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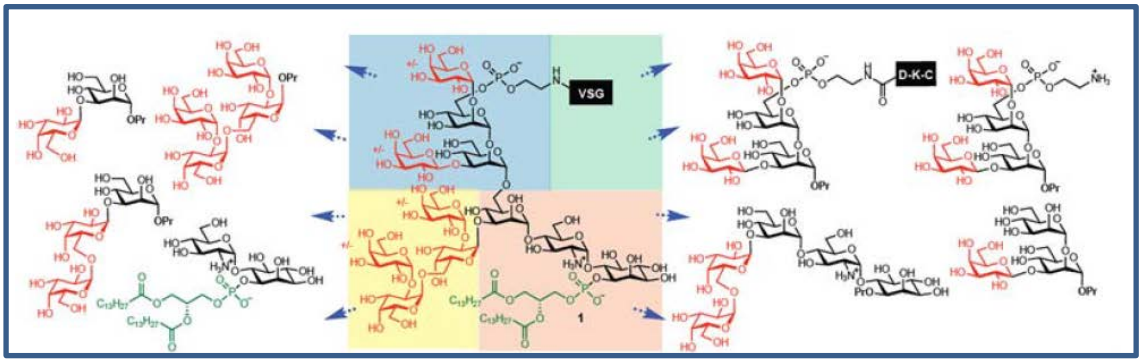

Glycan libraries: Methods to assemble a series of glycosylphosphatidylinositols derivatives containing α- and β-galactosylations were established. These molecules present key modifications for an immunmmodulatory epitope mapping of GPI from Trypanosoma brucei VSGs.

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Synthesis of Galactosylated Glycosylphosphatidylinositol Derivatives from *Trypanosoma brucei*

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Abstract: *Trypanosoma brucei* uses variant surface glycoproteins (VSGs) to evade the host immune system and ensure parasitic longevity in animals and humans. VSGs are attached to the cell membrane by complex glycosylphosphatidylinositol anchors (GPI). Distinguishing structural feature of VSG GPIs are multiple α– and β–galactosides attached to the conserved GPI core structure. *T. brucei* GPIs have been associated with macrophage activation and alleviation of parasitemia during infection, acting as disease onset delaying antigens. Literature reports that link structural modifications in the GPIs to changes in biological activity are contradictory. We have established a synthetic route to prepare structurally overlapping GPI derivatives bearing different T. brucei characteristic structural modifications. The GPI collection will be used to assess the effect of galactosylation and phosphorylation on *T. brucei* GPI immunomodulatory activity, and to perform a epitope mapping of this complex glycolipid as potential diagnostic marker for Trypanosomiasis. A strategy for the synthesis of a complete α–tetragalactoside using the 2-naphthylmethyl protecting group and for subsequent attachment of GPI fragments to peptides is presented.

Introduction

Human African Trypanosomiasis (HAT) and Nagana in animals are two devastating neglected diseases in sub-Saharan Africa caused by subspecies of the extracellular parasite *Trypanosoma brucei*.[1] These diseases remain a significant public health problem in rural areas of some African countries due to limitations in diagnosis and treatment, lack of trained personnel and restricted access to medical facilities.[2] During the first infection, or haemolymphatic phase, the disease is characterized by unspecific symptoms, such as weakness and fever, and often remains undiagnosed in animals and humans. Entering the second, the neurologic stage, which is characterized by severe anemia along with sleep cycle disruption and progressive mental deterioration, HAT becomes lethal if not treated by chemotherapy.[3]

To sustain infection, *T. brucei* parasites rely on several mechanisms that involve cell surface molecules and evasion of the host immune system.[4] At any given timepoint, the surface of a trypanosome is densely covered by one phenotype of variant surface glycoprotein (VSGs) which is attached to a glycosylphosphatidylinositol (GPI) anchor.[5] The main task of these glycoproteins is antigenic variation of the parasite's surface. Participation in complement inhibition, installation of a diffusion barrier, antibody scavenging, masking of other surface proteins, *e.g.* sialidases, and acting as an autoantibody have also been verified (Figure 1).[6] Antigen variation is achieved by random expression of one VSG out of several hundred genes encoded in the parasite's genome, and by the capability of switching the responsible gene between generations.[7] The parasite can engage in segmental gene conversion, a process where several random genes encode in the same reading frame are translated to generate a new N-terminal domain of the VSG with a unique phenotype.[8] Mosaicism, the equivalent process for the C-terminal domain (CTD), is less frequent.[9] The vast amount of different surface specific antigens significantly hampers the diagnosis of *T. brucei* infections and the development of vaccines against *T. brucei* parasites.

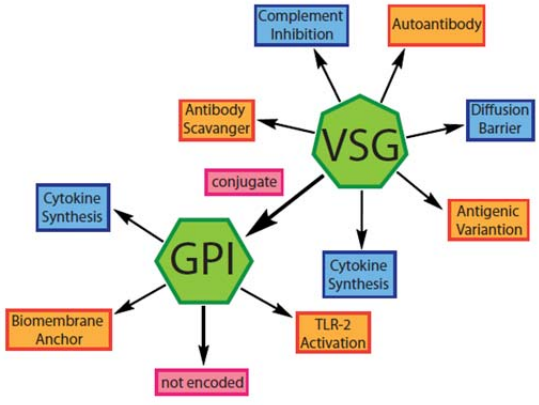

Figure 1. Summary of activities assigned tf the VSG-GPI-complex during *T. brucei* parasite infections.

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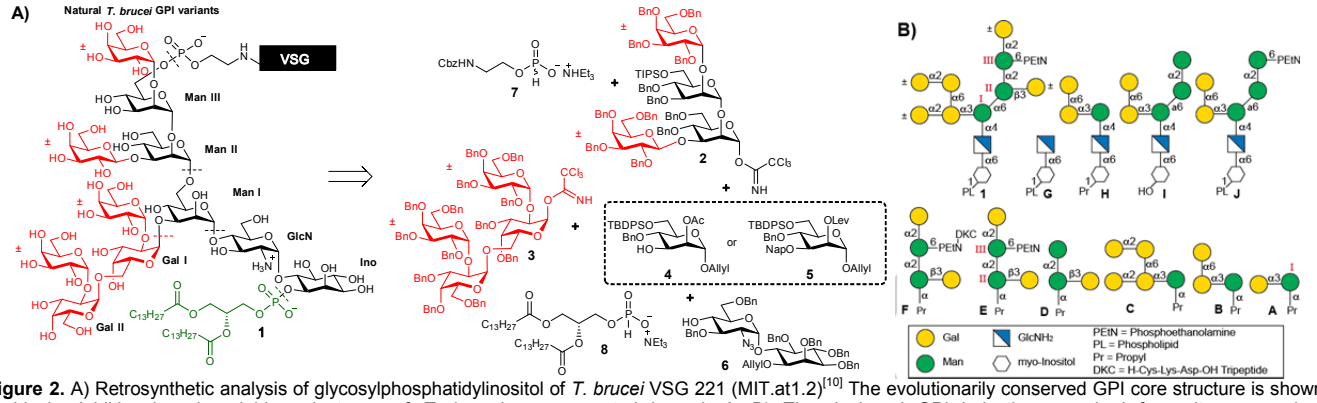

Figure 2. A) Retrosynthetic analysis of glycosylphosphatidylinositol of *T. brucei* VSG 221 (MIT.at1.2)[10] The evolutionarily conserved GPI core structure is shown in black. Additional and variable galactoses of *T. brucei* are presented in red. **1**. B) The designed GPI-derivatives required for epitope mapping.

A single VSG, sVSG117, was reported as an immunodiagnostic HAT antigen.[11] The assay was carried out by incorporation of isolated sVSG117 into a dual-antigen lateral flow test alongside a cell lysate protein rISG65[12] achieving robust test specificity against a set of human sera. Considering the mechanism of antigenic variation outlined above, these results suggest sVSG117 as either a dominant mother gene in segmental gene conversion and mosaicism, or the presence of a reactive epitope at the C-terminal domain of the glycoprotein, *i.e.* the CTD-GPI interface.

In contrast to the protein part of VSGs, the GPI structure depends only on the biosynthetic glycosylation machinery and is therefore not prone to structural variation beyond the addition of galactose units to the conserved backbone.[13] However, each VSG class is attached to GPI structures that may differ in the degree of galactosylation.[10]

GPIs can activate the immune system by interaction with toll-like receptors 2 and 4.[14] More specifically, GPIs isolated from *T. brucei* were able to trigger the release of tumor necrosis factor α (TNF-α) owing to distinct structural features such as the unique α-galactoside branch and the phospholipid attached to the glycosylated *myo*-inositol unit.[15] Isolated GPIs alleviate immune pathologies when they are incorporated into liposomes and injected into mice. Thereby, heavy parasitemia, onset of infection and death were significantly delayed as compared to a control group.[16]

We recently described the synthesis of diverse GPI glycolipids using a general convergent strategy that relies on a set of fully orthogonal protecting groups.[17] Triisopropylsilyl ether (TIPS), levulinic ester (Lev), 2-naphtylmethyl (Nap) and allyl ether (All) were used to mask potential phosphorylation and glycosylation sites in a fully benzylated GPI glycan.[17] Synthetic GPI derivatives obtained by this strategy can be used to identify the binding patterns of antibodies found in sera of patients infected with *Toxoplasma gondii* and *Plasmodium falciparum*.[17-18]

The distinct structural features of C-terminal domain of VSGs, including the features of GPI glycolipid, may determine their interactions with the immune system. Therefore, they may serve as a basis for the development of a carbohydrate-based tools for HAT and Nagana diagnosis and as candidates for the development of intervention that would prevent or combat these diseases.

Synthesis represents the only means to obtain the homogeneous GPI-derivatives in sufficient quantities. Despite the sizable body of work in the field,[19] target oriented synthesis of all possible glycan structures of *Trypansosoma brucei* VSG GPI 221 and structurally related VSG GPIs is still too challenging (Figure 2a). We therefore focused on GPI fragments with modifications characteristic for *T. brucei* including the synthetic fragments of VSG 117 GPI (**J**, Figure 2b)[17] and the GPI core structure[20] as suitable alternative for evaluation of their biological activity. Here, we report the synthesis of a set of *T. brucei* GPIs fragments featuring key modifications of the core structure (**A**-**I**, Figure 2b). In this context, a strategy to obtain the challenging α-tetragalactoside was developed and reactions were optimized to selectively form α- and β-galactoside derivatives. The synthetic fragments are instrumental for activation assays in murine macrophages and human peripheral blood monocytes that will map the relevant epitopes in an immune response during *T. brucei* infections and evaluate the binding of antibodies in sera from infected patients.

Results and Discussion

The upregulation of galactosyltransferases during *T. brucei* GPI biosynthesis results in the formation of GPI glycans with varying levels of galactosylation. Depending of the VSG class, α-linked galactosides can be attached to Man I and Man III. β-Galactosylation of Man II can be observed as well.[10, 21] Biological studies with isolated *T. brucei* GPIs attribute the capacity of GPIs to induce a macrophage inflammatory response to either α-galactosylation or the presence of dimiristoylglycerol.[15a] GPI fragments **A**-**H** featuring different levels of galactosylations at Man I, Man II and Man III are designed as tools to evaluate the role of galactosylation patterns in eliciting an immune response observed during trypanosomiasis infections, and to distinguish the effects of galactosylation from the potential roles of the lipid fragment of the GPI. Previously, we showed that conformational flexibility of the core GPI oligosaccharide is best described as two rigid fragments that are connected via a flexible 1-6-linkage between Man I and Man II.[22] Based on these results, we designed the fragments with key modifications attached to only one of the

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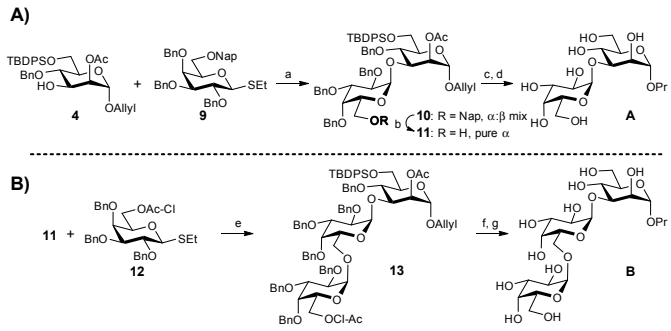
rigid units within the core structure (galactosylation at either ManI or ManII-ManIII unit). The fragments should be accessible using our convergent strategy (Figure 2a) by glycosylating the central Man I building blocks, **4** or **5**, with the galactosylated structures **2** and **3** or truncated versions thereof, or by using elongated Man I glycosyl donor to glycosylate the pseudodisaccharide inositol **6**.

Synthesis

The synthetic work commenced with the assembly of a series of structures containing one (**A**), two (**B**) and four (**C**) galactoside residues. Gal-Man disaccharide **A** and Gal-Gal-Man trisaccharide **B** required a galactose building block with an orthogonal protecting group at the C6 position. This group should be removable after glycosylation of Man I building block **4**.[23] The synthesis of Gal-Man structure started with galactosylation of **4** with building block **9**,[24] which due to the absence of a participating group gave disaccharide **10** as an inseparable mixture of anomers. Removal of the 2-naphthylmethyl (Nap) protecting group of **10** with DDQ in aqueous CH_2Cl_2 over two reaction cycles delivered the disaccharide **11** in 20% yield over two steps.[25] The acetyl and TBDPS group of alcohol **11** were cleaved with *in situ* generated HCl from acetyl chloride in methanol-dichloromethane mixture and the remaining protecting groups were removed by hydrogenolysis. Disaccharide **A** was isolated by purification using size exclusion chromatography (SEC) in 78 % yield (Scheme 1A).



Scheme 1. A) Synthesis of Disaccharide **A**: a) NIS, TMSOTf, -11 °C, DCM/Et_2O; b) DDQ, DCM, H_2O, 20% over two steps; c) AcCl, MeOH/DCM; d) H_2, Pd/C, MeOH/EtOAc, quant. over two steps; B) Synthesis of Trisaccharide **B**: e) **11**, NIS, TMSOTf, -11 °C, DCM/Et_2O, 35%; f) AcCl, MeOH/DCM; g) H_2, Pd/C, MeOH/EtOAc, 78% over two steps.

Due to the low α-selectivity obtained with donor **9** in the synthesis of **A**, the galactoside donor **12** bearing a chloroacetyl ester at the C6-position was used to improve the selectivity for formation of 1,2-*cis* galactoside in synthesis of **B** from disaccharide alcohol **11.**[26] The reaction of thioglycoside **12**[27] and **11** was carried out using NIS and TfOH for activation. Despite the use of the new donor, the trisaccharide **13** was only isolated in moderate yield of 35%. To complete the synthesis of trisaccharide **B**, all acid labile protecting groups were removed by *in situ* generated HCl and the intermediate triol was globally deprotected by hydrogenolysis. After SEC purification, **B** was isolated in 78% over two steps (Scheme 1b).

The homologous tetragalactosylated mannoside **C**, frequently present in the GPI of *T. brucei* VSGs, is a highly demanding synthetic target, having multiple 1,2-*cis* galactose connections. In order to obtain the repetitive α-1,2 digalactoside motif, an initial strategy involving a [2+2] glycosylation was designed (Figure 3). This strategy required the synthesis of disaccharide **14** from thioglycoside **15**[28] and highly versatile galactose building block **16**. The isolated intermediate could then be used as precursor of both acceptor **17** and donor **18** for the [2+2]-glycosylation reaction.

Allyl protection was selected to mask the anomeric position of **16** while former PMB-protected C2 position and TBDPS-protection at the C6 position guarantee orthogonal masking of the connection points. To ensure high α-selectivity during galactosylations, a remote participating benzoyl ester group was introduced at C4 position of the galactosyl donor.[26, 29]

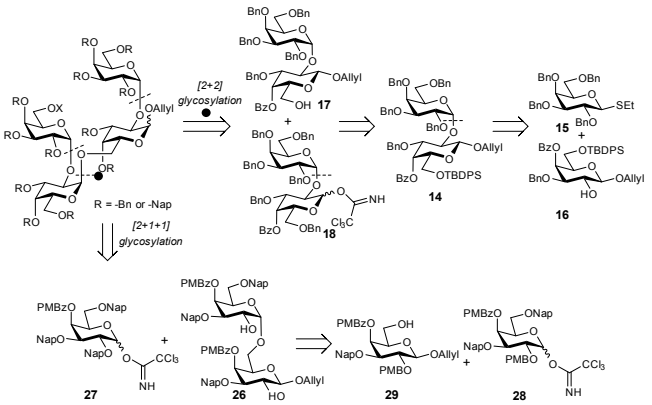
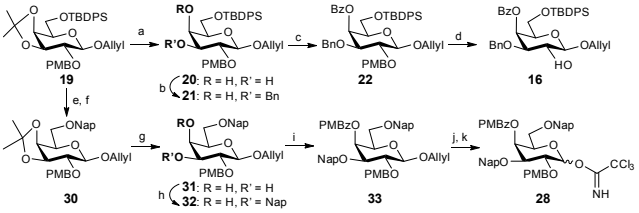


Figure 3. Strategies for assembling the tetragalactoside structure side chain. Two protecting groups patterns for two glycosylation approaches were considered.

The following sequence was employed for synthesis of galactoside **16**. Hydrolysis of acetal **19**[30] using CSA in MeOH gave diol **20** in 82% yield. Regioselective benzylation via stannylation and subsequent reaction with benzyl bromide delivered alcohol **21** in 71% yield. Protection of the C4 position with benzoyl chloride in pyridine at 50 °C gave fully protected galactose **22** in 86% yield. The PMB group at the C2 position of **22** was removed without TBDPS cleavage using a 10:1 mixture of DCM and TFA at 0 °C for 15 minutes, giving **16** in excellent yield of 90% (Scheme 2).



Scheme 2. Synthesis of galactose building blocks **16** and **28** : a) CSA, MeOH, 82%; b) *i.* Bu_2SnO, MeOH; *ii.* BnBr, TBAI, DMF, 71%; c) BzCl, Pyr, 50 °C, 86%; d) TFA, DCM, 0 °C, 15 min, 90%; e) TBAF, THF, 90%; f) NapBr, NaH, DMF, 89%; g) CSA, MeOH, 91%; h) *i.* Bu_2SnO, MeOH; *ii.* NapBr, TBAI, DMF, 60 °C, 84%; i) PMBzCl, pyr, 80 °C, 70%; j) $PdCl_2$, MeOH, 80%; k) CCl_3CN, DBU, DCM, 85%;

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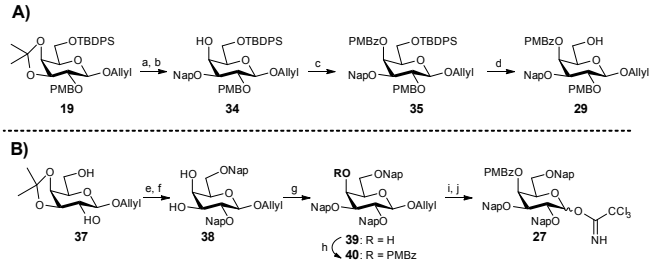
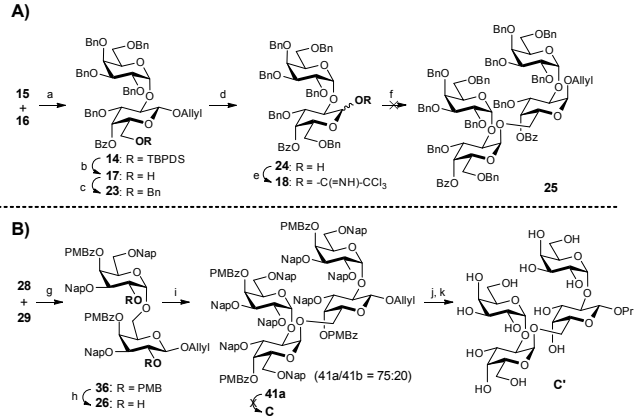
Next, the assembly of the tetra-α-galactoside was investigated according to the [2+2]-glycosylation (Scheme 4a). An initial glycosylation of **15** and **16** was carried out under optimized conditions using TfOH and NIS in ether at -11 ºC with 59% yield based on recovery starting material (brsm) as separable 1.6:1 mixture of α/β-anomers, (other tested conditions are summarized in Table 1, SI). α-Disaccharide **14** was used to obtain glycosyl acceptor **17** and imidate donor **18**.

The TBDPS group in **14** was removed in 69% yield using HF-pyridine in THF. A portion of alcohol **17** was kept in hand as future acceptor and the rest was benzylated at the C6 position to deliver **23** in 54% yield. The allyl group in **23** was removed via a two-step process. First, the allyl group was isomerized to the corresponding enol ether employing hydrogen and $[Ir(COD)(PMePh_2)_2]PF_6$ as catalyst. In a second step, acid hydrolysis of the enol ether with an aqueous solution of mercury(II) oxide and mercury(II) chloride in acetone/water delivered the hemiacetal **24** in 95% yield.[31] Formation of desired imidate **18** using trichloracetonitrile and DBU in DCM at 0 °C delivered only trace amounts of the product. Further increase of reaction temperature induced a fast rearrangement of the donor giving the trichloroacetamide as the main product. Several repetitions of the reaction were carried out to accumulate enough imidate donor **18** so that synthesis of tetrasaccharide **25** could be evaluated. Unfortunately, independent of the conditions used, formation of product **25** was not observed and the glycosyl imidate was hydrolyzed or rearranged into the corresponding amide. A possible explanation for the decreased reactivity of **18** in this glycosylation is the steric hindrance from the α-galactose attached at the C2-position and from the participation of the benzoyl group that blocks an alternative attack of the nucleophile from the equatorial side.



Scheme 3: A) Synthesis of galactose building block **28**: a) CSA, MeOH, 82%; b) *i.* Bu_2SnO, MeOH, reflux; *ii.* NapBr, TBAI, DMF, 60 °C, 88%; c) PMBz-Cl, pyr, 80 ºC, 73%; d) HF-Py, THF, 94%; B) Synthesis of galactose building block **27**: e) NapBr, NaH, DMF, 92%; f) CSA, MeOH, 91%; g) *i.* Bu_2SnO, MeOH; *ii.* NapBr, TBAI, DMF, 60 °C, 86%; h) PMBzCl, pyr., 80 °C, 70%; i) $PdCl_2$, MeOH, 83%; j) CCl_3CN, DBU, DCM, 77 %.

An alternative approach to the tetragalactoside structure was inspired by GPI biosynthesis, where Gal I and Gal II are glycosylated with additional galactose residues after the *T. brucei* GPI core structure is completely assembled.[21] Translating this observation into the synthesis of the desired tetragalactoside, we considered double galactosylation of disaccharide acceptor **26** using monosaccharide donor **27** to be a suitable strategy to reduce the steric hindrance coming from the α–(1-2)-galactosylation at the donor (Figure 3). Instead of using benzyl ethers as permanent protecting group, we considered a synthesis of the tetragalactose fragment **C** using 2-naphthylmethyl (Nap) ethers for permanent protection. Recently, we demonstrated the utility of Nap-protection for the synthesis of GPI derivatives and highlighted it's advantages over benzyl ethers in the synthesis of challenging targets. Notably, this switch in protecting group does not affect the reactivity of the building blocks but gives good selectivity and glycosylation yields in the formation of 1,2-cis products.[32]



Scheme 4. A) Attempted synthesis of tetragalactosylated structure **C** using a [2+2]-approach: a) NIS, TMSOTf, Et_2O, 59%; b) HF-Pyridine, THF, 69%; c) NaH, BnBr, DMF, 54%; d) *i.* H_2, $[Ir(COD)(PMePh_2)_2]PF_6$, THF; *ii.* HgO, $HgCl_2$, acetone, water, 5:1, 95%; e) CCl_3CN, DBU, DCM, 0 °C, <10%; f) **17**, TMSOTf, DCM, 0 °C, no reaction; B) Synthesis of tetragalactoside structure **C'** using a [2+1+1]-approach: g) TMSOTf, Et_2O, -10 °C, 90%; h) TFA:DCM, 1:10, 15 min., 85%; i) **27**, TMSOTf, Et_2O/DCM, 0 ºC, 73%; j) H_2, Pd/C, MeOH/EtOAc; k) NaOMe, DCM/MeOH, 33% over two steps.

The synthesis of **C** began by preparation of galactose building blocks **28** and **29** having a C4 benzoyl ester group for remote participation and an orthogonal PMB-protection in the C2 position (Scheme 2 and 3A). To obtain donor **28**, the TBDPS group of ketal **19** was exchanged for a Nap ether in a two-step procedure giving **30** in 89% yield. Hydrolysis of acetal **30** was achieved with CSA in MeOH. The resulting diol **31** was regioselectively protected at the C3 position using one-pot stannylene acetal formation, followed by reaction with Nap-Br to give the galactoside alcohol **32** in 84% yield. A following esterification of the C4 position performed with p-methoxybenzoyl chloride (PMBzCl) in pyridine at 80 ºC delivered fully protected galactose **33**. Removal of the anomeric allyl group with $PdCl_2$ in MeOH and installation of an trichloroacetimidate leaving group using trichloroacetonitrile and DBU in DCM gave desired donor **28** (Scheme 2).

To generate the acceptor **29**, the ketal **19**[30] was hydrolyzed with CSA in MeOH to obtain a diol that was regioselective protected at the C3 position with a Nap group in 88% yield using stannylene acetal and NapBr. Following, the C4 hydroxyl was protected with PMBzCl in pyridine at 80 ºC to obtain **35**, which was treated with HF-pyridine to remove the TBDPS group giving the acceptor **29** in 94% yield (Scheme 3A).

The assembly of the tetragalactoside was initiated with glycosylation of acceptor **28** with imidate **29** in ether at -10 ºC and TMSOTf as promotor to exclusively obtain disaccharide **36** as α-product in excellent yield of 90%. The two PMB groups of

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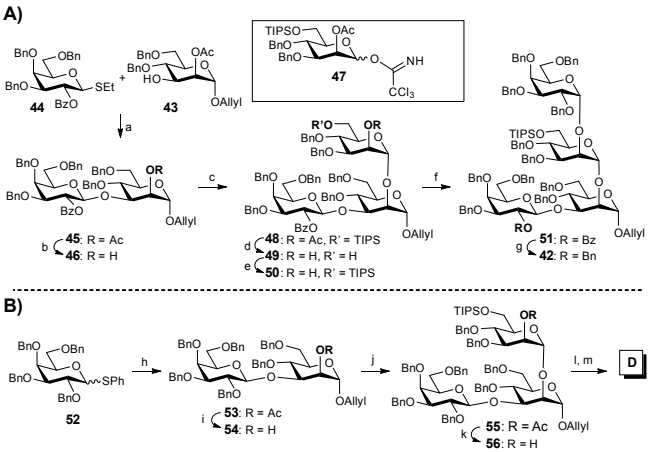
36 were removed with 10% TFA in DCM giving diol **26** in 85% yield (Scheme 4B).

Glycosyl donor **27**, required for a double glycosylation, was synthesized from **37**[33] in a five step protocol. First, the C2 and C6 positions of **37** were protected with a Nap group. Then, the resulting acetal was hydrolyzed with CSA in MeOH obtaining diol **38** in 82% yield. A regioselective napthylmethylation at C3 position in 86% yield and an esterification of the C4 position with PMBzCl gave fully protected building block **40**. Removal of anomeric allyl protection with $PdCl_2$ in MeOH and conversion of the obtained hemiacetal into an imidate delivered donor **27** (Scheme 3B).

Double glycosylation of acceptor **26** with donor **27** using TMSOTf, and diethyl ether/DCM as solvent resulted in formation of a mixture of four tetrasaccharides in 73% yield containing desired double α-product **41a** as major product and various isomers with β-linkages **41b**. The tetragalactoside was isolated by repetitive purification on silica gel column and a HPLC separation (Scheme 3B). Conversion of this tetragalactoside to a glycosyl donor suitable for glycosylation of Man I building block **4** proved challenging. Removal of the allyl group from **41a** could not be effected by the Ir-catalyzed isomerization and hydrolysis protocol or by the use of $PdCl_2$ or $Pd(OH)_2$ in methanol. These difficulties were also attributed to the steric hindrance due to the presence of α-galactosylation at the reducing end. Despite the presence of one β-linkage, deprotection of **41a** was sought to produce fragment **C'**. **C'** together with structures **A** and **B** may deliver information about the role of tetragalactoside in the GPI activity. Removal of the four ester from **41a** by saponification in a mixture of methanol and DCM failed despite the attempts at different temperature, concentration and nature of nucleophile. When naphthyl groups were removed by hydrogenolysis first, esters could be hydrolyzed by sodium methoxide in methanol. After purification by SEC, Tetrasaccharide **C'** was isolated in 33% yield over two steps (Scheme 4B).

A second unique structural motif of *T. brucei* GPI comprises galactosylation of Man II and Man III residues. To obtain compound **E**, the synthesis of tetrasaccharide **42** was pursued following a stepwise glycosylation strategy (Scheme 5A). The process started with the quantitative glycosylation of mannose **43**[34] using participating group bearing galactose building block **44**. To extend the resulting disaccharide, a deacetylation of **45** in the presence of benzoyl group using K_2CO_3 resulted in the saponification of both esters, while the use of a range of Zemplen conditions[35] led to mixtures of desired product **46** and the corresponding diol. In contrast, an acidic deacetylation using *in situ* generated anhydrous HCl gave desired alcohol **46** in good yield (84%). A [2+1] glycosylation reaction of **46** with 2-*O*-acetylated imidate **47**[23] using prolonged reaction times and consecutive addition of up to 5 eq. of donor delivered trisaccharide **48** in 95 % yield. Due to the acid lability of the TIPS ether, acceptor **50** for the following [3+1]-glycosylation was obtained in 85% yield over two steps by acidic deacetylation of **48** and reinstallation of TIPS. Diverse conditions were evaluated for the glycosylation of **50** with galactose donor **15** (Table 2, SI). Under optimized conditions, tetrasaccharide **51** was obtained in 10:1 α/β ratio at -11 °C in 88% yield (brsm). Finally, the remaining benzoyl ester was replaced by a benzyl ether in two steps to give desired tetrasaccharide **42** (Scheme 5A).



Scheme 5. A) Synthesis of Tetrasaccharide **42**: a) NIS, TMSOTf, 0 °C, DCM, quant.; b) AcCl, MeOH/DCM, 67%, c) 5 eq **47**, TMSOTf, 0 °C, DCM, 95%; d) AcCl, MeOH/DCM, 88%; e) TIPSCl, Imidazole, DMAP, 80 °C, 96%; f) **15**, NIS, TMSOTf, Et_2O, -11 °C, 88%, α,β-not separated; g) i. NaOMe, MeOH/DCM, 40 °C; ii. NaH, BnBr, DMF and $CCl_3C(=NH)$-OBn, TMSOTf, DMF, 44% over two steps; B) Synthesis of Trisaccharide **D:** h) **43**, NIS, TMSOTf, DCM, 0 °C, 66%; i) NaOMe, MeOH/DCM, 40 °C, 71%; j) 3 eq **47**, TMSOTf, DCM, 0 °C, 82%; k) two cycles: NaOMe, MeOH/DCM, quant; l) $Sc(OTf)_3$, ACN/DCM, H_2O, reflux; m) H_2, Pd/C, MeOH/EtOAc, 49% over two steps

Despite ensuring β-selectivity in the first glycosylation, the presence of benzoyl group in **44** added additional steps in the synthesis of **42.** Purification in the synthesis of structures **51** and **42** was problematic due to similar mobility of starting compounds and products of both steps during silica gel chromatography. Considering the relevance of this fragment for the total synthesis of GPI **1** or of larger galactosylated GPI fragments, an alternative synthesis of **42** was evaluated using thioglycoside donor **52** (SI) for the glycosylation of mannoside **43**. Due to the lack of a participating group, the control of the reaction was exerted by using low temperature and acetonitrile as a solvent, which are conditions reported to favor the formation of the β-products.[36] The reaction was performed at 0°C in CH_2Cl_2 and acetonitrile and delivered desired β-disaccharide **53** in 66% isolated yield. Following, deacetylation with NaOMe in a mixture of MeOH and DCM at 40 °C, acceptor **54** was obtained in 71% yield, which was glycosylated by multiple additions of imidate donor **47**. The poor reactivity of acceptor disaccharide **54** was comparable to the results observed for the stereo controlled strategy employing **46** (Scheme 5A). Deacetylation of trisaccharide **55** using NaOMe in Methanol and DCM yielded **56** in quantitative yield over two reaction cycles.

The β-galactose attached to the Man II residue in tetrasaccharide **42** is the only β-galactosylation found in *T. brucei* GPIs. To evaluate the influence of this galactosylation on biological activity, the assembled trisaccharide precursor **56** was deprotected in two steps by removal of TIPS with scandium triflate and hydrogenolysis. After SEC, the trisaccharide **D** was isolated in 49% yield over two steps (Scheme 5B).

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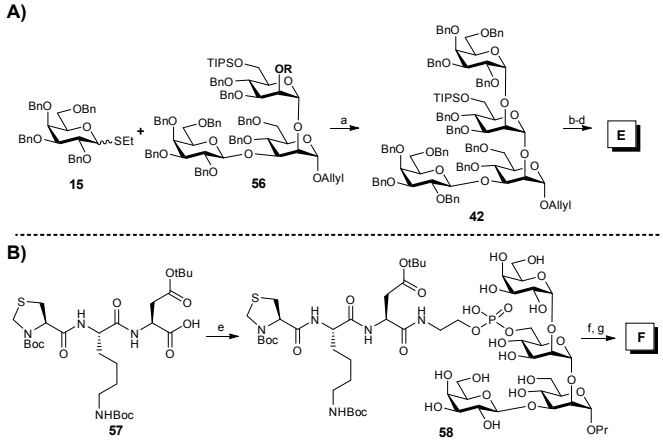
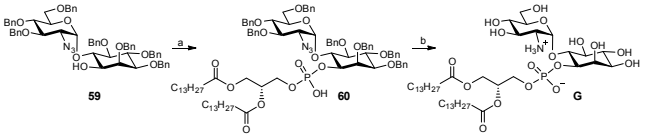


Scheme 6. A) Synthesis of Tetrasaccharide **E:** a) NIS, TfOH, Et_2O, -11°C, 60%; b) $Sc(OTf)_3$, H_2O, DCM, ACN, reflux, 44%; c) i. **7**, PivCl, pyr; ii. I_2, H_2O, pyr, 67% d) H_2, Pd/C, MeOH/EtOAc, 62%; B) Fragment condensation strategy to CKD-GPI-Fragment **F**: e) **58**, DIPEA, PyBOP, DMF, DMSO, 71%; f) 90:5:5, TFA, anisole, TIPS; g) NH_2-OMe-HCl; TCEP, guanidine, Na_2HPO_4

In a next step towards **E**, trisaccharide **56** was glycosylated with thiophenyl donor **15** giving desired tetrasaccharide **42** in 60% isolated yield. The TIPS group of **42** was removed with scandium triflate in a 3:1 mixture of ACN and DCM with 100 µL of water.[37] The intermediate alcohol was directly used for phosphitylation with H-phosphonate **7** using pivaloyl chloride in pyridine.[38] Oxidation of the phosphonate diester by iodine and a 9:1 mixture of pyridine and water at 0 °C gave the corresponding phosphorylated tetrasaccharide which was deprotected in a third step using Pd/C and H_2 in a mixture of MeOH and EtOAc. Hydrogenolysis was performed under elevated pressure (1.5 bar) for 6 h followed by reaction at atmospheric pressure for 40 h. Purification on a Sephadex and a Hypercarb column gave phosphorylated tetrasaccharide **E** in 62% yield (Scheme 6A).

To study the interphase epitope formed by the C-terminal residues of *T. brucei* VSGs and the GPI-glycans, a tripeptide was conjugated to GPI fragment **E** (Scheme 6B). Tripeptide H_2N-Cys-Lys-Asp-H **57** was synthesized on a Trityl-OH ChemMatrix® with cysteine being protected as a thioproline, raising the possibility of peptide elongation using native chemical ligation.[39] After cleavage and purification, peptide **57** was coupled to tetrasaccharide **E** in a fragment condensation mediated by DIPEA and PyBOP giving **58** in 71% yield. Deprotection over two steps using a 90:5:5 mixture of TFA, anisole and TIPS and methoxyamine delivered CKD-GPI-fragment **F**.

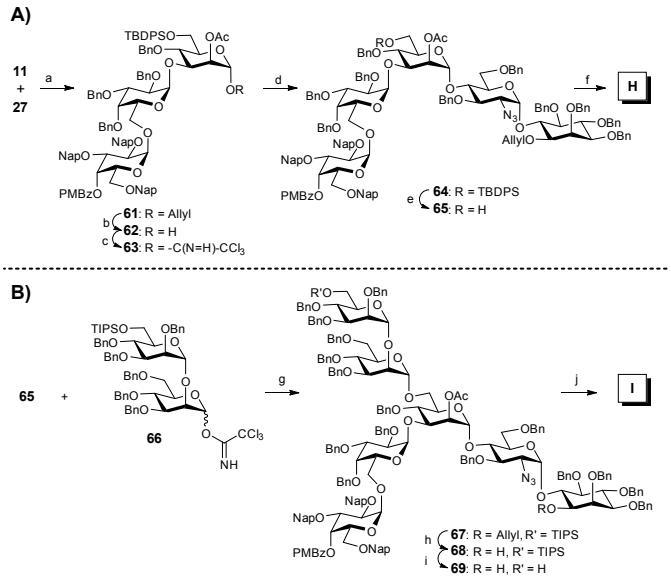
Macrophage activation has also been attributed to specifically lipidated glycan structures of *T. brucei* GPIs.[15] This effect could be a consequence of specific binding to the appropriately lipidated GPI or a consequence of aggregation of multiple GPI molecules in the membrane.[40] To address this hypothesis, lipidated fragment **G** was pursued. the conserved pseudodisaccharide **59** was phosphytilated with H-phosphonate **8**[17] in presence of pivaloyl chloride. The intermediate phosphonate was oxidized with iodine in wet pyridine to give **60** in 74% yield. Subsequent removal of the protecting groups by hydrogenolysis and purification by SEC gave **G** containing a dimirystoylglycerol lipid moiety (Scheme 7).



Scheme 7: Synthesis of phospholipidated structure **G**: a) *i.* **8,** PivCl, pyr; *ii.* I_2, 0ºC, 9:1 pyr: H_2O; 74%; b) H_2, Pd/C, MeOH/EtOAc; 59%.

To be able to compare the implicated activity of **G** against the complete phosphorylated GPI structure **J**[17], two structures containing the conserved *T. brucei* digalactoside branch attached to either a pseudotrisaccharide or a pseudopentasaccharide backbone but lacking both phosphorylations were synthesized (Scheme 8).

The synthesis of pseudopentasaccharide **H** was initiated by glycosylation of disaccharide **11** with 4-*O*-benzoylated donor **27**. Using DCM and ether as solvents and TMSOTf as a promotor at 0 ºC resulted in an inseparable mixture of product **61** and hydrolyzed donor. The allyl group was removed using the isomerization and hydrolysis sequence to give pure hemiacetal **62** in 75% after isolation over three steps. A quantitative installation of imidate leaving group completed the synthesis of trisaccharide donor **63** (Scheme 8A).



Scheme 8. A) Synthesis of pseudopentasaccharide **H**: a) TMSOTf,DCM/Ether, 0ºC; b) *i.* H_2, $[Ir(COD)(PMePh_2)_2]PF_6$, THF; *ii.* HgO, $HgCl_2$, acetone, water, 5:1, 75% over two steps; c) CCl_3CN, DBU, DCM, 0 ºC, quant.; d) **6**, TMSOTf, DCM, 0 °C, 28%; e) HF-Pyridine, THF, 53%; f) NH_3(*liq.*), Na, MeOH, -78 °C, 98%; B) Synthesis of pseudoheptasaccharide **I**: g) TBSOTf, thiophene:toluene 2:1, rt, 61%; h) *i.* H_2, $[Ir(COD)(PMePh_2)_2]PF_6$, THF; *ii.* HgO, $HgCl_2$, acetone, water, 5:1, 82%; i) $Sc(OTf)_3$, ACN/DCM, H_2O, reflux; j) $NH_{3(l)}$, Na, MeOH, -78 °C, 44% over two steps.

Glycosylation of pseudodisaccharide **6**[23] with 2-*O*-acetylated trisaccharide imidate **63** using TMSOTf in DCM at 0 °C, gave **64** in 28% yield. The yield can be explained with isolation of hydrolyzed donor **62** along with inseparable acceptor **6**. A removal of TBDPS with HF-pyridine in THF provided alcohol **65** in 53% yield after four days. Removal of all protecting groups from **65** under birch conditions[41] delivered pseudopentasaccharide **H** in excellent yield.

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To complete the synthesis of the library and to generate pseudoheptasaccharide **I**, glycosylation of **65** with dimannoside donor **66**[23] was performed using TBSOTf in a 2:1 mixture of thiophene and toluene at room temperature. Earlier reports of α-selective glycosylation exerted by Lewis basic solvents, such as dioxane[17] and thiophene,[42] showed reliable results and were adapted and used for this specific glycosylation step. This protocol delivered pseudoheptasaccharide **67** in 61% yield. To deprotect the structure, allyl was removed via the isomerization and hydrolysis protocol in 82% yield. Finally, removal of TIPS with scandium triflate and treatment under Birch conditions delivered pseudoheptasaccharide **I** in 44% yield over two steps and after purification by SEC (Scheme 8B).

Conclusions

The physical properties together with the size and complexity of glycosylphosphatidylinositols hamper investigation of their biological activity. Despite significant contributions in synthesis of these glycolipids, comprehensive libraries designed to investigate the role of GPI core modifications in biological activity are still rare. We have established the process to synthesize a set of galactosylated GPI fragments (**A**-**I**) related to the complex GPIs that anchor VSGs 221 and 117 in the membrane of the extracellular parasite *T. brucei*. For the synthesis of these GPI fragments, we developed a series of building blocks (monosaccharides **16**, **27**, **28**, **29**, and **19**; disaccharides **14** and **26**) and used them in combination with other reported building blocks to obtain GPI structures covering the individual relevant epitopes.

In addition to generating structures comprised of the conserved core structure of *T. brucei* GPIs, different series of homologous galactosylated fragments having α- and β-galactosides were generated. A milestone in this process, was the assembly of the synthetically challenging tetragalactoside fragment **C'**, which, was successfully prepared using a [2+1+1]-glycosylation strategy that relies on remote participation of C4-PMBz group and relying on Nap ethers as permanent protecting groups. A second unique structure of *T. brucei* GPIs, tetrasaccharide **42** which contains both α- and β-galactosyl substituents, was synthesized by two strategies, with and without protecting group participation. Further coupling of **42** and appropriate tripeptide resulted in fragment **F**, a mimic of GPI anchored VSG structures. The structures **G**-**I** including a lipidated pseudodisaccharide and a pure glycan structure completed the library of fragments, which together with the VSG117 GPI **J** constitute a valuable tool for evaluation of the immunomodulatory effects of *T. brucei* GPI modifications.

Currently, the fragments are being used to perform diverse biological experiments. Specifically, structures **A**-**J** are investigated in *in vitro* studies with murine macrophages and human monocytes. Here, the release of cytokines, specifically TNF-α, during *T. brucei* infections is evaluated and quantified in a dose dependent manner with and without IFN-γ priming. Further, an analogue set of linker modified structures (synthesis not shown), is used to perform binding experiments of antibodies with the immobilized structures supported on microarrays. This will give detailed information about implications of GPI-galactosylation in activation and modulation of the host immune system through formation of anti-GPI antibodies.

Experimental Section

General method for global deprotection by hydrogenolysis: The compound was dissolved in methanol and few drops of ethyl acetate. 10 mg palladium on carbon was added and the suspension was stirred in hydrogen atmosphere. Hydrogen was replaced with argon and the suspension was filtered. The filtrate was removed *in vacuo* and the residue purified by size exclusion chromatography. Collected fractions were lyophilized and characterized.

Propyl D-galactopyranosyl-α-(1→3)-α-D-mannopyranoside (**A**)**:** Disaccharide **11** (10 mg, 0.011mmol) was dissolved in methanol and DCM and 0.1 mL acetyl chloride was added. and the reaction was stirred at room temperature until TLC indicated full conversion. The reaction was quenched with saturated $NaHCO_3$-solution, extracted three times with DCM, dried with $MgSO_4$, filtered and reduced *in vacuo*. The residue was purified by silica gel chromatography using an eluent of hexane and ethyl acetate. The obtained product was submitted to hydrogenolysis to give disaccharide **A** as white solid in quantitative yield (0.020 mmol, 7.800 mg) after purification on a Sephadex G15 column using 5% EtOH in H_2O. ^{1}H-NMR (400 MHz, D_2O): δ = 5.23 (d, *J* = 4.1 Hz, 1H, Gal-1), 4.82 (s, 1H, Man-1), 4.11 (s, 1H), 4.04 (s, 1H), 3.96 (s, 1H), 3.92 – 3.59 (m, 9H), 3.51 – 3.45 (m, 1H), 1.59 (q, *J* = 7.8 Hz, 2H), 1.18 – 1.12 (m, 1H), 0.89 (td, *J* = 8.0, 7.1, 2.5 Hz, 3H) ppm; ^{13}C-NMR (101 MHz, D_2O): δ = 100.5 (Gal-1), 99.3 (Man-1), 78.4, 72.6, 71.3 69.7, 69.4, 69.1, 68.6, 66.0, 61.2, 60.7, 57.3, 21.8, 16.6, 9.8 ppm; ESI-MS: m/z M_{calcd} for $C_{15}H_{28}O_{11}$ = 384.1632; M_{found} = 407.1536 $[M+Na]^+$.

Propyl (D-galactopyranosyl-α-(1→6)-D-galactopyranosyl-α-(1→3)-α-D-mannopyranoside (**B**): Trisaccharide **13** (7.180 µmol, 0.011 g) was dissolved in methanol and few drops of DCM. 0.1 mL acetyl chloride was added and the reaction was stirred at room temperature until TLC indicated full conversion. The reaction was quenched with saturated $NaHCO_3$-solution, extracted three times with DCM, dried with $MgSO_4$, filtered and reduced *in vacuo*. The residue was purified by silica gel chromatography using an eluent of hexane and ethyl acetate. The triol was obtained in quantitative yield (7.180 µmol, 8.440 mg) as colorless oil and was direclty submitted to hydrogenolysis. The final product was purified on a Sephadex G15 column using 5% EtOH in H_2O as eluent giving **B** in 78% yield (5.860 µmol, 3.200 mg) as white solid. ^{1}H-NMR (400 MHz, D_2O): δ = 5.14 (s, 1H, Man-1), 4.92 (s, 2H, Gal-1, Gal'-1), 4.32 – 4.21 (m, 2H), 4.02 – 3.34 (m, 31H), 3.17 (s, 2H), 1.25 (s, 5H), 1.15 (s, 3H), 0.90 (s, 4H) ppm; ^{13}C-NMR from HSQC (101 MHz, D_2O): δ = 102.1 (Gal'-1), 99.8 (Man-1), 99.1 (Gal-1), 81.0, 71.2, 70.1, 69.9, 69.8, 69.7, 69.3, 68.8, 68.6, 68.3, 68.2, 67.9, 67.9, 66.9, 22.5, 10.3, 8.5 ppm; ESI-MS: m/z M_{calcd} for $C_{21}H_{38}O_{16}$ = 546.2160; M_{found} = 569.2067 $[M+Na]^+$.

Propyl (D-galactopyranosyl-α-(1→2)-(-(D-galactopyranosyl-α-(1→2)-D-galactopyranosyl-α-(1→6)-β-D-galactopyranoside (**C'**): Tetrasaccharide **41a** (4.250 µmol, 10.0 mg) was debenzylated by hydrogenolysis under standard conditions. The resulting product was dissolved in a 1:1 mixture of chloroform and methanol and 0.1 ml of freshly prepared 1M NaOMe solution in methanol was added. The reaction mixture was stirred at 40°C for 16 hours. The reaction was quenched with Amberlite H^+ resin

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and filtered. The solvents were removed and the residue was purified using a Sephadex G15 column (5% EtOH in H_2O). The tetrasaccharide **C'** was obtained in 33% yield (1.411 µmol, 1.000 mg) as white solid over two steps. ^{1}H-NMR (400 MHz, D_2O): δ = 5.26 (d, *J* = 4.0 Hz, 1H, Gal'-1), 5.09 (d, *J* = 2.5 Hz, 1H, Gal^t-1), 4.99 (d, *J* = 3.9 Hz, 1H, Gal^t-1), 4.40 (d, *J* = 7.8 Hz, 1H, Gal-1), 4.11 (t, *J* = 6.4 Hz, 1H), 3.99 (t, *J* = 6.3 Hz, 1H), 3.88 – 3.45 (m, 22H), 1.49 (h, *J* = 7.2 Hz, 2H), 1.02 (t, *J* = 7.1 Hz, 1H), 0.77 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C-NMR (101 MHz, D_2O): δ = 103.2 (Gal-1), 97.7 (Gal'-1), 95.6 (Gal^t-1), 95.4 (Gal^t-1), 74.8, 72.8, 72.6, 72.6, 72.5, 71.9, 71.4, 70.9, 70.8, 70.6, 70.5, 70.4, 69.2, 69.1, 69.0, 69.0, 68.8, 68.1, 68.1, 67.8, 67.8, 66.2, 61.0, 60.8, 57.3, 22.4, 16.6, 9.8 ppm; ESI-MS: m/z M_{calcd} for $C_{27}H_{48}O_{21}$ = 708.2688; M_{found} = 731.2437 $[M+Na]^+$.

Propyl (D-galactopyranosyl-β-(1→3)-(D-mannopyranosyl-α-(1→2)-α-D-mannoypyranoside (**D**): Trisaccharide **56** (0.016 mmol, 22.7 mg) was dissolved in a 5:2 mixture of ACN and DCM. Scandium triflate (0.032 mmol, 15.7 mg) and 100 µl water were added and the reaction mixture was refluxed for three hours. The solvents were removed *in vacuo* and the residue was dissolved in DCM, washed with saturated $NaHCO_3$-solution, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dried under high vacuum and directly deprotected by hydrogenolysis. The product was purified using Sephadex G15 column using 5% EtOH in H_2O. The trisaccharide **D** was obtained in 49% yield (7.690 µmol, 4.200 mg) as white solid over two steps. ^{1}H-NMR (400 MHz, D_2O): δ = 5.01 (d, *J* = 1.7 Hz, 1H, Man-1), 4.95 (d, *J* = 1.7 Hz, 1H, Man'-1), 4.33 (d, *J* = 7.7 Hz, 1H, Gal-1), 4.15 – 4.12 (m, 1H), 3.86 – 3.33 (m, 22H), 1.51 – 1.44 (m, 2H), 0.78 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C-NMR (101 MHz, D_2O): δ = 102.0 (Gal-1), 100.3 (Man-1), 97.9 (Man'-1), 78.8, 76.9, 75.1, 73.1, 72.6, 72.5, 72.3, 70.4, 70.3, 70.3, 70.2, 70.1, 69.5, 69.5, 68.5, 66.9, 61.1, 60.8, 60.5, 60.4, 60.4, 21.8, 9.8 ppm; ESI-MS: m/z M_{calcd} for $C_{21}H_{38}O_{16}$ = 546.2160; M_{found} = 569.1926 $[M+Na]^+$.

Propyl α-D-galactopyranosyl-(1 → 2)-6-O-(2-amino-ethyl-phosphatidyl)-β-D-mannopyranosyl-(1 → 2)-3-O-(β-D-galactopy-ranosyl)-α-D-mannoypyranoside (**E**): In a round bottom flask equipped with an air condenser, tetrasaccharide **42** (30.0 µmol, 58.4 mg) was dissolved in a 5:2 mixture of ACN and DCM. 10 µL water and scandium triflate (60 µmol, 29.5 mg) were added and the reaction was stirred at 45°C until TLC indicated full conversion. The reaction was quenched by adding sat. $NaHCO_3$ solution. The resulting mixture was extracted five times with DCM and the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica column chromatography using hexane and ethyl acetate as eluent. In a second step, H-phosphonate **7** (14.54 µmol, 5.2 mg) and the isolated intermediate (13.22 µmol, 23.6 mg) were co-evaporated three times with pyridine. After drying the compound mixture under high vacuum, it was dissolved in pyridine. Pivaloylchloride (19.83 µmol, 2.4 µl) were added and the reaction was stirred at room temperature until TLC indicated full conversion. Iodine (19.83 µmol, were added to a 9:1 mixture of pyridine and water. The solution was added to the reaction mixture and stirred at 0°C. When the TLC indicated full conversion, the reaction was quenched by adding sat. $Na_2S_2O_3$ solution. The mixture was extracted three times with DCM and the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica column chromatography using DCM and methanol as eluent. The intermediate Phosphate (4.890 µmol, 10.0 mg) was suspended in methanol. A few drops of ethyl acetate were added to dissolve the compound. Pd/C (0.49 µmol, 0.5 µg) were added and the reaction was placed in a hydrogenation chamber. The reaction was stirred for one day at room temperature. The reaction was filtered and concentrated under reduced pressure. The residue was purified by size exclusion using 5% ethanol in water as eluent. Product **E** was obtained in 62% yield (3.010 µmol, 2.500 mg) as white solid. ^{1}H-NMR (400 MHz, D_2O): δ = 5.30 (d, *J* = 1.9 Hz, 1H, Galα-1), 5.01 (d, *J* = 3.8 Hz, 1H, Man-1), 4.95 (d, *J* = 1.6 Hz, 1H, Man'-1), 4.37 (d, *J* = 7.8 Hz, 1H, Galβ-1), 4.03 – 3.95 (m, 8H), 3.87 – 3.38 (m, 21H), 3.15 (ddd, *J* = 6.2 Hz, 3.6 Hz, 1.0 Hz, 2H), 1.48 (h, *J* = 7.2 Hz, 2H), 1.16 – 1.00 (m, 1H), 0.78 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C-NMR (101 MHz, D_2O): δ = 101.1 (Man'-1), 100.5 (Galα-1), 100.5 (Galβ-1), 98.0 (Man-1), 79.7, 79.6, 77.3, 77.3, 77.3, 76.4, 76.3, 75.2, 72.9, 72.6, 72.0, 71.9, 71.4, 71.3, 70.6, 70.5, 70.0, 69.7, 69.6, 69.2, 69.1, 68.7, 68.6, 66.4, 65.3, 65.2, 61.7, 61.6, 61.5, 61.5, 61.4, 61.0, 60.9, 60.7, 40.0, 39.9, 21.9, 9.8 ppm; ^{31}P-NMR (162 MHz, D_2O): δ = 0.25 ppm; ESI-MS: m/z M_{calcd} for $C_{29}H_{54}NO_{24}P$ = 831.2773; M_{found} = 830.2719 $[M]^-$.

Propyl α-D-galactopyranosyl-(1 → 2)-6-O-(2-(N-(N-(N-L-cysteinyl)-L-lysinyl)-L-aspartatyl)-aminoethyl-phosphatidyl)-α-D-mannopyranosyl-(1 → 2)-3-O-(β-D-galactopyranosyl)-α-D-mannopyranoside (**F**): Tripeptide conjugate **58** (1.10 µmol, 1.6 mg) was dissolved in a deprotection mixture of TFA (5.84 mmol, 450 µL), TIPS (1.10 µmol, 25 µL) and Anisole (0.229 mmol, 25 µL). After stirring for 30 minutes at room temperature, the mixture was diluted with toluene, concentrated *in vacuo* and dried under high vacuum. 25 mL buffer containing TCEP (0.125 mmol, 0.031 g), methoxyamine hydrochloride (5.0 mmol, 418.0 mg), guanidine hydrochloride (150 mmol, 14.33 g), disodium monophosphate (2.5 mmol, 355.0 mg) with pH 4 was added and the mixture was stirred over night. After lyophilization, the residue was purified by size exclusion chromatography (sephadex G25) using 5% EtOH in water as eluent. **F** was isolated as white solid after performing RP-HPLC (YMC hydrosphere RP C18 [250x10 mm; 5 µm] 5% ACN to 35% ACN in H_2O (0.1% TFA) in 35 min), eluting with the injection peak. MALDI-TOF: m/z M_{calcd} for $C_{42}H_{76}N_5O_{29}PS$ = 1177.4084; M_{found} = 1176.969 $[M]^-$

1-O-(2,3-O-Di-myristoyl-sn-glyceryl)-phosphatidyl (2-azido-2-deoxy-D-glucopyranosyl-α-(1 → 6)-myo-inositol (**G**): The phosphorylated pseudodisaccharide **60** (18.0 mg, 0.011 mmol) *was* submitted to hydrogenolysis to give glycolipid **G** in 59% yield (8.0 µmol, 5.9 mg) as white solid after G15 SEC (3:3:1, $CHCl_3$, MeOH, H_2O, 0.5% NEt_3) and hydrogenolysis. ^{1}H-NMR (400 MHz, $CDCl_3$, MeOD, D_2O): δ = 5.24 – 4.92 (m, 1H), 3.91 – 2.82 (m, 17H), 2.12 – 1.85 (m, 4H), 1.30 (s, 4H), 1.02 – 0.91 (m, 40H), 0.60 (s, 6H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 172.8 (C=O), 172.7 (C=O), 95.1 (GlcN-1), 76.2, 75.6, 73.9, 72.7, 72.2, 70.6, 69.9, 69.4, 64.4, 53.8, 33.7, 31.5, 29.2, 29.0, 28.9, 28.7, 24.5, 24.5, 22.4, 13.5 ppm; ^{31}P-NMR (162 MHz, $CDCl_3$): δ = 4.26, 3.91 ppm; ESI-MS: m/z M_{calcd} for $C_{43}H_{82}NO_{17}P$ = 915.5320; M_{found} = 914.5486 $[M]^-$.

α-D-galactopyranosyl-(1 →6)-α-D-galactopyranosyl-(1 →3)-α-D-mannopyranosyl-(1 →4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1 →6)-1-O-propyl-myo-inositol (**H**):

Approximately 10 ml ammonia were condensed in a flask and methanol (2 drops) was added. Sodium was added in small pieces until a dark blue color established. Pseudopentasaccharide **65** (1.797 µmol, 5.410 mg) was dissolved in THF and added to the ammonium solution at -78°C. At this temperature the reaction was stirred for 1 h. The reaction was quenched by adding methanol and ammonia was blown off using a stream of nitrogen. The pH of the resulting solution was adjusted to 7-8 using glacial acetic acid. The reaction was concentrated under reduced pressure and the residue was purified by size exclusion using 5% ethanol in water as eluent. A final HPLC purification on a hypercarb column (150×10mm, ThermoFisher, 5 µ) using a 0-100% gradient of acetonitrile in

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water in 60 min delivered product **H** in 98% yield (1.758 µmol, 1.800 mg) over two steps as white solid. ^{1}H-NMR (400 MHz, D_2O): δ = 5.39 (d, *J* = 1.8 Hz, 1H, GlcN-1), 5.04 (d, *J* = 3.5 Hz, 1H, Gal-1), 4.92 (d, *J* = 1.4 Hz, 1H, Gal'-1), 4.39 (d, *J* = 7.6 Hz, 1H, Man-1), 4.05 – 3.33 (m, 38H), 1.55 – 1.47 (m, 4H, -CH_2-), 1.18 – 1.12 (m, 4H, -CH_2-) ppm; 13C-NMR from HSQC: (101 MHz, D2O, coupled): δ = 101.1, 100.8, 99.9, 98.2, 80.0. 77.7, 75.2, 75.1, 73.6, 73.4, 72.8, 71.3, 70.7, 70.1, 69.6, 69.5, 69.1, 69.0,68.7, 67.3, 66.9, 65.4,62.9, 61.5, 60.9, 60.8, 22.1, 17.33, 9.9, ESI-MS: m/z M_{calcd} for $C_{33}H_{59}NO_{25}$ = 869.3376; M_{found} = 887.3883 $[M+NH_4]^+$

D-mannopyranosyl-α-(1 → 2)-D-mannopyranosyl-α-(1 → 6)-3-O-(D-galactopyranosyl-α-(1 → 6)-D-galactopyranosyl-α-)-D-mannopyranosyl-α-(1 → 4)-2-amino-2-deoxy-D-glucopyranosyl-α-(1-6)-myo-inositol (**I**): Alcohol **69** (2.372 µmol, 8.0 mg) was dissolved in a 5:2 mixture of ACN and DCM. Scandium triflate (4.744 µmol, 2.3 mg) and 100 µl water were added and the mixture was refluxed for three hours. The solvent was removed *in vacuo*, the residue dissolved in DCM, washed with saturated $NaHCO_3$-solution, dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography using an eluent of hexane and ethyl acetate and diretly used for the next step. R_f = 0.4 (2:1; Hex/EtoAc). Approximately 10 mL of ammonia were condensed in a flask and methanol (2 drops) was added. Sodium was added in small pieces until a dark blue color established. The diol was dissolved in THF and added to the ammonium solution at -78°C. At this temperature the reaction was stirred for 1 h. The reaction was quenched by adding methanol and ammonia was blown off using a stream of nitrogen. The pH of the resulting solution was adjusted with glacial acetic acid to 7-8. The reaction was concentrated under reduced pressure and the residue was purified on a Sephadex G 15 column using 5% ethanol in water as eluent. After lyophilization, product **I** was obtained in 44% yield (1.042 µmol, 1.200 mg) as white solid over two steps. ^{1}H-NMR (400 MHz, D_2O): δ = 5.26 (s, 1H, GlcN-1), 5.10 (d, *J* = 3.7 Hz, 1H, Gal-1), 5.06 (d, *J* = 4.0 Hz, 1H, Gal'-1), 5.04 (d, *J* = 1.6 Hz, 1H, Man-1), 4.92 (d, *J* = 1.8 Hz, 1H, Man'-1), 4.86 (d, *J* = 3.6 Hz, 1H, Man''-1), 4.29 – 4.25 (m, 1H), 4.22 (d, *J* = 2.8 Hz, 1H), 4.01 – 3.44 (m, 37H), 3.41 (dd, *J* = 10.0 Hz, 2.9 Hz, 1H), 3.26 (t, *J* = 9.1 Hz, 1H), 2.68 (dd, *J* = 10.2 Hz, 3.7 Hz, 1H) ppm; ^{13}C-NMR (151 MHz, D_2O): δ = 102.2 (Man'-1), 101.5 (Gal'-1), 101.0 (GlcN-1), 99.8 (Gal-1), 98.7 (Man''-1), 98.1 (Man-1), 80.8, 80.7, 78.6, 78.4, 78.4, 76.3, 74.2, 73.7, 73.1, 72.9, 72.6, 72.4, 72.4, 71.4, 70.9, 70.9, 70.7, 70.2, 70.2, 69.9, 69.7, 69.7, 69.5, 69.3, 69.2, 69.1, 68.7, 68.1, 66.8, 66.7, 61.0 ppm; ESI-MS: m/z M_{calcd} for $C_{42}H_{73}NO_{35}$ = 1151.3963; M_{found} = 1152.4642 $[M]^+$.

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