The Glc_2Man_2-fragment of the *N*-glycan precursor – a novel ligand for the glycan-binding protein *malectin*?†‡

Lisa N. Müller,[a] Claudia Muhle-Goll[b] and Moritz B. Biskup*[a]

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The Glcα(1→3)Glcα(1→3)Manα(1→2)Man tetrasaccharide (Glc_2Man_2-fragment), a substructure of the natural *N*-glycan precursor, was synthesized. The interaction of this fragment with the protein *malectin*, a carbohydrate binding protein localized in the endoplasmatic reticulum, was investigated by $^1H^{15}N$ HSQC experiments and isothermal calorimetry. The chemical shift perturbations of nuclei in the protein's backbone caused by the binding of the Glc_2Man_2-fragment to *malectin* suggest a binding mode like the known ligand nigerose.

Introduction

The majority of all proteins expressed in living organisms is subjected to posttranslational modifications; among the most widely spread of those modifications is the addition of carbohydrate moieties to the protein's side chains to form glycoproteins.[1] Over 90% of the glycan structures present in eukaryotic proteins are glycosidically linked to the side chains of asparagines (→ *N*-glycans). These *N*-glycan structures participate in a multitude of functions *e.g.* as ligands in various recognition processes, mediators in pathogen interactions, stabilizers against enzymatic degradation, solubility enhancers, or modifiers of an enzyme's charge, structure or orientation. This plethora of adopted roles is matched by an equally diverse multitude of *N*-glycan structures.[2]

While the glycan structures present in an organism are highly diverse, the first steps of their biogenesis are highly conserved. *N*-Glycan structures in eukaryotic organisms are derived from a common lipid-bound, tetradecasaccharidic precursor ($Glc_3Man_9GlcNAc_2$-glycan; **A** in Fig. 1A), that is co-translationally transferred onto the nascent polypeptide chain while still in the endoplasmic reticulum (ER).

Subsequently the glycan moiety is trimmed to a common core region present in all *N*-glycans. The early stages of this trimming process occur in the ER and the resultant *N*-glycan structures are closely associated with the correct folding process of the newly formed protein.[3] Only recently *malectin*, a membrane-bound ER protein, was identified to exhibit preferential binding to an early stage form of *N*-glycan trimming. By carbohydrate microarray experiments a selective binding of *malectin* to the $Glc_2Man_7GlcNAc_2$-form (Glc_2-*N*-glycan; **A** without the residues G1, D2 and D3) was detected. Furthermore, the structure of a

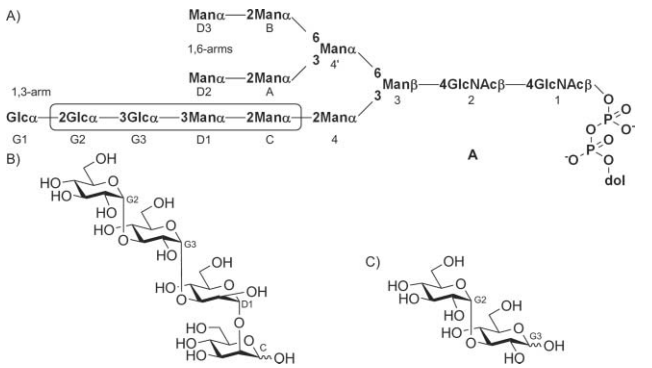


Fig. 1 (A) Lipid-bound $Glc_3Man_9GlcNAc_2$-*N*-glycan precursor **A** (dol = dolichol); (B) Glc_2Man_2-fragment, assumed binding region for the carbohydrate binding protein *malectin*; (C) known *malectin* ligand nigerose (Glcα1→3Glc).

complex of *malectin* with nigerose (Glcα1-3Glc; Fig. 1C), was determined by NMR experiments. *Malectin's* location in the ER, its remarkable selectivity for the Glc_2-*N*-glycan and its wide conservation across different species, has led to the assumption that *malectin* is closely involved in the quality assurance of *N*-glycan biogenesis.[4] However, the exact mode of *malectin's* binding to the Glc_2-*N*-glycan has not yet been determined. Despite the fact that a binding of *malectin* to mannose could not be observed, the interaction with the Glc_2-*N*-glycan appeared to be much stronger than only with nigerose (deduced from the carbohydrate microarray data). This leads to the assumption that a cooperative binding effect to one or several of the adjacent mannose residues of the Glc_2-*N*-glycan takes place.

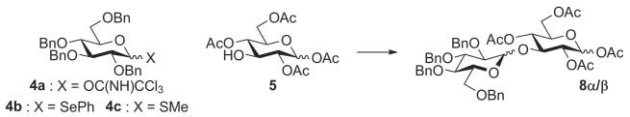
While the synthesis of fully processed *N*-glycan structures has been of interest for the last 30 years, the synthesis of early stage *N*-glycan trimming products or substructures of these has not received such widespread attention. Some syntheses in this field have been reported, *e.g.* the group of Ito has recently reported on their synthesis of the complete $Glc_3Man_9GlcNAc_2$-glycan, as well as the trimmed $Glc_2Man_9GlcNAc_2$-glycan,[5] and Fairbanks *et al.* have recently accomplished a synthesis of the 1,3-arm's terminal Glc_3Man moiety.[6] However, to our knowledge, there is no literature precedent for the preparation of small substructures of the trimmed 1,3-arm.

[a]*Karlsruher Institut für Technologie – KIT, Institut für Organische Chemie, Fritz-Haber-Weg 6, 76131, Karlsruhe, Germany. E-mail: biskup@kit.edu; Fax: +49 721 608-5637; Tel: +49 721 608-8795*
[b]*Karlsruher Institut für Technologie – KIT, Institut für Biologische Grenzflächen (IBG-2), POB 3640, 76021, Karlsruhe, Germany*

† Dedicated to Professor Dr Richard R. Schmidt on the occasion of his 75th birthday.

‡ Electronic supplementary information (ESI) available: $^1H^{15}N$ HSQC spectra of *malectin* with/without **1**, experimental details for compounds **2c**, **4c** and **6–11** , and NMR spectra for compounds **1–3**, **4c** and **6–12α**. See DOI: 10.1039/c004502k

Table 1 Formation of disaccharide **8α/β** from different donors



	Donor[a]	Acceptor	Conditions	Yield[b]	α/β ratio[c]
1	**4a**	**5**	TMSOTf, Et_2O, RT, 25 min	~65%	2 : 1–3 : 1
2[d]	**4b**	**5**	NIS, AgOTf, 4 Å MS, CH_2Cl_2, Et_2O, RT, 30 min	~75%	1.2 : 1–1.5 : 1
3[d]	**4c**	**5**	NIS, AgOTf, 4 Å MS, CH_2Cl_2, Et_2O, RT, 30 min	70–80%	1 : 1
4	**4c**	**5**	$CuBr_2$, $^nBu_4NBr$, 4 Å MS, DCE, DMF, RT, 3–5 d	65–75%	6 : 1–8 : 1

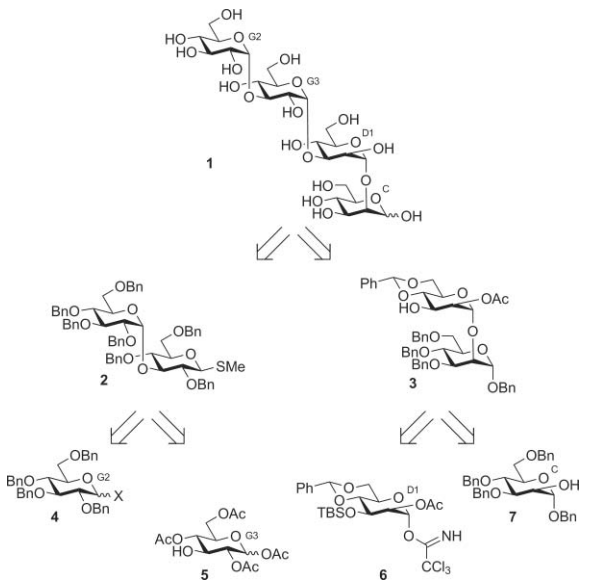
[a] **4a**: trichloroacetimidyl α-glucoside; **4b**: phenyl β-selenoglucoside; **4c**: methyl β-thioglucoside. [b] Determined from the isolated mass of epimers **8α/β**. [c] Estimated from ^{1}H NMR spectra. [d] Substitution of Et_2O by THF did not lead to an increase in diastereoselectivity.

Here, we report on our chemical synthesis of the G2-G3-D1-C motif of the *N*-glycan precursor (Glc_2Man_2-fragment; highlighted region in Fig. 1A or chemical structure depicted in Fig. 1B). Using this defined carbohydrate structure in NMR-based chemical shift perturbation experiments, we hope to gain further information on the Glc_2-*N*-glycan's binding epitope with respect to *malectin* and a possible participation of the adjacent mannose units.

Results and discussion

Synthesis of the Glc_2Man_2-fragment

Inspecting the Glc_2Man_2-fragment **1** (Scheme 1) it is evident that the main challenge in preparing **1** resides in the glucose α1→3-linkages between the G2- and G3-residues and the G3- and D1-residues of the *N*-glycan precursor, respectively.



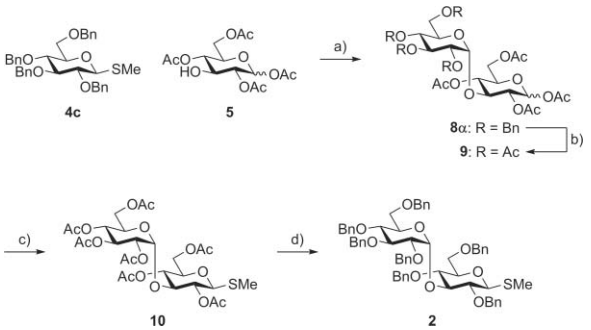
Scheme 1 Retrosynthetic analysis of the *N*-glycan precursor's Glc_2Man_2-fragment **1**, which can be traced to the monosaccharide building blocks **4–7** (X = OC(NH)CCl_3 (**4a**), SePh (**4b**), SMe (**4c**)).

The formation of these 1,2-*cis*-configured glycosides cannot be aided by classical neighbouring group assistance.[7] However, the α-1→2-linkage between the mannose residues D1 and C is readily accessible by making use of the participation of an *O*-acyl residue located at the C2-position of the D1-building block during the glycosylation. Thus, our approach to the tetrasaccharide **1** was based on a block glycosylation strategy relying on building blocks carrying a general *O*-benzyl based protecting group scheme (Scheme 1). This leads to nigerose building block **2**[8] and dimannose **3** as key intermediates in the synthesis. Retrosynthetically these disaccharides can be traced to the corresponding monosaccharide derivatives **4–7**, which are accessible from D-glucose and D-mannose, respectively. The preparation of the building blocks **4–7** closely followed known procedures and is detailed in the ESI.‡

In our initial attempts to efficiently prepare the diglucose donor **2**, we investigated a variety of glucose donors (**4a–c**; *cf.* Table 1) in the coupling of **4** and **5** with regard to the yields and the ratio of α/β-epimers obtained. The trichloroacetimidate donor **4a**[9] and the seleno glycoside **4b**[6,10] afforded the desired disaccharide **8** in useful yields of 65–75% upon rapid activation of the glycoside donor. However, the selectivity with regard to the α/β-configuration of the newly formed glycosidic bond was moderate (at best α/β ratios of 3 : 1 were obtained); the use of different coordinating solvents[11] (*e.g.* diethylether or THF) did not increase the diastereoselectivity of the reaction (Table 1 entries 1 and 2).

When thioglycoside **4c** was used under reaction conditions as employed for glycosylations with **4a** or **4b**, that is a rapid activation of the donor and reliance on the solvent effect to ensure a stereoselective reaction, disaccharide **8α/β** was obtained in good yields with no diastereoselectivity observed (Table 1 entry 3). Based on literature precedent[8] we switched the mode of activation to a metal bromide mediated variant[12] of Lemieux's *in situ* anomerisation methodology,[13] thus we were able to obtain the desired disaccharide **8α/β** in yields of 65–75% and with diastereoselectivities of α/β > 7 : 1 (Table 1 entry 4). To our delight, the epimers could easily be separated at this stage by conventional chromatography on silica gel, allowing an efficient access of up to several tens of grams of **8α**.

The conversion of disaccharide **8α** to the desired thioglycosyl donor **2** has previously been described by Takeo *et al.*;[8] however, that route requires the handling of toxic tin species to introduce the methyl thioglycoside. Thus we decided to investigate an alternative approach to accomplish the conversion of the *O*-acetyl group at the reducing end into an *S*-methyl group. Our approach (Scheme 2) requires the installation of a uniform *O*-acetyl protecting group

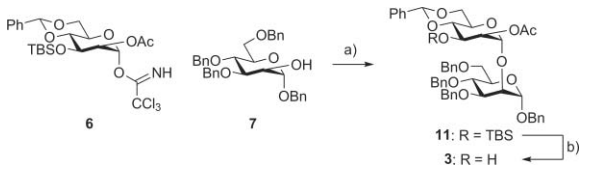


Scheme 2 Preparation of the G2-G3-glycosyl donor **2** from D-Glucose precursors **4c** and **5**; (a) $CuBr_2$, $^nBu_4NBr$, 4 Å MS, DCE, DMF, RT, 5 d, 68%; (b) i. H_2, $Pd(OH)_2/C$, MeOH, RT, 12 h; ii. Ac_2O, pyridine, RT, over night, 88% over 2 steps; (c) i. HBr, HOAc, 0 °C → RT, 120 min; ii. thiourea, acetone, reflux, 60 min; iii. Na_2SO_3, K_2CO_3, $CHCl_3$–H_2O, RT, 60 min; iv. Et^iPr_2N, MeI, CH_2Cl_2, RT, 60 min, 90% over 4 steps; (d) i. NaOMe (~0.02 M), MeOH, RT, 4 h; ii. BnBr, NaH, DMF, RT, over night, 71% over two steps.

pattern as a first step. This was achieved by catalytic cleavage of the *O*-benzyl groups by hydrogenolysis in the presence of Pearlman's catalyst[14] and subsequent treatment with acetic anhydride in pyridine to afford the fully *O*-acetylated **9** in 88% yield in two synthetic steps.

An easily scalable route was chosen to form the *S*-methyl thioglycoside **10** in a 4-step sequence[15] from **9** in 90% yield (the purification of intermediates was not required). This approach compares favourably with Takeo's tin-based methodology, for which yields of ~85% were reported. At this stage the final *O*-benzyl protecting groups in **2** are installed in 71% yield by removing the ester groups in fully *O*-acetylated **10** under Zemplén conditions,[16] followed by exhaustive *O*-benzylation.

The synthesis of the glycosyl acceptor, dimannose **3** (Scheme 3), was achieved by glycosylating the D-mannose acceptor **7** carrying a free 2-OH group with the trichloroacetimidate donor **6,** which carried a 2-*O*-acetyl group to ensure the formation of the desired 1,2-*trans*-configured glycosidic bond.

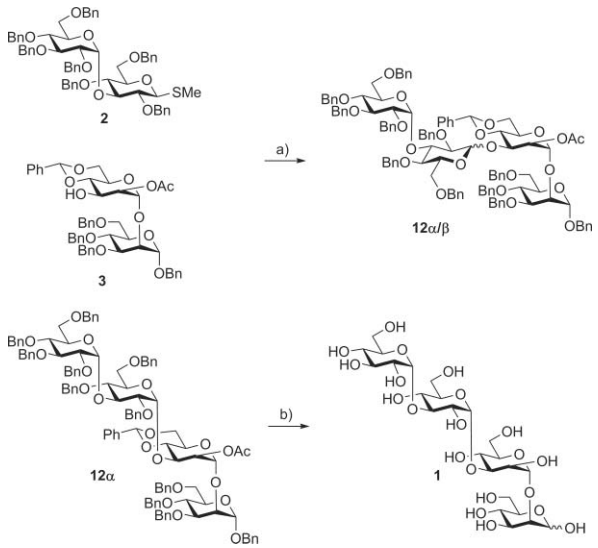


Scheme 3 Preparation of the D1-C-glycosyl acceptor **3** from the mannose building blocks **6** and **7**; (a) TMSOTf, CH_2Cl_2, 0 °C → RT, 30 min, 90%; (b) $(HF)_x$·pyr, pyridine, RT, overnight, 79%.

This glycosylation was effected by the action of trimethylsilyl triflate as a promoter in dichloromethane to afford the disaccharide **11** in excellent yield (90%). Subsequent cleavage of the 3-*O*-*tert*-butyldimethylsilyl protecting group from the terminal mannose moiety with hydrogen fluoride·pyridine complex in pyridine allowed an efficient access to the 3-OH free glycosyl acceptor **3** in 79% yield.

The glycosylation of the G2-G3 moiety **2** with the D1-C moiety **3** to afford the fully protected tetrasaccharide **12** was anticipated to proceed under conditions similar to those reported for the glycosylation of **4c** and **5** (*vide supra*). While the formation of a mixture of diastereoisomeric tetrasaccharides **12α/β** was observed under these reaction conditions in moderate yields of <50% (ratio of the diastereoisomers was not determined), we were not able to isolate the desired tetrasaccharide **12α** as a uniform compound. Instead we observed that the glycosyl acceptor **3** is consumed slower than in the corresponding glycosylation of the monosaccharide **5** with the *S*-methyl thioglycoside **4c** to afford **8α/β** (Scheme 2). After seven days some amounts of the acceptor **3** still remained, while the donor **2** had partially decomposed.

To overcome this mismatch of reactivities, we reconsidered the activation of the *S*-methyl thioglycoside **2** with methyl triflate.[17] These reactions were carried out in the presence of diethylether and 1,2-dimethoxyethane as coordinating solvents[11] to effect preferential formation of the 1,2-*cis* glycosidic linkage. This more S_N1-like glycosylation reaction proceeded decidedly faster and afforded a mixture of the diastereoisomers **12α/β** (*e.g.* 61% yield, α/β ratio 2.5 : 1; Scheme 4). Fortunately a separation of the obtained mixture of isomers was possible by standard column chromatography on silica gel, directly affording **12α** in amounts of several hundred milligrams.



Scheme 4 Glycosylation towards the fully protected G2-G3-D1-C moiety and liberation of title tetrasaccharide **1**; (a) MeOTf, 4 Å MS, Et_2O/1,2-dimethoxyethane, 0 °C → RT, 3.5 h, 61% (α/β = 2.5 : 1); (b) i. Na, NH_3/THF, −78 °C, 120 min; ii. NH_4Cl, NH_3 −78 °C → RT; iii. gel filtration, >90% from **12α**.

Subjecting fully protected **12α** to Birch-like conditions[18] removed the *O*-benzyl groups as well as the *O*-benzylidene group. Under these reaction conditions the concomitant cleavage of the 3-*O*-acetyl group on the D1-mannose moiety allowed complete deprotection in one step to afford the fully deprotected tetrasaccharide. After purification by desalting and gel filtration of the title compound **1**, the Glc_2Man_2-fragment, was afforded in over 90% yield as a mixture of the anomers at the reducing end.

Interaction studies

The chemical shifts of NMR-active nuclei are sensitive to alterations of their electronic and/or conformational environments as *e.g.* induced by binding of a ligand molecule. Consequently, we investigated the interaction between *malectin* and the

tetrasaccharide **1** by chemical shift perturbation in $^{1}H^{15}N$ HSQC spectra. The pattern of the corresponding chemical shift changes per residue is comparable to the one obtained for the known ligand nigerose[4] (Fig. 2). A superposition of the HSQC spectra of the free and Glc_2Man_2-bound form of *malectin* is depicted in the ESI.‡

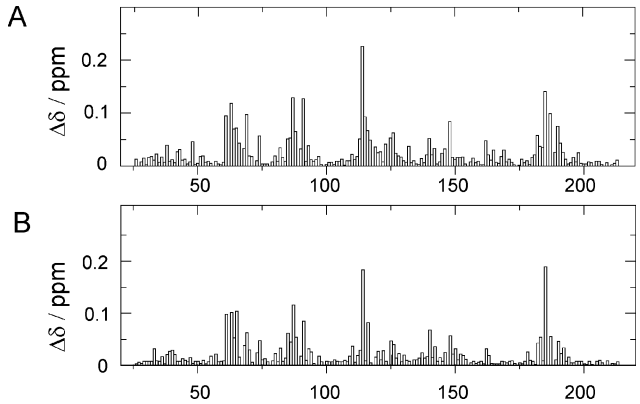


Fig. 2 Comparison of the chemical shifts changes $\Delta\delta$ induced by the addition of (A) Glc_2Man_2-fragment **1** and (B) nigerose; abscissa labelled with amino acid numbers in the *malectin* sequence.

It was expected that the additional mannose residues present at the reducing end of the Glc_2Man_2-fragment would mainly affect the region close to the nigerose binding site. According to the determined *malectin*-nigerose complex structure (PBD accession number 2K46)[19] the region following Tyr89 or Tyr185 was most strongly influenced by the binding of nigerose. Consequently, we explicitly inspected the chemical shift changes in these areas (Fig. 2A). However, significant chemical shift changes additional to those already observed for nigerose (Fig. 2B) could not be detected. These observations lead us to the assumption, that the Glc_2Man_2-fragment is bound by *malectin* and it is bound by the same region of *malectin* as nigerose is.

However, earlier results[4] had shown that at least in the case of the *malectin*-nigerose interaction, large chemical shift changes cannot necessarily be interpreted as a direct interaction with the bound ligand. For example, Phe117 had a very low chemical shift change despite measurable nOE contacts to the glucose residue at the reducing end of the nigerose, whereas Glu114 displayed the highest observed shift change without any direct contact to the bound carbohydrate as evidenced by nOE contacts. The lack of additional chemical shift changes, thus offered no additional information with regard to *malectin's* binding epitope for the Glc_2Man_2-fragment **1**.

To substantiate the results obtained from NMR experiments the binding affinity of *malectin* to tetrasaccharide **1** was determined by isothermal calorimetry. The determined dissociation constant ($K_D = 12.3$ μM) is comparable to the value previously determined for nigerose ($K_D = 26.3$ μM).[4] With observed binding affinities that lie in the same order of magnitude the existence of significant, additional interactions between the D1- and C-residues of the Glc_2Man_2-fragment and *malectin* seems unlikely. This leads us to the assumption, that *malectin* reliably recognizes only the nigerose motif in the Glc_2Man_2-fragment and that the stronger interaction observed with the Glc_2-*N*-glycan, may be mediated by residues on the 1,6-arms of the Glc_2-*N*-glycan.

Conclusion

In conclusion, we have established a concise, preparative access to the Glc_2Man_2-fragment **1** of the *N*-glycan precursor. Hallmark features of our synthesis are the successful construction of Glcα(1→3)-linkages between the rings G2-G3 and G3-D1, and the global deprotection of fourteen hydroxyl groups in one single step to afford title compound **1** in high yield and purity.

At the current stage, without further knowledge of the structure of the *N*-glycan-*malectin*-complex, we find no evidence for interactions of the additional two D-mannose residues in **1** with the protein *malectin*.

Experimental section

General methods

Solvents were purified by distillation and dried by standard procedures. Solvents for flash chromatography were purchased in technical quality and redistilled. Organic solvents for HPLC (MeCN, MeOH) were purchased from Fischer Scientific. Thin layer chromatography (TLC) was performed on E. Merck Silica Gel 60 F_{254} plates (0.2 mm) and E. Merck RP-18 F_{254} plates (0.2 mm). The plates were visualized by immersion in mostain (200 mL 10% H_2SO_4, 10 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 200 mg $Ce(SO_4)_2$), 10% H_2SO_4 or $KMnO_4$ solution (1% in water, 1% $NaHCO_3$) followed by heating (165 °C). Preparative flash chromatography was carried out on Macherey-Nagel Silica Gel 60 (43–60 μm) at a positive pressure of 0.2–0.4 bar. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker WM 250 Cryospec, AM 400, DRX 500 or Avance 600 instruments, respectively. Proton chemical shifts are reported in ppm relative to signal from residual solvent protons or Me_4Si as internal standard. Assignments of protons and carbons were carried out with the aid of 400 or 600 MHz spectra: COSY, HMQC, nOESY, ROESY, TOCSY. Multiplicities are reported as they appear in the spectra. They are designated as: s = single, d = doublet, t = triplet, q = quartet, qu = quintet, dd = doublet of doublets, m = multiplet, bs = broad single. Diastereotopic geminal protons are designated with subscripts a and b; non-distinguishable diastereotopic protons are designated n.d. Carbohydrate residues in disaccharides and larger are differentiated with superscripts (*e.g.* ′, ″, …); monosaccharides or the carbohydrate residue at the reducing end carry no superscripts. Measurements of optical rotations were performed on a Perkin-Elmer polarimeter 241 MC (1 dm cell). MALDI-MS were obtained on a Bruker Biflex MALDI-TOF instrument with 2,5-dihydroxybenzoic acid (DHB), 2′,4′,6′-trihydroxyacetophenone monohydrate (THAP) or α-cyano-4-hydroxycinnamic acid (CHCA) as matrix (positive mode).

NMR experiments

^{15}N-labelled His_6-*malectin* was prepared as previously described.[4] NMR spectra were acquired at 22 °C in 20 mM potassium phosphate buffer pH 6.8, 150 mM KCl and 2 mM DTT. $^{1}H^{15}N$ HSQC spectra of 250 μM His_6-*malectin* in the absence or presence of 1 mM Glc_2Man_2-fragment **1** were acquired on a Bruker Avance II 600 spectrometer equipped with a broadband triple resonance probe, with 8 scans per increment and a total of 128 increments in the indirect dimension. Details on the experiments used for

chemical shift assignment of the protein have been previously published.[4] Data were processed with NMRPIPE[20] and analyzed using NMRVIEW.[21] The difference in chemical shift $\Delta\delta$ was calculated with the formula $\Delta\delta = \sqrt{((\Delta\delta_{HN})^2 + (\Delta\delta_N/10)^2)}$.

Isothermal microcalorimetry

ITC measurements were carried out using a VP-ITC Microcalorimeter (Microcal, Northhampton, MA, USA) in 20 mM phosphate buffer pH 6.8, 150 mM KCl and 1 mM TCEP. A typical titration consisted of sequential injections (10 µl each) of a Glc_2Man_2-solution (1.5 mM) into a *malectin* sample (50 µM), at time intervals of five minutes, to ensure that the titration peak returned to the baseline.

α-D-Glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-D-mannopyranose (1)

Into a two-neck Schlenk flask equipped with a glass stirbar and a Dewar-type condenser ammonia (~20 mL) was condensed at −78 °C. Sodium sand (50 mg, 2.18 mmol, 130 eq.) was added, whereupon the solution turned a deep blue colour. It was stirred for 10 min at −78 °C until all sodium was dissolved. Then a solution of tetrasaccharide (30 mg, 17 µmol, 1 eq.) in anhydrous THF (2.00 mL) was added dropwise *via* a syringe and the reaction was stirred for 2 h at −78 °C. Solid NH_4Cl (200 mg) was added and the solution was stirred until the blue colour disappeared. The cooling bath was removed so that the ammonia could volatilize. The resulting white solid was dried under high vacuum for 1 h and then purified by gel filtration on a Bio-Rad P-2 gel filtration column (~15 × 1.5 cm, H_2O : MeOH 20%). The remaining salts were removed by precipitating them with MeOH. After lyophilization the unprotected tetrasaccharide **1** (11 mg, 16.5 µmol, 98%) was obtained as a mixture of the anomers at the reducing end; in aqueous solution the α-configured compound predominates (TLC: $R_f = 0.03$ (silica gel, EtOAc : MeOH 20%); $[\alpha]_D^{20} = +60.9$ ($c = 1.2$, H_2O); δ_H(600 MHz; D_2O) 5.43 (d, $^3J_{1,2} = 1.9$ Hz, 1H, H-1), 5.41 (d, $^3J_{1,2} = 3.9$ Hz, 1H, H-1‴), 5.33 (d, $^3J_{1,2} = 3.9$ Hz, 1H, H-1″), 5.09 (d, $^3J_{1,2} = 2.0$ Hz, 1H, H-1′), 4.30 (dd, $^3J_{2,3} = 3.4$ Hz, $^3J_{1,2} = 1.9$ Hz, 1H, H-2′), 4.06 (ddd, $^3J_{4,5} = 10.0$ Hz, $^3J_{5,6a} = 4.2$ Hz, $^3J_{5,6b} = 2.0$ Hz, 1H, H-5‴), 4.01 (1H, H-2), 4.01 (1H, H-3′), 4.00 (n.d., 2H, H-$6_{a,b}$), 3.96 (1H, H-3″), 3.95-3.83 (n.d., 2H, H-$6'_{a,b}$), 3.91 (1H, H-5″), 3.89 (1H, H-3), 3.88 (1H, H-4′), 3.88-3.77 (n.d., 2H, H-$6''_{a,b}$), 3.86 (1H, H-4), 3.84 (n.d., 2H, H-$6'''_{a,b}$), 3.82 (1H, H-5′), 3.80 (1H, H-3‴), 3.71 (1H, H-2‴), 3.75 (1H, H-5), 3.68 (1H, H-4″), 3.62 (dd, $^3J_{2,3} = 10.0$ Hz, $^3J_{1,2} = 4.0$ Hz, 1H, H-2‴), 3.50 (t, $^3J_{3,4} \approx {}^3J_{4,5} = 9.7$ Hz, 1H, H-4‴); δ_C(101 MHz; D_2O) 102.5 (C1′), 101.0 (C1″), 99.7 (C1‴), 93.0 (C1), 80.2, 79.6, 78.8, 73.7, 73.4, 73.0, 72.6, 72.2, 72.2, 70.9, 70.7, 70.5, 70.3, 69.9, 67.6, 66.6, 61.5, 61.4, 61.0, 60.9 ppm; non-decoupled $^1H^{13}C$ HMQC (151 MHz; $CDCl_3$): $J_{C1,H-1} = 172$ Hz, $J_{C1',H-1'} = 172$ Hz, $J_{C1'',H-1''} = 173$ Hz, $J_{C1''',H-1'''} = 172$ Hz; MALDI-MS (pos. mode, THAP): $[M+Na]^+$ calcd.: 689.2, found: 689.3; $[M+K]^+$ calcd.: 705.2, found: 705.2; $C_{24}H_{42}O_{21}$ (666.6)).

Benzyl (2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (3)

In a Teflon flask disaccharide **11** (1.03 g, 1.09 mmol, 1 eq.) was dissolved in dry pyridine (12 mL). $(HF)_x$·pyridine (65–70% in pyridine, 1.09 mL, 8.16 mmol) was added and the mixture was stirred overnight. Saturated aqueous $NaHCO_3$ and Et_2O were added, the layers were separated and the aqueous layer was extracted with Et_2O (2 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified by column chromatography (silica gel, Hx : EtOAc 33%). The disaccharide **3** (720 mg, 0.87 mmol, 79%) was obtained as colourless oil (TLC: $R_f = 0.49$ (silica gel, Hx : EtOAc 50%); $[\alpha]_D^{20} = +58.8$ ($c = 1.0$, $CHCl_3$); δ_H(600 MHz; $CDCl_3$) 7.47-7.44 (m, 2H, ar), 7.38-7.21 (m, 21H, ar), 7.16-7.13 (m, 2H, ar), 5.54 (s, 1H, benzylidene), 5.42 (dd, $^3J_{2',3'} = 3.5$ Hz, $^3J_{1',2'} = 1.3$ Hz, 1H, H-2′), 5.01 (d, $^3J_{1',2'} = 1.3$ Hz, 1H, H-1′), 4.92 (d, $^3J_{1,2} = 1.5$ Hz, 1H, H-1), 4.82 (d, $^2J = 10.7$ Hz, 1H, CH_2Ph), 4.71 (d, $^2J = 11.8$ Hz, 1H, CH_2Ph), 4.69 (d, $^2J = 11.7$ Hz, 1H, CH_2Ph), 4.68 (d, $^2J = 12.1$ Hz, 1H, CH_2Ph), 4.63 (d, $^2J = 11.7$ Hz, 1H, CH_2Ph), 4.55 (d, $^2J = 12.1$ Hz, 1H, CH_2Ph), 4.51 (d, $^2J = 10.7$ Hz, 1H, CH_2Ph), 4.47 (d, $^2J = 11.8$ Hz, 1H, CH_2Ph), 4.25 (dt, $^3J_{3',4'} = 9.5$ Hz, $^3J_{2',3'} \approx {}^3J_{3',OH} = 3.1$ Hz, 1H, H-3′), 4.07 (dd, $^2J = 10.3$ Hz, $^3J_{6a',5'} = 4.7$ Hz, 1H, H-6a'), 3.98 (t, $^3J_{1,2} \approx {}^3J_{2,3} \approx 2$ Hz, 1H, H-2), 3.95 (dd, $^3J_{2,3} = 2.8$ Hz, $^3J_{3,4} = 9.2$ Hz, 1H, H-3), 3.93 (t, $^3J_{3,4} \approx {}^3J_{4,5} = 9.2$ Hz Hz, 1H, H-4), 3.90 (dd, $^3J_{5',4'} = 9.5$ Hz, $^3J_{5',6a,b'} = 4.6$ Hz, 1H, H-5′), 3.85 (t, $^3J_{3',4'} \approx {}^3J_{4',5'} = 9.5$ Hz, 1H, H-4′), 3.81 (ddd, $^3J_{4,5} = 9.3$ Hz, $^3J_{5,6a} = 4.6$ Hz, $^3J_{5,6b} = 1,3$ Hz, 1H, H-5), 3.78 (dd, $^2J = 10.7$ Hz, $^3J_{5,6a} = 4.7$ Hz, 1H, H-6a), 3.72 (d, $^2J = 10.3$ Hz, 1H, H-6b'), 3.69 (dd, $^2J = 10.7$ Hz, $^3J_{5,6b} = 1.3$ Hz, 1H, H-6b), 2.23 (bs, 1H, OH), 2.12 (s, 3H, CH_3CO) ppm; δ_C(151 MHz; $CDCl_3$) 170.2, 138.4, 138.2, 137.1, 137.0, 129.2, 128.4, 128.4, 128.3, 128.3, 128.3, 128.0, 127.9, 127.9, 127.7, 127.6, 127.5, 127.5, 127.5, 126.3, 102.2 (benzylidene), 100.0 (C1′), 97.9 (C1), 79.7, 78.8, 75.2, 75.1, 74.6, 73.3, 72.3, 72.1, 71.8, 69.0, 68.9, 68.5, 67.2, 63.7, 21.0; non-decoupled $^1H^{13}C$ HMQC (151 MHz, $CDCl_3$): $J_{C,Hbenzylidene} = 161.5$ Hz, $J_{C1,H-1} = 170.1$ Hz, $J_{C1',H-1'} = 173.7$ Hz; MALDI-MS (pos. mode, DHB): $[M+Na]^+$ calcd.: 855.3, found: 855.1; $[M+K]^+$ calcd.: 871.3, found: 871.0; $C_{49}H_{52}O_{12}$ (832.9)).

Benzyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (12α)

Donor **2** (668 mg, 0.67 mmol, 1.15 eq.) and acceptor **3** (482 mg, 0.58 mmol, 1 eq.) were dried together under high vacuum. After dissolving under argon atmosphere in anhydrous diethyl ether (20 mL) and anhydrous 1,2-dimethoxyethane (1.33 mL) 4 Å molecular sieve (3 g) was added and the mixture was stirred for 30 min. The solution was cooled to 0 °C and methyl triflate (459 µL, 4.05 mmol, 7 eq.) was added. The ice bath was removed and the mixture was stirred for 3.5 h at room temperature. Et_3N (1 mL) was added and the solution was diluted with diethyl ether. After stirring for a few minutes the mixture was filtered over a pad of Celite. The solids were washed successively with ether and the combined filtrates were concentrated in vacuo. The crude product was purified by column chromatography on (silica gel, Hx : EtOAc 16%). Three fractions were collected: pure β-epimer (80 mg, 47 µmol), followed by a mixture of the epimers (120 mg, 67 µmol, α/β ~ 1 : 7) and finally the pure α-epimer (430 mg, 240 µmol) as colourless oils. In total the glycosylation reaction afforded **12α/β** (630 mg, 352 µmol, 61% α/β ~ 2.5 : 1). (TLC: **12α**: $R_f = 0.53$; **12β**: $R_f = 0.56$ (silica gel, Hx : EtOAc 33%); **12α**: $([\alpha]_D^{20} = +49.1$

(c = 1.1, $CHCl_3$); δ_H(600 MHz; $CDCl_3$) 7.46-7.02 (m, 60H, ar), 5.58 (d, $^3J_{1''',2'''}$ = 3.3 Hz, 1H, H-1‴), 5.55 (d, $^3J_{2',3'}$ = 3.4 Hz, $^3J_{1',2'}$ = 1.1 Hz, 1H, H-2′), 5.51 (dd, $^3J_{1'',2''}$ = 3.4 Hz, 1H, H-1″), 5.30 (s, 1H, benzylidene), 5.13 (d, $^3J_{1',2'}$ = 1.1 Hz, 1H, H-1′), 5.02 (d, $^3J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.96-4.90 (m, 3H, CH_2Ph), 4.85 (d, ^2J = 10.4 Hz, 1H, CH_2Ph), 4.84 (d,^2J = 10.6 Hz, 1H CH_2Ph), 4.79 (d, ^2J = 12.0 Hz, 1H, CH_2Ph), 4.79 (d, ^2J = 11.2 Hz, 1H, CH_2Ph), 4.74-4.70 (m, 3H, CH_2Ph), 4.67 (d, ^2J = 12.5 Hz, 1H, CH_2Ph), 4.62 (m, 2H, CH_2Ph), 4.55 (d, ^2J = 11.6 Hz, 1H, CH_2Ph), 4.55 (d, ^2J = 10.6 Hz, 1H, CH_2Ph), 4.54 (d, ^2J = 12.0 Hz, 1H, CH_2Ph), 4.53 (d, ^2J = 9.3 Hz, 1H, CH_2Ph), 4.51-4.45 (m, 2H, CH_2Ph, H-3′), 4.45-4.35 (m, 3H, CH_2Ph, H-5‴), 4.30 (d, ^2J = 12.2 Hz, 1H, CH_2Ph), 4.25 (d, ^2J = 11.6 Hz, 1H, CH_2Ph), 4.24 (d, ^2J = 11.2 Hz, 1H, CH_2Ph), 4.19 (t, $^3J_{2'',3''} \approx {}^3J_{3'',4''}$ = 9.3 Hz, 1H, H-3″), 4.15 (t, $^3J_{3',4'} \approx {}^3J_{4',5'}$ = 9.9 Hz, 1H, H-4′), 4.12 (m, 2H, H-$6_{a,b}$), 4.10 (d, $^3J_{1,2}$ = 1.9 Hz, 1H, H-2), 4.03 (m, 1H, H-3), 4.02 (m, 1H, H-3‴), 4.00 (m, 1H, H-5′), 3.93-3.89 (m, 2H, H-4, H-5″), 3.87 (dd, $^3J_{4'',5''}$ = 10.1 Hz, $^3J_{3'',4''}$ = 9.3 Hz, 1H, H-4″), 3.78 (m, 1H, H-5), 3.76 (m, 2H, H-$6_{a,b}$'), 3.66 (t, $^3J_{3''',4'''} \approx {}^3J_{4''',5'''}$ = 9.4 Hz, 1H, H-4‴), 3.60 (dd, ^2J = 10.9 Hz, $^3J_{5'',6a''}$ = 2.1 Hz, 1H, H-6_a''), 3.56 (m, 1H, H-6_b''), 3.53 (dd, $^3J_{2''',3'''}$ = 9.8 Hz, $^3J_{1''',2'''}$ = 3.7 Hz, 1H, H-2‴), 3.47 (dd,$^3J_{2'',3''}$ = 9.6 Hz, $^3J_{1'',2''}$ = 3.5 Hz, 1H, H-2″), 3.25 (dd, ^2J = 11.0 Hz, $^3J_{5''',6a'''}$ = 1.8 Hz, 1H, H-6_a'''), 3.23 (dd, ^2J = 10.9 Hz, $^3J_{5''',6a'''}$ = 2.5 Hz, 1H, H-6_b'''), 2.21 (s, 3H, CH_3CO); δ_C(151 MHz; $CDCl_3$) 169.6, 138.8 (2C), 138,7, 138.2, 138.1, 138.1, 138.0, 137.9, 137.9, 137.8, 136.9 (2C), 129.1, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.4, 127.3, 127.2, 127.2, 126.8, 126.3, 126.2, 102.2 ($C_{benzylidene}$), 100.1 (C1′), 97.8 (C1), 97.4 (C1‴), 96.3 (C1″), 82.2, 79.7, 79.4, 79.2, 78.0, 77.9, 76.7, 75.4, 75.2, 75.1, 74.8, 74.7, 74.4, 73.3, 73.3, 73.2, 72.6, 72.1, 71.9, 70.2, 70.2, 70.0, 69.6, 69.0, 68.9, 68.5, 68.0, 63.7, 20.8 ppm; non-decoupled ^{1}H,^{13}C HMQC (151 MHz, $CDCl_3$): $J_{C,Hbenzylidene}$ = 163 Hz, $J_{C1,H\text{-}1}$ = 172 Hz, $J_{C1',H\text{-}1'}$ = 175 Hz, $J_{C1'',H\text{-}1''}$ = 173 Hz, $J_{C1''',H\text{-}1'''}$ = 175 Hz; MALDI-MS (pos. mode, DHB): $[M+Na]^+$ calcd.: 1809.8, found: 1809.8; $[M+K]^+$ calcd.: 1825.7, found: 1825.8; $C_{110}H_{114}O_{22}$ (1788.1)).

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