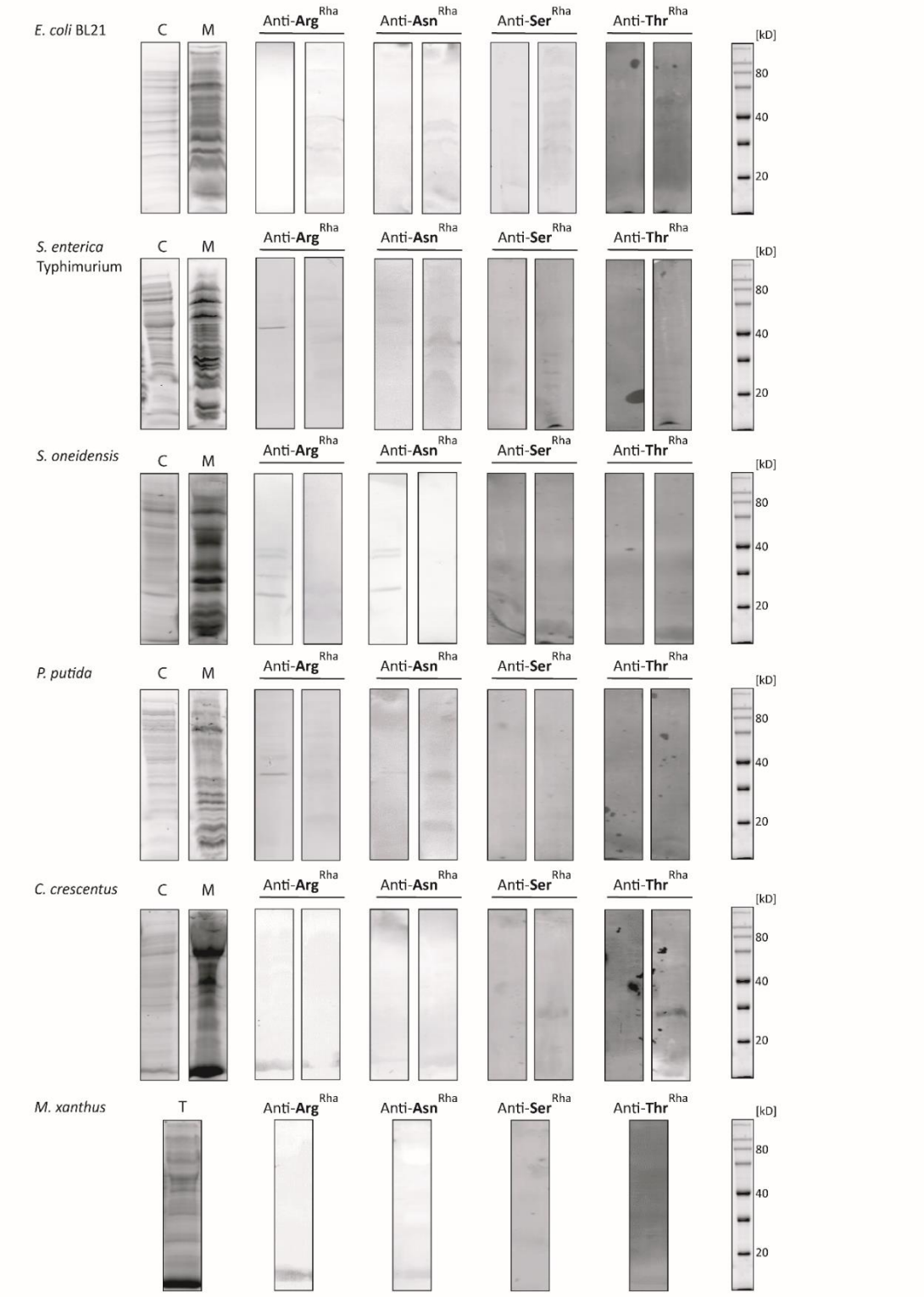


Figure 11: **A**) SDS-PAGE of purified flagellar proteins of swimming bacteria. Flagellar proteins were isolated from overnight cultures using ammonium sulphate precipitation. An *Escherichia coli* strain lacking the flagellar protein FliC (Δ*fliC*) was used as negative control. The expected size of the flagellar proteins is indicated (*Bacillus subtilis*: 33 kDa [556], *Caulobacter crescentus*: 22 kDa [556], *Geobacillus stearothermophilus*: 29 kDa [557], *Pseudomonas aeruginosa*: 52 kDa [558], *Shewanella oneidensis*: 28 kDa [556], *Salmonella enterica* Typhimurium: 52 kDa [556], *E. coli*: 52 kDa [556]). **B**) – **E**) Immunodetection of flagellar proteins with 0.2 mg/ml of the corresponding antibody.


H. volcanii
C
M
Anti-Arg Rha
Anti-Asn Rha
Anti-Ser Rha
Anti-Thr Rha
[kD]
80
40
20
S. acidocaldarius
E. coli MG1655
E. coli Nissle
E. coli BW25113


E. coli BL21
C
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Anti-Arg Rha
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Anti-Ser Rha
Anti-Thr Rha
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80
40
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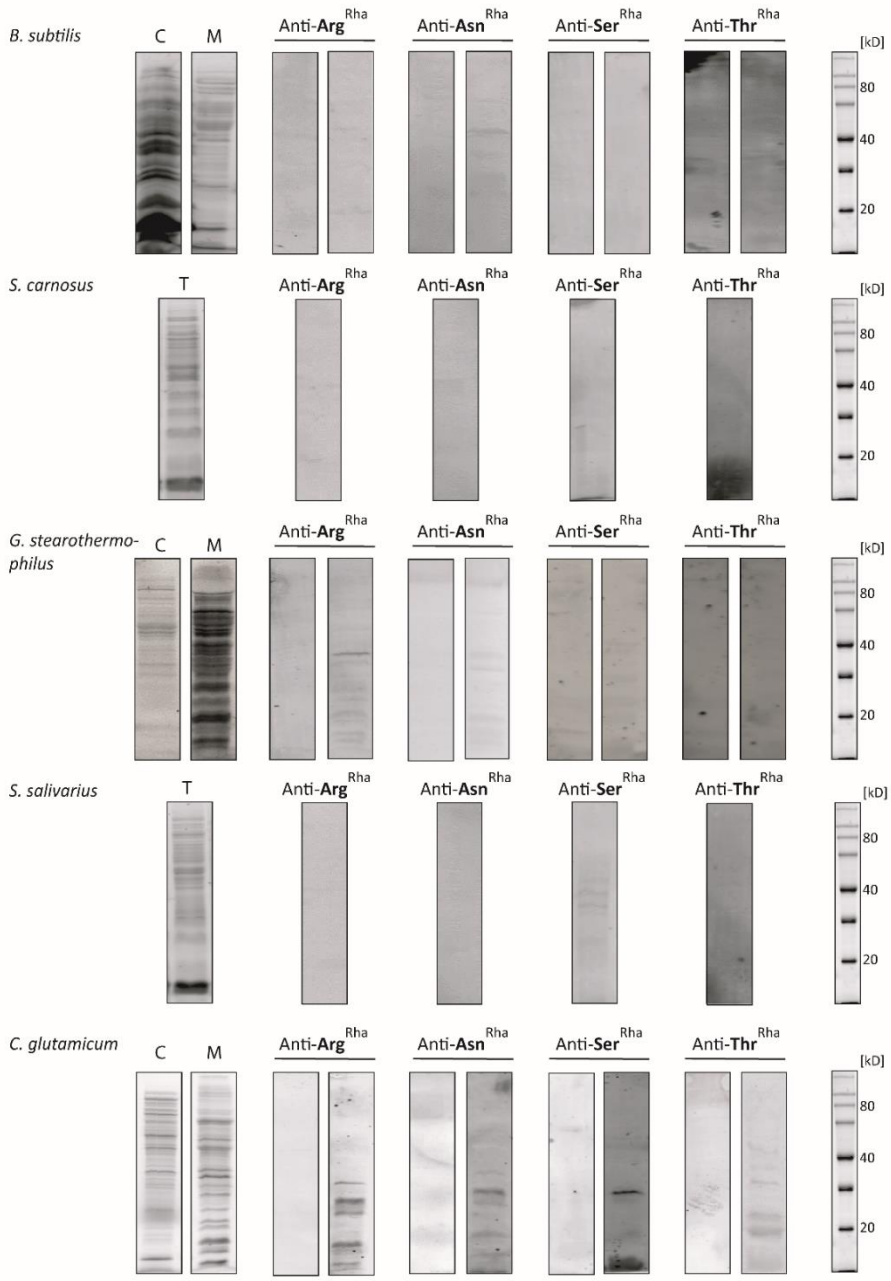
S. oneidensis
P. putida
C. crescentus
M. xanthus
T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C
M
Anti-Arg Rha
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G. stearothermo-philus
C
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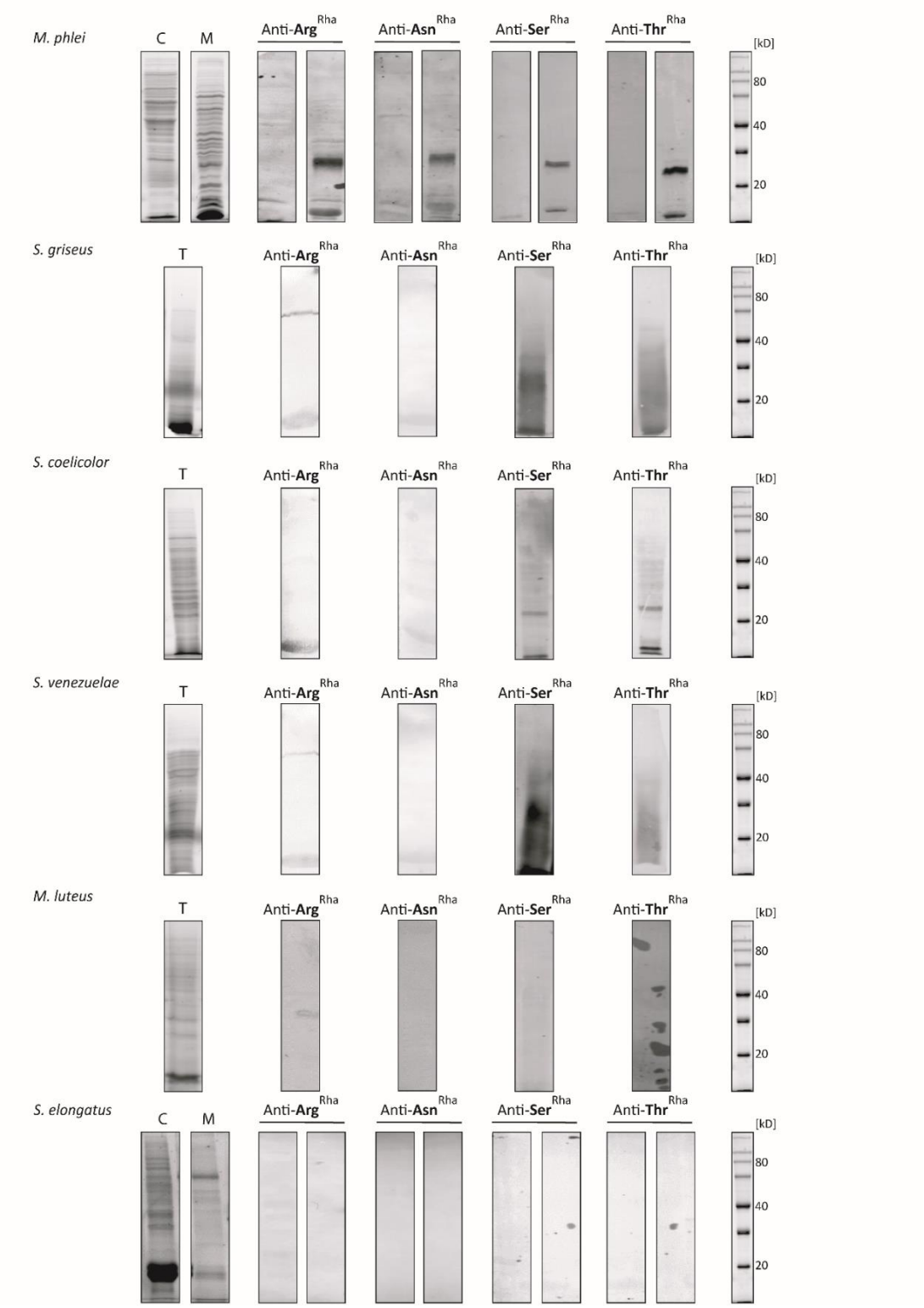

Figure 12: Immunodetection of rhamnosylated proteins using *anti*-Arg^{Rha}, *anti*-Asn^{Rha}, *anti*-Ser^{Rha} and *anti*-Thr^{Rha}. Prokaryotic samples were lysed (T) where indicated separated into cytosolic (C) and membrane (M) fractions. Total protein was visualized from SDS-PAGE (left) size separated proteins using 2,2,2-trichlorethanol. A subsequent Western Blot analysis with 0.2 mg/ml of the respective aa^{Rha} specific antibody is shown on the right.

III. Appendix

Appendix Part 1 and Part 2: Spectroscopic data and evaluation of antibodies and rhamnoprotein detection

Spectroscopic Data for part 1 can be found collectively in the electronic supplementary file “Spectroscopy data Part 1”. Spectroscopic data for Part 2 can be found collectively in the electronic supplementary file “*Spectroscopic and HPLC data part 2*”. In addition, electronic supplementary file of part 2 includes experimental procedures and characterisation of literature known amino acid derivatives and rhamnosyl donor building blocks. Both files can be found in additional files of the electronic version or on the memory stick in the back of the print version of this thesis.

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