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FULL PAPER

Synthesis of asparagine derivatives harboring a Lewis X type DC-SIGN ligand and evaluation of their impact on immunomodulation in multiple sclerosis

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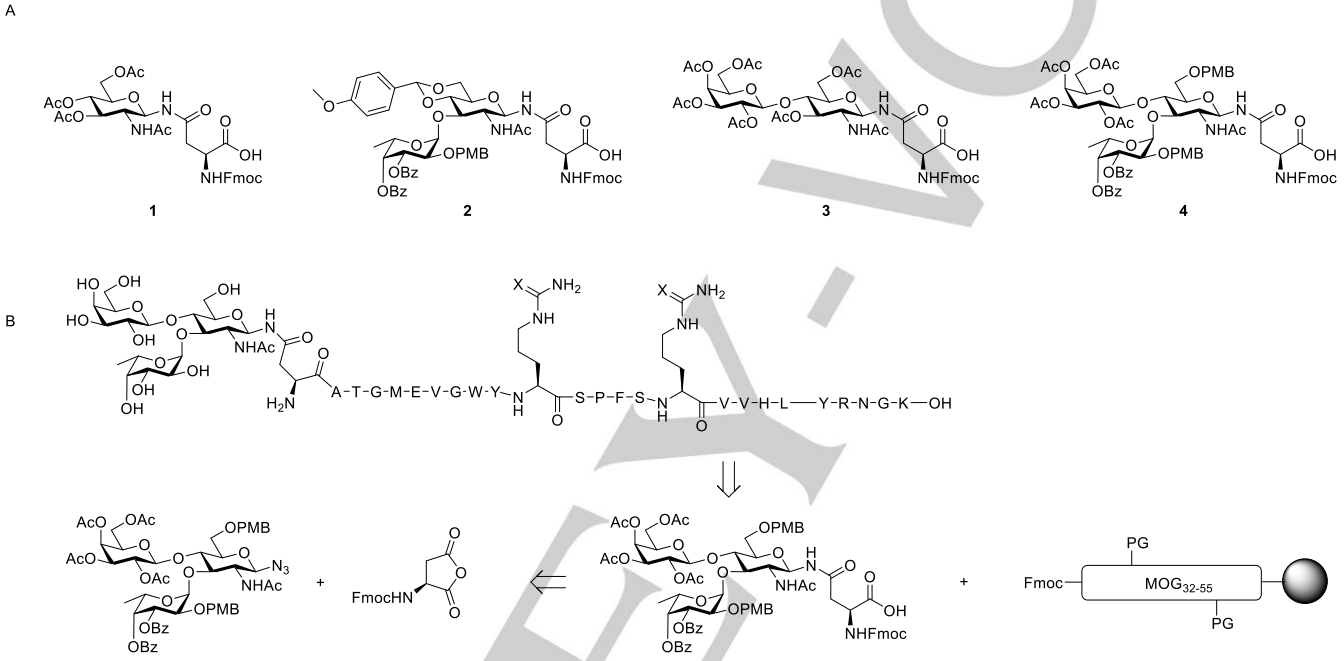
Abstract: The protein myelin oligodendrocyte glycoprotein (MOG) is a key component of myelin and an autoantigen in the disease multiple sclerosis (MS). The posttranslational *N*-glycosylation of Asn_{31} of MOG seems to play a key role in modulating the immune response towards myelin. This is mediated by the interaction of Lewis type glycan structures on the *N*-glycan of MOG to the DC-SIGN receptor on dendritic cells (DCs). Here, we report the synthesis of an unnatural Lewis X (Le^X) containing Fmoc-SPPS compatible asparagine building block, as well as asparagine building blocks containing two Le^X derived oligosaccharides: LacNAc and Fucα1-3GlcNAc. These building blocks were utilized for the synthesis of glycosylated MOG (MOG_{31-55}) and were analyzed with respect to their ability to bind to DC-SIGN in different biological setups, as well as their ability to inhibit the citrullination induced aggregation of MOG_{31-55}. Finally, a cytokine secretion assay was carried out on human moDCs, showing the ability of a neoglycopeptide decorated with a single Le^X to alter the balance of pro- and antiinflammatory cytokines, inducing a tolerogenic response.

Introduction

Multiple Sclerosis (MS) is a group of auto-immune neurodegenerative diseases, characterized by the formation of lesions in the patient's brain that lead to loss of functions.[1] The pathology of MS is not fully understood, but degradation of myelin sheath seems to be a critical step in the process.[2] Myelin sheaths are comprised of myelin, an insulating substance consisting of lipids, proteins and other molecules, and are responsible for fast information transfer through axons.[3] Indeed, some proteinogenic components of myelin sheath have been shown to become antigenic upon their degradation.[4] For example, myelin oligodendrocyte glycoprotein (MOG), an exclusively CNS-resident protein found on the surface of oligodendrocytes and myelin sheaths, acts as an autoantigen in an MS-like animal model, experimental autoimmune encephalomyelitis (EAE).[5]
MOG is a glycoprotein, decorated with an *N*-glycan[6] on Asn_{31}, with a molecular mass of 26-28 kDa.[7,8] It comprises 245 amino acids (AA) and belongs to the immunoglobulin superfamily (Ig). Over the last few decades, it has been shown that antibodies against MOG are circulating in the bloodstream of patients suffering from various demyelinating diseases such as MS and *N*-methyl-D-aspartate receptor-encephalitis[9], and that a peptide fragment comprising AAs 35-55, MOG_{35-55}, is a key T-cell epitope in EAE.[10,11]
We have recently discovered a potential reason for the pathogenicity of this MOG_{35-55}-peptide in EAE: after post-translational citrullination (deimination of guanidine on arginine), the peptide can form amyloid-like aggregates intracellularly, where they appear to be cytotoxic.[12,13] Citrullination of myelin proteins is considered to be critical in MS. For example, another antigenic myelin protein, myelin basic protein (MBP), has been shown to have increased citrullination in myelin samples from MS patients.[14] Together, these advances led to the hypothesis that post-translational citrullination of MOG could be in part responsible for the disease pathogenesis in EAE to be shifted towards neurodegeneration rather than autoimmunity.
In light of the above findings, we wanted to explore whether the native *N*-glycan at position 31 has an effect on the aggregation behavior of the citrullinated peptide, as *O*-glycosylation of serine or threonine residues has previously been shown to be inhibitory on the aggregation of a tau derived peptide, a highly aggregation-prone protein family involved in Alzheimer's disease.[15] The introduction of *N*-glycans and mimics thereof on peptides derived from prion protein[16] and the full-length prion protein[17] itself, has also been shown to decrease or even abrogate aggregation.
Furthermore, previous studies on the glycosylation of MOG suggest that the nature of the carbohydrate structures of the *N*-glycan plays an important role in the modulation of immunological tolerance through glycan interaction with the dendritic cell-specific intercellular adhesion molecule-3–grabbing nonintegrin (DC-SIGN) receptor.[18] This receptor has been shown to recognize the fucose-containing Lewis-type glycans[19], especially the trisaccharide Galβ1-4(Fucα1-3)GlcNAc, better known as Lewis^X (Le^X), which has been shown to be highly abundant on natively glycosylated MOG.[20] Hence, studies using synthetic neoglycopeptides bearing DC-SIGN binding *N*-glycan mimics may shed light on the role of a putative interaction between DC-SIGN and MOG in MS. We synthesized MOG_{31-55}-peptides decorated with Le^X and Le^X derived oligosaccharides (LacNAc and Fucα1-3GlcNAc) on the N-terminal asparagine (Asn_{31}) and

assess the effect of these modifications on the aggregation proneness of the peptides. It was our aim to link the glycans to the peptides via amide linkages, to minimize artefacts stemming from various non-native linkers.[21–23] To achieve this, we extended our recently published method for the synthesis of glycosylated asparagine derivatives using larger oligosaccharides.[24] By using these asparagine building blocks with our previously established model peptide, MOG_{31-55}[12], we were able to evaluate the effect of glycosylation on citrullination-dependent aggregation of MOG. Subsequently, using the binding of Le^X decorated neoglycopeptides to DC-SIGN was confirmed by solid-phase immunoassays. Finally, a cytokine secretion assay in monocyte derived dendritic cells (moDCs) from human donors was utilized to analyze the degree of modulation for IL-10 (anti-inflammatory) and IL-12p70 (pro-inflammatory) production by Le^X decorated peptides.

The outcome of these biochemical and immunological studies suggested that i) aggregation behavior of citrullinated MOG_{31-55} can be halted or abrogated depending on the glycan; ii) our amide linked Le^X ligand indeed binds to DC-SIGN in an ELISA and iii) the Le^X decorated neoglycopeptide has an *in vitro* tolerogenic effect (cytokine secretion assay) thus potentially prevents inflammation. Hence, we report the first synthesis of MOG_{31-55} derivatives that are site-specifically decorated with DC-SIGN ligands and analyze the immunological consequences of exposure of moDCs to the Le^X decorated peptide.



Scheme 1. A) Structures of glycosylated asparagine derivatives **1-4** B) Retrosynthetic analysis of the synthesis of Le^X decorated MOG_{31-55} peptides. X = NH (Arg) or X = O (Cit).

Results and Discussion

While *N*-glycosylation of asparagine is of prime importance for a variety of protein functions such as signaling and folding[25], the typical size and complexity of an *N*-glycan poses a considerable synthetic challenge. *N*-glycosylated peptides have been generated using semisynthetic methods involving synthesis and/or isolation of carbohydrate segments which can be linked covalently using endohexosaminidases[26,27], or extended via specific glycosyltransferases as recently demonstrated by Boons and colleagues.[28,29] Synthetic preparation of an entire peptide bearing a natural *N*-glycan has also been reported.[30]

Previous work from our group and others[31–35] has demonstrated that fucosylated glycans interact with DC-SIGN without the need for an *N*-glycan core structure. This inspired us to synthesize a Le^X *N*-glycan derivative similar to the one developed by von dem Bruch and Kunz.[36] We designed and synthesized three glycosyl amide derivatives of asparagine as Fmoc-SPPS (solid phase peptide synthesis) compatible building blocks, containing Le^X and two Le^X derivatives, LacNAc and Fucα1-3GlcNAc, attached to the asparagine side chain via the reducing ends of respective sugars (Scheme 1A, **1-4**). The LacNAc construct (**3**) served as a negative control for DC-SIGN binding, as the interaction of Le^X with the receptor has been shown to be fucose dependent.[32]

We chose to base our synthesis on acid labile *para*-methoxybenzyl (PMB) and *para*-methoxybenzylidene groups, which would be removed during global peptide deprotection in standard Fmoc-based solid phase peptide synthesis, and on esters, which can be selectively removed using hydrazine in methanol after the acidic global deprotection of the peptide. By including these protecting groups from the start of the oligosaccharide synthesis, late-stage protecting group manipulation could mostly be avoided.

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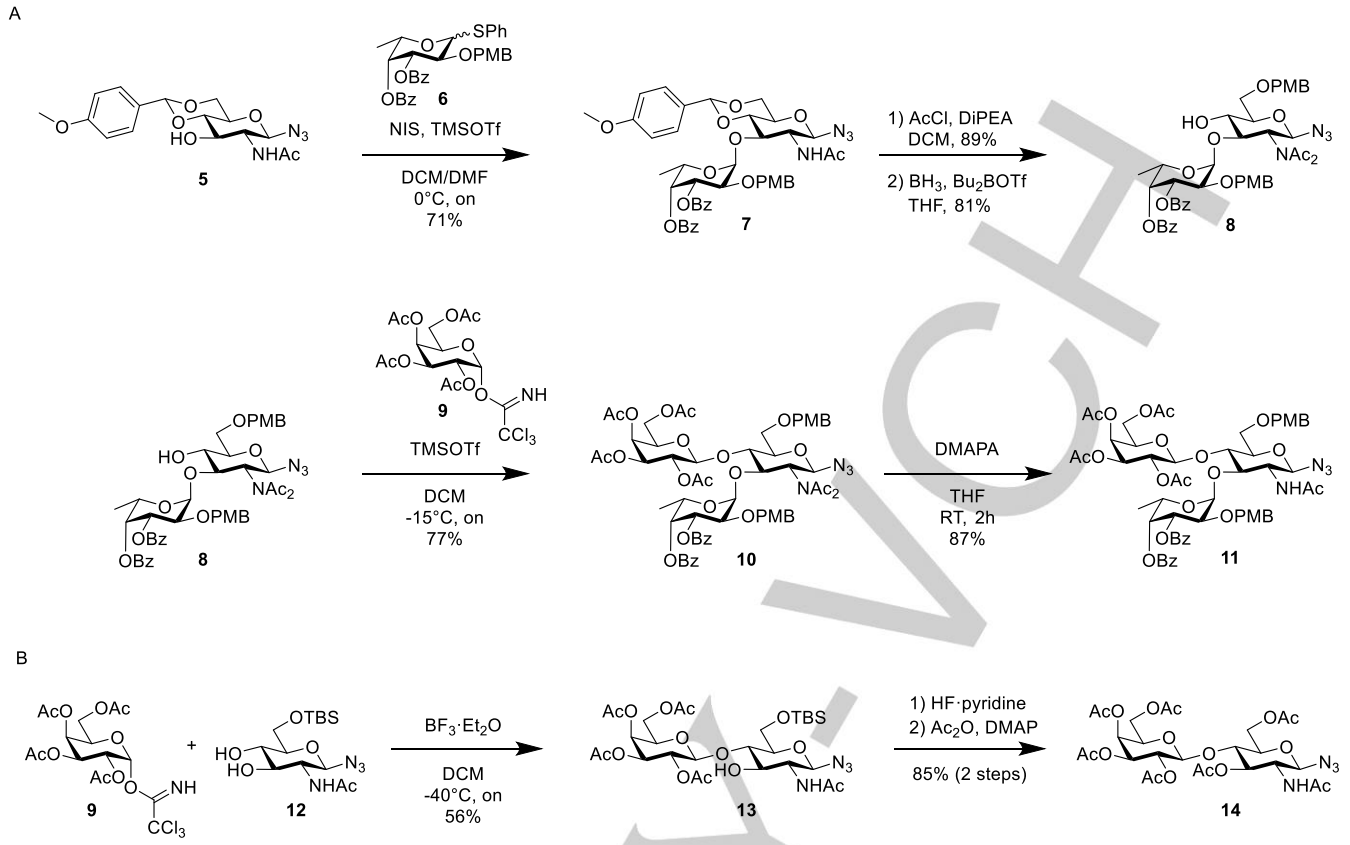

Scheme 2. Synthesis of Lewis X azide (A) **11** and LacNAc azide **14** (B)

The linkages between the oligosaccharides and the asparagine side chain were installed using our recently developed two-step one-pot approach for the synthesis of glycosylated asparagine derivatives.[24] Here, we combine a Staudinger reduction to transform a glycosyl azide into a glycosyl amine, as reported by many others[37–39], followed by aspartic anhydride ring-opening, generating a protected glycosyl asparagine derivative (Scheme 1B). The synthesis of the protected Le^X glycosyl azide **11** (Scheme 2) was initiated from the *para*-methoxy benzylidene protected glycosyl azide **5** (synthesis in SI) by means of a NIS/TMSOTf-promoted fucosylation reaction with the thioglycoside **6** (synthesis in SI) to afford disaccharide **7** in 71% yield. The presence of the acetamido group was detrimental to the results of the following glycosylation, an often encountered problem with *N*-acetyl-glucosamine derived acceptors.[40] Accordingly, disaccharide **7** was treated with an excess of acetyl chloride and diisopropylethylamine (DiPEA) to convert the amide functionality to the less interfering imide in 89% yield.[41] Reductive opening of the *para*-methoxybenzylidene with BH_3/Bu_2BOTf was performed as described[42], affording compound **8** in 81% yield. Finally, galactosylation with the trichloroacetimidate donor **9** (synthesis in SI) yielded the desired protected trisaccharide **10** in 77% yield. Chemoselective deacetylation of **10** using N,N-dimethylaminopropylamine (DMAPA)[43] afforded **11** in 87% yield.

The protected lactosaminyl azide **14** was prepared using a literature protocol for regioselective glycosylation of 1,6-protected GlcNAc derivatives.[44,45] Silyl ether protected glycosyl azide **12** was subjected to $BF_3{\cdot}Et_2O$ promoted galactosylation with trichloroacetimidate donor **9**, affording the partially protected disaccharide **13** in a 56% yield (Scheme 2). This compound was treated with HF·pyridine for removal of the *tert*-butyldimethylsilyl (TBS) group, followed by acetylation to afford the desired peracetylated glycosyl azide **14** in 85% yield over 2 steps.

Asparagine derivatives **1-4** were prepared following a general synthetic strategy involving the Staudinger reduction of a glycosyl azide followed by direct ligation of the resulting glycosyl amine with Fmoc aspartic anhydride, a sequence reported recently by us (Scheme 3A).[24] Accordingly, Fmoc-Asn(GlcNAc)-OH (**1**) was synthesized from easily obtained glycosyl azide **15**[46] in three steps by PMe_3-mediated azide reduction, followed by addition of H_2O to the crude iminophosphorane to obtain the intermediate glycosyl amine. The desired asparagine derivative was formed by redissolving the crude glycosyl amine in DMSO followed by addition of Fmoc aspartic anhydride. Precipitation directly afforded the desired SPPS building block **1** in 69% yield.

The above sequence proved similarly useful for the preparation of the other desired glycosylated asparagine building blocks (**2**-**4**, Scheme 3A). However, precipitation or extraction were found to be less efficient for small scale purification of the more complex carbohydrates, and therefore we subjected these compounds to silica gel chromatography for purification. Using this approach, the fucosylated glycosyl azide **7** was converted to its corresponding SPPS building block **2** in 65% yield, while lactosyl compound **14** was similarly converted to compound **3** in 63% yield. (Scheme 3A).

For the trisaccharide glycosyl azide, transformation of the NAc_2 functionality to the acetamide was required, as Staudinger reduction of **10** afforded conversion to an unknown side product.

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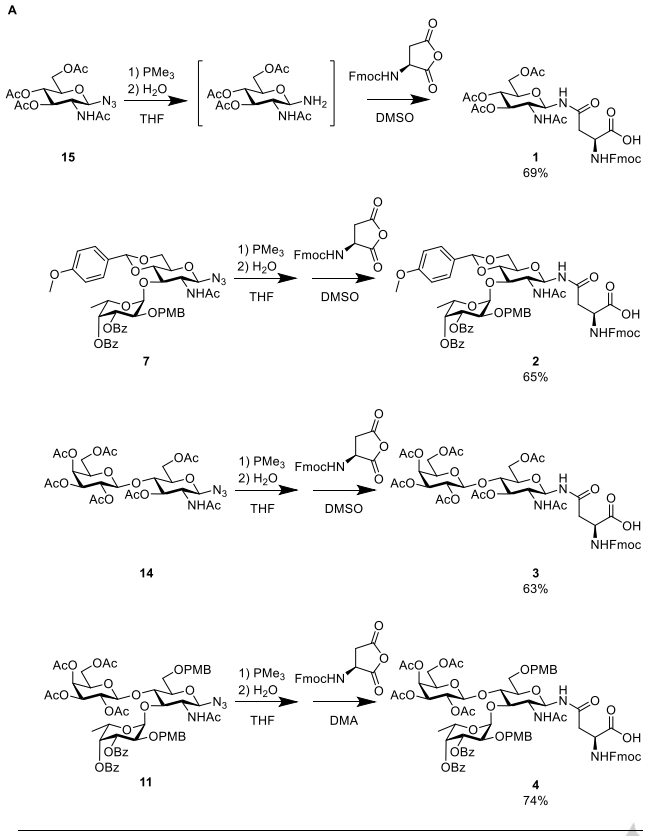
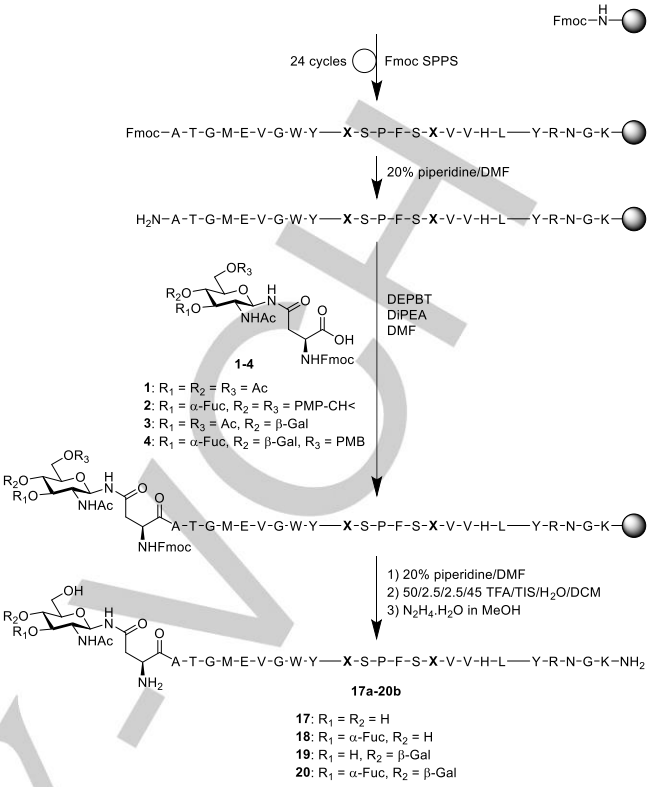


Scheme 3: A) Synthesis of glycosylated Fmoc-asparagine derivatives **1**-**4** via the two step Staudinger reduction/aspartic anhydride coupling approach. B) observed reaction when performing Staudinger reduction of diacetylimide **16**

Acetyl migration is a likely explanation, as Staudinger reduction of the more simple NAc_2 protected glycosyl azide **16** afforded clean conversion to the more readily assignable glycosyl acetamide **16a** (Scheme 3B). Glycosyl azide **11** was coupled to Fmoc aspartic anhydride yielding the desired Le^X SPPS building block **4** as an inseparable 10:1 mixture with its corresponding iso-asparagine isomeric product. It has been shown that dimethylacetamide (DMA) gives similar regioselectivity as DMSO when used as solvent for aspartic anhydride ring opening reactions.[47] However, the lower melting point of this solvent allows for aspartic anhydride ring-opening at 0°C, potentially increasing regioselectivity. Indeed, this solvent and temperature change resulted in the desired Le^X asparagine **4** being formed in 74% yield with complete regioselectivity.

The syntheses of the desired glycopeptides were initiated with automated SPPS of the MOG_{32-55} peptide on Tentagel®S-RAM resin, using HCTU as the coupling reagent. These peptides where then manually elongated at the N-terminus with glycosylated asparagines **1**-**4** using DEPBT as the coupling reagent to prevent aspartimide formation, as described by Yamamoto *et al*.[48] The general synthetic strategy used for the synthesis of the glycopeptides is outlined in Scheme 4.



Scheme 4. Synthetic strategy employed for the synthesis of MOG_{31-55} glycopeptides. X = Arg (**17a**-**20a**) or Cit (**17b**-**20b**)

The peptides were cleaved from the solid support under acidic conditions (95:2.5:2.5 TFA:TIS:H_2O mixture for 2 hours) for the non-fucosylated peptides, and more dilute acidic conditions (50:2.5:2.5:45 TFA:TIS:H_2O:DCM mixture for 4 hours) for the fucose containing ones, to prevent hydrolysis of the acid labile α-fucosyl bond.[49] The reaction time under these less acidic conditions had to be extended to ensure complete removal of the 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) protecting groups, which are more acid stable than the usual side chain protecting groups (Boc/tBu) in Fmoc-SPPS.[50]
To remove the remaining ester protecting groups on the carbohydrates, the crude peptides were treated with 10% hydrazine monohydrate in methanol. Crude glycopeptides were purified by preparative reverse-phase (RP) HPLC. All four different glycosylated asparagine building blocks exhibited good coupling efficiencies under the conditions used here (Scheme 4) and the neoglycopeptides **17a**-**20a** were isolated in moderate to good yields after RP-HPLC (Table 1).

In order to test whether glycosylation has an impact on the aggregation behavior of citrullinated MOG-derived peptides, we prepared MOG_{31-55} peptides carrying both post-translational modifications, namely citrullination and glycosylation. For the citrullination pattern we chose to replace both Arg_{41} and Arg_{46} with citrullines, since we have previously shown that this citrullination pattern enhances aggregation behavior.[12] Furthermore, the location of the modifications in the putative MHC-I restricted non-human primate epitope MOG_{40-48}[51] was interesting, as citrullination of one of these positions was demonstrated to exacerbate ongoing EAE.[52]

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Table 1. Yields of glycopeptides obtained using the synthetic strategy outlined in Scheme 4 after preparative HPLC. The number for each compound is given together with the HPLC yield based on crude mass.

Amino acid	X = Arg	X = Cit
GlcNAc (**1**)	**17a** (4.0%)	**17b** (8.6%)
Fucα1-3GlcNAc (**2**)	**18a** (5.6 %)	**18b** (2.1 %, 5.7 %[a])
LacNAc (**3**)	**19a** (5.8 %)	**19b** (5.6 %)
Lewis X (**4**)	**20a** (4.1 %)	**20b** (6.1 %, 4.8%[a])

[a] The product containing methionine oxidation was isolated separately.

The citrullinated peptides (**17b**-**20b**) were synthesized using the same methodology as for their non-citrullinated counterparts, using Fmoc-citrulline as the 41st and 46th amino acid. Similar levels of glycosyl amino acid incorporation and similar RP-HPLC yields were achieved during the synthesis of these glycopeptides (Table 1).

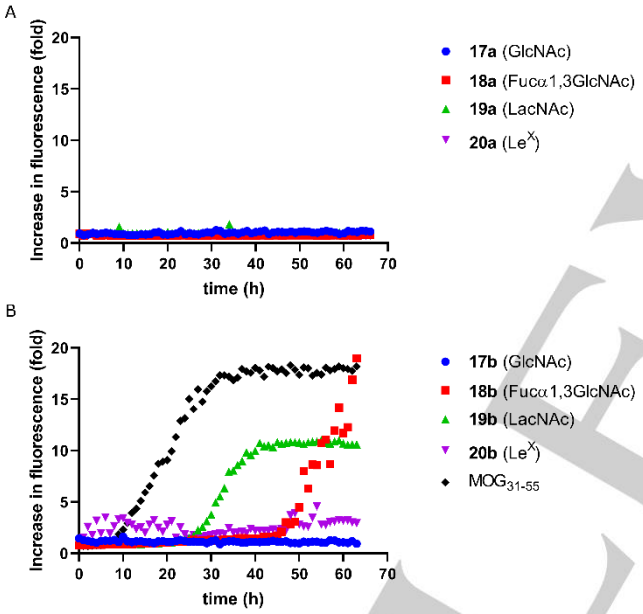

Figure 1. ThT aggregation assay of non-citrullinated (A) and citrullinated (B) glycosylated MOG_{31-55} peptides **17a**-**20b**. Peptides were tested at a concentration of 10 µM. Positive control (black diamonds) is nonglycosylated MOG_{31-55} citrullinated at positions 41 and 46. All data were recorded at an excitation wavelength of 444 ± 9 nm and an emission wavelength of 485 ± 9 nm. All samples were used at a pH of 5.0 and aggregation assays were performed at least three times and with experimental triplicates.

To assess the influence of glycosylation on immune-relevant MOG_{31-55}, we opted for different biophysical and biochemical experiments. First, we determined the secondary structure in solution via circular dichroism (CD). All peptides showed a pre-dominantly random-coiled structure. The effect of addition of the α-helix stabilizer TFE (50% v/v in PBS) or SDS at non-micellar concentrations (4 mM) was also evaluated (Figure S1). These results indicate the peptides are not prone to β-sheet formation.

Next, inspired by the recently published aggregation behavior of citrullinated MOG_{31-55} peptides, we evaluated the susceptibility of all glycopeptides to amyloid-like aggregation using the previously described ThT fluorescence assay.[12] In this assay, a fluorogenic substrate, Thioflavin T, with a selectivity towards cross β-sheet structures as found in amyloid-like aggregates, is used to detect whether such aggregation occurs. Non-citrillunated peptides did not show aggregation at physiologically relevant concentrations (10 µM, Figure 1A). For the citrullinated peptides, the effect of glycosylation seemed to be structure dependent.

Whilst all forms of glycosylation had an inhibitory effect on aggregation (Figure 1B), the inclusion of a single GlcNAc modification (**17b**) was sufficient to completely abrogate the aggregation, displaying the powerful effect glycosylation can have on peptide aggregation. The DC-SIGN ligand Le^X (**20b**) showed a similar inhibition of aggregation to that of GlcNAc suggesting the potential in controlling immune household and not the neurodegenerative mechanism in MS. However, other glycosylation patterns tested, Fucα1-3GlcNAc (**18b**) and LacNAc (**19b**), did not fully inhibit aggregation delaying only its onset (Figure 1B). In our previous studies[12] , we have shown that citrullinated MOG_{35-55} peptides are cytotoxic to murine bone marrow derived dendritic cells (BMDCs). Citrullinated MOG_{31-55} however was not yet tested. To analyze whether native, glycosylated or citrullinated MOG_{31-55} variants show similar cytotoxicity to those of citrullinated MOG_{35-55}, we conducted cell viability assays using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) as described previously (Figure S1).[12] BMDCs were treated with citrullinated peptides **18b-20b** as well as their non-glycosylated counterpart (cit_MOG_{31-55}) at four different concentrations (40, 20, 10 and 5 µM). None of the tested peptides showed significant decrease in viability of BMDCs at any concentration tested. Remaining glycosylated MOG_{31-55} derivatives as well as the native variant did also not exhibit any significant drop in cell viability in BMDCs.

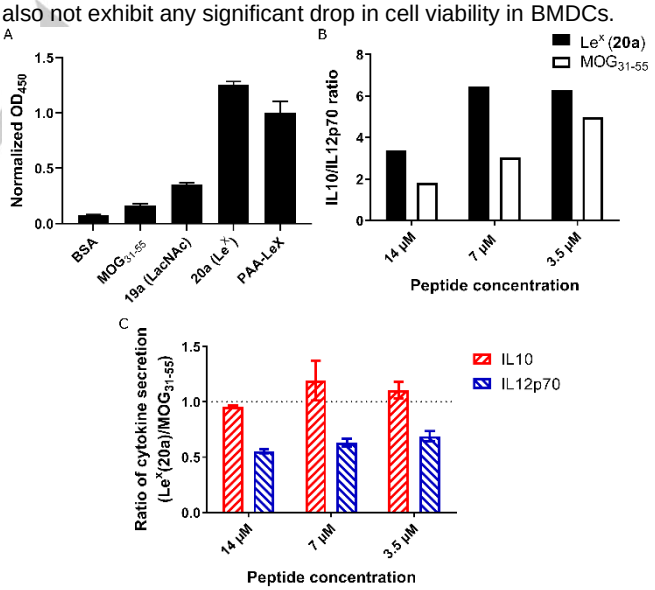

Figure 2. In vitro DC-SIGN binding assay (A) and moDC cytokine profiling upon exposure to **20a** (B,C). A) DC-SIGN-FC ELISA. Lewis X decorated polymer (PAA-LeX) was used as the positive control, while for the negative control no peptide was added, meaning they are fully blocked with BSA. The DC-SIGN ELISA has been performed three times showing similar results. The graph shows data of one representative experiment out of three independent experiments performed in duplicate. Error bars represent standard deviation. B) Ratio of IL10/IL12p70 secretion measured upon moDC stimulation with either **20a** or non-glycosylated control in the presence of 10 ng/mL of LPS. This graph is a representative plot from one donor (N=3). C) Normalized ratios for IL-10 and IL-12p70 secretion between non-glycosylated peptide MOG_{31-55} and peptide **20a** harboring Le^X incubated with

moDCs at different concentrations in the presence 10 ng/mL LPS. Here a ratio of 1 means cytokine production is the same for both peptides, while a ratio of 0.5 means cytokine production is halved for **20a** compared to non-glycosylated peptide. The results are the average of three experiments performed using cells from three separate donors, each measured in duplicate.

Overall, it could be concluded that glycosylation of MOG_{31-55} does not alter its biophysical properties as measured by CD, whereas GlcNAc and LeX modifications abrogate amyloid like behavior of MOG_{31-55}. Moreover, no major cytotoxic effects were observed for citrullinated and glycosylated MOG_{31-55} derivatives in BMDCs, which renders them useful for subsequent studies to explore the impact of DC-SIGN binding on moDCs.

Next, we investigated the physiological relevance of our simplified *N*-glycan structures. To assess the ability of the model *N*-glycans to bind DC-SIGN, a DC-SIGN binding ELISA was carried out.[53,54] Briefly, peptides were coated on the bottom of high-binding plates. DC-SIGN binding was assessed by incubating with recombinant DC-SIGN-Fc construct (N-terminally truncated extracellular domain (K_{62}-A_{404}) of human DC-SIGN expressed with the fused Fc region of human IgG1 at the N-terminus) followed by an HRP-conjugated secondary antibody for qualitative readout of binding. The results of this assay are displayed in Figure 2.

As expected[19], Le^X peptides **20a** (Figure 2A) and **20b** (Figure S3) were recognized by DC-SIGN-Fc, while the other glycopeptides were recognized to a lesser extent (**18a-b,** Figure S3) or not at all (**19a-b,** Figure 2A and Figure S3). Note that we observed an increase in binding affinity of GlcNAcylated peptide **17a** to DC-SIGN (Figure S3). This can be explained by GlcNAc being a weak binder to DC-SIGN with an IC_{50} of 5 mM *in vitro*.[55] Citrullination of this peptide, **17b**, inhibited the increase in binding affinity (Figure S3).

Finally, we investigated the downstream effects of stimulation of human monocytes-derived dendritic cells (moDCs) with Le^X decorated peptide **20a**. Since DC-SIGN is absent on murine DCs[56], human dendritic cells, derived from donor blood, are an useful alternative. Furthermore, the pathophysiology of MS is not completely mimicked by murine EAE[57], further necessitating the use of human model systems when feasible. We utilized a well-established assay[58] measuring the release of anti- and pro-inflammatory cytokines, IL-10 and IL-12p70 respectively. It has been shown that stimulation of DC-SIGN with fucosylated glyco-conjugates (in presence of TLR4 ligands) induce an upregulation of IL-10 and a down-regulation of IL-12p70, switching the immune response towards tolerance instead of inflammation. In this assay moDCs, derived from peripheral blood monocytes (PBMCs) of three donors, are stimulated with peptide **20a** or non-glycosylated MOG_{31-55} at distinct concentrations (14, 7 and 3.5 µM) in presence or absence of the TLR4 ligand LPS (from *E. coli* at 10 ng/mL), and their cytokine secretion levels are measured.[59] As expected, no cytokine production was observed upon stimulation of moDCs with peptide in the absence of LPS (Figure S4). However, upon co-stimulation with LPS, we observed a Le^X-dependent effect for MOG_{31-55} on IL-12p70 secretion at all concentration tested for peptide **20a**. In Figure 2B the ratio of IL-10/IL-12p70 secretion is plotted for a single donor (representative for three independent experiments, N=3), showing an increase for the Le^X decorated neoglycopeptide **20a** over the non-glycosylated control at all concentrations tested. This increase in IL10/IL12p70 ratio shows that stimulation with peptide **20a** leads to a more tolerogenic response compared to non-glycosylated MOG_{31-55}. In Figure 2C we plotted the ratio of cytokine secretion between stimulation of moDCs with **20a** and non-glycosylated MOG_{31-55} for all donors (N=3). A reduction in secretion of pro-inflammatory cytokine IL-12p70 is observed, while secretion of anti-inflammatory IL-10 remains unchanged. Since the DC-SIGN-Fc binding ELISA shows a binding interaction between the Le^X decorated peptide and not the non-glycosylated peptide, a DC-SIGN driven process is strongly suggested.

Conclusion

We have developed a synthetic route for three novel SPPS compatible glycosylated Fmoc-asparagine building blocks, including an asparagine derivative of the important DC-SIGN ligand Le^X. These building blocks have been synthesized from the glycosyl-azides using our Staudinger-reduction/aspartic anhydride ring-opening approach. By careful choice of protecting groups during the oligosaccharide assembly, the amount of protecting group manipulations could be kept to a minimum, while glycopeptide deprotection was accomplished in a straightforward manner. To demonstrate this, we have synthesized glycosylated derivatives of the peptide MOG_{31-55} in good yields and purity, as well as derivatives that are both glycosylated and citrullinated.

Using these synthetic neoglycopeptides, we have demonstrated that glycosylation has a powerful effect on citrullination driven aggregation of this model peptide. Interestingly, the effect glycosylation has on citrullination driven aggregation seems to be also dependent on oligosaccharide structure. Furthermore, we have shown that Le^X, while linked to asparagine directly via an amide bond, is capable of binding to DC-SIGN, *via* ELISA. Finally, we showed that a peptide, decorated with Le^X on asparagine, was able to elicit a tolerogenic response (reduced IL12p70 secretion compared to non-glycosylated counterpart), when used to stimulate moDCs.

Experimental Section

General methods for SPPS An automated synthesizer (PTI Tribute UV-IR synthesizer, Gyros Protein Technologies) was utilized. If not stated otherwise, peptides were synthesized on Tentagel S RAM resin (Rapp Polymere GmbH, Germany) on a 100 µmol scale using 5.0 equiv of each amino acid (AA) with respect to the resin loading. Fmoc protected amino acids were purchased from either Novabiochem or Sigma-Aldrich. For the amino acids that require sidechain protection, the following protecting groups were used: tBu for Ser, Thr and Tyr; OtBu for Asp and Glu; Trt for Asn, Gln and His; Boc for Lys and Trp; Pbf for Arg; An equimolar quantity of 2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) was used as activator. Coupling cycles of 1 h were utilized, and unreacted amines were capped after each cycle using a solution of 500 µL of acetic anhydride, 250 µL of DIPEA, and 4.25 mL of DMF for 5 min at room temperature twice. Fmoc deprotection was accomplished with 20% piperidine in DMF (3 x 5 min). Cleavage of non-glycosylated peptides was accomplished using a 95:2.5:2.5 mixture of TFA:TES:H_2O for 3 hours, followed by precipitation from cold diethyl ether and recovery of the precipitate by centrifugation. Peptides were characterized using electrospray ionization mass spectrometry (ESI-MS) on a Thermo Finnigan LCQ Advantage Max LC-MS instrument with a Surveyor PDA plus UV detector on an analytical C18 column (Phenomenex, 3 µm, 110 Å, 50 mm × 4.6 mm) in combination with buffers A (H_2O), B (MeCN), and C (1% aq TFA). Quality of crude peptides was evaluated with a linear gradient of 10-50% B with a constant 10% C over

10 minutes, while final peptide quality was evaluated using a linear gradient of 5-65% B with a constant 10% C over 30 minutes.

Incorporation of glycosylated amino acids Synthesis of glycopeptides was carried out at 25 µmol scale. Fmoc group was removed from the resin bound peptide using 2 x 2 mL of 20% piperidine in DMF (3 + 7 min). After Fmoc deprotection, the resin was washed five times with DMF (5 x 5 mL). Fully protected glycosylated asparagine (2 eq, 50 µmol) was dissolved in 500 µL of a 0.3 M solution of 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT) in DMF by the addition of DIPEA (8.7 µL, 2 eq, 50 µmol). The mixture was agitated for at least 5 minutes or until all amino acid had been dissolved. The solution containing the activated amino acid was added to the resin and the resin was incubated overnight under mild agitation. After overnight coupling, the resin was washed with DMF (5 x 5 mL) and a small portion was deprotected to confirm incorporation of the glycosylated amino acid. Fmoc deprotection was carried out as normal using a freshly prepared piperidine solution. Full cleavage of the peptide was achieved using 2 mL of 95:2.5:2.5 mixture of TFA:TES:H_2O for 2 hours or 50:2.5:2.5:45 mixture of TFA:TES:H_2O:DCM for 4 hours for fucose containing peptides. The deprotected peptide was precipitated in cold diethyl ether (10 mL) and the resin was washed with DCM (1 mL) which was added to the ether phase. After centrifugation, the pellet was washed with a small amount of diethyl ether (3-5 mL) and centrifugated again. To facilitate the removal of the ester protection groups, the peptide was suspended in methanol (2.25 mL) in a roundbottom flask and placed under N_2 atmosphere, followed by the addition of hydrazine monohydrate (0.25 mL). After stirring overnight, the reaction progress was checked by LC-MS. When complete deprotection was confirmed the volatiles were removed *in vacuo* to yield the crude glycopeptide. Preparative reverse phase HPLC on a Waters AutoPurification system (eluent A: H_2O + 0.2% TFA; eluent B: ACN) with a preparative Gemini C18 column (5 µm, 150 x 21.2 mm) yielded the final products.

***N*$^{\gamma}$-[3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-d-glucopyranosyl]-*N*$^{\alpha}$-fluorenylmethoxycarbonyl-l-asparagine (1)** Glycosyl azide **15** (200 mg, 0.54 mmol) was dissolved in THF (0.76 mL) and the solution was cooled in an icebath. 0.54 mL of a 1 M solution of trimethylphosphine (1.0 eq, 0.54 mmol) in THF was added dropwise over 2 minutes, during which gas evolution was observed. The icebath was removed, and the reaction was stirred for 5 minutes before H_2O (10 eq, 97 µL, 5.4 mmol) was added. The reaction was stirred at room temperature for 1.5 hours, after which it was concentrated. The residue containing the crude glycosyl amine was redissolved in DMSO (1.8 mL) and Fmoc-aspartic anhydride[60] (1.0 eq, 181 mg, 0.54 mmol) was added. The reaction was stirred for 2 hours at room temperature. The DMSO solution was added dropwise to a centrifuge tube containing 30 mL of a 2:1 mixture of diethyl ether and ethyl acetate and a precipitate started to form. The compound was left to fully precipitate for 16 hours at room temperature, after which it was collected by centrifugation. The supernatant was discarded and the pellet was washed with a small amount of the diethyl ether/ethyl acetate mixture. After removing the volatiles under reduced pressure, the title compound was obtained as a white amorphous solid (255 mg, 0.37 mmol, 69%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.60 (d, *J* = 9.8 Hz, 1H, N$^{\gamma}$H), 7.99 – 7.78 (m, 3H, NHC(O)CH_3), 7.71 (d, *J* = 7.5 Hz, 2H, Fmoc-Ar), 7.51 (d, *J* = 8.5 Hz, 1H, N$^{\alpha}$H), 7.41 (t, *J* = 7.5 Hz, 2H, Fmoc-Ar), 7.32 (t, *J* = 7.4 Hz, 2H, Fmoc-Ar), 5.18 (t, *J* = 9.8 Hz, 1H, H1), 5.10 (t, *J* = 9.8 Hz, 1H, H3), 4.82 (t, *J* = 9.8 Hz, 1H, H4), 4.38 (q, *J* = 7.5 Hz, 1H, Asn-CH), 4.33 – 4.13 (m, 4H, Fmoc-CH_2, Fmoc-CH, H6a), 3.94 (d, *J* = 11.3 Hz, 1H, H6b), 3.88 (q, *J* = 9.8 Hz, 1H, H2), 3.84 – 3.78 (m, 1H, H5), 2.66 (dd, *J* = 16.3, 5.4 Hz, 1H), 1.99 (s, 3H, OC(O)CH_3), 1.96 (s, 3H, OC(O)CH_3), 1.90 (s, 3H, OC(O)CH_3), 1.72 (s, 3H, NHC(O)CH_3). **^{13}C NMR** (126 MHz, DMSO-d_6) δ = 173.0 (C=O), 170.1 (C=O), 169.9 (C=O), 169.6 (C=O), 169.6 (C=O), 169.4 (C=O), 155.9 (C=O), 143.8 (Fmoc-Ar), 143.8 (Fmoc-Ar), 140.7 (Fmoc-Ar), 127.7 (Fmoc-Ar), 127.1 (Fmoc-Ar), 125.3 (Fmoc-Ar), 120.2 (Fmoc-Ar), 78.1 (C1), 73.4 (C3), 72.3 (C5), 68.4 (C4), 65.8 (Fmoc-CH_2), 61.9 (C6), 52.2 (C2), 50.0 (Asn-CH), 46.6 (Fmoc-CH), 36.9 (Asn-CH_2), 22.6 (NHC(O)CH_3), 20.6 (OC(O)CH_3), 20.4 (OC(O)CH_3), 20.4 (OC(O)CH_3). **HRMS** (ESI) m/z: [M + H^+] calcd for $C_{33}H_{37}N_3O_{13}H$ 684.23991, found 684.23920.

***N*$^{\gamma}$-[3,4-di-O-benzoyl-2-O-(4-methoxybenzyl)-α-l-fucopyranoside-(1→3)-4,6-O-(4-methoxybenzylidene)-2-deoxy-2-acetamido-β-d-glucopyranosyl]-*N*$^{\alpha}$-fluorenylmethoxycarbonyl-l-asparagine (2)** Glycosyl azide **7** (168 mg, 0.2 mmol) was dissolved in dry THF (2 mL) and trimethylphosphine was added as a 1 M solution in THF (1.1 eq, 220 µL, 0.22 mmol). The reaction was stirred for 10 minutes at room temperature and H_2O (50 eq, 180 µL, 10 mmol) was added. After stirring for 1 hour at room temperature, the reaction was concentrated and the residue was dissolved in DMSO (2 mL). Fmoc-aspartic anhydride[60] (1.0 eq, 67 mg, 0.2 mmol) was added and the reaction mixture was stirred for 1 hour at room temperature. The solvent was removed *in vacuo* and the crude was subjected to silica gel column chromatography (0 → 8% MeOH in DCM, Δ = 1%). This yielded the title compound (150 mg, 0.13 mmol, 65%). **[α]$_D^{25}$** = -73.3 (c 1.00 in $CHCl_3$). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.46 (d, J = 9.4 Hz, 1H, N$^{\gamma}$H), 8.16 (d, J = 9.0 Hz, 1H, NHC(O)CH_3), 7.87 (t, J = 8.0 Hz, 3H, CH_{arom}), 7.81 – 7.64 (m, 5H, CH_{arom}), 7.64 – 7.47 (m, 5H, CH_{arom}), 7.47 – 7.26 (m, 8H, N$^{\alpha}$H, CH_{arom}), 7.18 – 7.04 (m, 2H, CH_{arom}), 6.97 – 6.89 (m, 2H, CH_{arom}), 6.73 – 6.62 (m, 2H, CH_{arom}), 5.71 (s, 1H, PMP-CH_{acetal}), 5.42 – 5.33 (m, 2H, H1', H3'), 5.23 (d, J = 3.5 Hz, 1H, H4'), 5.15 (t, J = 9.5 Hz, 1H, H1), 4.55 – 4.43 (m, 2H, H5', PMB-CHH), 4.39 – 4.30 (m, 2H, PMB-CHH, Asn-CH), 4.30 – 4.17 (m, 4H, Fmoc-CH_2, H5, Fmoc-CH), 4.13 (t, J = 9.5 Hz, 1H, H3), 3.99 (dd, J = 10.7, 3.5 Hz, 1H, H2'), 3.95 – 3.84 (m, 1H, H2), 3.76 – 3.60 (m, 9H, H6, H4, OCH_3, OCH_3), 2.66 (dd, J = 16.1, 5.6 Hz, 1H, Asn-CHH), 1.82 (s, 3H, NHC(O)CH_3), 0.46 (d, J = 6.4 Hz, 3H, H6'). **^{13}C NMR** (101 MHz, DMSO-d_6) δ = 170.2 (C=O), 169.7 (C=O), 165.6 (C=O), 164.8 (C=O), 159.7 (C_q), 158.8 (C_q), 155.9 (C=O), 143.9 (Fmoc-Ar), 143.8 (Fmoc-Ar), 140.7 (Fmoc-Ar), 133.7 (CH_{arom}), 133.5 (CH_{arom}), 130.0 (C_q), 129.2 (CH_{arom}), 129.1 (C_q), 129.0 (CH_{arom}), 128.8 (CH_{arom}), 128.5 (CH_{arom}), 127.8 (CH_{arom}), 127.8 (CH_{arom}), 127.7 (CH_{arom}), 127.1 (CH_{arom}), 125.3 (CH_{arom}), 120.1 (CH_{arom}), 113.4 (CH_{arom}), 100.9(PMP-CH), 96.2 (C1'), 79.4 (C1, C4), 75.3 (C3), 72.3 (C4'), 71.5 (C2'), 70.0 (PMB-CH_2), 69.6 (C3'), 68.0 (C5), 67.8 (C6), 65.8 (Fmoc-CH_2), 63.9 (C5'), 55.1 (OCH_3, C2), 55.0 (OCH_3), 50.4 (Asn-CH), 46.6 (Fmoc-CH), 37.3 (Asn-CH_2), 23.1 (NHC(O)CH_3), 15.2, (C6'). **HRMS** (ESI) m/z: [M + H^+] calcd for $C_{63}H_{63}N_3O_{18}H$ 1150.41794, found 1150.41741.

***N*$^{\gamma}$-[2,3,4,6-tetra-O-acetyl-β-d-galactopyranoside-(1→4)-6,3-di-O-acetyl-2-deoxy-2-acetamido-β-d-glucopyranosyl]-*N*$^{\alpha}$-fluorenylmethoxycarbonyl-l-asparagine (3)** Azidosugar **14** (0.74 mmol, 488 mg) was dissolved in THF (7.4 mL) and a 1M solution of trimethylphosphine in THF (1.5 eq, 1.1 mL, 1.1 mmol) was added and the reaction was stirred at room temperature. H_2O (50 eq, 0.67 mL, 37 mmol) was added and the reaction was further stirred for 60 minutes. The volatiles were removed *in vacuo* and the crude glycosyl amine was redissolved in DMSO (7.4 mL). Fmoc-aspartic anhydride (1 eq, 0.74 mmol, 249 mg) was added and the reaction was stirred for 75 minutes. The solvent was removed *in vacuo* and the crude was subjected to silica gel column chromatography (0 → 8% MeOH in DCM, Δ = 1%) to yield the title product (455 mg, 0.47 mmol, 63%). **[α]$_D^{20}$** = +0,2 (c 1.00 in MeOH) **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.58 (d, J = 9.1 Hz, 1H, N$^{\gamma}$H), 7.89 (d, *J* = 7.7 Hz, 2H, Fmoc-Ar), 7.86 (d, J = 9.5 Hz, 1H, NHC(O)CH_3), 7.71 (d, J = 7.5 Hz, 2H, Fmoc-Ar), 7.42 (t, J = 7.3 Hz, 3H, Fmoc-Ar, N$^{\alpha}$H), 7.33 (t, J = 7.4 Hz, 2H, Fmoc-Ar), 5.23 (d, J = 3.7 Hz, 1H, H4'), 5.16 (dd, J = 10.3, 3.6 Hz, 1H, H3'), 5.10 (t, J = 9.5 Hz, 1H, H1), 4.97 (t, J = 9.5 Hz, 1H, H3), 4.84 (dd, J = 10.3, 8.0 Hz, 1H, H2'), 4.70 (d, J = 8.0 Hz, 1H, H1'), 4.36 – 4.15 (m, 6H, Asn-CH, Fmoc-CH_2, H6a, H5', Fmoc-CH), 4.09 – 3.95 (m, 3H, H6b, H6'), 3.81 (q, J = 9.5 Hz, 1H, H2), 3.73 – 3.55 (m, 2H, H4, H5), 2.63 (dd, J = 16.3, 5.3 Hz, 1H, Asn-CH_2), 2.11 (s, 3H, C(O)CH_3), 2.07 (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 1.94 (s, 3H, C(O)CH_3), 1.90 (s, 3H, C(O)CH_3), 1.71 (s, 3H, NH(CO)CH_3). **^{13}C NMR** (101 MHz, DMSO-d_6) δ = 173.1 (C=O), 170.4 (C=O), 170.0 (C=O), 169.9 (C=O), 169.6 (C=O), 169.5 (C=O), 169.3 (C=O), 169.2 (C=O), 155.8 (C=O), 143.8 (Fmoc-Ar), 140.7 (Fmoc-Ar), 127.7 (Fmoc-Ar), 127.1 (Fmoc-Ar), 125.3 (Fmoc-Ar), 120.2 (Fmoc-Ar), 99.9 (C1'), 77.9 (C1'), 76.2 (C4), 73.8 (C3), 73.5 (C5), 70.4 (C3'), 69.7 (C5'), 68.9 (C2'), 67.1 (C4'), 65.7 (Fmoc-CH_2), 62.5 (C6), 60.9 (C6'), 52.3 (C2), 50.3 (Asn-CH), 46.6 (Fmoc-CH), 37.1 (Asn-CH_2), 22.7 (NHC(O)CH_3), 20.7 (C(O)CH_3), 20.6 (C(O)CH_3), 20.5 (C(O)CH_3), 20.4 (C(O)CH_3), 20.4 (C(O)CH_3).**HRMS** (ESI) m/z: [M + H^+] calcd for $C_{45}H_{53}N_3O_{21}H$ 972.32443, found 972.32357.

Nγ-{2,3,4,6-tetra-O-acetyl-β-d-galactopyranoside-(1→4)-[3,4-di-O-benzoyl-2-O-(4-methoxybenzyl)-α-l-fucopyranoside-(1→3)]-6-O-(4-methoxybenzyl)-2-deoxy-2-acetamido-β-d-glucopyranosyl}-Nα-fluorenylmethoxycarbonyl-l-asparagine (4) Glycosyl azide **11** (53 mg, 45 µmol) was dissolved in dry THF (0.45 mL) and cooled to 0°C in an icebath. 75 µL of a 1 M trimethylphosphine solution in THF was added dropwise. The reaction was stirred for 5 minutes at 0°C and for 5 minutes at room temperature. H_2O (50 eq, 40 µL, 2.25 mmol) was added and the reaction was stirred for 2 hours at room temperature. The volatiles were removed *in vacuo* and the crude glycosyl amine was redissolved in DMA (450 µL). The reaction mixture was again cooled in an icebath and aspartic anhydride[60] (1 eq, 15 mg, 45µmol) was added. The reaction was stirred and allowed to warm to room temperature overnight. The solvent was removed by evaporation and the crude glycoaminoacid was subjected to silicagel column chromatography (0 → 25% acetone in DCM + 0.5% acetic acid, $\Delta_{acetone}$ = 5%) to yield the title compound (49 mg, 33 µmol, 73%). Traces of acetic acid were removed by sequential co-evaporation with dioxane (3 x 2 mL), toluene (3 x 2 mL) and $CHCl_3$ (3 x 2 mL). **$[\alpha]_D^{25}$** = -94.2 (c 1.00 in $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.98 – 7.89 (m, 2H, CH_{arom}), 7.78 – 7.64 (m, 5H, NᵞH, CH_{arom}), 7.64 – 7.51 (m, 3H, CH_{arom}), 7.51 – 7.40 (m, 3H, CH_{arom}), 7.40 – 7.19 (m, 9H, NHC(O)CH_3, CH_{arom}), 7.07 (d, J = 8.6 Hz, 2H, CH_{arom}), 6.91 (d, J = 8.6 Hz, 2H, CH_{arom}), 6.66 (d, J = 8.3 Hz, 2H, CH_{arom}), 6.41 (d, J = 8.4 Hz, 1H, NᵅH), 5.63 – 5.54 (m, 2H, H4', H3'), 5.47 (d, J = 3.4 Hz, 1H, H1'), 5.33 (d, J = 3.7 Hz, 1H, H4'), 5.09 (dd, J = 10.4, 8.0 Hz, 1H, H2''), 4.99 (t, J = 7.8 Hz, 1H, H1), 4.85 (dd, J = 10.4, 3.6 Hz, 1H, H3''), 4.81 – 4.70 (m, 1H, H5'), 4.69 – 4.43 (m, 5H, PMB-CH_2, Asn-CH, Fmoc-CH, H1''), 4.39 – 4.22 (m, 5H, Fmoc-CH_2, PMB-CHH, H6''), 4.22 – 4.03 (m, 4H, PMB-CHH, H2, H2', H4), 3.95 (t, J = 8.4 Hz, 1H, H3), 3.82 – 3.63 (m, 8H, OCH_3, 6H, OCH_3), 3.57 – 3.44 (m, 2H, H5, H5''), 2.90 – 2.72 (m, 2H, Asn-CH_2), 2.17 (s, 3H, C(O)CH_3), 2.07 – 1.91 (m, 12H, 4 x C(O)CH_3), 1.24 (d, J = 6.5 Hz, 3H, H6'). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 173.5 (C=O), 173.2 (C=O), 171.8 (C=O), 170.5 (C=O), 170.4 (C=O), 170.0 (C=O), 169.8 (C=O), 165.9 (C=O), 165.3 (C=O), 159.6 (C_q), 159.5 (C_q), 156.4 (C=O), 143.9 (Fmoc-Ar), 143.7 (Fmoc-Ar), 141.2 (Fmoc-Ar), 141.2 (Fmoc-Ar), 133.3 (CH_{arom}), 133.1 (CH_{arom}), 130.3 (CH_{arom}), 129.8 (CH_{arom}), 129.7 (CH_{arom}), 129.6 (CH_{arom}), 129.6 (C_q), 129.5 (C_q), 128.9 (C_q), 128.5 (CH_{arom}), 128.3 (CH_{arom}), 127.7 (CH_{arom}), 127.1 (CH_{arom}), 125.3 (CH_{arom}), 125.2 (CH_{arom}), 119.9 (CH_{arom}), 114.1 (CH_{arom}), 114.0 (CH_{arom}), 99.4 (C1''), 97.4 (C1'), 79.7 (C1), 76.0 (C5, C3) , 73.3 (C4), 73.3 (C2'), 73.3 (PMB-CH_2), 72.7 (PMB-CH_2), 72.5 (C4'), 71.0 (C5''), 70.8 (C3''), 70.1 (C3'), 69.3 (C2''), 67.8 (C6), 67.2 (Fmoc-CH_2), 66.9 (C4''), 65.8 (C5'), 61.0 (C6''), 55.3 (OCH_3), 55.2 (OCH_3), 53.6 (C2), 50.5 (Asn-CH), 47.1 (Fmoc-CH), 37.9 (Asn-CH_2), 22.8 (NHC(O)CH_3), 20.8 (C(O)CH_3), 20.8 (C(O)CH_3), 20.7 (C(O)CH_3), 20.6 (C(O)CH_3), 16.1 (C6'). **HRMS** (ESI) m/z: [M + Na^+] calcd for $C_{77}H_{83}N_3O_{27}Na$ 1504.51061, found 1504.51004.

Azido 3,4-di-O-benzoyl-2-O-(4-methoxybenzyl)-α-l-fucopyranoside-(1→3)-4,6-O-(4-methoxybenzylidene)-2-deoxy-2-acetamido-β-d-glucopyranoside (7) Donor **6** (1.5 eq., 1.76 mg, 3.0 mmol) and acceptor **5** (728 mg, 2.0 mmol) were co-evaporated 3 times with toluene, backfilling the flask with N_2 after every co-evaporation round, and placed under a N_2 atmosphere. The sugars were dissolved in dry DCM (36 mL) with dry DMF (4 mL). Activated 4Å molecular sieves (1 g) were added and the solution was stirred for 90 minutes. The reaction mixture was then cooled in an icebath and NIS (2.0 eq., 900 mg, 4.0 mmol) and TMSOTf (0.1 eq., 37 µL) were added. The reaction was stirred and allowed to warm to room temperature overnight. The reaction was filtered, diluted with DCM and washed with a 1:1 mixture of 10% $Na_2S_2O_3$ (aq) and saturated $NaHCO_3$ (aq). The organic layer was dried over $MgSO_4$, filtered and concentrated. Silica gel column chromatography (30% → 40% → 50% → 60% EtOAc in pentane) yielded the title compound (1.19 g, 1.42 mmol, 71%). **$[\alpha]_D^{25}$** = -144.0 (c 1.00 in $CHCl_3$). **ν_{max}/cm^{-1}** 2117.80 (N_3), 1724.29 (CO) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.00 – 7.89 (m, 2H, CH_{arom}), 7.82 – 7.75 (m, 2H, CH_{arom}), 7.64 – 7.56 (m, 1H, CH_{arom}), 7.54 – 7.40 (m, 5H, CH_{arom}), 7.33 – 7.27 (m, 2H, CH_{arom}), 7.15 – 7.08 (m, 2H, CH_{arom}), 6.88 (d, J = 8.8 Hz, 2H, CH_{arom}), 6.74 (d, J = 8.6 Hz, 2H, CH_{arom}), 6.05 (d, J = 6.9 Hz, 1H, NH), 5.73 (dd, J = 10.5, 3.3 Hz, 1H, H3'), 5.53 (s, 1H, PMP-CH_{acetal}), 5.50 (dd, J = 3.4, 1.4 Hz, 1H, H4'), 5.28 (d, J = 9.3 Hz, 1H, H1), 5.15 (d, J = 3.5 Hz, 1H, H1'), 4.61 (d, J = 11.4 Hz, 1H, PMB-CHH), 4.54 (d, J = 11.4 Hz, 1H, PMB-CHH), 4.51 – 4.42 (m, 2H, H3, H5'), 4.38 (dd, J = 10.5, 4.2 Hz, 1H, H5), 4.13 (dd, J = 10.5, 3.4 Hz, 1H, H2'), 3.84 – 3.70 (m, 7H, PMB-OCH_3,PMP-OCH_3, H6a), 3.70 – 3.57 (m, 2H, H6b, H4), 3.25 (td, J = 9.3, 6.9 Hz, 1H, H2), 1.81 (s, 3H, NHC(O)CH_3), 0.73 (d, J = 6.5 Hz, 3H, H6'). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.4 (C=O), 166.0 (C=O), 165.7 (C=O), 160.4 (C_q), 159.6 (C_q), 133.4 (CH_{arom}), 133.2 (CH_{arom}), 129.8 (CH_{arom}), 129.7 (CH_{arom}), 129.7 (CH_{arom}), 129.6 (C_q), 129.6 (C_q), 128.6 (CH_{arom}), 128.4 (CH_{arom}), 127.8 (CH_{arom}), 114.1 (CH_{arom}), 113.7 (CH_{arom}), 102.1 (PMP-CH), 98.6 (C1'), 87.9 (C1), 80.7 (C4), 75.5 (C3), 74.2 (C2'), 73.4 (PMB-CH_2), 72.6 (C4'), 70.9 (C3'), 68.6 (C6), 68.5 (C5), 65.6 (C5'), 58.4 (C2), 55.4 (OCH_3), 55.3 (OCH_3), 23.4 (NHC(O)CH_3), 15.6 (C6'). **HRMS** (ESI) m/z: [M + H^+] calcd for C44H46N4O13H 839.31341, found 839.31311.

Azido 3,4-di-O-benzoyl-2-O-(4-methoxybenzyl)-α-l-fucopyranoside-(1→3)-6-O-(4-methoxybenzyl)-2-deoxy-2-(*N*-acetylacetamido)-β-d-glucopyranoside (8) Dissacharide **7** (436 mg, 0.52 mmol) was dissolved in anhydrous DCM and DiPEA (10 eq., 870 µL, 5 mmol) and acetyl chloride (50 eq., 1.8 mL, 25 mmol) were added. The reaction was stirred for 2 hours at room temperature, after which TLC (10% EtOAc in DCM) indicated full conversion. The reaction mixture was diluted with DCM and the organic layer was washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated. Silica gel column chromatography (30% → 40% → 50% Et_2O in pentane) yielded the diacetylated intermediate (406 mg, 0.46 mmol, 89%). **$[\alpha]_D^{25}$** = -105.2 (c 0.50 in $CHCl_3$). ν_{max}/cm^{-1} 2119.23 (N_3), 1727.15 (CO) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.94 – 7.87 (m, 2H, CH_{arom}), 7.78 – 7.71 (m, 2H, CH_{arom}), 7.63 – 7.55 (m, 1H, CH_{arom}), 7.52 – 7.39 (m, 5H, CH_{arom}), 7.32 – 7.26 (m, 2H, CH_{arom}), 7.12 – 7.04 (m, 2H, CH_{arom}), 6.91 – 6.83 (m, 2H, CH_{arom}), 6.68 (d, J = 8.7 Hz, 2H, CH_{arom}), 5.75 – 5.66 (m, 2H, H1, H3'), 5.51 (s, 1H, PMP-CH_{acetal}), 5.42 (dd, J = 3.3, 1.4 Hz, 1H, H4'), 4.79 (dd, J = 9.6, 8.6 Hz, 1H, H3), 4.74 (d, J = 3.5 Hz, 1H, H1'), 4.52 – 4.37 (m, 4H, PMB-CH_2, H5', H5), 4.06 (dd, J = 10.6, 3.5 Hz, 1H, H2'), 3.85 – 3.70 (m, 8H, PMB-OCH_3, PMP-OCH_3, H6), 3.70 – 3.61 (m, 2H, H4, H2), 2.50 (s, 3H, N(C(O)CH_3)C(O)CH_3), 2.30 (s, 3H, N(C(O)CH_3)C(O)CH_3), 0.52 (d, J = 6.4 Hz, 3H, H6'). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 175.2 (C=O), 174.6 (C=O), 165.9 (C=O), 165.8 (C=O), 160.5 (C_q), 159.5 (C_q), 133.3 (CH_{arom}), 133.2 (CH_{arom}), 130.5 (CH_{arom}), 129.8 (CH_{arom}), 129.7 (CH_{arom}), 129.4 (C_q), 129.2 (C_q), 128.5 (CH_{arom}), 128.4 (CH_{arom}), 128.0 (CH_{arom}), 113.8 (CH_{arom}), 113.7 (CH_{arom}), 102.5 (PMP-CH), 98.8 (C1'), 87.5 (C1), 80.9 (C4), 73.6 (C3), 73.4 (PMB-CH_2), 72.6 (C4'), 71.8 (C2'), 71.3 (C3'), 68.6 (C6), 68.0 (C5), 65.4 (C5'), 64.1 (C2), 55.4 (OCH_3), 55.2 (OCH_3), 28.6 (N(C(O)CH_3)C(O)CH_3), 25.6 (N(C(O)CH_3)C(O)CH_3), 15.2 (C6'). HRMS (ESI) m/z: [M + Na^+] calcd for C46H48N4O14Na 903.30592, found 903.30478. The 4-methoxybenzylidene protected disaccharide (461 mg, 0.52 mmol) was dissolved in dry THF and cooled to -70°C. BH_3·THF was added as a 1.0 M solution in THF (5 eq, 2.6 mmol, 2.6 mL) and the reaction was stirred for 15 minutes at this temperature. Then Bn_2BOTf was added as a 1.0 M solution in DCM (2 eq, 1 mmol, 1 mL) and the reaction was stirred for an additional 15 minutes at -70°C. The reaction was then heated to -50°C and stirred overnight. The reaction was quenched by careful addition of 0.5 mL of Et_3N followed by 15 mL MeOH and was stirred at room temperature for 30 minutes. The reaction mixture was concentrated *in vacuo* and subjected to silica gel column chromatography (40% → 50% →60 % Et_2O in pentane). This yielded the title compound (370 mg, 0.42 mmol, 81%). **$[\alpha]_D^{25}$** = -93.2 (c 1.00 in $CHCl_3$). **ν_{max}/cm^{-1}** 2117.80 (N_3), 1724.29 (CO). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.95 – 7.88 (m, 2H, CH_{arom}), 7.80 – 7.73 (m, 2H, CH_{arom}), 7.67 – 7.59 (m, 1H, CH_{arom}), 7.54 – 7.42 (m, 3H, CH_{arom}), 7.35 – 7.26 (m, 4H, CH_{arom}), 7.11 – 7.04 (m, 2H, CH_{arom}), 6.90 (d, *J* = 8.6 Hz, 2H, CH_{arom}), 6.74 (d, *J* = 8.6 Hz, 2H, CH_{arom}), 5.67 – 5.59 (m, 3H, H1, H3', H4'), 4.93 (d, *J* = 3.6 Hz, 1H, H1'), 4.66 – 4.39 (m, 6H, PMB-CH_2, PMB-CH_2, H3, H5'), 4.10 (dd, *J* = 10.3, 3.6 Hz, 1H, H2'), 4.01 (s, 1H, 4-OH), 3.85 – 3.79 (m, 4H, H6a, OCH_3), 3.78 – 3.72 (m, 4H, H6b, OCH_3), 3.71 – 3.62 (m, 3H, H2, H4, H5), 2.41 (s, 3H, N(C(O)CH_3)C(O)CH_3), 2.37 (s, 3H, N(C(O)CH_3)C(O)CH_3)), 1.21 (d, *J* = 6.5 Hz, 3H, H6'). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 175.4 (C=O), 174.3 (C=O), 165.8 (C=O), 165.4 (C=O), 159.5 (C_q), 159.4 (C_q), 133.5 (CH_{arom}), 133.3 (CH_{arom}), 130.0 (CH_{arom}), 129.9 (CH_{arom}), 129.7 (CH_{arom}), 129.5 (CH_{arom}), 129.2 (C_q), 128.6 (CH_{arom}), 128.4 (CH_{arom}), 113.9 (CH_{arom}), 99.7 (C1'), 86.8 (C1), 82.6 (C3), 76.7 (C4), 73.4 (PMB-CH_2), 72.7 (PMB-CH_2), 72.1 (C4'), 71.4 (C5), 71.3 (C2'), 70.3 (C3'), 68.7

(C6), 66.7 (C5'), 62.3 (C2), 55.4 (OCH_3), 55.3 (OCH3), 28.4 ($N(C(O)C\underline{H}_3)C(O)CH_3$), 25.6 ($N(C(O)CH_3)C(O)C\underline{H}_3$), 16.2 (C6'). **HRMS** (ESI) m/z: [M + NH_4^+] calcd for C46H50N4O14NH4 900.36618, found 900.36581.

Azido 2,3,4,6-tetra-O-acetyl-β-d -galactopyranoside(1→4)-[3,4-di-O-benzoyl-2-O-(4-methoxybenzyl)-α-l-fucopyranoside-(1→3)]-6-O-(4-methoxybenzyl)-2-deoxy-2-(*N*-acetylacetamido)-β-d -glucopyranoside (10) Donor **9** (5 eq., 737 mg, 1.5 mmol) and acceptor **8** (266 mg, 0,3 mmol) were co-evaporated 3 times with toluene, backfilling the flask with N_2 after every co-evaporation round, and placed under a N_2 atmosphere. The sugars were dissolved in dry DCM and activated 4Å molecular sieves (300 mg) were added. The mixture was stirred 30 minutes at room temperature and subsequently cooled to -10°C. TMS triflate (0.1 eq, 5.6 µl, 0.03 mmol) was added and the reaction was stirred over night at -10°C. The reaction was quenched by addition of TEA (0.1 mL) and allowed to warm to room temperature. The reaction mixture was diluted with DCM, filtered, further diluted with toluene and concentrated *in vacuo*. Silica gel column chromatography (40 → 70% Et_2O in pentane, Δ=5%) yielded the title compound (283 mg, 0.23 mmol, 77%). **$[\alpha]_D^{25}$** = -104.4 (c 1.00 in $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.99 – 7.92 (m, 2H, CH_{arom}), 7.78 – 7.71 (m, 2H, CH_{arom}), 7.65 – 7.58 (m, 1H, CH_{arom}), 7.50 – 7.42 (m, 3H, CH_{arom}), 7.36 – 7.30 (m, 2H, CH_{arom}), 7.26 (dd, J = 8.3, 7.4 Hz, 3H, CH_{arom}), 7.13 (d, J = 8.6 Hz, 2H, CH_{arom}), 6.97 (d, J = 8.7 Hz, 2H, CH_{arom}), 6.70 (d, J = 8.6 Hz, 2H, CH_{arom}), 5.66 (dd, J = 3.3, 1.4 Hz, 1H, H4'), 5.64 – 5.54 (m, 2H, H3', H1), 5.38 (dd, J = 3.6, 1.0 Hz, 1H, H4''), 5.14 (q, J = 6.5 Hz, 1H, H5'), 5.04 (dd, J = 10.3, 8.3 Hz, 1H, H2''), 4.86 – 4.67 (m, 5H, H3'', PMB-C$\underline{H}$H, H1', H1'', H3), 4.60 (dd, J = 11.5, 6.1 Hz, 1H, H6''a), 4.50 (s, 2H, PMB-CH_2), 4.46 – 4.38 (m, 2H, PMB-CH$\underline{H}$, H6''b), 4.11 (dd, J = 10.6, 3.7 Hz, 1H, H2'), 4.05 (dd, J = 10.0, 8.9 Hz, 1H, H4), 3.88 – 3.68 (m, 8H, OCH_3, H6, OCH_3), 3.59 (t, J = 9.4 Hz, 1H, H2), 3.56 – 3.49 (m, 2H, H5, H5''), 2.51 (s, 3H, $N(C(O)CH_3)C(O)C\underline{H}_3$), 2.25 (s, 3H, $N(C(O)C\underline{H}_3)C(O)CH_3$), 2.24 (s, 3H, $C(O)CH_3$), 2.10 (s, 3H, $C(O)CH_3$), 2.00 (s, 3H, $C(O)CH_3$), 1.98 (s, 3H, $C(O)CH_3$), 1.24 (d, J = 6.6 Hz, 3H, H6'). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 175.5 (C=O), 174.8 (C=O), 170.9 (C=O), 170.5 (C=O), 170.2 (C=O), 168.9 (C=O), 166.1 (C=O), 165.4 (C=O), 159.8 (C_q), 159.5 (C_q), 133.3 (CH_{arom}), 133.0 (CH_{arom}), 130.8 (CH_{arom}), 130.0 (C_q), 130.0 (CH_{arom}), 129.9 (CH_{arom}), 129.8 (C_q), 129.6 (CH_{arom}), 129.4 (C_q), 128.5 (CH_{arom}), 128.3 (CH_{arom}), 114.3 (CH_{arom}), 113.7 (CH_{arom}), 99.7 (C1''), 97.8 (C1'), 86.9 (C1), 76.6 (C5''), 74.3 (C4), 73.7 (PMB-CH_2), 73.5 (PMB-CH_2), 72.9 (C4'), 71.8 (C3', C3, C2'), 71.3 (C3''), 71.1 (C5), 69.2 (C2''), 67.0 (C4''), 66.9 (C6), 64.9 (C5'), 64.3 (C2), 61.1 (C6''), 55.4 (OCH_3), 55.3 (OCH_3), 28.8 ($N(C(O)CH_3)C(O)\underline{C}H_3$), 25.8 ($N(C(O)\underline{C}H_3)C(O)CH_3$), 21.0 ($C(O)\underline{C}H_3$), 20.9 ($C(O)\underline{C}H_3$), 20.8 ($C(O)\underline{C}H_3$), 20.7 ($C(O)\underline{C}H_3$), 16.0 (C6'). **HRMS** (ESI) m/z: [M + Na^+] calcd for $C_{60}H_{68}N_4O_{23}Na$ 1235.41666, found 1235.41654.

Azido 2,3,4,6-tetra-O-acetyl-β-d-galactopyranoside-(1→4)-[3,4-di-O-benzoyl-2-O-(4-methoxybenzyl)-α-l-fucopyranoside-(1→3)]-6-O-(4-methoxybenzyl)-2-deoxy-2-acetamido-β-d-glucopyranoside (11) Protected trisaccharide **10** (61 mg, 50 µmol) was dissolved in dry THF (1 mL) and N,N-dimethylaminopropylamine (10 eq, 63 µL, 0.5 mmol) was added. The reaction was stirred for 30 minutes at room temperature and another portion of N,N-dimethylaminopropylamine (10 eq, 63 µL, 0.5 mmol) was added. After further stirring for 1 hour, TLC (15% EtOAc in DCM) indicated full conversion. The reaction mixture was diluted with DCM and washed with 1 M HCl (aq). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Silica gel column chromatography (0% → 10% → 15% → 20% EtOAc in DCM) yielded the title compound (51 mg, 42 µmol, 87%). **$[\alpha]_D^{25}$** = -76.0 (c 1.00 in $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.02 – 7.94 (m, 2H, CH_{arom}), 7.79 – 7.73 (m, 2H, CH_{arom}), 7.65 – 7.58 (m, 1H, CH_{arom}), 7.51 – 7.44 (m, 3H, CH_{arom}), 7.33 – 7.28 (m, 3H, CH_{arom}), 7.17 (d, J = 8.6 Hz, 2H, , CH_{arom}), 6.95 (d, J = 8.6 Hz, 2H, CH_{arom}), 6.76 (d, J = 8.7 Hz, 2H, CH_{arom}), 6.03 (d, J = 7.5 Hz, 1H, NH), 5.68 – 5.61 (m, 2H, H4', H3'), 5.38 (dd, J = 3.6, 1.1 Hz, 1H, H4''), 5.26 (d, J = 8.2 Hz, 1H, H1), 5.21 (d, J = 3.6 Hz, 1H, H1'), 5.08 (dd, J = 10.4, 8.1 Hz, 1H, H2''), 4.99 – 4.86 (m, 2H, H5', H3''), 4.73 – 4.67 (m, 2H, PMB-C$\underline{H}$H, H1''), 4.64 (d, J = 11.6 Hz, 1H, PMB-C$\underline{H}$H), 4.57 (d, J = 11.7 Hz, 1H, PMB-CH$\underline{H}$), 4.46 – 4.31 (m, 4H, PMB-CH$\underline{H}$, H6'', H3), 4.18 (dd, J = 9.7, 3.5 Hz, 1H, H1'), 4.06 (t, J = 8.3 Hz, 1H, H4), 3.85 – 3.78 (m, 5H, OCH_3, H6), 3.75 (s, 3H, OCH_3), 3.64 – 3.55 (m, 2H, H5'', H5), 3.33 (q, J = 8.1 Hz, 1H, H2), 2.21 (s, 3H, $C(O)CH_3$), 2.07 (s, 3H, $C(O)CH_3$), 2.02 (s, 3H, $C(O)CH_3$), 1.98 (s, 3H, $C(O)CH_3$), 1.89 (s, 3H, $NHC(O)C\underline{H}_3$), 1.25 (d, J = 6.6 Hz, 3H, H6'). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.0 (C=O), 170.6 (C=O), 170.5 (C=O), 170.2 (C=O), 169.4 (C=O), 166.1 (C=O), 165.3 (C=O), 159.6 (C_q), 159.6 (C_q), 133.3 (CH_{arom}), 133.0 (CH_{arom}), 129.9 (CH_{arom}), 129.8 (C_q), 129.8 (CH_{arom}), 129.7 (CH_{arom}), 129.7 (CH_{arom}), 128.6 (CH_{arom}), 128.3 (CH_{arom}), 114.1 (CH_{arom}), 114.0 (CH_{arom}), 99.6 (C1''), 97.3 (C1'), 87.2 (C1), 76.7 (C5), 73.6 (C2', C4), 73.5 (C3), 73.4 (PMB-CH_2), 73.1 (PMB-CH_2), 72.8 (C4'), 71.1 (C5''), 71.0 (C3''), 71.0 (C3'), 69.2 (C2''), 67.4 (C6), 67.0 (C4''), 65.2 (C5'), 61.1 (C6''), 57.0 (C2), 55.4 (OCH_3), 55.3 (OCH_3), 23.5 ($NHC(O)C\underline{H}_3$), 20.9 ($C(O)CH_3$), 20.9 ($C(O)CH_3$), 20.7 ($C(O)CH_3$), 16.1 (C6'). **HRMS** (ESI) m/z: [M + Na^+] calcd for $C_{58}H_{66}N_4O_{22}Na$ 1193.40609, found 1193.40573.

Azido 2,3,4,6-tetra-O-acetyl-β-d-galactopyranoside-(1→4)-6-(*t*-butyldimethylsilyl)-2-deoxy-2-acetamido-β-d -glucopyranoside (13) Donor **9** (1.5 eq, 368 mg, 0.75 mmol) and acceptor **12** (180 mg, 0.5 mmol) were co-evaporated 3 times with toluene and put under N_2. The sugars were dissolved in dry DCM (5 mL) and stirred with activated 4 Å molecular sieves (0.5 g) for 2 hours at room temperature. The reaction was cooled to -40°C and $BF_3{\cdot}Et_2O$ (1.6 eq, 100 µL, 0.8 mmol) was added. The reaction was stirred at -40°C overnight and formation of disaccharide product was confirmed by TLC (70% EtOAc in pentane). The reaction was quenched with Et_3N (0.5 mL), diluted with DCM, filtered, diluted with toluene and concentrated. Silica gel column chromatography (60% → 70% → 80% EtOAc in pentane) yielded the title compound (193 mg, 0.28 mmol, 56%). **$[\alpha]_D^{20}$** = +5,8 (c 1.00 in $CHCl_3$) **ν_{max}/cm^{-1}** 2115.65 (N_3), 1752.19 (CO). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.17 (d, J = 8.5 Hz, 1H, NH), 5.40 (dd, J = 3.4, 1.0 Hz, 1H, H4'), 5.22 (dd, J = 10.5, 8.0 Hz, 1H, H2'), 4.99 (dd, J = 10.5, 3.4 Hz, 1H, H3'), 4.69 – 4.61 (m, 2H, H1, H1'), 4.15 (d, J = 6.5 Hz, 2H, H6'), 4.06 (bs, 1H, 3-OH), 4.01 (t, J = 6.5 Hz, 1H, H5'), 3.90 – 3.72 (m, 3H, H6, H3), 3.69 – 3.57 (m, 2H, H4, H2), 3.43 (ddd, J = 9.6, 3.4, 1.5 Hz, 1H, H5), 2.17 (s, 3H, $C(O)C\underline{H}_3$), 2.08 (s, 3H, $C(O)C\underline{H}_3$), 2.07 (s, 3H, $C(O)C\underline{H}_3$), 2.03 (s, 3H, $NHC(O)C\underline{H}_3$), 1.99 (s, 3H, $C(O)C\underline{H}_3$), 0.92 (s, 9H, tBu), 0.11 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.0 (C=O), 170.6 (C=O), 170.2 (C=O), 170.1 (C=O), 169.4 (C=O), 101.6 (C1'), 87.9 (C1), 80.5 (C4), 76.7 (C5), 71.9 (C3), 71.4 (C5'), 70.9 (C3'), 68.7 (C2'), 66.8 (C4'), 61.4 (C6'), 61.2 (C6), 55.6 (C2), 25.9 (tBu), 23.4 ($NHC(O)\underline{C}H_3$), 20.7 ($C(O)\underline{C}H_3$), 20.6 ($C(O)\underline{C}H_3$), 20.6 ($C(O)\underline{C}H_3$), 20.6 ($C(O)\underline{C}H_3$), 18.3 (Si-$\underline{C}$), -5.0 (Si-CH_3), -5.2 (Si-CH_3). **HRMS** (ESI) m/z: [M + Na^+] calcd for $C_{28}H_{46}N_4O_{14}SiNa$ 713.2672, found 713.2695.

Azido 2,3,4,6-tetra-O-acetyl-β-d-galactopyranoside-(1→4)-6,3-di-O-acetyl-2-deoxy-2-acetamido-β-d-glucopyranoside (14) Silyl protected disaccharide **13** (517 mg, 0.75 mmol) was dissolved in dry THF (7.5 mL) in a plastic tube. HF·pyridine complex (16 eq, 310 µL, 12 mmol) was added and the reaction was stirred overnight. Completion of the reaction was assessed by TLC (100% EtOAc) and the reaction mixture was diluted with DCM. The organic layer was washed with aqueous saturated $NaHCO_3$ (3:1 ratio of DCM:H_2O) and the aqueous layer was back extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated, yielding 380 mg (0.66 mmol) of crude intermediate. The crude desilylated disaccharide was dissolved in dry pyridine (6.6 mL) and cooled to 0°C in an ice bath. Acetic anhydride (10 eq, 620 µL, 6.6 mmol) and DMAP (0.1 eq, 9 mg, 0.07 mmol) were added. The reaction was stirred overnight at room temperature and reaction completion was confirmed by TLC (100% EtOAc). The reaction was quenched with methanol and concentrated. Pyridine traces were removed with toluene co-evaporation. Silica gel column chromatography (70% → 80% → 90% EtOAc in pentane) yielded the title compound (421 mg, 0.64 mmol, 85%). **$[\alpha]_D^{20}$** = -26,4 (c 1.00 in $CHCl_3$) **ν_{max}/cm^{-1}** 2116.37 (N_3), 1744.32 (CO)**. ^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.53 (d, J = 9.6 Hz, 1H, NH), 5.37 (dd, J = 3.4, 1.2 Hz, 1H, H4'), 5.19 – 5.03 (m, 2H, H3, H2'), 4.99 (dd, J = 10.5, 3.4 Hz, 1H, H3'), 4.64 – 4.50 (m, 3H, H1, H1', H6a), 4.21 – 4.01 (m, 4H, H6', H6b, H2), 3.93 (t, J = 7.1 Hz, 1H, H5'), 3.84 (t, J = 9.1 Hz, 1H, H4), 3.73 (ddd, J = 9.1, 5.0, 2.2 Hz, 1H, H5), 2.17 (s, 3H, $C(O)C\underline{H}_3$), 2.14 (s, 3H, $C(O)C\underline{H}_3$), 2.11 (s, 3H, $C(O)C\underline{H}_3$), 2.07 (s, 3H, $C(O)C\underline{H}_3$), 2.07 (s, 3H, $C(O)C\underline{H}_3$), 1.99 (s, 3H, $NHC(O)C\underline{H}_3$), 1.97 (s, 3H, $C(O)C\underline{H}_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ =

171.0 (C=O), 170.5 (C=O), 170.4 (C=O), 170.3 (C=O), 170.1 (C=O), 170.0 (C=O), 169.3 (C=O), 101.3 (C1'), 88.3 (C1), 76.1 (C4), 74.5 (C5), 73.1 (C3), 70.8 (C3'), 70.7 (C5'), 69.0 (C2'), 66.6 (C4'), 61.9 (C6), 60.6 (C6'), 53.0 (C2), 23.0 (NHC(O)$\underline{C}H_3$), 20.9 (C(O)$\underline{C}H_3$), 20.8 (C(O)$\underline{C}H_3$), 20.6 (C(O)$\underline{C}H_3$), 20.6 (C(O)$\underline{C}H_3$), 20.5 (C(O)$\underline{C}H_3$), 20.5 (C(O)$\underline{C}H_3$). **HRMS** (ESI) m/z: [M + Na^+] calcd for $C_{26}H_{36}N_4O_{16}Na$ 683.2019, found 683.2029.

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[1] R. Dobson, G. Giovannoni, *Eur. J. Neurol.* **2019**, *26*, 27–40.

[2] L. Steinman, *Nat. Immunol.* **2001**, *2*, 762–764.

[3] N. Snaidero, M. Simons, *J. Cell Sci.* **2014**, *127*, 2999–3004.

[4] J. N. Whitaker, *J. Neuroimmunol.* **1982**, *2*, 201–207.

[5] M. Adelmann, J. Wood, I. Benzel, P. Fiori, H. Lassmann, J. M. Matthieu, M. V. Gardinier, K. Dornmair, C. Linington, *J. Neuroimmunol.* **1995**, *63*, 17–27.

[6] J. Jung, E. Dudek, M. Michalak, *Biochim. Biophys. Acta - Mol. Cell Res.* **2014**, *1853*, 2115–2121.

[7] D. Pham-Dinh, M. G. Mattei, J. L. Nussbaum, G. Roussel, P. Pontarotti, N. Roeckel, I. H. Mather, K. Artzt, K. F. Lindahl, A. Dautigny, *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 7990–4.

[8] C. Linnington, M. Webb, P. L. Woodhams, *J. Neuroimmunol.* **1984**, *6*, 387–396.

[9] P. Peschl, M. Bradl, R. Höftberger, T. Berger, M. Reindl, *Front. Immunol.* **2017**, *8*, 529.

[10] I. Mendel, N. K. de Rosbo, A. Ben-Nun, *Eur. J. Immunol.* **1995**, *25*, 1951–1959.

[11] S. A. Jagessar, I. R. Holtman, S. Hofman, E. Morandi, N. Heijmans, J. D. Laman, B. Gran, B. W. Faber, S. I. van Kasteren, B. J. L. Eggen, B. A. 't Hart, *J. Immunol.* **2016**, *197*, 1074–88.

[12] C. Araman, M. E. Van Gent, N. J. Meeuwenoord, N. Heijmans, M. H. S. Marqvorsen, W. Doelman, B. W. Faber, B. A. T'Hart, S. I. Van Kasteren, *Biochemistry* **2019**, *58*, 763–775.

[13] D. Eisenberg, M. Jucker, *Cell* **2012**, *148*, 1188–1203.

[14] D. D. Wood, M. A. Moscarello, J. M. Bilbao, P. O'Connors, *Ann. Neurol.* **1996**, *40*, 18–24.

[15] M. Frenkel-Pinter, M. Richman, A. Belostozky, A. Abu-Mokh, E. Gazit, S. Rahimipour, D. Segal, *Chem. - A Eur. J.* **2016**, *22*, 5945–5952.

[16] C. J. Bosques, B. Imperiali, *Proc. Natl. Acad. Sci.* **2003**, *100*, 7593–7598.

[17] C. Araman, R. E. Thompson, S. Wang, S. Hackl, R. J. Payne, C. F. W. Becker, *Chem. Sci.* **2017**, *8*, 6626–6632.

[18] T. B. H. Geijtenbeek, S. J. van Vliet, E. A. Koppel, M. Sanchez-Hernandez, C. M. J. E. Vandenbroucke-Grauls, B. Appelmelk, Y. van Kooyk, *J. Exp. Med.* **2003**, *197*, 7–17.

[19] E. van Liempt, C. M. C. Bank, P. Mehta, J. J. García-Vallejo, Z. S. Kawar, R. Geyer, R. A. Alvarez, R. D. Cummings, Y. van Kooyk, I. van Die, *FEBS Lett.* **2006**, *580*, 6123–6131.

[20] J. J. García-Vallejo, J. M. Ilarregui, H. Kalay, S. Chamorro, N. Koning, W. W. Unger, M. Ambrosini, V. Montserrat, R. J. Fernandes, S. C. M. Bruijns, J. R. T. van Weering, N. J. Paauw, T. O'Toole, J. van Horssen, P. van der Valk, K. Nazmi, J. G. M. Bolscher, J. Bajramovic, C. D. Dijkstra, B. A. 't Hart, Y. van Kooyk, *J. Exp. Med.* **2014**, *211*, 1465–1483.

[21] T. Buskas, Y. Li, G.-J. Boons, *Chem. - A Eur. J.* **2004**, *10*, 3517–3524.

[22] H. Cai, R. Zhang, J. Orwenyo, J. Giddens, Q. Yang, C. C. LaBranche, D. C. Montefiori, L.-X. Wang, *ACS Cent. Sci.* **2018**, *4*, 582–589.

[23] M. H. S. Marqvorsen, C. Araman, S. I. Van Kasteren, *Bioconjug. Chem.* **2019**, *30*, 2715–2726.

[24] M. H. S. Marqvorsen, S. Paramasivam, W. Doelman, A. J. Fairbanks, S. I. van Kasteren, *Chem. Commun.* **2019**, *55*, 5287–5290.

[25] M. Aebi, *Biochim. Biophys. Acta - Mol. Cell Res.* **2013**, *1833*, 2430–2437.

[26] J. D. McIntosh, M. A. Brimble, A. E. S. Brooks, P. R. Dunbar, R. Kowalczyk, Y. Tomabechi, A. J. Fairbanks, *Chem. Sci.* **2015**, *6*, 4636–4642.

[27] Y. Tomabechi, G. Krippner, P. M. Rendle, M. A. Squire, A. J. Fairbanks, *Chem. - A Eur. J.* **2013**, *19*, 15084–15088.

[28] L. Liu, A. R. Prudden, C. J. Capicciotti, G. P. Bosman, J. Y. Yang, D. G. Chapla, K. W. Moremen, G. J. Boons, *Nat. Chem.* **2019**, *11*, 161–169.

[29] Z. Wang, Z. S. Chinoy, S. G. Ambre, W. Peng, R. McBride, R. P. De Vries, J. Glushka, J. C. Paulson, G. J. Boons, *Science (80-.).* **2013**, *341*, 379–383.

[30] X. Geng, V. Y. Dudkin, M. Mandal, S. J. Danishefsky, *Angew. Chemie Int. Ed.* **2004**, *43*, 2562–2565.

[31] B. J. Appelmelk, I. van Die, S. J. van Vliet, C. M. J. E. Vandenbroucke-Grauls, T. B. H. Geijtenbeek, Y. van Kooyk, *J. Immunol.* **2003**, *170*, 1635–1639.

[32] Y. Guo, H. Feinberg, E. Conroy, D. A. Mitchell, R. Alvarez, O. Blixt, M. E. Taylor, W. I. Weis, K. Drickamer, *Nat. Struct. Mol. Biol.* **2004**, *11*, 591–598.

[33] C. M. Fehres, H. Kalay, S. C. M. Bruijns, S. A. M. Musaafir, M. Ambrosini, L. Van Bloois, S. J. Van Vliet, G. Storm, J. J. Garcia-Vallejo, Y. Van Kooyk, *J. Control. Release* **2015**, *203*, 67–76.

[34] N. Frisont, M. E. Taylor, E. Soilleux, M. T. Bousser, R. Mayer, M. Monsigny, K. Drickamer, A. C. Roche, *J. Biol. Chem.* **2003**, *278*, 23922–23929.

[35] G. Timpano, G. Tabarani, M. Anderluh, D. Invernizzi, F. Vasile, D. Potenza, P. M. Nieto, J. Rojo, F. Fieschi, A. Bernardi, *ChemBioChem* **2008**, *9*, 1921–1930.

[36] K. von dem Bruch, H. Kunz, *Angew. Chemie Int. Ed. English* **1994**, *33*, 101–103.

[37] T. Inazu, K. Kobayashi, *Synlett* **1993**, *1993*, 869–870.

[38] A. Bianchi, A. Bernardi, *J. Org. Chem.* **2006**, *71*, 4565–4577.

[39] C. Toonstra, M. N. Amin, L. X. Wang, *J. Org. Chem.* **2016**, *81*,

6176–6185.

[40] L. Liao, F.-I. Auzanneau, *J. Org. Chem.* **2005**, *70*, 6265–6273.

[41] L. Liao, F.-I. Auzanneau, *Org. Lett.* **2003**, *5*, 2607–2610.

[42] J. M. Hernández-Torres, J. Achkar, A. Wei, *J. Org. Chem.* **2004**, *69*, 7206–7211.

[43] S. M. Andersen, M. Heuckendorff, H. H. Jensen, *Org. Lett.* **2015**, *17*, 944–947.

[44] S. Sato, Y. Ito, T. Nukada, Y. Nakahara, T. Ogawa, *Carbohydr. Res.* **1987**, *167*, 197–210.

[45] S. I. van Kasteren, H. B. Kramer, H. H. Jensen, S. J. Campbell, J. Kirkpatrick, N. J. Oldham, D. C. Anthony, B. G. Davis, *Nature* **2007**, *446*, 1105–1109.

[46] Y. Tomabechi, M. A. Squire, A. J. Fairbanks, *Org. Biomol. Chem.* **2014**, *12*, 942–955.

[47] C. P. Yang, C. S. Su, *J. Org. Chem.* **1986**, *51*, 5186–5191.

[48] N. Yamamoto, A. Takayanagi, T. Sakakibara, P. E. Dawson, Y. Kajihara, *Tetrahedron Lett.* **2006**, *47*, 1341–1346.

[49] L. Urge, L. Otvos, E. Lang, K. Wroblewski, I. Laczko, M. Hollosi, *Carbohydr. Res.* **1992**, *235*, 83–93.

[50] A. Isidro-Llobet, M. Álvarez, F. Albericio, *Chem. Rev.* **2009**, *109*, 2455–2504.

[51] S. A. Jagessar, N. Heijmans, E. L. A. Blezer, J. Bauer, J. H. Blokhuis, J. A. M. Wubben, J. W. Drijfhout, P. J. van den Elsen, J. D. Laman, B. A. '. Hart, *Eur. J. Immunol.* **2012**, *42*, 217–227.

[52] A. Carrillo-Vico, M. D. Leech, S. M. Anderton, *J. Immunol.* **2010**, *184*, 2839–46.

[53] T. B. H. Geijtenbeek, G. C. F. Van Duijnhoven, S. J. Van Vliet, E. Krieger, G. Vriend, C. G. Figdor, Y. Van Kooyk, *J. Biol. Chem.* **2002**, *277*, 11314–11320.

[54] F. Chiodo, M. Marradi, B. Tefsen, H. Snippe, I. van Die, S. Penadés, *PLoS One* **2013**, *8*, e73027.

[55] V. Porkolab, E. Chabrol, N. Varga, S. Ordanini, I. Sutkevičiute, M. Thépaut, M. J. García-Jiménez, E. Girard, P. M. Nieto, A. Bernardi, F. Fieschi, *ACS Chem. Biol.* **2018**, *13*, 600–608.

[56] J. J. Garcia-Vallejo, Y. van Kooyk, *Trends Immunol.* **2013**, *34*, 482–486.

[57] H. Lassmann, M. Bradl, *Acta Neuropathol.* **2017**, *133*, 223–244.

[58] J. J. García-Vallejo, M. Ambrosini, A. Overbeek, W. E. van Riel, K. Bloem, W. W. J. Unger, F. Chiodo, J. G. Bolscher, K. Nazmi, H. Kalay, Y. van Kooyk, *Mol. Immunol.* **2013**, *53*, 387–397.

[59] S. I. Gringhuis, J. den Dunnen, M. Litjens, M. van der Vlist, T. B. H. Geijtenbeek, *Nat. Immunol.* **2009**, *10*, 1081–1088.

[60] F. M. Ibatullin, S. I. Selivanov, *Tetrahedron Lett.* **2009**, *50*, 6351–6354.

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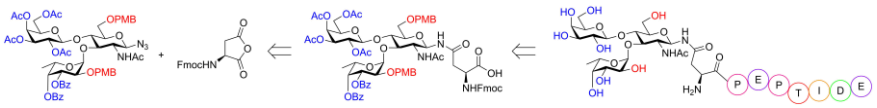

A novel asparagine SPPS building block harboring a Lewis X type DC-SIGN ligand was synthesized and incorporated into the immunodominant portion of Myelin Oligodendrocyte Glycoprotein (MOG_{31-55}). This glycopeptide was evaluated for its ability to inhibit citrullination induced aggregation of MOG_{35-55}, to bind to DC-SIGN *in vitro* and for its immunomodulatory effects on human dendritic cells, with the Lewis X yielding a tolerogenic response.

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