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Oligosaccharides

Synthesis of High-Mannose Oligosaccharide Analogues through Click Chemistry: True Functional Mimics of Their Natural Counterparts Against Lectins?

Marc François-Heude,[a] Alejandro Méndez-Ardoy,[b] Virginie Cendret,[a] Pierre Lafite,[c] Richard Daniellou,[c] Carmen Ortiz Mellet,[b] José M. García Fernández,[d] Vincent Moreau,*[a] and Florence Djedaïni-Pilard*[a]

Abstract: Terminal "high-mannose oligosaccharides" are involved in a broad range of biological and pathological processes, from sperm-egg fusion to influenza and human immunodeficiency virus infections. In spite of many efforts, their synthesis continues to be very challenging and actually represents a major bottleneck in the field. Whereas multivalent presentation of mannopyranosyl motifs onto a variety of scaffolds has proven to be a successful way to interfere in recognition processes involving high-mannose oligosaccharides, such constructs fail at reproducing the subtle differences in affinity towards the variety of protein receptors (lectins) and antibodies susceptible to binding to the natural ligands. Here we report a family of functional high-mannose oligosaccharide mimics that reproduce not only the terminal mannopyranosyl display, but also the core structure and the branching pattern, by replacing some inner mannopyranosyl units with triazole rings. Such molecular design can be implemented by exploiting "click" ligation strategies, resulting in a substantial reduction of synthetic cost. The binding affinities of the new "click" high-mannose oligosaccharide mimics towards two mannose specific lectins, namely the plant lectin concanavalin A (ConA) and the human macrophage mannose receptor (rhMMR), have been studied by enzyme-linked lectin assays and found to follow identical trends to those observed for the natural oligosaccharide counterparts. Calorimetric determinations against ConA, and X-ray structural data support the conclusion that these compounds are not just another family of multivalent mannosides, but real "structural mimics" of the high-mannose oligosaccharides.

Introduction

"High-mannose" type *N*-glycans play a pivotal role in the quality control of *N*-glycoprotein biosynthesis as well as in their intracellular transportation.[1] Moreover, they are involved in many biological recognition phenomena at the surface of the cell-membrane. For instance, they cover the surface of many pathogenic microorganisms and are implicated in bacteria and virus adhesion to host-cells,[1b] being a target of the immune system. All high-mannose glycans share the common pentasaccharide core Manα-(1→3)[Manα-(1→6)]Manβ-(1→4)GlcNAcβ-(1→4)GlcNAc ($Man_3GlcNAc_2$), bearing up to six additional mannose residues (Figure 1).

Fragments of the mannooligosaccharide moiety, Man_3 to Man_9, behave as the recognition motifs (glycotopes) towards different receptor partners. Given their biological significance, high-mannose oligosaccharides have inspired numerous works focused on their partial or total synthesis and on their applications in carbohydrate-based vaccines or glyco-targeted drug delivery systems, among others. The preparation of such molecules remains very challenging, however, and the design of more readily accessible artificial mimics that are able to efficiently emulate Man_{8-9} in molecular recognition events is highly sought-after in the field of glycobiology.

Inspired by the work of Crich and Yang[2] and of Dondoni and co-workers,[3] which suggested that replacing internal monosaccharide units by 1,2,3-triazole rings preserves the overall shape of their natural oligosaccharide analogues, we recently reported the preparation of a "click" Man_8 mimic (pseudo-Man_8) by exploiting the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC).[4] Encouraged by the strong af-

[a] *Dr. M. François-Heude, Dr. V. Cendret, Dr. V. Moreau, Prof. F. Djedaïni-Pilard*
LG-2A FRE-CNRS 3517, Institut de Chimie de Picardie
Université de Picardie Jules Verne, 33 Rue Saint-Leu
80039 Amiens Cedex 1 (France)
E-mail: vincent.moreau@u-picardie.fr
florence.pilard@u-picardie.fr

[b] *Dr. A. Méndez-Ardoy, Prof. C. Ortiz Mellet*
Departamento de Química Orgânica, Facultad de Química
Universidad de Sevilla, Profesor García González 1
41012 Sevilla (Spain)

[c] *Dr. P. Lafite, Prof. R. Daniellou*
ICOA, UMR CNRS 7311, Université d'Orléans
rue de Chartres, BP6759, 45067 Orléans cedex 2 (France)

[d] *Prof. J. M. García Fernández*
Instituto de Investigaciones Quımicas, CSIC-Universidad de Sevilla
Americo Vespucio 49, Isla de la Cartuja
41092 Sevilla (Spain)

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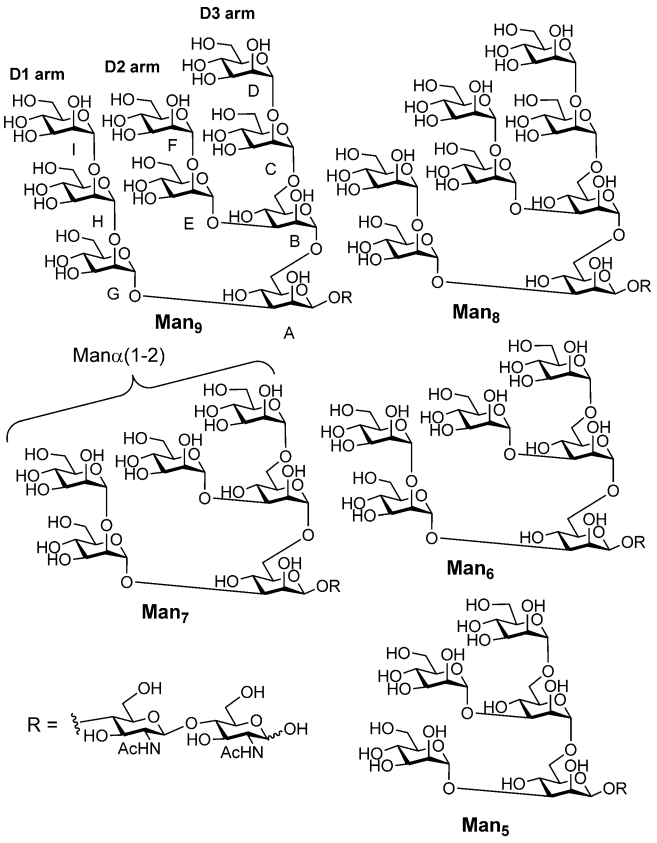


Figure 1. Structure of high-mannoses glycans Man_{5-9}.

finity displayed by this compound towards the mannose-specific lectin concanavalin A (ConA), we have now accomplished the synthesis of a series of "click" Man_n mimics family including pseudo-Man_{3-5} and pseudo-Man_{8-9} (**1**–**5**; Figure 2).

The final compounds are equipped with an aminoethyl spacer arm at the reducing end to highlight their suitability as building blocks for the construction of multivalent systems after clustering onto appropriately functionalized scaffolds. A study of their binding capabilities towards ConA and the biomedically relevant human macrophage mannose receptor (rhMMR) in comparison with the natural mannooligosaccharides has been conducted by undertaking enzyme-linked lectin assays (ELLA) and isothermal titration calorimetry (ITC) measurements. Both lectins displayed comparable affinity for Man_n and pseudo-Man_n homologues, which strongly supports the validity of the approach. The crystal structure of a ConA:pseudo-Man_9 complex at 2.0 Å resolution provided further evidence to support this conclusion. The pseudo-Man_{8-9} prototypes **4** and **5** keep the three peripheral units **H**, **F**, **D** or **I**, **F**, **D** and the disaccharide **AB** or the trisaccharide **ABG** in relative positions that are homotopic to those encountered in the natural Man_8 and Man_9 oligosaccharides (Figure 1). We reasoned that the inner branched mannopyranosyl trisaccharide moiety in the latter could act as a scaffold that preserves the overall orientation of the D1, D2, and D3 arms. Pseudo-Man_3 **1** and pseudo-Man_4 **2** mimic the D1 arm, whereas pseudo-Man_5 **3** is conceived to imitate the pentasaccharide Man_5 entangling the D2 and D3 arms. A simultaneous multiple "click-coupling" approach involving the known α-D-mannopyranosyl azide **6**[5] or the dimannosyl azide derivative **7** and the alkynyl partners **8**–**11** was implemented to generate triazole connectors at the position of the inner carbohydrate units **G**, **E**, **C**, and **H** (Figure 3).

Results and Discussion

Synthesis of "high-mannose" oligosaccharide mimics 1–5

The required *O*-alkynyl derivatives **8**–**11** were prepared from known phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**12**),[6] and mannopyranosyl donors **13**[4] and **14** (Figure 3).[7] Preparation of **8** and **9** first required reductive ring-opening of the 4,6-*O*-benzylidene group[8] of **12** to give diol **15**[9] (85% yield), which was subsequently acetylated at O-6 and O-3 (→**16**) and engaged in glycosylation of *N*,*N*-dibenzylaminoethanol, affording **17**. It is worth mentioning that insertion of the aminoethyl arm proved to be problematic when performed at a later stage in the reaction sequence, for example, when propargyl or triazole moieties were already present in the precursor, and thus had to be carefully planned. The use of *N*,*N*-dibenzylethanolamine as the aglyconic moiety was motivated by the fact that carbamate protection of the amino group led to N-alkylation during the O-propargylation step. De-O-acetylation of **17** afforded the pivotal intermediate

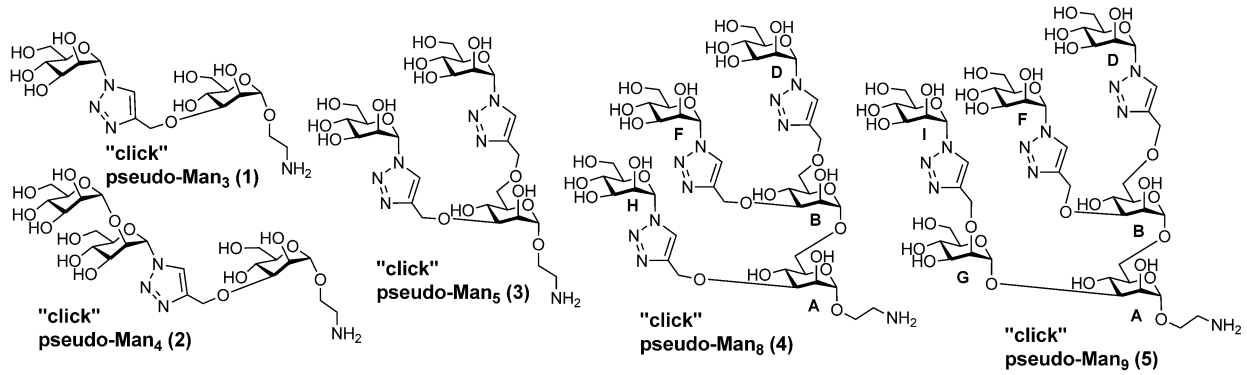


Figure 2. Structure of targeted "High-Mannose" type oligosaccharides mimics **1**–**5**.

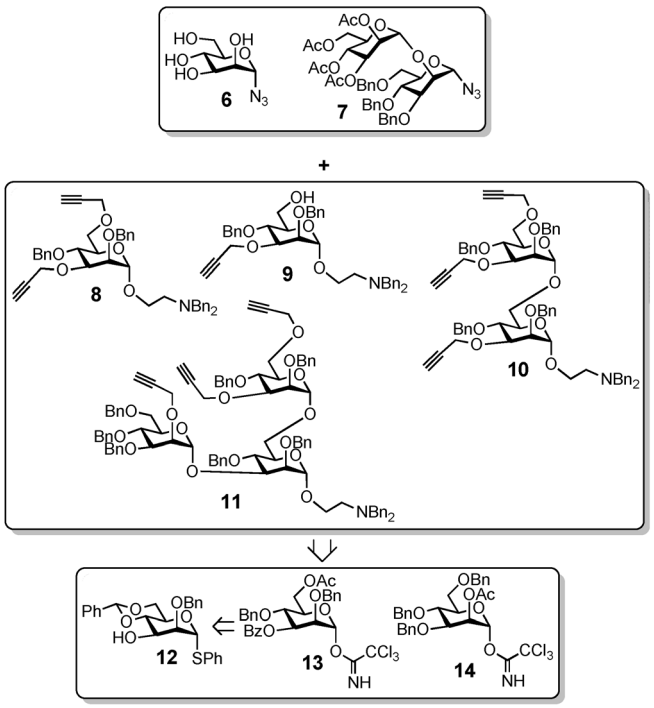
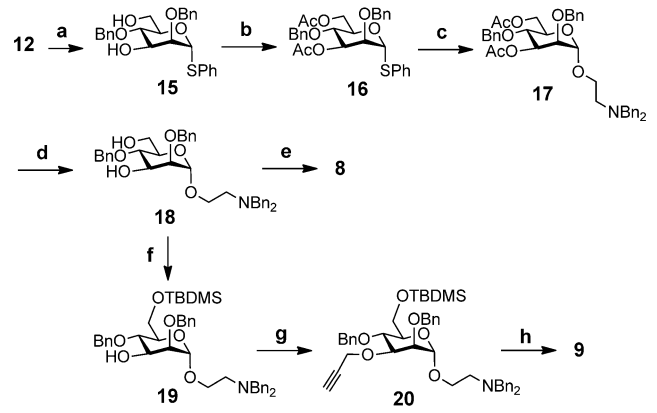


Figure 3. Synthetic strategy and key intermediates.

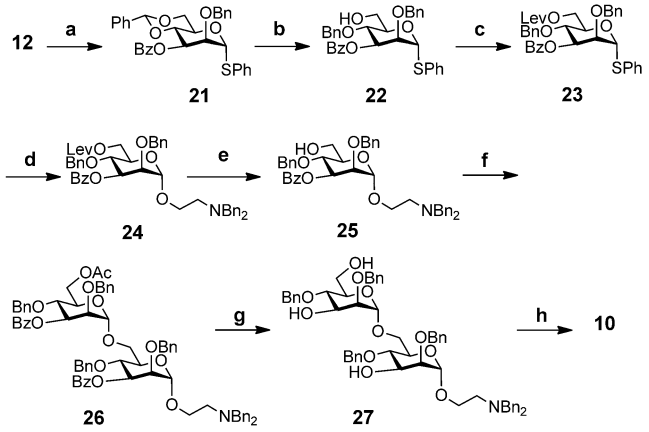
18 (49% overall yield from **12**). Treatment of **18** with propargyl bromide in the presence of sodium hydride gave the doubly-clickable precursor **8**, whereas regioselective *tert*-butyldimethylsilylation at O-6 (→**19**), propargylation of the remaining hydroxyl OH-3 (→**20**), and final tetrabutylammonium fluoride-promoted desilylation provided the mono-clickable derivative **9** (81% yield from **17**; Scheme 1).

Synthesis of disaccharide **10** starts with benzoylation of **12** (→**21**) followed by the reductive ring-opening of the 4,6-*O*-benzylidene group to afford **22**. The primary hydroxyl was then protected as the corresponding levulinic ester, giving **23**, which was used as glycosyl donor to incorporate the *N*,*N*-dibenzylethanolamine aglycone (→**24**; 67% yield). The levulinyl group was selectively removed by treatment with hydrazine to afford alcohol **25**, which was glycosylated with donor **13**,[4] following the trichloroacetimidate method.[10] The reaction led exclusively to α(1-6)-disaccharide **26** in high yield (73%), in agreement with the α-directing character of acyl substituents at O-3 and O-6 in mannopyranosylation reactions.[11] Base-catalyzed methanolysis of the disaccharide **26** (→**27**) followed by treatment with propargyl bromide gave disaccharidic tripropargyl ether scaffold **10** (Scheme 2).

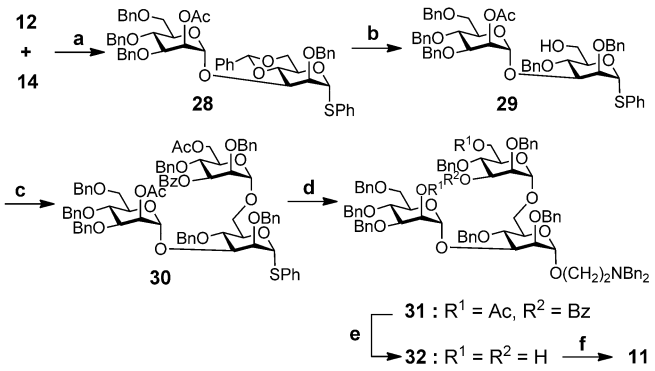
Compound **12** was also used as the starting material in the route towards the triply-clickable trisaccharide **11** (Scheme 3). Glycosylation of **12** with known trichloroacetimidate donor **14**[7] led to α(1-3)-disaccharide **28** in good yield (73%). Subsequent reductive opening of the benzylidene acetal afforded ac-



Scheme 1. Synthesis of **8** and **9**. Reagents and conditions: a) BH_3·THF, Bu_2BOTf·CH_2Cl_2, 0 °C to RT, 1 h, 85%; b) Ac_2O, pyridine, 0 °C to RT, 3 h, 81%; c) *N*,*N*-dibenzylethanolamine, NIS, TfOH, CH_2Cl_2, 0 °C, 1 h, 79%; d) NH_3/MeOH, RT, 2 d, 90%; e) propargyl bromide, NaH, DMF, 0 °C to RT, 2 h, 80%; f) TBDMSCl, pyridine, RT, 12 h, 97%; g) propargyl bromide, NaH, DMF, 0 °C to RT, 2 h, 91%; h) TBAF (1 M in THF), 0 °C to RT, 12 h, 92%.



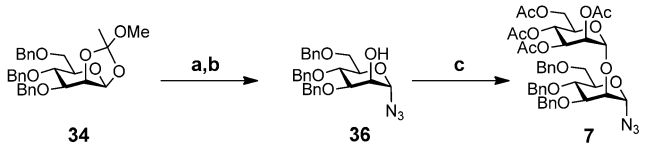
Scheme 2. Synthesis of propargylated disaccharide **10**. Reagents and conditions: a) BzCl, pyridine, 0 °C to RT, 2 h, 91%; b) BH_3·THF, Bu_2BOTf·CH_2Cl_2, RT, 1 h, 94%; c) Levulinic acid, DMAP, DCC, CH_2Cl_2, RT, 6 h, 90%; d) *N*,*N*-dibenzylethanolamine, NIS, TfOH, CH_2Cl_2, 0 °C, 1 h, 67%; e) NH_2NH_2, AcOH, pyridine, RT, 20 min., 80%; f) donor **13**, TMSOTf, CH_2Cl_2, −40 °C, 2 h, inverse proc. 73%; g) MeONa, MeOH, RT, 12 h, 84%; h) propargyl bromide, NaH, DMF, 0 °C to RT, 2 h, 91%.



Scheme 3. Synthesis of propargylated trisaccharide **11**. Reagents and conditions: a) TMSOTf, CH_2Cl_2, −40 °C, 1 h, 73%; b) BH_3·THF, Bu_2BOTf·CH_2Cl_2, 0 °C to RT, 1 h, 94%; c) donor **13**, TMSOTf, CH_2Cl_2, −40 °C, 1 h, inverse proc., 70%; d) *N*,*N*-dibenzylethanolamine, NIS, TfOH, CH_2Cl_2, 0 °C, 1 h, 76%; e) MeONa, MeOH, RT, 6 h, 85%; f) propargylbromide, NaH, DMF, 0 °C to RT, 2 h, 60%.

ceptor **29**, which was glycosylated with donor **13**, using the inverse procedure[12] to ensure a good yield (→**30**, 70%). Installation of the spacer (→**31**), followed by base-catalyzed methanolysis (→**32**) and treatment with propargyl bromide provided **11**.

Disaccharide azide **7**, which is an intermediate required for the synthesis of pseudo-Man_4 **2**, was obtained in 66% yield by glycosylation reaction between the known 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**33**)[13] and azide **36**[14] {prepared in two steps from the 1,2-orthoester derivative **34**,[15a] using $FeCl_3$ as catalyst in the azido glycosylation reaction[15b]}, thereby completing the whole set of building blocks to undertake the preparation of the target click high-mannose oligosaccharide mimics (Scheme 4).



Scheme 4. Synthesis of azide **7**. Reagents and conditions: a) $TMSN_3$, $FeCl_3$, CH_2Cl_2, RT, 15 h, 63%; b) MeONa, MeOH, RT, 2 h, 80%; c) **33**, TMSOTf, CH_2Cl_2, −40 °C, 1 h, 66%.

The CuAAC reactions between **8**–**11** and azide **6** were carried out under microwave heating, using sodium ascorbate and copper(II) sulfate to generate the Cu^I catalyst in situ in a 4:1 mixture of *N*,*N*-dimethylformamide (DMF) and water.[16] The protected pseudo-Man_3 **37** (76%), pseudo-Man_5 **38** (75%), pseudo-Man_8 **39** (72%), and pseudo-Man_9 **40** (75%) were thus obtained. The CuAAC reaction of **7** and **9** (→**41**), followed by base-catalyzed methanolysis, afforded pentabenzylated pseudo-Man_4 **42** (71%, 2 steps; Scheme 5).

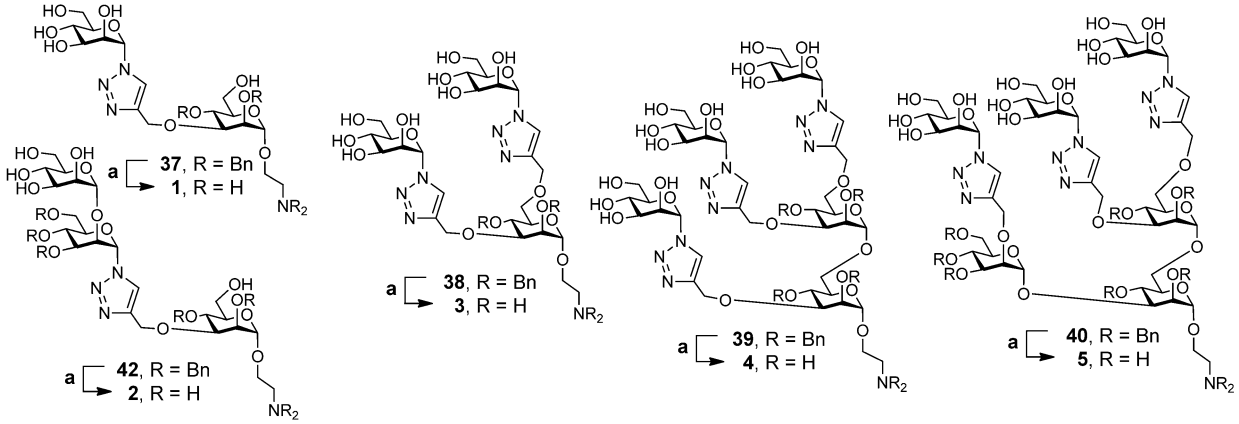
We[4] and others[17] have already observed that the final de-O-benzylation step of derivatives containing triazole rings failed under catalytic hydrogenation conditions. Gin and coworkers[18] previously described the simultaneous de-O-benzylation of 18 benzyl ether groups in cyclooligosaccharide mimics by transfer hydrogenolysis using ammonium formate and palladium on charcoal. In our hands, this procedure afforded rather modest yields of the desired fully unprotected pseudo-Man derivatives under classical heating. However, we were delighted to see that the use of microwave heating in the presence of resin-supported ammonium formate led cleanly to the target fully unprotected pseudo "high-mannose" type oligosaccharides **1**–**5**, which were isolated in 77, 92, 80, 81, and 74% yield, respectively, after HPLC purification.

All Man_n mimics **1**–**5** were fully characterized by NMR spectroscopy. In the case of pseudo-Man_9 **5**, a mass spectrometry fragmentation study was also performed to confirm the linking pattern. The MS/MS spectrum (negative mode) obtained is presented in Figure 4. The $[M-H]^-$ ion of **5** (*m*/*z* 1275.53) generates ions coming from the successive loss of the peripheral mannosidic units **D** (*m*/*z* 1113.45), **F** (*m*/*z* 951.38), and **I** (*m*/*z* 789.34). The loss of triazole rings is monitored by the presence of the fragments of *m*/*z* 870.36, 708.30, 627.26, and 546.24. The latter fragment is characteristic of the trisaccharidic scaffold **ABG**, from which the disaccharidic (*m*/*z* 384.18) and the monosaccharidic (*m*/*z* 222.10) fragments are finally generated.

Evaluation of the lectin binding affinities of "high-mannose" oligosaccharide mimics 1–5

Evaluation of the binding affinity of the oligomannoside mimics towards ConA and rhMMR lectins was first carried out by conducting enzyme-linked lectin assays (ELLA). In this technique, inhibition of the binding between yeast mannan immobilized in a microtiter plate and the corresponding lectin in solution by increasing concentrations of the ligands is determined.[19] The resulting IC_{50} values are then taken to be proportional to the corresponding binding constants. Data for the new click mimics were compared with those obtained for their natural partners. Interestingly, affinities of the same order of magnitude were obtained for pseudo-Man_n/Man_n pairs in the case of the higher homologues against both lectins, which strongly supports their functional equivalency (Table 1).

ELLA results indicate that the lower mimics **1** and **2** are not recognized well by the lectins, with pseudo-Man_4 **2** exhibiting



Scheme 5. Partially benzylated pseudo-Man_{3-9} **37**–**40** and **42** and final deprotection: a) resin-supported ammonium formate, Pd/C, MW, 74–92%.

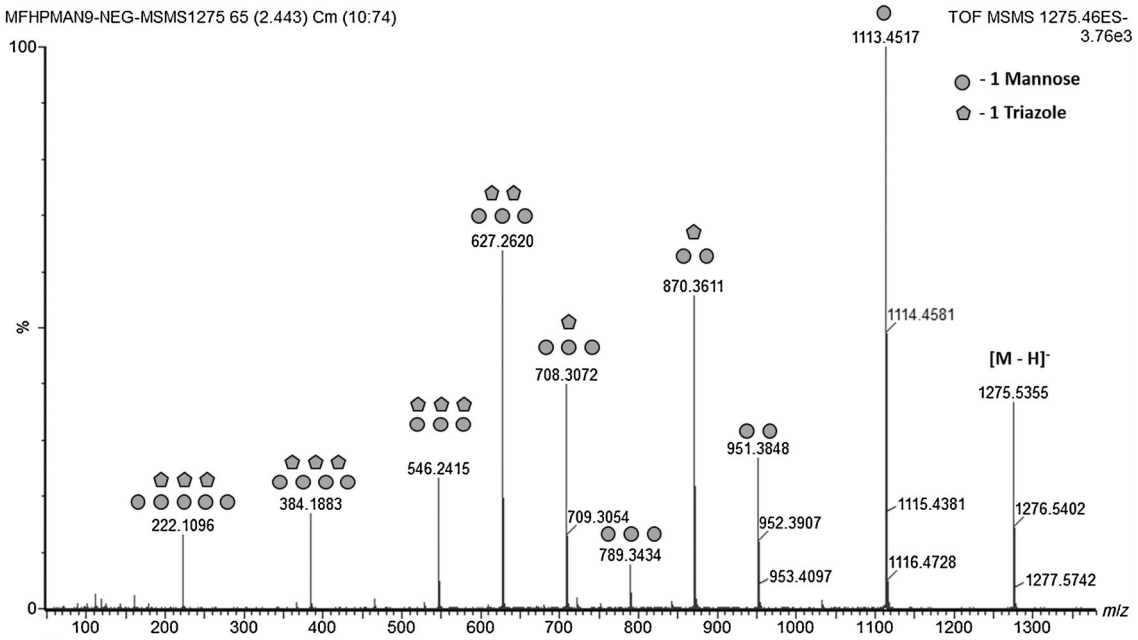


Figure 4. ESI-MS/MS spectrum of pseudo-Man_9 **5**, each gray pentagon and gray circle refer to the loss of, respectively, one triazole and one mannosidic unit.

Table 1. IC_{50} [μM] values for the inhibition of ConA-HRP (ConA) and rhMMR binding to yeast mannan (37 °C, pH 7.3)

Ligand	IC_{50} vs. ConA	Rel. potency	IC_{50} vs. rhMMR	Rel. potency
MeαMan (**I**)	875[b]	1	2850	1
triMan (**II**)[a]	81	11	296	10
Man_5	23[b]	38	62	45.9
Man_6	26[b]	33	52.5	54.3
Man_7	17[b]	51	36	79.2
Man_8	5.6[b]	156	7.2	395
Man_9	3.9[b]	224	4	715
Ps.Man_3 (**1**)	1000	0.87	2600	1.1
Ps.Man_4 (**2**)	230	3.8	1340	2.12
Ps.Man_5 (**3**)	42	20.8	93	30.6
Ps.Man_8 (**4**)	10.6	82.5	18.1	157
Ps.Man_9 (**5**)	7.1	123	5.5	518
III	144	6.7	950	3

[a] triMan (**II**) : 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranosyl azide (see the Supporting Information). [b] Work previously reported.[4]

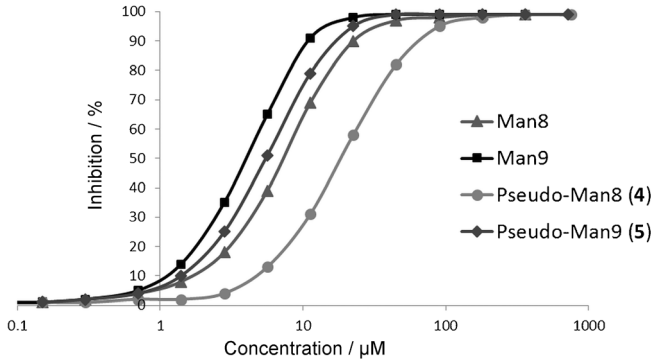


Figure 5. ELLA plots (logarithm scale) for the inhibition of rhMMR binding to yeast mannan by increasing concentrations of the Pseudo-Man_8 **4** and Pseudo-Man_9 **5** in comparison with the natural high-mannose glycans Man_8 and Man_9.

only 4- and 2-fold higher affinities towards ConA and rhMMR, respectively, compared with methyl α-D-mannopyranoside (MeαMan) **I**, which was used as reference. Binding capabilities of mimics **3**–**5** were much more noticeable and were very similar to those shown by their natural counterparts. For both lectins, pseudo-Man_5 **3** is a strong ligand, being only less than 2-fold weaker than the natural Man_5. Pseudo-Man_8 **4** is a stronger ligand than Man_7, but was found to be around 2-fold weaker than Man_8; pseudo-Man_9 **5** was established to be the strongest ligand of the series (Figure 5). Although the presence of the aminoethyl aglycone in the "click" mimics might be involved in lectin binding to some extent, for example, by establishing salt bridges with amino acid residues in the protein, the ConA affinity data obtained previously for an analogue of the pseudo-Man_8 derivative **4**, which lacks this moiety, removes a significant contribution to the binding energy.[4]

In principle, the inner disaccharide **AB** in pseudo-Man_8 **4** and the trisaccharide moiety **ABG** in pseudo-Man_9 **5** (Figure 2) might act as simple scaffolds, bearing three peripheral mannosidic ligands, with the observed lectin affinity enhancements being simply the result of a particularly favorable multivalent effect of the triad-type presentations.[20] To investigate this possibility, trivalent glycodendron **III** (Figure 6) was prepared (see the Supporting Information) and evaluated. The much weaker lectin affinity displayed by **III** as compared with **4** and **5** (by 52 and 172-fold, respectively), in spite of bearing an identical number of external mannosyl-triazole units, strongly suggests that the latter behave as real Man_n-type complementary structural motifs, providing an optimal overall shape and presentation or even participating in the binding process, as occurs in the natural Man_8 and Man_9 counterparts.

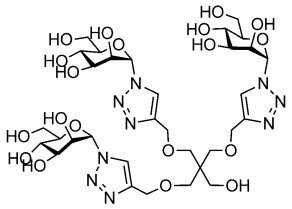


Figure 6. Glycodendrimer **III** used as trivalent mannosyl-triazole control in ELLA.

The thermodynamic signature ($-\Delta H^\circ$, $-T\Delta S$, and K_a values) for the binding of MeαMan **I**, 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl azide **II**, pseudo-Man_{3-5}, and pseudo-Man_{8-9} **1**–**5** to ConA was further studied by isothermal titration calorimetry (ITC), which is a technique used widely to investigate carbohydrate–lectin interactions.[21] After integrating the heat signal as a function of the ratio between the carbohydrate and the lectin, the isotherm was fitted to the one-site binding model (see the Supporting Information). The average values for the thermodynamic parameters are given in Table 2. Trisaccharide **II**, for which ConA possesses an extended binding site, exhibited a $-\Delta H^\circ$ value of 11.4 kcal mol^{-1} and a K_a value of 47.6×10^4 M^{-1}, which are consistent with reported data,[22,23] considering the slightly different experimental conditions, and are much more favorable compared with those for **I**. The $-\Delta H^\circ$, $-T\Delta S$, and K_a values for ConA-binding pseudo-Man_{3-5} **1**–**3** are also indicative of weaker affinities compared with those of **II**, which is consistent with the ELLA measurements.

Table 2. Thermodynamic parameters for ConA (25 °C, pH 7.2)

Ligand	N[a]	$-\Delta H^\circ$ [kcal mol^{-1}]	$-T\Delta S$ [kcal mol^{-1}]	K_a [M$^{-1}\times10^{-4}$]
MeαMan (**I**)	1	6.1	1.7	0.97
triMan (**II**)	1	11.4	12.3	47.6
Ps.Man_3 (**1**)	1	6.1	5.3	0.19
Ps.Man_4 (**2**)	1	7.7	3.7	8.62
Ps.Man_5 (**3**)	0.8	6.4	1.3	3.35
Ps.Man_8 (**4**)	0.23	17.2	32.6	37.03
Ps.Man_9 (**5**)	0.28	16.2	29.9	30.30

[a] Number of sites per monomer.

In contrast, ITC experiments provided evidence that pseudo-Man_{8-9} **4** and **5** are high affinity ligands for ConA, with K_a values similar to those of **II** but with higher $-\Delta H^\circ$ values (17.2 and 16.2 kcal mol^{-1}, respectively). Surprisingly the $-T\Delta S$ values are much more negative, with **4** and **5** exhibiting 18- and 2.5-fold higher $-T\Delta S$ values relative to **I** and **II**, respectively. This entropy penalty is consistent with cross-linking processes. Finally, ITC data also indicated that the number of binding sites (N) per monomer of ConA binding to **I**, **II**, and pseudo-Man_{3-5} **1**–**3** is close to 1.0, in agreement with reported data, which confirms the monovalency of these ligands for ConA. In contrast, **4** and **5** exhibit N values of 0.23 and 0.25, respectively, indicating that **4** and **5** are probably involved in multivalent binding with ConA.

Crystal structure of ConA:pseudo-Man_9 **5** complex was determined at 2.0 Å resolution (See Table 5 in the Supporting Information). Only one terminal mannosyl residue (**D**, **F**, or **I**) and the triazolyl aglycone could be modeled in the binding site of each subunit of dimeric ConA (Figure 7 A). However, structural alignment of the complex with a previously reported ConA:-Manα(1-6)[Manα(1-3)]Man trimannoside complex[24] gave similar interactions between the mannosyl moiety and the binding site residues (Figure 7 B).

As reported for other ConA:mannoside structures,[25] Arg228, Asn14, Asp208, and Leu99 interact with the terminal mannosyl residue through a network of hydrogen bonds. Tyr12 and Tyr100 complete the binding site and link the **B** or **G** unit at O-6 or O-3 (**B**) or O-2 (**G**) through hydrogen bonds involving a water molecule. Moreover, Tyr12, Leu99, and Tyr100 form a hydrophobic pocket in which the triazole ring can be stabilized and act as a good mimic of mannose.

Another striking aspect of the ConA:**5** structure is the formation of an unexpected oligomer, which led to crystallization of the complex in the trigonal $H3_2$ space group, instead of the reported monoclinic $P2_1$ groups for ConA:Manα(1-6)[Manα(1-3)]Man trimannoside that crystallized under the same condi-

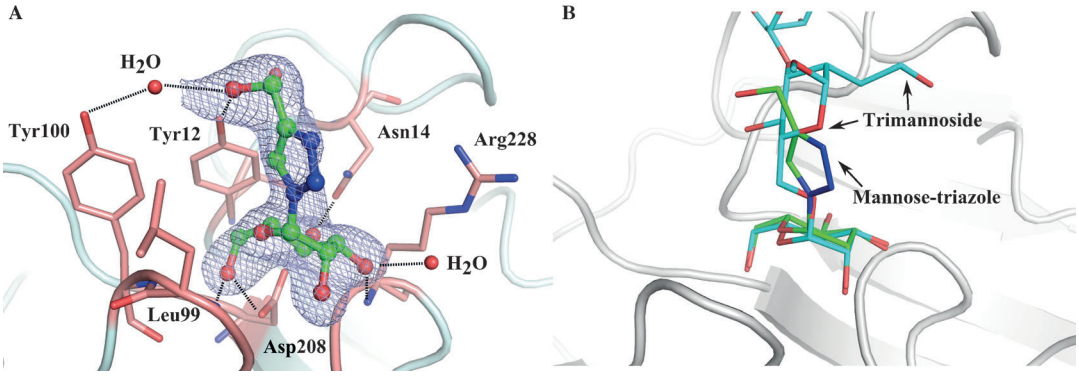


Figure 7. A) X-ray structure of the ConA:**5** complex (binding site region). **5** (colored green) is shown with the corresponding 2Fo-Fc omit map density contoured at 1.5σ. Residues interacting with the ligand are indicated. B) Overlay of the ConA:**5** complex (green) with the ConA:Manα(1-6)[Manα(1-3)]Man trimannoside complex (cyan) (PDB code 3D4K).

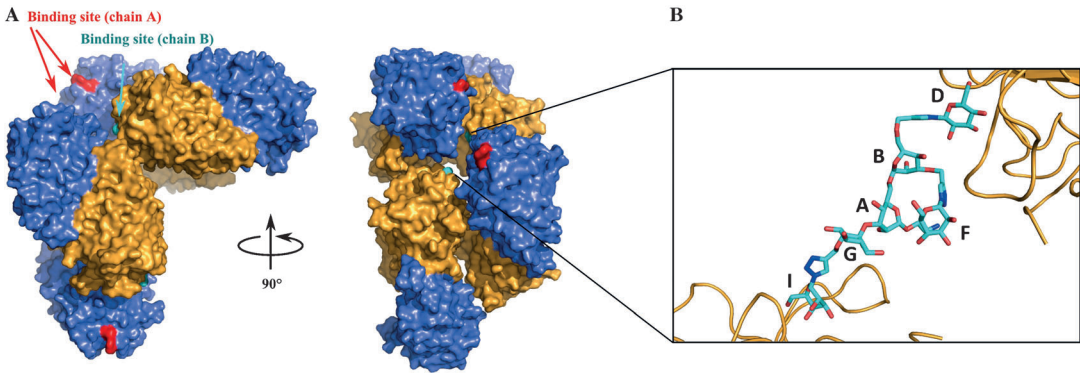


Figure 8. Modeling of ConA cross-linking by pseudoMan$_9$ **5**. A) Supramolecular assembly visible in crystal structure of ConA:**5** complex. In each of the four interacting dimers, Chain A (resp. B) is colored in purple (resp. orange). Mannoside binding site in each chain are in red (Chain A) and cyan (Chain B). B) Detailed view of the modeled full length **5** bridging two 'B' binding sites. Mannoside moieties are labeled according to Figure 2.

tions.[24] ConA:pseudo-Man$_9$ exists as an assembly of four dimers (Figure 8 A). This geometry allows two sites of Chain A (and two sites of Chain B) from different dimers to be in close contact. Using the terminal mannosyl residue as visible anchor point in the X-ray structure, a model of **5** was docked into each pair of binding sites (Figure 8 B).

This molecular modeling confirmed the possible bridging of two binding sites of ConA by the pseudo-Man$_9$ molecule. Similar results were obtained by Dimick et al.[25d] with a short dimannoside analogue that could crosslink two ConA binding sites. Thus, **5** interacts with ConA lectin through interactions that are similar to those reported for oligomannoside compounds and change the oligomeric state of the protein, possibly by crosslinking ConA dimers.

Conclusion

A new family of high-mannose oligosaccharide mimics that reproduce the peripheral presentation of mannopyranosyl glycotopes as well as the core structure and branching pattern has been developed. Replacement of some of the inner monosaccharide residues by triazole rings preserves the overall architecture of the natural compounds and allows click chemistry ligation strategies to be implemented to assemble the mimetics, which represents a very significant simplification compared with classical glycosylation methods. The new pseudo-Man derivatives displayed binding affinities towards the mannose-specific lectins ConA and the human macrophage mannose receptor that reproduced the trends encountered for the natural counterparts in enzyme-linked lectin assays, with binding potencies that also rivaled those of their respective natural partners. Isothermal titration calorimetry also produced data that was consistent with the ELLA measurements and indicate that Pseudo-Man$_8$ (**4**) and Pseudo-Man$_9$ (**5**) are involved in crosslinking processes with ConA. Most interestingly, the X-ray structure of the complex of ConA with the highest member of the series, pseudo-Man$_9$, provided evidence for contacts beyond the terminal mannopyranosyl residue and crosslinking abilities analogous to those observed in the corresponding complex with the natural Man$_9$. The ensemble of data strongly support the conclusion that the "click" high-mannose oligosaccharide analogues reported here behave as true functional mimics of the parent oligosaccharides.

Experimental Section

General Methods

Optical rotations were measured with a JASCO DIP-370 digital polarimeter, using a sodium lamp ($\lambda = 589$ nm) at 20 °C. NMR experiments were performed at 300.13 and 600.13 MHz with a Bruker AVANCE DPX300 or AVANCE III IPSO600 spectrometers equipped with a Z-gradient unit for pulsed-field gradient spectroscopy. Me_4Si was used as an external standard and calibration was performed by using the signal of the residual protons or of the carbon of the solvents as a secondary reference. Measurements were performed at 300 K with careful temperature regulation. The length of the 90° pulse was approximately 7 μs. 1D NMR data spectra were collected as 16 K data points. 2D experiments were run using 1 K data points and 512 time increments. The phase-sensitive (TTPI) sequence was used and processing resulted in a 1 K×1 K (real-real) matrix. Details concerning experimental conditions are given in the Figure captions. Low-resolution electrospray mass spectra were obtained with a hybrid quadrupole/time-of-flight (Q-TOF) instrument, equipped with a pneumatically assisted electrospray (Z-spray) ion source (Micromass). High-resolution mass spectra were recorded in positive mode with a ZabSpec TOF (Micromass, UK) tandem hybrid mass spectrometer with EBETOF geometry. The compounds were individually dissolved in a water–CH_3CN mixture (1:1) at a concentration of 10 μg cm^{-3} and then infused into the electrospray ion source at a flow rate of 10 mm^3 mn^{-1} at 60 °C. The mass spectrometer was operated at 4 kV while scanning the magnet at a typical range of 4000–100 Da. The mass spectra were collected as continuum profile data. Accurate mass measurement was achieved by using polyethylene glycol as internal reference masses with a resolving power set to a minimum of 10 000 (10% valley). HPLC analyses were performed with a Water PrepLC400 System with a PL-ELS 1000 detector (Waters and Polymer laboratories) detector (Method 1) or Waters 2545 and SQ mass detector (Waters) (Method 2). Analytical chromatography was performed with an Apollo C18 5 μm column (length: 250 mm; Ø: 4.6 mm) at 1 mL min^{-1} rate flow. Preparative chromatography was performed with a VisionHT C18 HL 5 μm column (length: 250 mm; Ø: 22 mm) at 23 mL min^{-1} rate flow. Analytical TLC were performed with Silica

Gel 60 F_{254} plates (Merck, Germany). Eluents used were mixtures of dichloromethane and methanol or cyclohexane and ethyl acetate; ratios are specified in each case. Products were visualized under UV light ($\lambda = 254$ nm) followed by charring with a mixture of vanillin in a 5% solution of H_2SO_4 in ethanol. For "manual" flash chromatography, a glass column filled with silica gel (GERUDAN SI 60, grading 0.040–0.062 mm MERCK) was used. For "automatic" flash chromatography a Reveleris iES Flash System device supplied by Grace was used as silica Grace Flash Cartridges (normal phase) or C-18 Grace Flash Cartridges (inverse phase). The resin-supported ammonium formate used in de-O-benzylation reactions of partially benzylated pseudo-Man_{3-9} **37**–**40** and **42** was prepared from Dowex marathon MSA resin (Sigma Aldrich) by treatment with formic acid (99%) for 30 min. The resin was washed with water (twice) and methanol (twice), and finally dried at 30 °C under vacuum for 4 h.

Enzyme-linked lectin assay (ELLA)

Horseradish peroxidase-labeled concanavalin A (HRP-Con A; Sigma), mannan from *Saccharomyces cerevisiae* (Sigma) recombinant human macrophage mannose receptor (rhMMR; R&D Systems), biotinylated anti-human MMR antibody (R&D Systems), NeutrAvidin (Thermo Scientific), biotin-conjugated HRP (Thermo Scientific), high-mannose oligosaccharides (Man_n; Ludger) and all other common reagents and materials were purchased from commercial sources and used without further purification unless otherwise stated. All aqueous solutions for lectin-binding assays were prepared from deionized water filtered with a Milli-Q purification system.

Nunc-Inmuno plates (MaxiSorp) were coated overnight with yeast mannan at 100 μL/well diluted from a stock solution of 10 μg mL^{-1} in 10 mM phosphate buffer saline (PBS, pH 7.3 containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+}) at RT. The wells were then washed three times with 300 μL washing buffer (PBST, containing 0.05% (v/v) Tween 20). The washing procedure was repeated after each of the incubations throughout the assay. The wells were then blocked with 150 μL/well of 1% BSA/PBS for 1 h at 37 °C.

For concanavalin A affinity evaluations, after washing, the wells were filled with 100 μL of serial dilutions of horseradish peroxidase labeled concanavalin A (ConA-HRP, Sigma) from 10^{-1} to 10^{-4} mg mL^{-1} in PBS, and incubated at 37 °C for 1 h. The plates were washed and 50 μL/well of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (0.25 mg mL^{-1}) in citrate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reaction was stopped after 20 min by adding 50 μL/well of 1 M H_2SO_4 and the absorbances were measured at 415 nm. Control wells contained citrate-phosphate buffer. The concentration of lectin-enzyme conjugate that displayed an absorbance between 0.8 and 1.0 was used for inhibition experiments.

To carry out the competitive lectin-binding inhibition experiments, each compound was added in a serial of 2-fold dilutions (60 μL per well) in PBS to 60 μL of the appropriate ConA-HRP concentration in PBS buffer on Nunclon (Delta) microlitre plates and incubated for 1 h at 37 °C. The above solutions (100 μL) were then transferred to the mannan-coated microplates, which were incubated for 1 h at 37 °C. The plates were washed and the ABTS substrate was added (50 μL per well). Color development was stopped after 20 min and the absorbances were measured.

For determination of recombinant human MMR (rhMMR) binding affinities, the wells were filled with 100 μL of serial dilutions of rhMMR from a 10 μg mL^{-1} stock solution in PBS (pH 7.3 containing 0.1 mm Ca^{2+} and 0.1 mm Mn^{2+}), and incubated at 37 °C for 1 h. The plates were washed three times with PBST as described above and 100 μL of a solution of biotinylated anti-human MMR antibody (0.2 mg mL^{-1}, R&D Systems) in PBS was added in each well, and the plates were further incubated for 1 h at 37 °C. The complex NeutrAvidin-biotinylated HRP was preformed separately by successively adding to Tris buffer (9.6 mL, 50 mM, pH 7.6) a solution of NeutrAvidin (100 μg mL^{-1} in Tris buffer, 1.2 mL, Thermo Scientific) and a solution of biotin-conjugated HRP (25 μg mL^{-1} in Tris buffer, 1.2 mL, Thermo Scientific). The mixture was shaken for 30 min at RT and the solution was immediately transferred into the plates (60 μL/well). After 1 h at 37 °C, these plates were washed twice with Tris (250 μL/well) and ABTS (0.25 mg mL^{-1}, 50 μL/well) in citrate buffer (0.2 m, pH 4.0 with 0.015% H_2O_2) was added. After 5 min at RT, the optical density was measured at 415 nm. Control wells were processed with anti-human MMR antibody as well as NeutrAvidin-biotinylated HRP. The concentration of rhMMR that displayed an absorbance between 0.8 and 1.0 was used for inhibition experiments. For the competitive lectin-binding inhibition experiment, MeαMan (**I**) or the click high-mannose mimics **1**–**5** mixed in a serial of 2-fold dilutions (60 mL per well) in HEPES buffer (20 mm, pH 7.4) with 60 mL of the appropriate rhMMR concentration in PBS buffer on Nunclon (Delta) microtitre plates and incubated for 1 h at 37 °C. The above solutions (100 μL) were then transferred to the mannan-coated titer plates, which were incubated for 1 h at 37 °C. The plates were washed and the solution of biotinylated anti-human MMR antibody in PBS (100 μL) was added in each well, and the plates were further incubated for 1 h at 37 °C. The NeutrAvidin solution was then transferred into the plates (60 μL/well). After 1 h at 37 °C, these plates were washed twice with Tris (250 μL/well) and ABTS was added (50 μL/well). Optical density at 415 nm was determined after 5 min. The percent of inhibition was calculated as follows: $\%\,\text{Inhibition} = 100 \times (A_{\text{no inibithor}} - A_{\text{with inhibitor}})/A_{\text{no inibithor}}$.

Results in triplicate were used to plot the inhibition curves for each individual ELLA experiment. Typically, the IC_{50} values (concentration required for 50% inhibition of the ConA-yeast mannan or the rhMMR-yeast mannan association) obtained from several independently performed tests were in the range of ±12%. Nevertheless, the relative inhibition values calculated from independent series of data were highly reproducible.

Isothermal calorimetric measurements

Concanavalin A (ConA) was purchased from Sigma–Aldrich (C2010–250MG, Sigma). Protein concentration was determined spectrophotometrically at 280 nm by using the Beer–Lambert law with a molar extinction coefficient of 32430 cm^{-1} M^{-1} and is expressed in terms of the monomer.[26] The protein purity (94%) was checked at three concentrations (0.095, 0.047, 0.025 mM) and plotted versus absorbance to monitor linearity. ITC was performed with a Microcal ITC200 system (GE Healthcare). All experiments were performed at 25 °C in 0.1 M Hepes buffer containing NaCl (0.9 M), $CaCl_2$ (0.1 mM), and $MnCl_2$ (0.1 mM) at pH 7.2. Briefly, 1 μL of saccharide solution was injected successively by using a computer-controlled 40 μL syringe at an interval of 180 s into the ConA solution (Cell Volume 204.6 μL). For the Man8 and Man9 mimics, the first four injections were of 0.5 μL. Additional parameters were as follows: reference power 8 mV, initial delay 60 s, rotation speed 1000 rpm. Control experiments were performed by the dilution of each saccharide solution in buffer and showed insignificant heats of dilution. These experiments were then subtracted from each titration to remove heats of dilution of ligand. The experimental data were then fitted to a theoretical titration curve by using Origin 7.0-Microcal Software with the single-site model. During this

fitting, enthalpy (ΔH), stoichiometry (N) and association constants (K_a) were adjustable parameters. All experiments were performed with C values $1 < C < 90$ in the present study. The linear trigalactose Galβ(1–4)Galβ(1–4)Galα(1-*O*Me), used as negative control, did not shown any binding to the lectin (see Table 4 and Figure S5 in Supporting Information). In the case of MeαMan and PseudoMan$_3$ ($C < 5$), the data were fitted with N fixed and K_a and ΔH as floating parameters.

Crystallographic experiments

ConA was purified by affinity chromatography on a Superdex 200 GL column (GE Healthcare), using glucose for elution. Protein was concentrated to 20 mg mL^{-1} in 20 mM Tris buffer pH 7, 50 mM NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, in the presence of 5 mM pseudo-Man$_9$. Crystals suitable for diffraction experiments were obtained by sitting-drop vapor diffusion in 7 μL drops containing 2 μL ConA:pseudo-Man$_9$ solution and 5 μL of the precipitation solution (10% polyethylene glycol 6000, and 0.1 M Sodium Cacodylate pH 6.8).[24] After equilibration of the drops against the crystallization solution, crystals were cryoprotected in 20% polyethylene glycol 400, before freezing in liquid nitrogen. Data were collected at the beamline Proxima-1 at the Soleil Synchrotron Facility, reduced with the X-Ray Detector Software XDS,[27] and scaled with SCALA[28] by using the CCP4 program suite.[29] ConA:trimannoside crystal structure (pdb 3D4K)[24] was then used as a template for molecular replacement in the PHASER program.[30] The model was completed by iterative cycles of manual model building and real space refinement using the program Coot[31] and crystallographic refinement using Phenix refine.[32] Structure validation with MOLPROBITY[33] was integrated as part of the iterative rebuild and refinement procedure. Model and structure factors have been deposited at the Protein Data Bank (PDB code 4PF5). Pymol software was used to generate all images.[34] NAMD software[35] was used to perform all molecular dynamics simulations of pseudo-Man$_9$ in complex with ConA. The topology and parameters files for the ligand were obtained by using the Antechamber program[36] and AM1-BCC charges[37] in the Ambertools 13 program.[38] Pseudo-Man$_9$ was originally placed and aligned so that the terminal mannose would fit the ligand density in the ligand structure. Two initial models of pseudo-Man$_9$ were thus generating, either bridging the ConA between the terminal mannose D and E (model named pMan9-DE) or D and F (pMan9-DF). Each model was equilibrated by several cycles, alternating minimization (10000 steps, steepest descent), and molecular dynamics (200 ps, 200 K). Long simulations were then run (1 ns), keeping the backbone of ConA restrained to its position in the X-ray structure.

Synthesis of pseudo-Man$_{8\text{-}9}$ 4 and 5

Syntheses of mimics **1**–**3** and azide **7** are fully described in the Supporting Information. A, B, C letters refer to each pyranosidic rings and are used to indicate their associated protons and carbons in the NMR data. Arabic numerals 1–6 refer to each proton and carbon of the pyranosidic rings according to the nomenclature of carbohydrates. Arabic numerals higher than 6 refer to protons and carbons of the side chains. For spectra see the Supporting Information.

Phenyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (21): Benzoyl chloride (4.26 mL, 36.62 mmol, 2 equiv) was added to a solution of **12** (8.5 g, 18.35 mmol) in anhydrous pyridine (200 mL) at 0 °C. The mixture was stirred at RT for 4 h, then the reaction was quenched with MeOH (10 mL). Pyridine was removed under reduce pressure and the residue was diluted in EtOAc. The organic layer was washed with saturated aq. $KHSO_4$, $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by "automatic" flash chromatography (50 min, cyclohexane/EtOAc 1:0 to 8:2 (v/v)) to give **21** (9.24 g, 91%). $[\alpha]_D^{20}$ +48.4 ($c = 1$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.13$–8.03 (m, 2H; ArH), 7.65–7.08 (m, 18H, ArH), 5.65 (s, 1H; H-7), 5.60 (d, $J(1,2) = 1.4$ Hz, 1H; H-1), 5.56 (dd, $J(2,3) = 3.4$ Hz, $J(3,4) = 9.0$ Hz, 1H; H-3), 4.67 (d, $J = 11.9$ Hz, 1H; OCH_2Ph), 4.56 (d, $J = 11.9$ Hz, 1H; OCH_2Ph), 4.53–4.39 (m, 2H; H-4 and H-5), 4.38 (dd, $J(1,2) = 1.4$ Hz, $J(1,2) = 3.4$ Hz, 1H; H-2), 4.28 (dd, $J(5,6a) = 4.4$ Hz, $J(6a,6b) = 10.3$ Hz, 1H; H-6a), 3.93 ppm (t, $J(5,6b) = 10.3$ Hz, 1H; H-6b); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.9$ (PhC(O)O), 137.4–126.3 (Ar), 101.9 (C-7), 86.7 (C-1), 77.9 (C-2), 76.5 (C-4), 73.3 (OCH_2Ph), 71.3 (C-3), 68.7 (C-6), 65.5 ppm (C-5); HRMS (ESI): m/z calcd for $C_{33}H_{30}O_6SNa$: 577.1661 $[M+Na]^+$; found: 577.1681.

Phenyl 3-*O*-benzoyl-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (22): Compound **21** (8.4 g, 15.1 mmol) was treated with BH_3 (1 M in THF, 48.5 mL, 48.5 mmol, 3.2 equiv) and Bu_2BOTf solution (1 M in CH_2Cl_2, 16.1 mL, 16.1 mmol, 1.06 equiv) at 0 °C under an inert atmosphere. The reaction mixture was stirred for 2 h at 0 °C then the reaction was quenched by the dropwise addition of MeOH (until gas release ceased) and Et_3N (until pH reached 10). The solvent was evaporated under reduced pressure and the crude product was purified by "automatic" flash chromatography (40 min, cyclohexane/EtOAc 1:0 to 8:2 (v/v)) to afford **22** (7.90 g, 94%). $[\alpha]_D^{20}$ +43.3 ($c = 1$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 8.11–8.07 (m, 2H; ArH), 7.52–7.19 (m, 18H; ArH), 5.60 (d, $J(1,2) =$ 1.8 Hz, 1H; H-1), 5.54 (dd, $J(2,3) = 3.0$ Hz, $J(3,4) = 9.3$ Hz, 1H; H-3), 4.83 (d, $J = 10.8$ Hz, 1H; OCH_2Ph), 4.72 (d, $J = 10.8$ Hz, 1H; OCH_2Ph), 4.68 (d, $J = 12.6$ Hz, 1H; OCH_2Ph), 4.55 (d, $J = 12.6$ Hz, 1H; OCH_2Ph), 4.40–4.24 (m, 3H; H-2, H-4 and H-5), 3.89 (s, 2H; H-6a and H-6b), 2.04 ppm (br s, 1H; OH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.7$ (PhC(O)O), 137.9–124.7 (Ar), 85.9 (C-1), 77.4 (C-2), 75.1 (OCH_2Ph), 74.6 (C-3), 73.3, 73.2 (C-4 and C-5), 72.7 (OCH_2Ph), 62.0 ppm (C-6); HRMS (ESI): m/z calcd for $C_{33}H_{32}O_6SNa$: 579.1817 $[M+Na]^+$; found: 579.1804.

Phenyl 3-*O*-benzoyl-2,4-di-*O*-benzyl-6-*O*-levulinoyl-1-thio-α-D-mannopyranoside (23): DCC (1.01 g, 4.92 mmol, 1.6 equiv) was added to a solution of **22** (1.7 g, 3.07 mmol), DMAP (64 mg, 0.52 mmol, 0.17 equiv), and levulinic acid (0.57 g, 4.92 mmol, 1.6 equiv) in anhydrous dichloromethane (40 mL) under an inert atmosphere at RT. The mixture was stirred for 24 h at RT then filtered over Celite, washed with dichloromethane, and concentrated. The residue was dissolved in ethyl acetate and the organic layer was successively washed with a saturated solution of aq. $KHSO_4$, $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by "manual" flash chromatography (cyclohexane/EtOAc 1:0 to 7:3 (v/v)) to afford **23** (1.81 g, 90%). $[\alpha]_D^{20}$ +14.1 ($c = 1$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.16$–8.02 (m, 2H; ArH), 7.69–7.08 (m, 18H; Ar), 5.64 (d, $J(1,2) = 1.5$ Hz, 1H; H-1), 5.52 (dd, $J(2,3) = 3$ Hz, $J(3,4) = 9.3$ Hz, 1H; H-3), 4.81 (d, $J = 10.8$ Hz, 1H; OCH_2Ph), 4.75–4.60 (m, 2H; OCH_2Ph), 4.54 (d, $J = 12.3$ Hz, 1H; OCH_2Ph), 4.51–4.31 (m, 3H; H-5, H-6a and H-6b), 4.29 (dd, 1H; H-2), 4.22 (t, $J(4,5) =$ 9.3 Hz, 1H; H-4), 2.80–2.67 (m, 2H; H-8 or H-9), 2.66–2.57 (m, 2H; H-8 or H-9), 2.16 ppm (s, 3H; H-11); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 206.5 (C-10), 172.5, 165.6 (OC(O)Ph, OC(O)CH_2), 137.6–127.7 (Ar), 85.5 (C-1), 77.2 (C-2), 75.1 (OCH_2Ph), 74.6 (C-3), 73.6 (C-4), 72.5 (OCH_2Ph), 70.9 (C-5), 63.5 (C-6), 37.9 (C-8 or C-9), 29.9 (C-11), 28.0 ppm (C-8 or C-9); HRMS (ESI): m/z calcd for $C_{38}H_{38}O_8SNa$: 677.2185 $[M+Na]^+$; found: 677.2174.

(*N*,*N*-Dibenzyl-2-aminoethyl) 3-*O*-benzoyl-2,4-di-*O*-benzyl-6-*O*-levulinoyl-α-D-mannopyranoside (24): *N*-Iodosuccinimide (0.755 g,

3.36 mmol, 1.3 equiv) followed by TfOH (0.32 mL, 3.61 mmol, 1.4 equiv) were added to a solution of **23** (1.69 g, 2.58 mmol), *N*,*N*-dibenzyl amino ethanol (0.685 g, 2.84 mmol, 1.1 equiv), and molecular sieves (1 g) in anhydrous CH_2Cl_2 (50 mL) under an inert atmosphere at 0 °C. The mixture was stirred at 0 °C for 2 h, quenched with triethylamine, and concentrated to dryness. The residue was diluted in EtOAc, washed with saturated aq. $NaHCO_3$ and $Na_2S_2O_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by "automatic" flash chromatography (45 min, cyclohexane/EtOAc 1:0 to 7:3 (v/v) with 1% Et_3N) to afford **24** as the α anomer (1.37 g, 67%). $[\alpha]_D^{20}$ +14.1 (c = 1 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.07 (m, 2H; ArH), 7.62–7.14 (m, 23H; ArH), 5.55 (dd, J(2,3) = 3.6 Hz, J(3,4) = 9.6 Hz, 1H; H-3), 5.85 (d, J(1,2) = 1.2 Hz, 1H; H-1), 4.74 (dd, J = 10.8 Hz, 1H; OCH_2Ph), 4.63–4.52 (m, 3H; OCH_2Ph), 4.35 (dd, J(5,6a) = 4.8 Hz, J(6a,6b) = 12 Hz, 1H; H-6a), 4.28 (dd, J(5,6b) = 1.8 Hz, J(6a,6b) = 12 Hz, 1H; H-6b), 4.11 (t, J(3,4) = J(4,5) = 9.6 Hz, 1H; H-4), 3.97 (dd, J(1,2) = 1.2 Hz, J(2,3) = 3.6 Hz, 1H; H-2), 3.91 (m, 1H; H-5), 3.78 (m, 1H; H-13a), 3.67 (d, J = 13.8 Hz, 2H; NCH_2Ph), 3.64 (d, J = 13.8 Hz, 2H; NCH_2Ph), 3.51 (m, 1H; H-13b), 2.76–2.53 (m, 6H; H-8, H-9 and H-12), 2.13 ppm (s, 3H; H-11); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 206.5 (C-10), 172.6, 165.6 (O*C*(O)Ph and C-7), 139.4–127.1 (Ar), 97.1 (C-1), 76.2 (C-2), 75.0 (OCH_2Ph), 74.6 (C-3), 73.3 (C-4), 73.2 (OCH_2Ph), 69.9 (C-5), 66.0 (C-13), 63.6 (C-6), 59.0 (NCH_2Ph), 52.7 (C-12), 38.0 (C-8 or C-9), 29.9 (C-11), 28.0 ppm (C-8 or C-9); HRMS (ESI): m/z calcd for $C_{48}H_{52}O_9N$: 786.3642 $[M+H]^+$; found: 786.3633.

(*N*,*N*-Dibenzyl-2-aminoethyl) 3-*O*-benzoyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (25): A solution of **24** (1.32 g, 1.68 mmol) in pyridine (5 mL) was treated for 10 min at RT with a solution of pyridine/AcOH/hydrazine hydrate (12:8:1, 20 mL). The reaction medium was concentrated, then the residue was dissolved in CH_2Cl_2 and the organic layer was successively washed with a saturated solution of aq. NaOAc, $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by "manual" flash chromatography (cyclohexane/EtOAc 1:0 to 7:3 (v/v)) to afford **25** (0.924 g, 80%). $[\alpha]_D^{20}$ +6.5 (c = 0.63 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.13–7.99 (m, 2H; ArH), 7.64–7.07 (m, 23H; ArH), 5.55 (dd, J(2,3) = 3.3 Hz, J(3,4) = 9.9 Hz, 1H; H-3), 4.81 (d, J(1,2) = 1.8 Hz, 1H; H-1), 4.76 (d, J = 10.8 Hz, 1H; OCH_2Ph), 4.65 (d, J = 11.1 Hz, 1H; OCH_2Ph), 4.60–4.56 (m, 2H; OCH_2Ph), 4.19 (t, J(3,4) = J(4,5) = 9.9 Hz, 1H; H-4), 3.97 (dd, J(1,2) = 1.8 Hz, J(2,3) = 3.3 Hz, 1H; H-2), 3.84–3.69 (m, 4H; H-5, H-6a, H-6b and H-8a), 3.69–3.61 (m, 4H; NCH_2Ph), 3.48 (m, 1H; H-8b), 2.71 (t, J = 6 Hz, 2H; H-7), 1.94 ppm (app. t, J = 12.6 Hz, 1H; OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.7 (O*C*(O)Ph), 139.7–127.0 (Ar), 98.3 (C-1), 76.4 (C-2), 75.1 (OCH_2Ph), 74.7 (C-3), 73.4 (C-4 and OCH_2Ph), 72.1 (C-5), 66.1 (C-8), 62.2 (C-6), 59.2 (2×NCH_2Ph), 52.8 ppm (C-7); HRMS (ESI): m/z calcd for $C_{43}H_{46}O_7N$: 688.3275 $[M+Na]^+$; found: 688.3253.

(*N*,*N*-Dibenzyl-2-aminoethyl) 6-*O*-(6-*O*-acetyl-3-*O*-benzoyl-2,4-di-*O*-benzyl-α-D-mannopyranosyl)-3-*O*-benzoyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (26): A solution of **13**[4] (0.94 g, 1.44 mmol, 1.1 equiv) in CH_2Cl_2 (25 mL) was added dropwise to a solution of **25** (0.9 g, 1.31 mmol) and TMSOTf (0.33 mL, 1.83 mmol, 1.4 equiv) in anhydrous CH_2Cl_2 (25 mL) under an inert atmosphere at −40 °C. The mixture was stirred at −40 °C for 2 h, then the reaction was quenched with triethylamine and concentrated to dryness. The crude product was purified by "automatic" flash chromatography (70 min, cyclohexane/EtOAc 1:0 to 7:3 (v/v) with 1% Et_3N) to afford **26** (1.13 g, 73%). $[\alpha]_D^{20}$ +12.1 (c = 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.13–7.99 (m, 4H; ArH), 7.65–6.95 (m, 36H; ArH), 5.62 (dd, J(2,3) = 3.3 Hz, J(3,4) = 9.3 Hz, 1H; H-3B), 5.55 (dd, J(2,3) = 3.3 Hz, J(3,4) = 9.7 Hz, 1H; H-3A), 5.17 (d, J(1,2) = 1.7 Hz, 1H; H-1B), 4.82 (d, J = 11.1 Hz, 1H; OCH_2Ph), 4.75 (d, J(1,2) = 1.7 Hz, 1H; H-1A), 4.69 (d, J = 10.8 Hz, 1H; OCH_2Ph), 4.67 (d, J = 13.1 Hz, 1H; OCH_2Ph), 4.58–4.41 (m, 4H; OCH_2Ph), 4.35 (dd, J(5,6a) = 2.3 Hz, J(6a,6b) = 11.8 Hz, 1H; H-6Ba), 4.32 (d, J = 11.9 Hz, 1H; OCH_2Ph), 4.26 (dd, J(5,6b) = 1.7 Hz, J(6a,6b) = 11.8 Hz, 1H; H-6Bb), 4.25 (t, J(3,4) = J(4,5) = 9.7 Hz, 1H; H-4A), 4.11 (t, J(3,4) = J(4,5) = 9.3 Hz, 1H; H-4B), 4.04 (dd, J(1,2) = 1.7 Hz, J(2,3) = 3.3 Hz, 1H; H-2B), 3.99 (m, 1H; H-5B), 3.94 (dd, J(1,2) = 1.7 Hz, J(2,3) = 3.3 Hz, 1H; H-2A), 3.91–3.71 (m, 4H; H-5A, H-6Aa, H-6Ab and H-8a), 3.67 (d, J = 13.9 Hz, 2H; NCH_2Ph), 3.60 (d, J = 13.9 Hz, 2H; NCH_2Ph), 3.48 (m, 1H; H-8b), 2.70 (t, J = 4.6 Hz, 2H; H-7), 2.04 ppm (s, 3H; OC(O)CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.8 (O*C*(O)CH$_3$), 165.6, 165.1 (O*C*(O)Ph), 139.7, 138.3, 138.1, 137.9, 137.7, 133.2, 133.1 (Ar), 130.4–126.7 (Ar), 98.2 (C-1B), 98.0 (C-1A), 76.7 (C-2B), 76.5 (C-2A), 75.1 (OCH_2Ph), 74.9 (C-3A), 74.5 (C-3B), 73.5, 73.4 (C-4A, C-4B, and OCH_2Ph), 72.8 (OCH_2Ph), 72.0 (C-5A), 70.1 (C-5B), 66.0, 65.8 (C-6A and C-8), 63.5 (C-6B), 59.0 (2×NCH_2Ph), 52.8 (C-7), 21.0 ppm (OC(O)CH_3); HRMS (ESI): m/z calcd for $C_{72}H_{74}O_{14}N$: 1176.5110 $[M+H]^+$; found: 1176.5062.

(*N*,*N*-Dibenzyl-2-aminoethyl) 6-*O*-(2,4-di-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (27): A solution of **26** (1.96 g, 1.67 mmol) in methanol (15 mL) was added to a freshly prepared solution of sodium methoxide (0.6 M in MeOH, 15 mL). The mixture was stirred 20 h at RT, then neutralized with acetic acid and concentrated in vacuo. The crude product was purified by "automatic" flash chromatography (40 min, cyclohexane/EtOAc 1:0 to 8:2 (v/v) with 1% Et_3N) to afford **27** (1.32 g, 86%). $[\alpha]_D^{20}$ +39.7 (c = 1.2 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.5–7.15 (m, 30H; ArH), 5.03 (d, J(1,2) = 1.5 Hz, 1H; H-1B), 4.96 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.81 (d, J(1,2) = 1.3 Hz, 1H; H-1A), 4.72–4.57 (m, 4H; OCH_2Ph), 4.54 (d, J = 11.8 Hz, 1H; OCH_2Ph), 4.41 (d, J = 11.7 Hz, 1H; OCH_2Ph), 3.87 (dd, J(5,6a) = 4.1 Hz, J(6a,6b) = 11.2 Hz, 1H; H-6Aa), 4.01–3.53 (m, 16H; NCH_2Ph, H-2A, H-2B, H-6Ab, H-6Ba, H-6Bb, H-5A, H-5B, H-8a, H-3A, H-3B, H-4A and H-4B), 3.46 (m, 1H; H-8b), 2.66 (app. t, J = 5.8 Hz, 2H; H-7), 2.29 ppm (br. s, 2H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 139.7–127.1 (Ar), 97.4 (C-1B), 96.9 (C-1A), 78.8, 78.6 (C-2A and C-2B), 76.6–76.5 (C-3A or C-3B or C-4A or C-4B), 75.1, 74.7 (OCH_2Ph), 73.1, 73.0 (OCH_2Ph), 72.1, 71.9 (C-3A or C-3B or C-4A or C-4B), 71.5, 70.9 (C-5A and C-5B), 66.3 (C-6A), 65.9 (C-8), 62.4 (C-6B), 59.1 (2×NCH_2Ph), 52.9 ppm (C-7); HRMS (ESI): m/z calcd for $C_{56}H_{64}O_{11}N$: 926.4479 $[M+H]^+$; found: 926.4471.

(*N*,*N*-Dibenzyl-2-aminoethyl) 6-*O*-(3,6-di-*O*-propargyl-2,4-di-*O*-benzyl-α-D-mannopyranosyl)-3-*O*-propargyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (10): NaH (60% in mineral oil, 400 mg, 9.98 mmol, 7 equiv) and propargyl bromide (80% in toluene, 1.4 mL, 12.83 mmol, 9 equiv) were added to a solution of **27** (1.32 g, 1.43 mmol) in anhydrous DMF (20 mL) cooled to 0 °C under an inert atmosphere. The reaction mixture was stirred for 1 h at 0 °C and then slowly warmed to RT for 2 h. The reaction mixture was neutralized with MeOH and diluted with H_2O (50 mL) and aqueous phase was extracted with EtOAc (3×50 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by "automatic" flash chromatography (65 min, cyclohexane/EtOAc 1:0 to 7:3 (v/v) with 1% Et_3N) to afford **10** (1.35 g, 91%); $[\alpha]_D^{20}$ +23.6 (c = 0.8 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45–7.13 (m, 30H; ArH), 5.05 (d, J(1,2) = 1.8 Hz, 1H; H-1B), 4.93 (d, J = 11 Hz, 1H; OCH_2Ph), 4.88 (d, J = 11 Hz, 1H; OCH_2Ph), 4.73 (d, J = 12.3 Hz, 1H; OCH_2Ph), 4.73 (d, J(1,2) = 1.7 Hz, 1H; H-1A), 4.69–4.55 (m, 5H; OCH_2Ph), 4.30–4.06 (m, 6H; OCH_2CCH), 3.99 (dd, J(2,3) = 2.9 Hz, J(3,4) = 9.2 Hz, 1H; H-3B), 3.96–3.84 (m, 5H; H-2B, H-3A, H-5A, H-6Ba, and H-5B), 3.83–3.75 (m, 3H; H-2A, H-6Aa and H-4A or H-4B), 3.75–3.65 (m, 4H; H-6Ab, H-6Bb, H-8a and H-4A or H-4B),

3.64 (d, $J=13.7$ Hz, 2H; NCH_2Ph), 3.58 (d, $J=13.7$ Hz, 2H; NCH_2Ph), 3.49–3.35 (m, 1H; H-8b), 2.63 (app. t, $J=5.7$ Hz, 2H; H-7), 2.36 (t, $J=2.4$ Hz, 1H; OCH$_2$CC*H*), 2.32 (t, $J=2.3$ Hz, 1H; OCH$_2$CC*H*), 2.31 ppm (t, $J=2.3$ Hz, 1H; OCH$_2$CC*H*); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=139.7$–127.2 (ArH), 98.5 (C-1B), 98.0 (C-1A), 80.2 (C-3A), 80.1 (OCH$_2$*C*CH), 80.1 (C-3B), 75.8 (C-2B), 75.5 (C-2A), 75.1 (O*C*H$_2$Ph), 74.8–74.5 (OCH$_2$*C*CH, C-5A and C-5B), 73.1, 72.6 (O*C*H$_2$Ph), 71.8, 71.7 (C-4A and C-4B), 68.8 (C-6A), 66.3 (C-6B), 65.9 (C-8), 59.1 (2× NCH$_2$Ph), 58.6, 57.8, 57.6 (O*C*H$_2$CCH), 52.7 ppm (C-7); HRMS (ESI): *m/z* calcd for $C_{65}H_{70}O_{11}N$: 1040.4949 $[M+H]^+$; found: 1040.5001.

Phenyl 3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (28): TMSOTf (0.45 mL, 4.52 mmol, 0.5 equiv) was added to a solution of **14**[7] (5.76 g, 9.04 mmol) and **12** (4.08 g, 9.04 mmol, 1 equiv) in anhydrous CH_2Cl_2 (125 mL) under inert atmosphere at −40 °C. The mixture was stirred at −40 °C for 1 h, quenched with triethylamine, and concentrated to dryness. The crude product was purified by "automatic" flash chromatography (55 min, cyclohexane/EtOAc 1:0 to 7:3 (v/v)) to afford **28** (6.65 g, 79%). $[\alpha]_D^{20}$ +83.9 ($c=1$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.52$–7.12 (m, 30H; ArH), 5.65 (s, 1H; H-7), 5.63 (dd, $J(1,2)=1.8$ Hz, $J(2,3)=3.3$ Hz, 1H; H-2B), 5.51 (d, $J(1,2)=1.4$ Hz, 1H; H-1A), 5.35 (d, $J(1,2)=1.8$ Hz, 1H; H-1B), 4.89 (d, $J=10.9$ Hz, 1H; OCH_2Ph), 4.76–4.62 (m, 4H; OCH_2Ph), 4.57–4.44 (m, 3H; OCH_2Ph), 4.35–4.28 (m, 3H; H-3A, H-5A and H-5B), 4.24 (dd, $J(5,6a)=3.6$ Hz, $J(6a,6b)=10.3$ Hz, 1H; H-6Ba), 4.07 (br. s, 1H; H-2A), 3.98 (dd, $J(2,3)=3.3$ Hz, $J(3,4)=8.9$ Hz, 1H; H-3B), 3.93–3.78 (m, 3H; H-4A, H-4B and H-6Bb), 3.77–3.69 (m, 2H; H-6Aa and H-6Ab), 2.11 ppm (s, 3H; OC(O)CH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=$ 170.1 (O*C*(O)CH$_3$), 138.7–128.1 (Ar), 101.4 (C-7), 99.1 (C-1B), 87.1 (C-1A), 79.3 (C-2A), 79.2 (C-3A), 78.1 (C-3B), 75.2 (O*C*H$_2$Ph), 74.5 (C-4B), 73.6 (O*C*H$_2$Ph), 73.3 (C-5A or C-5B), 73.2 (O*C*H$_2$Ph), 72.4 (C-4A), 71.8 (O*C*H$_2$Ph), 69.2 (C-6A), 68.5 (C-6B), 68.3 (C-2B), 65.5 (C-5A or C-5B), 21.2 ppm (OC(O)*C*H$_3$); HRMS (ESI): *m/z* calcd for $C_{55}H_{56}O_{11}SNa$: 947.3441 $[M+Na]^+$; found: 947.3467.

Phenyl 3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (29): Compound **28** (6.6 g, 7.1 mmol) was treated with BH_3 (1 M in THF, 22.8 mL, 22.8 mmol, 3.2 equiv) and Bu_2BOTf (1 M in CH_2Cl_2, 7.6 mL, 7.6 mmol, 1.06 equiv) at 0 °C under an inert atmosphere. The reaction mixture was stirred for 3 h at 0 °C then the reaction was quenched by the addition of MeOH dropwise (until gas release ceased) and Et_3N (until pH reached 10). The solvent was evaporated under reduced pressure and the crude product was purified by "automatic" flash chromatography (45 min, cyclohexane/EtOAc 1:0 to 1:1 (v/v)) to afford **29** (6.29 g, 94%). $[\alpha]_D^{20}$ +81.2 ($c=1$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.42$–7.16 (m, 30H, ArH), 5.50 (dd, $J(1,2)=1,8$ Hz, $J(2,3)=3.3$ Hz, 1H; H-2B), 5.49 (d, $J(1,2)=1.5$ Hz, 1H; H-1A), 5.25 (d, $J(1,2)=1,8$ Hz, 1H; H-1B), 4.89 (d, $J=11.1$ Hz, 1H; OCH_2Ph), 4.82 (d, $J=10.8$ Hz, 1H; OCH_2Ph), 4.72–4.45 (m, 9H; OCH_2Ph), 4.19–4.04 (m, 4H; H-2A, H-3A, H-4A and H-5A), 4.00 (dd, $J(2,3)=3.3$ Hz, $J(3,4)=9$ Hz, 1H; H-3B), 3.95–3.87 (m, 1H; H-5B), 3.85 (t, $J(3,4)=J(4,5)=9$ Hz, 1H; H-4B), 3.79 (m, 2H; H-6Aa and H-6Ab), 3.74–3.65 (m, 2H; H-6Ba and H-6Bb), 2.12 (s, 3H; OC(O)CH$_3$), 1.85 ppm (br. s, 1H; OH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=170.2$ (O*C*(O)CH$_3$), 138.7–127.7 (Ar), 99.9 (C-1B), 85.5 (C-1A), 79.2, 78.9 (C-2A and/or C-3A and/or C-4A and/or C-5A), 78.0 (C-3B), 75.4, 75.1 (OCH$_2$Ph), 75.0 (C-2A or C-3A or C-4A or C-5A), 74.6 (C-4B), 73.7 (OCH$_2$Ph), 73.4 (C-2A or C-3A or C-4A or C-5A), 72.5 (C-5B), 72.0 (OCH$_2$Ph), 69.3 (C-6B), 69.0 (C-2B), 62.1 (C-6A), 21.1 ppm (OC(O)*C*H$_3$); HRMS (ESI): *m/z* calcd for $C_{55}H_{58}O_{11}SNa$: 949.3597 $[M+Na]^+$; found: 949.3643.

Phenyl 3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-(6-*O*-acetyl-3-*O*-benzoyl-2,4-di-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (30): Compound **13**[4] (3.13 g, 4.82 mmol, 1.6 equiv) in anhydrous CH_2Cl_2 (75 mL) was added dropwise to a solution of **29** (2.79 g, 3.01 mmol) and TMSOTf (0.24 mL, 2.41 mmol, 0.5 equiv) in anhydrous CH_2Cl_2 (75 mL) under an inert atmosphere at −40 °C. The mixture was stirred at −40 °C for 1 h, quenched with triethylamine, and concentrated to dryness. The crude product was purified by "automatic" flash chromatography (70 min, cyclohexane/EtOAc 1:0 to 0:1 (v/v)) to afford **30** (2.95 g, 70%): $[\alpha]_D^{20}$ +54.3 ($c=1$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta=8.07$ (d, $J=7.4$ Hz, 2H; ArH), 7.63–6.98 (m, 43H; ArH), 5.59 (dd, $J(2,3)=3.3$ Hz, $J(3,4)=9.5$ Hz, 1H; H-3C), 5.51 (m, 1H; H-2A), 5.46 (s, 1H; H-1A), 5.25 (s, 1H; H-1B), 5.10 (d, $J(1,2)=1.4$ Hz, 1H; H-1C), 4.90 (d, $J=10.4$ Hz, 1H; OCH_2Ph), 4.87 (d, $J=10.8$ Hz, 1H; OCH_2Ph), 4.75–4.42 (m, 11H; OCH_2Ph), 4.33 (d, $J=12.1$ Hz, 1H; OCH_2Ph), 4.31 (d, $J(5,6)=3.3$ Hz, 2H; H-6C), 4.24 (dd, $J(2,3)=4.1$ Hz, $J(3,4)=9.2$ Hz, 1H; H-3B), 4.13–4.05 (m, 4H; H-2B, H-4B, H-4C and H-5A), 4.03 (dd, $J(1,2)=1.4$ Hz, $J(2,3)=3.3$ Hz, 1H; H-2C), 4.01 (dd, $J(2,3)=3.3$ Hz, $J(3,4)=9.2$ Hz, 1H; H-3A), 3.96–3.90 (m, 2H; H-5C and H-6Aa), 3.89–3.85 (m, 1H; H-5B), 3.84–3.75 (m, 2H; H-4A and H-6Ab), 6.69–6.62 (m, 2H; H-6Ba and H-6Bb), 2.10 (s, 3H; OC(O)CH$_3$), 2.04 ppm (s, 3H; OC(O)CH$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta=171.0$, 170.2 (O*C*(O)CH$_3$), 165.5 (O*C*(O)Ph), 138.8–127.5 (Ar), 100.0 (C-1B), 98.2 (C-1C), 85.6 (C-1A), 79.2 (C-2B or C-4C or C-4B or C-5A), 78.1 (C-3A), 77.4 (C-2B or C-4C or C-4B or C-5A), 76.6 (C-2C), 75.5 (OCH$_2$Ph), 75.2 (C-2B or C-4C or C-4B or C-5A), 75.1, 75.0 (OCH$_2$Ph), 74.5, 74.4 (C-4A and C-3C), 73.6 (OCH$_2$Ph), 73.5 (C-2B or C-4C or C-4B or C-5A), 73.0 (OCH$_2$Ph and C-3B), 72.5 (C-5B), 72.1, 71.8 (OCH$_2$Ph), 70.0 (C-5C), 69.3 (C-6B), 69.0 (C-2A), 66.2 (C-6A), 63.4 (C-6C), 21.1, 21.0 ppm (OC(O)*C*H$_3$); HRMS (ESI): *m/z* calcd for $C_{84}H_{86}O_{18}SNa$: 1437.5432 $[M+Na]^+$; found: 1437.5372.

(*N*,*N*-Dibenzyl-2-aminoethyl) 3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-(6-*O*-acetyl-3-*O*-benzoyl-2,4-di-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (31): *N*-Iodosuccinimide (0.207 g, 0.92 mmol, 1.3 equiv) followed by TfOH (0.10 mL, 1.13 mmol, 1.6 equiv) were added to a solution of **30** (1.0 g, 0.71 mmol), dibenzylaminoethanol (0.188 g, 0.78 mmol, 1.1 equiv) and molecular sieves (1 g) in anhydrous CH_2Cl_2 (50 mL) under an inert atmosphere at 0 °C. The mixture was stirred at 0 °C for 2 h, then the reaction was quenched with triethylamine and concentrated to dryness. The residue was diluted in EtOAc, washed with saturated aq. $NaHCO_3$ and $Na_2S_2O_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by "automatic" flash chromatography (55 min, cyclohexane/EtOAc 1:0 to 7:3 (v/v) with 1% Et_3N) to afford **31** (0.827 g, 76%). $[\alpha]_D^{20}$ +26.4 ($c=$ 3.2 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta=8.08$ (d, $J=7.4$ Hz, 2H; ArH), 7.67–7.01 (m, 48H; ArH), 5.62 (dd, $J(2,3)=3.2$ Hz, $J(3,4)=$ 9.5 Hz, 1H; H-3C), 5.55 (dd, $J(1,2)=1.7$ Hz, $J(2,3)=3.2$ Hz, 1H; H-2B), 5.24 (d, $J(1,2)=1.7$ Hz, 1H; H-1B), 5.15 (d, $J(1,2)=1.7$ Hz, 1H; H-1C), 4.91 (d, $J=11.6$ Hz, 1H; OCH_2Ph), 4.89 (d, $J=11.4$ Hz, 1H; OCH_2Ph), 4.74 (d, $J(1,2)=1.2$ Hz, 1H; H-1A), 4.73–4.62 (m, 4H; OCH_2Ph), 4.60–4.44 (m, 6H; OCH_2Ph), 4.41–4.32 (m, 3H; OCH_2Ph and H-6Ca), 4.31 (dd, $J(5,6b)=4.2$ Hz, $J(6a,6b)=11.6$ Hz, 1H; H-6Cb), 4.18 (dd, $J(2,3)=2.8$ Hz, $J(3,4)=9.5$ Hz, 1H; H-3A), 4.13 (t, $J(3,4)=J(4,5)=9.5$ Hz, 1H; H-4C), 4.09–3.98 (m, 4H; H-3B, H-2C, H-4A and H-5C), 3.92–3.84 (m, 3H; H-4B, H-5A and H-6Aa), 3.80–3.66 (m, 4H; H-2A, H-5B, H-6Ab and H-8a), 3.66–3.52 (m, 6H; NCH_2Ph, H-6Ba and H-6Bb), 3.48–3.42 (m, 1H; H-8b), 2.71–2.60 (m, 2H; H-7), 2.11 (s, 3H; OC(O)CH$_3$), 2.06 ppm (s, 3H; OC(O)CH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=171.0$, 170.1 (O*C*(O)CH$_3$), 165.5 (O*C*(O)Ph), 139.8–126.9 (Ar), 99.8 (C-1B), 98.1 (C-1C), 97.4 (C-1A), 78.6 (C-3A), 78.1 (C-3B), 77.8 (C-2A), 76.7 (C-2C), 75.2 (OCH$_2$Ph and C-4A), 75.0, 74.9 (OCH$_2$Ph), 74.5 (C-3C), 74.4 (C-4B), 73.5 (OCH$_2$Ph and C-4C), 72.8, 72.6 (OCH$_2$Ph), 72.4 (C-5B), 72.2 (C-5A), 72.0 (OCH$_2$Ph), 70.0 (C-

5C), 69.1 (C-6B), 69.0 (C-2B), 65.9 (C-6A), 65.7 (C-8), 63.5 (C-6C), 58.9 (2 × NCH_2Ph), 52.8 (C-7), 21.1, 21.0 ppm ($OC(O)CH_3$); HRMS (ESI): *m/z* calcd for $C_{94}H_{100}O_{19}N$: 1546.6884 $[M+H]^+$; found: 1546.6853.

(*N*,*N*-Dibenzyl-2-aminoethyl) 3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-(2,4-di-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (32): A solution of **31** (0.136 g, 0.09 mmol) in methanol (20 mL) was added to a freshly prepared solution of sodium methoxide (0.8 M in MeOH, 20 mL). The mixture was stirred for 20 h at RT and then neutralized with acetic acid until pH 7, and concentrated in vacuo. The crude product was purified by "automatic" reverse phase chromatography (50 min, H_2O/acetonitrile 1:1 to 0:1 (v/v)) to afford **32** (102 mg, 85%): $[\alpha]_D^{20}$ +39.1 (*c* = 0.5 in $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ = 7.42–7.21 (m, 45H; ArH), 5.29 (d, *J*(1,2) = 1.2 Hz, 1H; H-1B), 5.14 (d, *J*(1,2) = 1.2 Hz, 1H; H-1C), 4.96 (d, *J* = 11.4 Hz, 1H; OCH_2Ph), 4.89 (d, *J* = 11.4 Hz, 1H; OCH_2Ph), 4.80 (d, *J* = 11.4 Hz, 1H; OCH_2Ph), 4.77 (d, *J*(1,2) = 1.2 Hz, 1H; H-1A), 4.72–4.63 (m, 9H; OCH_2Ph), 4.44 (d, *J* = 12.6 Hz, 1H; OCH_2Ph), 4.39 (d, *J* = 11.4 Hz, 1H; OCH_2Ph), 4.17 (dd, *J*(2,3) = 3 Hz, *J*(3,4) = 9.6 Hz, 1H; H-3A), 4.06 (dd, *J*(2,3) = 3.6 Hz, *J*(3,4) = 9.6 Hz, 1H; H-3C), 4.03 (dd, *J*(1,2) = 1.2 Hz, *J*(2,3) = 3 Hz, 1H; H-2B), 3.98 (t, *J*(3,4) = *J*(4,5) = 9.6 Hz, 1H; H-4A), 3.95 (dd, *J*(2,3) = 3 Hz, *J*(3,4) = 9 Hz, 1H; H-3B), 3.94–3.87 (m, 3H; H-4B, H-5C and H-6Aa), 3.86 (dd, *J*(1,2) = 1.2 Hz, *J*(2,3) = 9.6 Hz, 1H; H-2A), 3.84–3.96 (m, 3H; H-2C, H-6Ca and H-6Cb), 3.75–3.69 (m, 4H; H-4C, H-5A, H-5B and H-8a), 3.70–3.59 (m, 7H; H-6Ab, H-6Ba, H-6Bb and NCH_2Ph), 3.46 (m, 1H; H-8b), 2.69 (dt, *J* = 1.2, 6 Hz, 2H; H-7), 2.42 (d, *J*(2,OH) = 2.4 Hz, 1H; OH), 2.31 (d, *J*(3,OH) = 9 Hz, 1H; OH), 1.97 ppm (m, 1H; OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 139.6–137 (Ar), 129.5–126.2 (Ar), 101.5 (C-1B), 97.6 (C-1A and C-1C), 80.1 (C-3B), 78.8 (C-2C and C-3A), 77.8 (C-2A), 76.6 (C-4C or C-4B or C-5A), 75.1 and 74.9 (OCH_2Ph), 74.4 (C-5B or C-5C), 73.5, 72.8, 72.5 and 72.2 (OCH_2Ph), 72.1 (C-4C or C-4B or C-5A), 72.0 (C-5B or C-5C), 71.9 (C-3C), 71.5 (C-4C or C-4B or C-5A), 69.2 (C-6B), 68.8 (C-2B), 66.2 (C-6A), 65.9 (C-8), 62.3 (C-6C), 59.0 (2 × NCH_2Ph), 52.7 ppm (C-7); HRMS (ESI): *m/z* calcd for $C_{83}H_{92}O_{16}N$: 1358.6416 $[M+H]^+$; found: 1358.6428.

(*N*,*N*-Dibenzyl-2-aminoethyl) 3-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-propargyl-α-D-mannopyranosyl)-6-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-propargyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (11): NaH (60% in mineral oil, 81 mg, 2.02 mmol, 7 equiv) and propargyl bromide (80% in toluene, 0.3 mL, 2.6 mmol, 9 equiv) were added to a solution of **32** (0.392 g, 0.29 mmol) in anhydrous DMF (20 mL) and cooled to 0 °C under an inert atmosphere. The mixture was stirred for 1 h at 0 °C and then slowly warmed to RT for 1 h. The reaction mixture was neutralized with MeOH, diluted with H_2O (50 mL) and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by "automatic" flash chromatography (60 min, cyclohexane/EtOAc 1:0 to 7:3 (v/v) with 1% Et_3N) to afford **11** (253 mg, 60%). $[\alpha]_D^{20}$ +54.9 (*c* = 0.88 in $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ = 7.39–7.12 (m, 45H; ArH), 5.22 (d, *J*(1,2) = 1.2 Hz, 1H; H-1B), 5.01 (d, *J*(1,2) = 1.7 Hz, 1H; H-1C), 4.88, 4.87 (2d, *J* = 10.8, 12.2 Hz, 2H; OCH_2Ph), 4.79 (d, *J* = 11.5 Hz, 1H; OCH_2Ph), 4.71 (d, *J*(1,2) = 1.4 Hz, 1H; H-1A), 4.69 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.66–4.44 (m, 9H; OCH_2Ph), 4.37 (d, *J* = 12.1 Hz, 1H; OCH_2Ph), 4.28 (dd, $J(CH_2,CH)$ = 2.3 Hz, *J* = 16.3 Hz, 1H; OCH_2CCH), 4.23 (dd, $J(CH_2,CH)$ = 2.4 Hz, *J* = 16 Hz, 1H; OCH_2CCH), 4.17–4.09 (m, 5H; OCH_2CCH and H-3A), 4.07 (dd, *J*(1,2) = 1.2 Hz, *J*(2,3) = 2.8 Hz, 1H; H-2B), 3.99–3.85 (m, 6H; H-3B, H-3C, H-4A, H-4B, H-4C and H-5A or H-5B or H-5C), 3.86–3.83 (m, 2H; H-2C and H-6Aa), 3.82 (dd, *J*(1,2) = 1.4 Hz, $J_{2,3}$ = 3 Hz, 1H; H-2A), 3.81–3.74 (m, 2H; H-6Ca and H-5A or H-5B or H-5C), 3.70–3.64 (m, 3H; H-8a, H-6Cb and H-5A or H-5B or H-5C), 3.63–3.54 (m, 7H; NCH_2Ph, H-6Ba, H-6Bb and H-6Ab), 3.39 (m, 1H; H-8b), 2.60 (m, 2H; H-7), 2.35 (t, *J* = 2.4 Hz, 1H; OCH_2CCH), 2.26 (t, *J* = 2.4 Hz, 1H; OCH_2CCH), 2.16 ppm (t, *J* = 2.3 Hz, 1H; OCH_2CCH); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 140.0–138.0 (Ar), 128.9–127.0 (Ar), 100.2 (C-1B), 98.5 (C-1C), 97.2 (C-1A), 80.2, 80.1, 79.9 (OCH_2CCH), 79.8 (C-3B or C-3C), 79.5 (C-3A), 79.3 (C-3B or C-3C), 78.0 (C-2A), 75.7 (C-2C), 75.3–74.7 (C-4A, C-4B, C-4C, OCH_2CCH, OCH_2Ph), 74.6 (OCH_2Ph), 74.3 (C-2B), 73.5 (OCH_2Ph), 72.7 (C-5A or C-5B or C-5C), 72.6, 72.5, 72.4 (OCH_2Ph), 71.7, 71.6 (C-5A and/or C-5B and/or C-5C), 69.4 (C-6B), 68.7 (C-6C), 66.3 (C-6A), 65.9 (C-8), 59.0 (2×NCH_2Ph), 58.6, 58.1, 57.6 (OCH_2CCH), 52.7 ppm (C-7); HRMS (ESI): *m/z* calcd for $C_{92}H_{98}O_{16}N$: 1472.6880 $[M+H]^+$; found: 1472.6849.

(*N*,*N*-Dibenzyl-2-aminoethyl) 6-*O*-(3,6-di-*O*-[1-(α-D-mannopyranosyl)-1,2,3-triazol-4-yl]methyl-2,4-di-*O*-benzyl-α-D-mannopyranosyl)-3-*O*-[1-(α-D-mannopyranosyl)-1,2,3-triazol-4-yl]methyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (39): A freshly prepared solution of copper sulfate (16 mg, 0.07 mmol, 0.6 equiv) and sodium ascorbate (43 mg, 0.13 mmol, 1.2 equiv) in water (0.5 mL) was added to a vigorously stirred solution of **10** (114 mg, 0.11 mmol) and azide **6**[5] (90 mg, 0.44 mmol, 4 equiv) in anhydrous DMF (3 mL). The reaction mixture was placed under microwave irradiation at 100 °C for 30 min then concentrated. The crude product was purified by "automatic" reverse phase chromatography (50 min, water/acetonitrile, 7:3 to 0:1 (v/v)) to afford **39** (130 mg, 72%). $[\alpha]_D^{20}$ +55.1 (*c* = 0.83 in MeOH); ^1H NMR (600 MHz, MeOD): δ = 8.01, 7.97, 7.86 (3 × s, 3H; H-5 triazole), 7.40–7.12 (m, 30H, ArH), 5.95 (d, *J*(1,2) = 2.2 Hz, 1H; H-1C or D or E), 5.88 (d, *J*(1,2) = 2.5 Hz, 1H; H-1C or D or E), 5.87 (d, *J*(1,2) = 2.4 Hz, 1H; H-1C or D or E), 4.91 (d, *J*(1,2) = 1.5 Hz, 1H; H-1B), 4.89 (d, *J* = 12 Hz, 1H; OCH_2Ph), 4.88 (d, *J*(1,2) < 1 Hz, 1H; H-1A), 4.78–4.73 (2d, 2H; OCH_2Ph and $OCH_2(CCHN_3)$), 4.72–4.65 (2d, 2H; OCH_2Ph and $OCH_2(CCHN_3)$), 4.64–4.58 (m, 5H; H-2C to E, OCH_2Ph and $OCH_2(CCHN_3)$), 4.57–4.53 (m, 3H; OCH_2Ph), 4.53–4.49 (m, 2H; $OCH_2(CCHN_3)$), 4.48 (d, *J* = 11 Hz, 1H; OCH_2Ph), 4.43 (d, *J* = 12.4 Hz, 1H; $OCH_2(CCHN_3)$), 4.16 (dd, *J*(2,3) = 3.5 Hz, *J*(3,4) = 8.8 Hz, 1H; H-3C or D or E), 4.08 (dd, *J*(2,3) = 2 Hz, *J*(3,4) = 8.6 Hz, 1H; H-3C or D or E), 4.07 (dd, *J*(2,3) = 1.9 Hz, *J*(3,4) = 8.6 Hz, 1H; H-3C or D or E), 3.96–3.46 (m, 27H; H-2A, H-2B, H-3A, H-3B, H-4A to E, H-5A, H-5B, H-6Aa to Ea, H-6Ab to Eb, H-8 and NCH_2Ph), 3.30–3.21 (m, 3H; H-5C to E), 2.65 ppm (m, 2H; H-7); ^{13}C NMR (75 MHz, MeOD): δ = 146.4, 146.3, 146.0 (3×C-4 triazole), 140.8, 140.0, 139.8, 139.7 (Ar), 130.0–128.1 (Ar), 125.1, 125.0, 124.9 (3×C-5 triazole), 99.5 (C-1B), 99.2 (C-1A), 88.3, 88.2 (C-1C to E), 82.0 (C-3A), 81.1 (C-3B), 78.4, 78.3, 78.2 (C-5C to E), 76.6, 76.3 (C-2B and C-2A), 75.9, 75.8 (OCH_2Ph), 75.7, 75.6 (C-4A and C-4B), 74.3, 73.7 (OCH_2Ph), 73.0, 72.6 (C-5A and C-5B), 72.6, 72.5 (C-3C to E), 70.5 (C-6A or C-6B), 70.2, 70.1 (C-2C to E), 68.5, 68.4, 68.3 (C-4C to E), 67.6 (C-6A or C-6B), 66.6 (C-8), 65.2, 64.1, 63.7 (3 × $OCH_2(CCHN_3)$), 62.5, 62.4, 62.3 (C-6C to E), 60.0 (2×NCH_2Ph), 54 ppm (C-7); HRMS (ESI): *m/z* calcd for $C_{83}H_{103}O_{26}N_{10}$: 1655.7045 $[M+H]^+$; found: 1655.7061.

(*N*,*N*-Dibenzyl-2-aminoethyl) 3-*O*-(2-*O*-[1-(α-D-mannopyranosyl)-1,2,3-triazol-4-yl]methyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-(3,6-di-*O*-[1-(α-D-mannopyranosyl)-1,2,3-triazol-4-yl]methyl-2,4-di-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (40): A freshly prepared solution of copper sulfate (22 mg, 0.09 mmol, 0.6 equiv) and sodium ascorbate (58 mg, 0.18 mmol, 1.2 equiv) in water (0.5 mL) was added dropwise to a vigorously stirred solution of **11** (216 mg, 0.15 mmol) and azide **6**[5] (105 mg, 0.51 mmol, 3.5 equiv) in DMF (5 mL). The reaction mixture was placed under microwave irradiation at 100 °C for 30 min then concentrated. The crude product was purified by "automatic" reverse phase chromatography (50 min, water/acetonitrile 1:1 to 0:1 (v/v)) to afford **40** (229 mg, 75%). $[\alpha]_D^{20}$ +57.3 (*c* = 0.53 in

MeOH); ^{1}H NMR (600 MHz, MeOD): δ = 8.0, 7.95, 7.87 (3 s, 3×1 H; H-5 triazole), 7.36–7.03 (m, 45 H; ArH), 5.90 (d, J(1,2) = 2.5 Hz, 1 H; H-1D or H-1E or H-1F), 5.87 (d, J(1,2) = 2.5 Hz, 2 H; H-1D and/or H-1E and/or H-1F), 5.19 (d, J(1,2) = 1.1 Hz, 1 H; H-1B), 4.94 (d, J(1,2) = 1.3 Hz, 1 H; H-1C), 4.83 (d, J(1,2) = 1.1 Hz, 1 H; H-1A), 4.79–4.74 (m, 3 H; OCH_2Ph), 4.71–4.41 (m, 18 H; OCH_2Ph, OCH_2(CCHN$_3$) and H-2D to F), 4.37 (d, J = 11.9 Hz, 1 H; OCH_2Ph), 4.30 (d, J = 11.9 Hz, 1 H; OCH_2Ph), 4.15 (d, J(2,3) = 2.8 Hz, J(3,4) = 9.6 Hz, 1 H; H-3A), 4.14–4.07 (m, 3 H; H-3D to F), 3.97 (t, J(3,4) = J(4,5) = 9.6 Hz, 1 H; H-4A), 3.94–3.65 (m, 22 H; H-2A to C, H-3B, H-3C, H-4B to F, H-5A to C, H-6Aa, H-6Ca, H-6Da to Fa, H-6Db to Fb and H-8a), 3.64–3.49 (m, 8 H; H-6Ab, H-6Ba, H-6Bb, H-6Cb and NCH_2Ph), 3.45 (m, 1 H; H-8b), 3.33–3.25 (m, 3 H; H-5D to F), 2.57 ppm (m, 2 H; H-7); ^{13}C NMR (75 MHz, MeOD): δ = 146.5, 146.4, 146.3 (C-4 triazole), 140.8–139.5 (Ar), 130.2–127.6 (Ar), 124.9, 124.7 (C-5 triazole), 101.5 (C-1B), 99.6 (C-1C), 98.6 (C-1A), 88.3 (C-1D to F), 81.7 (C-3B or C-3C), 80.5 (C-3B or C-3C), 80.1 (C-3A), 79.3 (C-2A), 78.3 (C-5D to F), 77.8 (C-2B), 76.5, 76.4, 76.1, 75.8, 73.9, 73.0, 72.9 (C-2C, C-4A to C, C-5A to C); 75.9, 75.7, 75.6, 74.3, 73.7, 73.6, 73.1 (OCH_2Ph), 72.6 (C-3D to F); 70.5 (C-6B and C-6C), 70.1 (C-2D to F), 68.5 (C-4D to F), 67.6 (C-6A), 66.5 (C-8), 65.2, 64.3 (3×OCH_2(CCHN$_3$)), 62.4 (C-6D to F), 59.8 (2× NCH_2Ph), 59.8 ppm (C-7); HRMS (ESI): m/z calcd for $C_{110}H_{131}O_{31}N_{10}$: 2087.8922 $[M+H]^+$; found: 2087.8989.

2-Aminoethyl 6-*O*-(3,6-di-*O*-[1-(α-D-mannopyranosyl)-1,2,3-triazol-4-yl]methyl-α-D-mannopyranosyl)-3-*O*-[1-(α-D-mannopyranosyl)-1,2,3-triazol-4-yl]methyl-α-D-mannopyranoside hydroformate (4): Palladium on charcoal (10%, 30 mg, 0.28 mmol) and resin with ammonium formate (1.3 g) was added to a solution of **39** (93 mg, 0.056 mmol) in an isopropanol/H_2O mixture (5:1 (v/v), 12 mL). The reaction mixture was placed under microwave irradiation at 80 °C for 40 min, filtered over Celite and concentrated in vacuo. The crude product was then purified by HPLC (Method 2) to afford **4** as a white solid (51 mg; 81%). $[\alpha]_D^{20}$ + 60.5 (c = 0.43 in H_2O); ^{1}H NMR (600 MHz, D_2O): δ = 8.46 (s, 1 H; OC(O)*H*), 8.26, 8.24, 8.23 (3s, 3 H, H-5 triazole), 6.16 (d, J(1,2) = 2.4 Hz, 1 H; H-1C or H-1D or H-1E), 6.15 (d, J(1,2) = 1.8 Hz, 1 H; H-1C or H-1D or H-1E), 6.14 (d, J(1,2) = 2.4 Hz, 1 H; H-1C or H-1D or H-1E), 4.94 (d, J(1,2) = 1.8 Hz, 1 H; H-1B), 4.91 (d, J(1,2) = 1.8 Hz, 1 H; H-1A), 4.89 (d, J = 12.1 Hz, 1 H; OCH_2(CCHN$_3$)), 4.86 (d, J = 13.2 Hz, 1 H; OCH_2(CCHN$_3$)), 4.83–4.71 (m, 7 H; H-2C to E and OCH_2(CCHN$_3$)), 4.21 (dd, J(1,2) = 1.8 Hz, J(2,3) = 3 Hz, 1 H; H-2A), 4.18 (dd, J(1,2) = 1.8 Hz, J(2,3) = 2.4 Hz, 1 H; H-2B), 4.83–4.72 (m, 3 H; H-3C to E), 4.0–3.94 (m, 2 H; H-6Aa and H-8a), 3.90–3.69 (m, 19 H; H-3A, H-3B, H-4A to E, H-5A, H-5B, H-6Ab, H-6Ba to Ea, H-6Bb to Eb and H-8b), 3.37–3.30 (m, 3 H; H-5C to E), 3.0–3.22 ppm (m, 2 H; H-7); ^{13}C NMR (150 MHz, D_2O): δ = 171.0 (O*C*(O)H), 144.6, 144.5, 144.2 (C-5 triazole), 124.8, 124.7 (C-4 triazole), 100.1 (C-1B), 99.5 (C-1A), 86.7, 86.6 (C-1C to E), 78.6, 78.4 (C-3A and C-3B), 76.2, 76.1 (C-5C to E), 71.1, 71.0 (C-4A and/or C-4B and/or C-5A and/or C-5B), 70.5, 70.4 (C-3C to E), 68.9 (C-6B), 68.2 (C-2C to E), 66.6 (C-2A and C-2B), 66.6 (C-4C to E), 65.5 (C-8), 65.4 (C-4A or C-4B or C-5A or C-5B), 65.1 (C-4A or C-4B or C-5A or C-5B), 63.9 (C-6A), 63.1, 61.6, 61.5 (OCH_2(CCHN$_3$)), 60.4 (C-6C to E), 39.1 ppm (C-7); HRMS (ESI, amine form): m/z calcd for $C_{41}H_{67}O_{26}N_{10}$: 1115.4181 $[M+H]^+$; found: 1115.4228.

2-Aminoethyl 3-*O*-(2-*O*-[1-(α-D-mannopyranosyl)-1,2,3-triazol-4-yl]methyl-α-D-mannopyranosyl)-6-*O*-(3,6-di-*O*-[1-(α-D-mannopyranosyl)-1,2,3-triazol-4-yl]methyl-α-D-mannopyranosyl)-α-D-mannopyranoside hydroformate (5): Palladium on charcoal (10%, 68 mg, 0.64 mmol) and resin with ammonium formate (2.5 g) was added to a solution of **40** (149 mg, 0.071 mmol) in an isopropanol/H_2O mixture (1:5 (v/v), 12 mL). The reaction mixture was placed under microwave irradiation at 80 °C for 50 min, filtered over Celite and concentrated in vacuo. The crude product was then purified by HPLC (Method 2) to afford **5** as a white solid (67 mg; 74%). $[\alpha]_D^{20}$ + 63.5 (c = 0.32, H_2O); ^{1}H NMR (600 MHz, D_2O): δ = 8.44 (s, 1 H; HC(O)O), 8.26, 8.25, 8.24 (3s, 3×1 H; H-5 triazole), 8.15 (d, J(1,2) = 2.3 Hz, 1 H; H-1D or H-1E or H-1F), 8.14 (d, J(1,2) = 2.2 Hz, 1 H; H-1D or H-1E or H-1F), 8.13 (d, J(1,2) = 2.2 Hz, 1 H; H-1D or H-1E or H-1F), 5.19 (d, J(1,2) < 1 Hz, 1 H; H-1B), 4.92 (d, J(1,2) = 1.6 Hz, 1 H; H-1C), 4.89 (d, J(1,2) = 1.4 Hz, 1 H; H-1A), 4.89–4.72 (m, 9 H; H-2D to F and OCH_2(CCHN$_3$)), 4.19 (dd, J(1,2) = 1.6 Hz, J(2,3) = 2.6 Hz, 1 H; H-2C), 4.17–4.12 (m, 4 H; H-2A and H-3D to F), 4.02 (dd, J(1,2) < 1 Hz, J(2,3) = 3.2 Hz, 1 H; H-2B), 4.02–3.69 (m, 25 H; H-3A to C, H-4B to F, H-5A to C, H-6Aa to Fa, H-6Ab to Fb and H-8), 3.63 (t, J(3,4) = J(4,5) = 9.8 Hz, 1 H; H-4A), 3.37–3.25 ppm (m, 5 H; H-5D to F and H-7); ^{13}C NMR (150 MHz, D_2O): δ = 170.8 (HC(O)O), 145.2, 144.9, 144.8 (C-4 triazole), 125.5 125.4, 125.4 (C-5 triazole), 100.7 (C-1A), 100.6 (C-1B), 100.1 (C-1C), 87.3 (C-1D to F), 79.0, 78.9 (C-3A and C-3C), 78.3 (C-2B), 76.5, 76.4 (C-5D to F), 73.7 (C-3B), 71.7, 71.4, 70.4 (C-4B and/or C-4C and/or C-5A and/or C-5B and/or C-5C), 70.8 (C-3D to F), 69.8 (C-2A), 69.3 (C-6C), 68.5 (C-2D to F), 67.4 (C-4A), 66.9 (C-2C), 66.6 (C-4D to E), 66.8, 65.9 (C-4B and/or C-4C and/or C-5A and/or C-5B and/or C-5C), 65.7 (C-6A), 63.8 (C-8), 63.7, 63.5, 62.0 (OCH_2(CCHN$_3$)), 61.3 (C-6B), 60.8 (C-6D to F), 39.4 ppm (C-7); HRMS (ESI, amine form): m/z calcd for $C_{47}H_{77}O_{31}N_{10}$: 1277.4756 $[M+H]^+$; found: 1277.4742.

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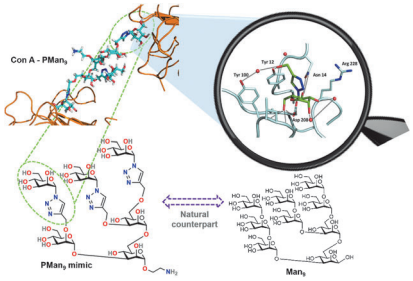
Simplifying the complex: "Click" emulators of "high-mannose oligosaccharides" containing triazole rings in the core act as functional mimics of the natural sugars towards the mannose specific lectins concanavalin A and the human macrophage mannose receptor. This strategy may prove of general use for the design of high affinity ligands of biologically relevant targets binding complex carbohydrates.



Oligosaccharides

M. François-Heude, A. Méndez-Ardoy, V. Cendret, P. Lafite, R. Daniellou, C. Ortiz Mellet, J. M. García Fernández, V. Moreau, F. Djedaïni-Pilard**

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Synthesis of High-Mannose Oligosaccharide Analogues through Click Chemistry: True Functional Mimics of Their Natural Counterparts Against Lectins?