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Synthesis and Immunological Screening of β-Linked Mono- and Divalent Mannosides

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Three different β-linked divalent mannosides, along with their corresponding monovalent counterparts, have been designed and chemically synthesized by coupling the corresponding propargyl (propargyl alcohol in the case of the monovalent compounds) and 2-azidoethyl glycosides by using an efficient click chemistry protocol. Crich's β-mannosylation methodology was applied to the construction of the β-mannosidic linkages. All the glycosylation reactions gave moderate-to-good yields with high selectivities. A competitive inhibition enzyme-linked immunosorbent assay (ELISA) was performed to determine the inhibition, by the synthesized mannosides, of specific human IgG binding to low-molecular-weight *Candida albicans* mannan; moderate inhibition capacity was observed for some of the compounds.

Introduction

Candida albicans is an opportunistic fungal pathogen causing systemic candidiasis mostly in immunocompromised patients undergoing long-term antibiotic treatment.[1] The chemical structure of the cell wall phosphomannan of *C. albicans* is comprised of β-(1,2)-oligomannosides, which are linked to an α-mannan chain either through glycosidic bonds or through α-linked phosphodiester bonds (Figure 1).[2,3] The *C. albicans* β-(1,2)-mannans are immunogenic and elicit specific antibodies, which have been shown to be protective against *C. albicans* in animal models of systemic and vaginal candidiasis.[4,5] Antibodies recognize β-mannans on the cell wall of *C. albicans* depending on the properties and chain lengths of these antigenic structures.[6–13]

Bundle and co-workers[14] have previously shown that short β-(1,2)-homo-oligosaccharides from disaccharides up to hexasaccharides inhibit the binding of β-(1,2)-mannan-specific monoclonal antibodies. The maximum inhibitory activity was observed for di- and trisaccharides, diminishing significantly for the corresponding tetra-, penta-, and hexasaccharides. It has also been recently shown that a tetravalent β-(1,2)-mannan disaccharide cluster, when linked to a protein, produces a good antibody response against *C. albicans*.[15] It is now well understood that molecular recognition events in biological systems are prone to multivalent ligand–receptor interactions and that such interactions often result in higher activities than their monovalent counterparts.[16–19] The chemical synthesis of saccharides with

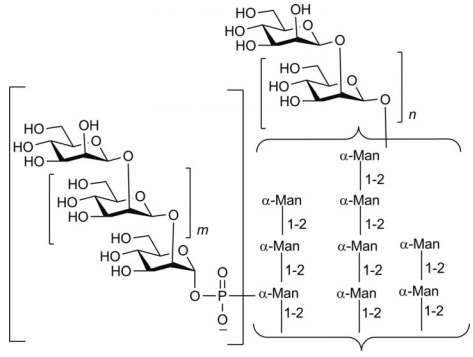

Figure 1. Partial chemical structure of the cell wall phosphomannan of *C. albicans* with an α-mannan backbone.[2,3]

known reactive groups and structures provides simple tools for studying these interactions. Overall, synthetic molecules have been shown to retain the biological properties and antigenicities of their native counterparts.[20,21] In this context we became interested in studying the biological effects of the multivalency of different β-linked/β-(1,2)-linked mannan constructs of the cell wall of *C. albicans*, along with their monovalent counterparts, to develop new tools for modulating the protective humoral and cellular immune responses towards *Candida*.

Divalent model compounds, being the simplest form of multivalent structures, were selected as a starting point for this study. To investigate the influence of divalency in biological systems, comparisons should be made with the corresponding monovalent counterparts. For this purpose we also aimed to synthesize monovalent model compounds. Various chemical approaches towards divalent sugar derivatives can be envisioned based on, for example, 1) the reaction of 2 equiv. of a sugar unit with a scaffold unit containing two points of attachment,[22–26] 2) homodimeriza-

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tion,[26–28] 3) multicomponent reactions,[29] 4) glycosylation,[25] and 5) heterocoupling between two sugar units.[30–32] The last-mentioned method has successfully been used for the synthesis of divalent carbohydrate model compounds by using click chemistry[30,33–37] as the synthetic tool. A beneficial feature of this click approach is its excellent compatibility with a vast range of functional groups, including alcohols, amines, and carboxylic acids in a number of solvent systems.[38] As an additional benefit, the resulting triazole ring is very stable to hydrolytic cleavage as well as inert towards oxidation and reduction reactions.[39,40] Furthermore, it can also be very effective in controlling the flexibility of the overall system. Triazole-containing compounds also differ from those with other types of passive linkers and may instead readily associate with biological targets through hydrogen bonding and dipole interactions.[39] A few literature reports have appeared recently in which the divalent molecules were synthesized by click coupling of the corresponding propargyl or 3-butynyl and azidomethyl or 2-azidoethyl glycosides.[41–45] Saccharide ligands, such as mannose (α-linked), glucose, or lactose units, were used to construct the corresponding 1,2,3-triazole-bridged dimeric compounds. To the best of our knowledge, similar coupling reactions using β-linked mannose or mannobiose units have not been explored in detail.

From another perspective, the chemical synthesis of β-mannosides has, until recently, remained a challenging task due to the α-directing anomeric effect and the presence of an axial substituent at the C-2 position causing steric hindrance for the incoming nucleophile. Owing to the pioneering work of Crich and co-workers and others, several methods have now become available for the synthesis of β-mannosides, either by direct glycosylation[46–51] or by indirect approaches.[52–58] We successfully used the Crich protocol in previous work as a direct approach to β-selective mannosylation for the construction of several β-linked manno-oligosaccharides.[28,59,60] In this work, we successfully used this method for the synthesis of carbohydrate building blocks suitable for click coupling. Thus, herein we report the chemical synthesis of several mono- and divalent β-linked mannosides. Furthermore, a competitive inhibition enzyme-linked immunosorbent assay (ELISA) of specific human IgG antibodies binding to low-molecular-weight *Candida albicans* mannan was performed to determine the inhibition by these synthesized mannosides.

Results and Discussion

Chemistry

Three different monovalent mannosides (**1**–**3**), along with their corresponding divalent counterparts (**4**–**6**), were selected as targets for the synthesis (Figure 2). The sugar moieties in each of the divalent molecules are connected through a triazole linker with a specific interconnecting chain length. Click chemistry was applied to the construction of the triazole-bridged mono- and divalent mannosides by reaction of the corresponding propargyl (propargyl alcohol in the case of the monovalent compounds) and 2-azidoethyl glycosides. Prior to the coupling reactions, the glycosides were synthesized by following previously reported literature procedures.

To synthesize the target mono- and divalent mannosides, three 2-azidoethyl glycosides (**10**, **12**, and **15**) and three propargyl glycosides (**20**, **21**, and **24**) were selected as building blocks (Figure 3). Monosaccharide building block **7**,[61] containing a 4,6-*O*-benzylidene group and a nonparticipating 2-*O*-(*p*-methoxybenzyl) group (PMB), was used as the glycosyl donor for introducing the initial β-linkage through Crich's methodology for β-mannosylation.

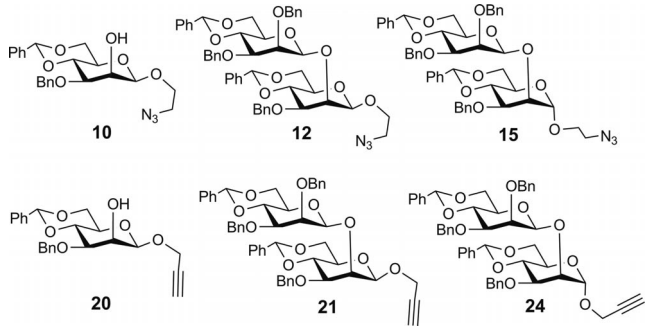


Figure 3. Chemical structures of the building blocks for the click reactions.

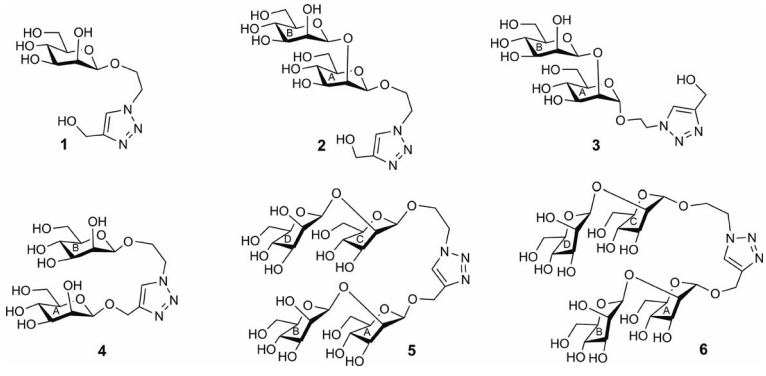
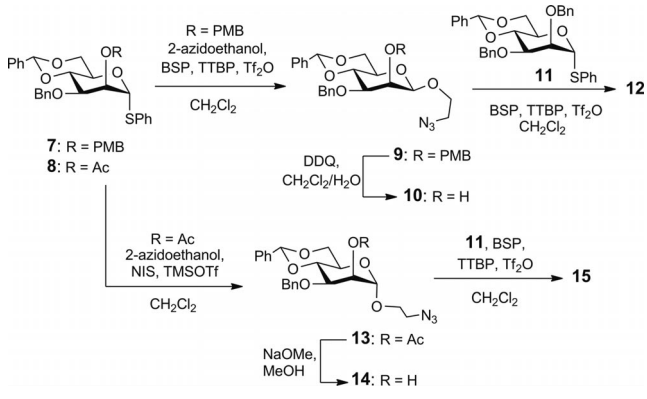


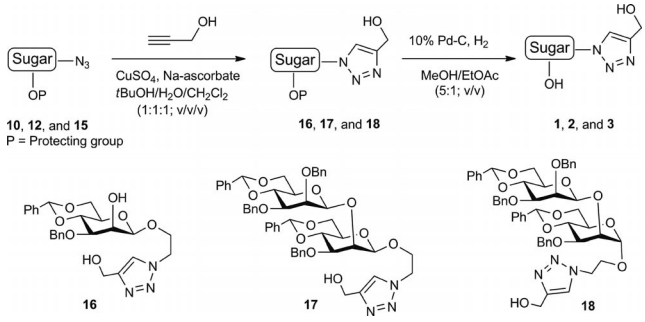
Figure 2. Chemical structures of the target mono- and divalent mannosides.

In line with this protocol, the glycosyl donor **7** was first activated in the presence of 1-benzenesulfinylpiperidine (BSP)/2,4,6-tri-*tert*-butylpyrimidine (TTBP)/Tf_2O system at –60 °C to yield a glycosyl triflate; subsequent in situ reaction with 2-azidoethanol[62] at –78 °C furnished the β-mannoside **9** in 48% yield (2-azidoethanol is potentially explosive and has to be handled at low temperatures). Unlike other glycosides, there is little difference in the 1-H→2-H coupling constants between the α- and β-mannosides, thus making difficult the anomeric assignment by ^{1}H NMR spectroscopy. The β stereochemistry of **9** was assigned on the basis of the upfield 5-H chemical shift at δ = 3.32 ppm and the $^1J_{CH}$ value of 154.4 Hz for the anomeric carbon.[46,63] Partial degradation of the product was observed during the course of the reaction for an unknown reason, which reduced the yield. Several precautions were taken and experiments performed to improve the yield led to an increase in yield of only 2–5%. Deprotection of the *p*-methoxybenzyl group in glycoside **9** was carried out by treatment with DDQ to furnish the glycosyl acceptor **10** in good yield. Again, BSP-mediated β-selective glycosylation of **10** with glycosyl donor **11**[61] afforded the disaccharide **12** in 92% yield. The formation of disaccharide **12** was confirmed on the basis of two upfield 5-H chemical shifts at δ = 3.34 (5-H_A) and 3.35 ppm (5-H_B) and the $^1J_{CH}$ values of 159.4 [$^1J_{CH(A)}$] and 156.5 Hz [$^1J_{CH(B)}$] for the two anomeric carbons. Building block **8**[61] was readily synthesized from commercially available D-mannose similarly to compound **7** but subjected to 2-*O*-acetylation instead of 2-*O*-*p*-methoxybenzylation. Next, α-selective glycosylation of glycosyl donor **8** with 2-azidoethanol in the presence of *N*-iodosuccinimide/TMSOTf in CH_2Cl_2 at –40 °C afforded the desired α-glycoside **13** in good yield. By Zemplén deacetylation, **13** was converted into the glycosyl acceptor **14** in 90% yield. Finally, BSP-mediated β-mannosylation of **14** with glycosyl donor **11** furnished the desired disaccharide **15** in 88% yield. An upfield 5-H chemical shift at δ = 3.33 ppm (5-H_B) and $^1J_{CH}$ values of 170.0 [$^1J_{CH(A)}$] and 154.7 Hz [$^1J_{CH(B)}$] for the anomeric carbons were used to verify the formation of **15** (Scheme 1).



Scheme 1. Synthesis of 2-azidoethyl glycosides **10**, **12**, and **15**.

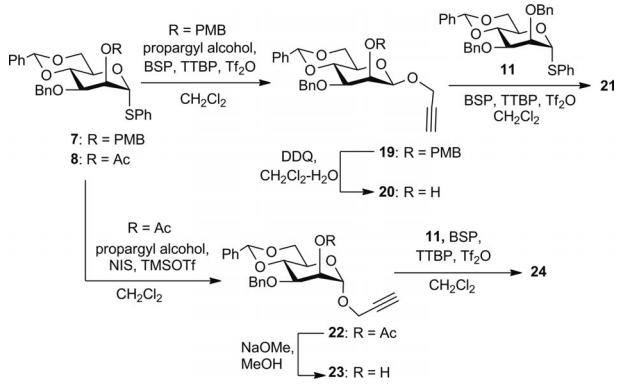
With the three desired 2-azidoethyl glycosides **10**, **12**, and **15** in hand, we next directed our efforts towards the coupling of each of the azides with propargyl alcohol according to a standard click chemistry protocol by using the copper sulfate/sodium ascorbate reagent system. Initially, a test reaction was performed in the solvent mixture *t*BuOH/H_2O,[64] but due to solubility problems the starting materials were first dissolved in CH_2Cl_2 before adding *t*BuOH/H_2O (1:1, v/v); the reactions proceeded readily in this *t*BuOH/H_2O/CH_2Cl_2 (1:1:1, v/v/v) solvent system. Following this procedure, the coupling products **16**–**18** were obtained in yields of 96, 81, and 86%, respectively. Finally, hydrogenolysis of the cycloaddition products with 10% Pd/C under hydrogen (2.76 bar) furnished the fully deprotected monovalent compounds **1**–**3** in yields of 84, 78, and 82%, respectively (Scheme 2).



Scheme 2. Synthesis of monovalent mannosides **1**–**3**.

BSP-mediated β-mannosylation of glycosyl donor **7** with propargyl alcohol afforded the β product **19** in 63% yield. The upfield 5-H chemical shift at δ = 3.36 ppm and the $^1J_{CH}$ value of 156.9 Hz for the anomeric carbon indicates the formation of the β product. Deprotection of the *p*-methoxybenzyl group of glycoside **19** by treatment with DDQ furnished the glycosyl acceptor **20** in 78% yield. Again, BSP-mediated β-selective glycosylation of **20** with glycosyl donor **11** afforded disaccharide **21** in 90% yield. The formation of the disaccharide **21** was confirmed on the basis of two upfield 5-H chemical shifts at δ = 3.38 (5-H_A) and 3.35 ppm (5-H_B) and the $^1J_{CH}$ values of 158.0 [$^1J_{CH(A)}$] and 157.7 Hz [$^1J_{CH(B)}$] for the anomeric carbons. Next, α-selective glycosylation of **8** with propargyl alcohol in the presence of *N*-iodosuccinimide/TMSOTf in CH_2Cl_2 at –40 °C afforded the desired α-glycoside **22** in 82% yield. Compound **22** was converted into **23** in 91% yield by Zemplén deacetylation. BSP-mediated β-mannosylation of glycosyl acceptor **23** with the glycosyl donor **11** furnished the desired disaccharide **24** in 64% yield. The upfield 5-H chemical shift at δ = 3.32 ppm (5-H_B) and the $^1J_{CH}$ values of 169 [$^1J_{CH(A)}$] and 154.6 Hz [$^1J_{CH(B)}$] for the anomeric carbons suggest the formation of **24** (Scheme 3).

To prepare the divalent mannosides by azido-alkyne cycloaddition, we first tested the reaction by using monosaccharide building blocks. A robust click coupling reaction between 2-azidoethyl glycoside **10** and propargyl glycoside **20** provided the expected triazole-fused product **25** in 83% yield. The coupled product **25** was then subjected to global deprotection under hydrogenolysis in the presence of 10% Pd/C and hydrogen gas (2.76 bar) to furnish the fully depro-



Scheme 3. Synthesis of the propargyl glycosides **20**, **21**, and **24**.

tected divalent **4** in 86% yield (Scheme 4). After successful preparation of the divalent mannoside from monosaccharide building blocks, we then applied the click coupling method to disaccharides. Accordingly, 2-azidoethyl glycoside **12** and propargyl glycoside **21** were coupled together to furnish the divalent compound **26** in 86% yield. Subsequent hydrogenolysis of **26** in the presence of 10% Pd/C under hydrogen pressure (2.76 bar) afforded the fully deprotected divalent compound **5** in 97% yield. The divalent molecule **6** was synthesized similarly; the disaccharides **15** and **24** were coupled to furnish the desired triazole-linked product **27** in 88% yield and subsequent hydrogenolysis of **27** in the presence of 10% Pd/C under hydrogen (2.76 bar) furnished the fully deprotected divalent product **6** in 76% yield (Scheme 4).

Biology

The IgG binding of the synthesized mannosides was studied through inhibition ELISA experiments with sera from vaginal candidiasis patients. All study subjects had elevated levels of IgG antibodies against *C. albicans* mannan. The binding of these antibodies to <3 kDa hydrolyzed *Candida albicans* Cetavlon mannan was inhibited by divalent mannosides **5** and **6** up to 50% and 70%, respectively (Figure 4). A similar inhibition pattern was observed by their monovalent counterparts **2** and **3**, respectively. However, relatively lower inhibition was noted for monovalent mannoside **1** and its divalent counterpart **4**.

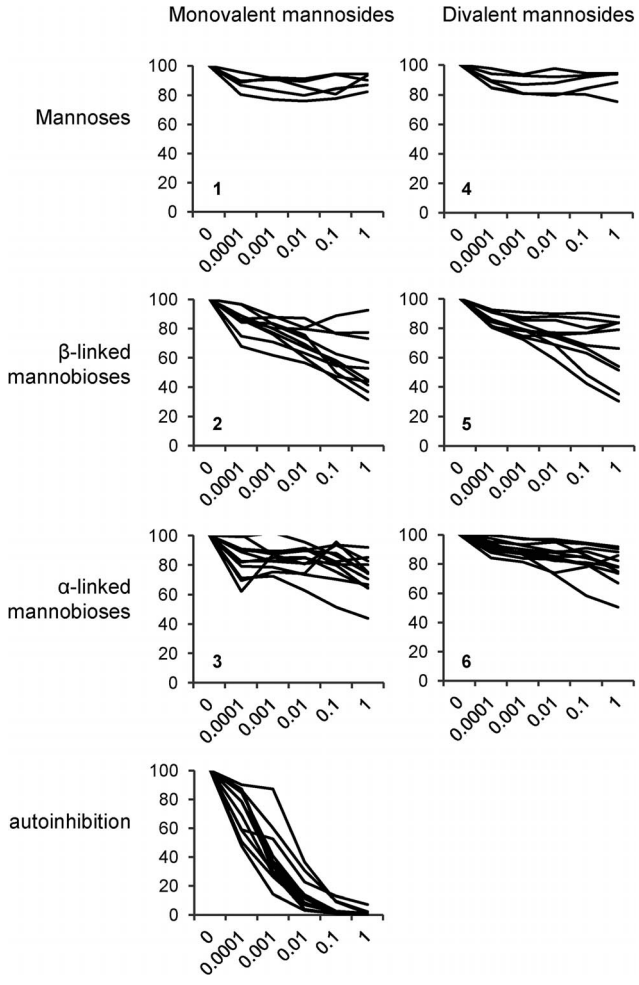
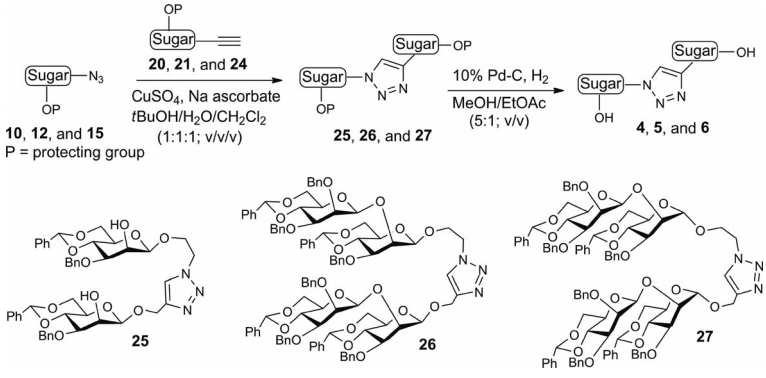


Figure 4. Inhibition of IgG binding to low-molecular-weight (<3 kDa) hydrolyzed Cetavlon *C. albicans* mannan by synthetic mono- and divalent mannosides in ELISA. *C. albicans* mannan (autoinhibition) is included as a control. The degree of inhibition in various concentrations is shown in the *y* axis and is defined by the formula 100 – {[1 – (absorbance of sera treated with various concentrations of saccharide inhibitors– absorbance of PBS)/absorbance of sera]} × 100. Each line represents the results from one study subject.



Scheme 4. Synthesis of divalent mannosides **4**–**6**.

Previous studies have shown that β-(1,2)-linked mannobiose, which consists of two β-anomeric linkages, is considered to be the minimum requirement for inhibiting the binding of *Candida*-specific antibodies.[14,15] In this study, divalent mannoside **5**, with two β-linkages, showed higher inhibitory activity than its corresponding α-linked (at the reducing terminus) counterpart **6**. It is also evident that divalency appears not to be essential for inhibition as no differences in the inhibition of IgG binding with divalent compounds were observed in comparison with monovalent compounds. In contrast, the β-linked monosaccharide compound **1** and its divalent analogue **4** showed low inhibitory activity. Thus, the biological activity declines sharply in the following order: disaccharide (with two β linkages) > disaccharide (with one α and one β linkage) > monosaccharide (β-linkage). Our results are in accordance with earlier findings and confirm the potential antigenic properties of β-(1,2)-linked *Candida albicans* derived mannans.

Conclusions

Three structurally unique divalent mannosides have been designed on the basis of immunopotent saccharides from *Candida albicans* mannan and synthesized for the first time by using click chemistry to study the effects of multivalency, length of saccharide chain, and linkage type on immune responses. Crich's β-mannosylation methodology was successfully used for the construction of the β-mannosidic linkages. Divalent compounds **5** and **6** were found to possess similar properties important in the antigenicity of *C. albicans*, as these compounds inhibited specific IgG antibody binding to <3 kDa hydrolyzed mannan up to 50% and 70%. The findings of this study confirm that the two synthesized divalent mannosides (compounds **5** and **6**) share some similarities with the immunopotent and antigenic epitopes of β-(1,2)-linked *Candida albicans* mannan. However, divalency appears not to strengthen the inhibition capacity of the mannosides. Further synthetic and biological studies on these and similar compounds are ongoing in our laboratories and will be reported in more detail in forthcoming papers.

Experimental Section

General Methods: All reaction solvents were distilled and dried under argon before use and all reactions were carried out under argon unless noted otherwise. Molecular sieves (4 Å) were activated under vacuum at 300 °C for 1 h prior to use in the reactions. All NMR spectra were recorded with a Bruker Avance spectrometer operating at 600.13 MHz for ^{1}H and 150.90 MHz for ^{13}C. All products were fully characterized by using 1D ^{1}H and ^{13}C NMR techniques in combination with 2D COSY, HSQC (both coupled and decoupled), and HMBC NMR techniques at 25 °C. Chemical shifts are reported downfield from TMS with residual chloroform or methanol as the internal reference. Computational analysis of the ^{1}H NMR spectra of all the mono- and disaccharide building blocks was achieved by using PERCH NMR software with the starting values and spectral parameters obtained from the various NMR techniques used.[65] HRMS were recorded by using either a Bruker Micro Q-TOF with ESI (electrospray ionization) spectrometer operated in positive mode or a Fison ZabSpecOaTOF spectrometer with EI (electron impact) ionization operated in positive mode. Optical rotations were measured with a Perkin–Elmer 241 polarimeter equipped with a Na lamp (589 nm). Reactions were monitored by TLC on aluminium sheets precoated with silica gel 60 F254 (Merck). The compounds were detected under UV light and by charring with H_2SO_4/MeOH (1:10, v/v). Column chromatography was performed on silica gel 60 (0.040–0.060 mm, Merck) by using different solvent systems based on the polar character of different compounds.

General Procedure A. Typical Procedure for the Synthesis of β-Mannosylation Reaction: Molecular sieves (4 Å, 500 mg) were added to a solution of compound **11** (164 mg, 0.30 mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred for 30 min at room temperature under argon. BSP (76 mg, 0.36 mmol) and TTBP (113 mg, 0.46 mmol) were added at –60 °C followed by the addition of Tf_2O (111 mg, 66 μL, 0.39 mmol). The resulting mixture was stirred under the same conditions for 30 min. Acceptor **10** (100 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was then added to the reaction mixture at –78 °C and stirred for 2 h at this temperature before quenching with Et_3N (200 μL). The reaction mixture was then filtered through a pad of Celite followed by washing with CH_2Cl_2 (30 mL). The eluted organic solvent was washed with water (15 mL), satd. aq. $NaHCO_3$ (15 mL), and brine (15 mL), dried (Na_2SO_4), concentrated, and purified over SiO_2 to give **12** (185 mg, 92%). Compounds **9**, **15**, **19**, **21**, and **24** were then synthesized following an analogous procedure.

General Procedure B. Typical Experimental Procedure for the Click Reaction: Copper sulfate (8 mg, 0.05 mmol) and sodium ascorbate (20 mg, 0.10 mmol) were added to a solution of compound **10** (74 mg, 0.17 mmol) and propargyl alcohol (20 μL, 19 mg, 0.34 mmol) in *t*BuOH/H_2O/CH_2Cl_2 (1:1:1, v/v/v, 2.4 mL). The resulting reaction mixture was stirred at room temperature for 16 h. Satd. NH_4Cl/water (1:1, v/v, 4 mL) was poured into the solution and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), and dried (Na_2SO_4), filtered, concentrated, and purified over SiO_2 to afford the title compound **16** (80 mg, 96%). Compounds **17**, **18**, **25**, **26**, and **27** were then synthesized following an analogous procedure.

General Procedure C. Typical Experimental Procedure for the Hydrogenolysis Reaction: Pd/C (10%, 120 mg) was added to a solution of compound **16** (60 mg, 0.12 mmol) in methanol/ethyl acetate (5:1, v/v, 3 mL) and the mixture was stirred under H_2 (2.76 bar) for 16 h at room temperature. The reaction mixture was filtered through a pad of Celite and washed with methanol (3 × 10 mL). The combined filtrate was concentrated under reduced pressure to afford pure **1** (32 mg, 84%). Compounds **2**–**6** were then synthesized following an analogous procedure.

2-Azidoethyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-(*p*-methoxybenzyl)-β-D-mannopyranoside (9): The glycosidation reaction was carried out starting from glycosyl donor **7** (700 mg, 1.23 mmol) and 2-azidoethanol (129 mg, 1.48 mmol) following the same reaction procedure as outlined in General Procedure A to afford compound **9** (320 mg, 48%). $[\alpha]_D^{25}$ = –74.3 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.51–7.49 (m, 2 H, Ar-H), 7.40–7.25 (m, 10 H, Ar-H), 6.86–6.84 (m, 2 H, Ar-H), 5.62 (s, 1 H, PhC*H*), 4.90, 4.79 (2 d, *J* = –11.9 Hz each, 2 × 1 H, PhCH_2), 4.65, 4.55 (2 d, *J* = –12.5 Hz, 2 × 1 H, PhCH_2), 4.53 (d, $J_{1,2}$ = 1.0 Hz, 1 H, 1-H), 4.30 (dd, $J_{6a,6b}$ = –10.3, $J_{5,6a}$ = 4.8 Hz, 1 H, 6-H$_a$), 4.19 (dd, $J_{3,4}$ =

9.9, $J_{4,5}$ = 9.2 Hz, 1 H, 4-H), 4.11 (ddd, $J_{OCH2a,OCH2b}$ = –10.6, $J_{OCH2a,CH2bN3}$ = 4.5, $J_{OCH2a,CH2aN3}$ = 3.5 Hz, 1 H, OCH_{2a}), 3.99 (dd, $J_{2,3}$ = 3.1 Hz, 1 H, 2-H), 3.93 (dd, $J_{5,6b}$ = 10.1 Hz, 1 H, 6-H_b), 3.80 (s, 3 H, OCH_3), 3.65 (ddd, $J_{OCH2b,CH2aN3}$ = 8.7, $J_{OCH2b,CH2bN3}$ = 3.2 Hz, 1 H, OCH_{2b}), 3.58 (dd, 1 H, 3-H), 3.56 (ddd, $J_{CH2aN3,CH2bN3}$ = –13.4 Hz, 1 H, CH_{2a}N$_3$), 3.34 (ddd, 1 H, CH_{2b}N$_3$), 3.33 (ddd, 1 H, 5-H) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 159.2–113.5 (Ar-C), 102.3 (C-1), 101.4 (Ph*C*H), 78.5 (C-4), 77.7 (C-3), 75.3 (C-2), 74.6 (Ph*C*H$_2$), 72.3 (Ph*C*H$_2$), 68.7 (O*C*H$_2$), 68.5 (C-6), 67.7 (C-5), 55.2 (O*C*H$_3$), 50.8 (*C*H$_2$N$_3$) ppm. HRMS (ESI): calcd. for $C_{30}H_{33}N_3O_7Na$ [M + Na]$^+$ 570.2216; found 570.2218.

2-Azidoethyl 3-*O*-Benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (10): DDQ (160 mg, 0.70 mmol) was added to a solution of compound **9** (320 mg, 0.58 mmol) in CH_2Cl_2/H_2O (1:1, v/v, 20 mL). The reaction mixture was stirred at room temperature for 2 h, diluted with CH_2Cl_2 (20 mL), and washed with water (2 × 20 mL) and satd. aq. $NaHCO_3$ (2 × 20 mL). The organic layer was then dried (Na_2SO_4), filtered, concentrated, and purified over SiO_2 to afford pure **10** (220 mg, 88%) as a colorless oil. $[\alpha]_D^{25}$ = –40.1 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.51–7.25 (m, 10 H, Ar-H), 5.61 (s, 1 H, PhC*H*), 4.86, 4.78 (2 d, *J* = –12.2 Hz each, 2 × 1 H, PhCH_2), 4.58 (d, $J_{1,2}$ = 1.1 Hz, 1 H, 1-H), 4.33 (dd, $J_{6a,6b}$ = –10.5, $J_{5,6a}$ = 4.9 Hz, 1 H, 6-H_a), 4.18 (ddd, $J_{2,3}$ = 3.3, $J_{2,2\text{-}OH}$ = 1.6 Hz, 1 H, 2-H), 4.16 (dd, $J_{3,4}$ = 9.5, $J_{4,5}$ = 9.4 Hz, 1 H, 4-H), 4.09 (ddd, $J_{OCH2a,OCH2b}$ = –10.8, $J_{OCH2a,CH2bN3}$ = 5.0, $J_{OCH2a,CH2aN3}$ = 3.8 Hz, 1 H, OCH_{2a}), 3.89 (dd, $J_{5,6b}$ = 10.1 Hz, 1 H, 6-H_b), 3.74 (ddd, $J_{OCH2b,CH2aN3}$ = 8.2, $J_{OCH2b,CH2bN3}$ = 3.6 Hz, 1 H, OCH_{2b}), 3.66 (dd, 1 H, 3-H), 3.54 (ddd, $J_{CH2aN3,CH2bN3}$ = –13.4 Hz, 1 H, CH_{2a}N$_3$), 3.38 (ddd, 1 H, CH_{2b}N$_3$), 3.36 (ddd, 1 H, 5-H), 2.56 (d, 1 H, 2-O*H*) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 137.8–126.0 (Ar-C), 101.5 (Ph*C*H), 100.6 (C-1), 78.2 (C-4), 76.5 (C-3), 72.5 (Ph*C*H$_2$), 69.7 (C-2), 68.6 (O*C*H$_2$), 68.5 (C-6), 67.0 (C-5), 50.6 (*C*H$_2$N$_3$) ppm. HRMS (ESI): calcd. for $C_{22}H_{25}N_3O_6Na$ [M + Na]$^+$ 450.1641; found 450.1637.

2-Azidoethyl (2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (12): $[\alpha]_D^{25}$ = –102.9 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.58–7.24 (m, 25 H, Ar-H), 5.61 (s, 1 H, PhC*H*), 5.43 (s, 1 H, PhC*H*), 5.08, 5.01 (2 d, *J* = –12.3 Hz each, 2 × 1 H, PhCH_2), 4.79 (d, $J_{1B,2B}$ = 0.9 Hz, 1 H, 1-H_B), 4.79, 4.77 (2 d, *J* = –12.6 Hz each, 2 × 1 H, PhCH_2), 4.66, 4.58 (2 d, *J* = –12.6 Hz each, 2 × 1 H, PhCH_2), 4.52 (s, 1 H, 1-H_A), 4.31 (dd, $J_{6aB,6bB}$ = –10.4, $J_{5B,6aB}$ = 4.8 Hz, 1 H, 6-H_{aB}), 4.28 (dd, $J_{6aA,6bA}$ = –10.4, $J_{5A,6aA}$ = 4.8 Hz, 1 H, 6-H_{aA}), 4.27 (dd, $J_{2A,3A}$ = 3.1 Hz, 1 H, 2-H_A), 4.23 (dd, $J_{3B,4B}$ = 10.0, $J_{4B,5B}$ = 9.2 Hz, 1 H, 4-H_B), 4.14 (dd, $J_{2B,3B}$ = 3.2 Hz, 1 H, 2-H_B), 4.07 (ddd, $J_{OCH2a,OCH2b}$ = –10.4, $J_{OCH2a,CH2bN3}$ = 4.9, $J_{OCH2a,CH2aN3}$ = 3.2 Hz, 1 H, OCH_{2a}), 4.06 (dd, $J_{3A,4A}$ = 9.9, $J_{4A,5A}$ = 9.3 Hz, 1 H, 4-H_A), 3.90 (dd, $J_{5B,6bB}$ = 10.2 Hz, 1 H, 6-H_{bB}), 3.75 (dd, $J_{5A,6bA}$ = 10.0 Hz, 1 H, 6-H_{bA}), 3.66 (ddd, $J_{OCH2b,CH2aN3}$ = 8.5, $J_{OCH2b,CH2bN3}$ = 2.8 Hz, 1 H, OCH_{2b}), 3.62 (dd, 1 H, 3-H_A), 3.59 (dd, 1 H, 3-H_B), 3.35 (ddd, 1 H, 5-H_B), 3.34 (ddd, 1 H, 5-H_A), 3.27 (ddd, $J_{CH2aN3,CH2bN3}$ = –13.4 Hz, 1 H, CH_{2a}N$_3$), 3.19 (ddd, 1 H, CH_{2b}N$_3$) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 139.0–126.0 (Ar-C), 103.7 (C-1$_B$), 101.5 (C-1$_A$), 101.5 (Ph*C*H), 101.3 (Ph*C*H), 78.4 (C-4$_B$), 78.1 (C-4$_A$), 77.3 (C-3$_B$), 76.8 (C-2$_A$), 75.9 (C-3$_A$), 75.8 (C-2$_B$), 74.5 (Ph*C*H$_2$), 71.1 (Ph*C*H$_2$), 68.7 (O*C*H$_2$), 68.7 (C-6$_B$), 68.6 (C-6$_A$), 67.6 (C-5$_B$), 67.5 (C-5$_A$), 50.8 (*C*H$_2$N$_3$) ppm. HRMS (ESI): calcd. for $C_{49}H_{55}N_4O_{11}$ [M + NH$_4$]$^+$ 875.3867; found 875.3841.

2-Azidoethyl 2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (13): Molecular sieves (4 Å, 3 g) were added to a solution of compound **8** (1.5 g, 3.05 mmol) and 2-azidoethanol (320 mg, 3.65 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was then stirred at room temperature for 30 min under argon. NIS (825 mg, 3.66 mmol) was added and the reaction mixture was cooled to –40 °C. TMSOTf (81 mg, 66 μL, 0.37 mmol) was then added and the reaction mixture was stirred for 1 h at the same temperature and quenched with Et_3N (200 μL), filtered through a pad of Celite, and washed with CH_2Cl_2 (60 mL). The eluted organic solvent was washed with water (30 mL), a satd. aq. $NaHCO_3$ solution (30 mL), a 10% aq. $Na_2S_2O_3$ solution (30 mL), and brine (2 × 30 mL). The organic layer was then dried (Na_2SO_4), concentrated, and purified over SiO_2 to afford pure **13** (1.23 g, 86%) as a colorless oil. $[\alpha]_D^{25}$ = +6.4 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.51–7.24 (m, 10 H, Ar-H), 5.63 (s, 1 H, PhC*H*), 5.41 (dd, $J_{2,3}$ = 3.5, $J_{1,2}$ = 1.6 Hz, 1 H, 2-H), 4.84 (d, 1 H, 1-H), 4.69, 4.66 (2 d, *J* = –12.1 Hz each, PhCH_2), 4.27 (dd, $J_{6a,6b}$ = –10.4, $J_{5,6a}$ = 4.9 Hz, 1 H, 6-H_a), 4.07 (dd, $J_{3,4}$ = 10.0, $J_{4,5}$ = 9.4 Hz, 1 H, 4-H), 4.04 (dd, 1 H, 3-H), 3.88 (ddd, $J_{5,6b}$ = 10.3 Hz, 1 H, 5-H), 3.86 (dt, $J_{OCH2a,OCH2b}$ = –10.6, $J_{OCH2a,CH2N3}$ = 5.0 Hz, 1 H, OCH_{2a}), 3.85 (dd, 1 H, 6-H_b), 3.60 (dt, $J_{OCH2b,CH2N3}$ = 5.0 Hz, 1 H, OCH_{2b}), 3.39 (dd, 2 H, CH_2N$_3$), 2.16 (s, 3 H, COCH_3) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 170.1 (*C*O), 137.8–126.0 (Ar-C), 101.6 (Ph*C*H), 98.9 (C-1), 78.1 (C-4), 73.6 (C-3), 72.2 (Ph*C*H$_2$), 69.5 (C-2), 68.6 (C-6), 66.9 (O*C*H$_2$), 64.2 (C-5), 50.3 (*C*H$_2$N$_3$) ppm. HRMS (ESI): calcd. for $C_{24}H_{27}N_3O_7Na$ [M + Na]$^+$ 492.1747; found 492.1730.

2-Azidoethyl 3-*O*-Benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (14): Sodium methoxide (1 M in MeOH, pH ca. 12) was added to a solution of compound **13** (460 mg, 0.98 mmol) in MeOH (10 mL) and the resulting mixture was stirred at room temperature for 2 h. Dowex 50WX8-100 (H^+) was then added to neutralize the solution. The reaction mixture was filtered and washed with methanol (3 × 20 mL). The combined organic layers were evaporated to dryness under reduced pressure and purified over SiO_2 to afford pure **14** (375 mg, 90%) as a white foam. $[\alpha]_D^{25}$ = +27.5 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.50–7.25 (m, 10 H, Ar-H), 5.62 (s, 1 H, PhC*H*), 4.92 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1-H), 4.86, 4.72 (2 d, *J* = –11.8 Hz each, 2 × 1 H, PhCH_2), 4.27 (dd, $J_{6a,6b}$ = –10.7, $J_{5,6a}$ = 5.3 Hz, 1 H, 6-H_a), 4.11 (dd, $J_{3,4}$ = 9.6, $J_{4,5}$ = 9.2 Hz, 1 H, 4-H), 4.10 (ddd, $J_{2,3}$ = 3.4, $J_{2,2\text{-}OH}$ = 1.4 Hz, 1 H, 2-H), 3.95 (dd, 1 H, 3-H), 3.90 (ddd, $J_{OCH2a,OCH2b}$ = –10.7, $J_{OCH2a,CH2aN3}$ = 6.5, $J_{OCH2a,CH2bN3}$ = 3.3 Hz, 1 H, OCH_{2a}), 3.86 (dd, $J_{5,6b}$ = 10.2 Hz, 1 H, 6-H_b), 3.86 (ddd, 1 H, 5-H), 3.63 (ddd, $J_{OCH2b,CH2bN3}$ = 6.8, $J_{OCH2b,CH2aN3}$ = 3.3 Hz, 1 H, OCH_{2b}), 3.42 (ddd, $J_{CH2aN3,CH2bN3}$ = –13.3 Hz, 1 H, CH_{2a}N$_3$), 3.38 (ddd, 1 H, CH_{2b}N$_3$), 2.70 (d, 1 H, 2-O*H*) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 137.9–126.0 (Ar-C), 101.6 (Ph*C*H), 100.1 (C-1), 78.6 (C-4), 75.4 (C-3), 73.1 (Ph*C*H$_2$), 69.8 (C-2), 68.8 (C-6), 66.7 (O*C*H$_2$), 63.6 (C-5), 50.4 (*C*H$_2$N$_3$) ppm. HRMS (ESI): calcd. for $C_{22}H_{25}N_3O_6Na$ [M + Na]$^+$ 450.1641; found 450.1646.

2-Azidoethyl (2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (15): Employing the same method as outlined in General Procedure A, the glycosylation reaction between glycosyl donor **11** (164 mg, 0.30 mmol) and glycosyl acceptor **14** (100 mg, 0.23 mmol) gave compound **15** (177 mg, 88%). $[\alpha]_D^{25}$ = –50.3 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.53–7.25 (m, 25 H, Ar-H), 5.60 (s, 1 H, PhC*H*), 5.50 (s, 1 H, PhC*H*), 5.04, 4.98 (2 d, *J* = –12.2 Hz each, 2 × 1 H, PhCH_2), 4.86 (d, $J_{1A,2A}$ = 1.7 Hz, 1 H, 1-H_A), 4.76, 4.73 (2 d, *J* = –12.2 Hz each, 2 × 1 H, PhCH_2), 4.71, 4.62 (2 d, *J* = –12.5 Hz each, 2 × 1 H, PhCH_2), 4.63 (s, 1 H, 1-H_B), 4.27 (dd, $J_{2A,3A}$ = 3.3 Hz, 1 H, 2-H_A), 4.27 (dd, $J_{6aB,6bB}$ = –10.5, $J_{5B,6aB}$ = 4.8 Hz, 1 H, 6-H_{aB}), 4.26 (dd, $J_{3B,4B}$ = 9.8, $J_{4B,5B}$ = 9.3 Hz, 1 H,

4-H_B), 4.25 (dd, $J_{6aA,6bA}$ = –10.3, $J_{5A,6aA}$ = 4.7 Hz, 1 H, 6-H_{aA}), 4.12 (dd, $J_{3A,4A}$ = 10.0, $J_{4A,5A}$ = 9.5 Hz, 1 H, 4-H_A), 3.99 (dd, $J_{2B,3B}$ = 3.2 Hz, 1 H, 2-H_B), 3.98 (dd, 1 H, 3-H_A), 3.88 (ddd, $J_{OCH2a,OCH2b}$ = –10.7, $J_{OCH2a,CH2bN3}$ = 5.7, $J_{OCH2a,CH2aN3}$ = 3.3 Hz, 1 H, OCH_{2a}), 3.87 (dd, $J_{5B,6bB}$ = 10.0 Hz, 1 H, 6-H_{bB}), 3.82 (ddd, $J_{5A,6bA}$ = 10.3 Hz, 1 H, 5-H_A), 3.77 (dd, 1 H, 6-H_{bA}), 3.60 (dd, 1 H, 3-H_B), 3.59 (ddd, $J_{OCH2b,CH2aN3}$ = 7.5, $J_{OCH2b,CH2bN3}$ = 3.2 Hz, 1 H, OCH_{2b}), 3.43 (ddd, $J_{CH2aN3,CH2bN3}$ = –13.3 Hz, 1 H, C$H_{2a}$$N_3$), 3.35 (ddd, 1 H, C$H_{2b}$$N_3$), 3.33 (ddd, 1 H, 5-$H_B$) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 138.8–126.0 (Ar-C), 101.6 (PhCH), 101.4 (PhCH), 100.9 (C-1_B), 98.7 (C-1_A), 78.5 (C-2_A), 78.4 (C-4_A), 77.6 (C-3_B), 75.9 (C-3_A), 75.0 (C-4_B), 74.5 (PhCH_2), 74.0 (C-2_B), 72.3 (PhCH_2), 71.5 (PhCH_2), 68.8 (C-6_A), 68.5 (C-6_B), 67.8 (C-5_B), 66.6 (OCH_2), 64.5 (C-5_A), 50.4 (C$H_2$$N_3$) ppm. HRMS (ESI): calcd. for $C_{49}H_{51}N_3O_{11}Na$ [M + Na]$^+$ 880.3421; found 880.3400.

1-(3-*O*-Benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyloxyethyl)-4-hydroxymethyl-1,2,3-triazole (16): $[\alpha]_D^{25}$ = –16.8 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.74 (s, 1 H, triazole-*H*), 7.49–7.46 (m, 2 H, Ar-H), 7.39–7.24 (m, 8 H, Ar-H), 5.58 (s, 1 H, PhC*H*), 4.80, 4.73 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 4.71 (s, 2 H, CH_{2ab}OH), 4.58–4.49 (m, 2 H, CH_2CH_{2ab} triazole), 4.42 (s, 1 H, 1-H), 4.27 (dd, $J_{6a,6b}$ = –10.4, $J_{5,6a}$ = 4.9 Hz, 1 H, 1-H), 4.21 (ddd, *J* = –10.9, 4.8, 3.7 Hz, 1 H, C$H_{2a}$$CH_2$ triazole), 4.10 (dd, $J_{3,4}$ ≈ $J_{4,5}$ ≈ 9.5 Hz, 1 H, 4-H), 4.07 (d, $J_{2,3}$ = 3.1 Hz, 1 H, 2-H), 3.90 (ddd, *J* = 8.0, 3.6 Hz, 1 H, C$H_{2b}$$CH_2$ triazole), 3.84 (dd, $J_{5,6b}$ = 10.3 Hz, 1 H, 6-H_b), 3.60 (dd, 1 H, 3-H), 3.30 (ddd, 1 H, 5-H) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 147.7 (C-4, triazole), 137.7–125.9 (12 C, Ar-C), 123.4 (C-5, triazole), 101.4 (PhCH), 100.7 (C-1), 78.2 (C-4), 76.6 (C-3), 72.4 (PhCH_2), 69.3 (C-2), 68.3 (C-6), 67.9 (C$H_2$$CH_2$ triazole), 66.9 (C-5), 56.0 (CH_2OH), 50.1 (CH_2CH_2 triazole) ppm. HRMS (ESI): calcd. for $C_{25}H_{29}N_3O_7Na$ [M + Na]$^+$ 506.1903; found 506.1886.

1-(β-D-Mannopyranosyloxyethyl)-4-hydroxymethyl-1,2,3-triazole (1): $[\alpha]_D^{25}$ = –22.8 (*c* = 1, H_2O). ^{1}H NMR (600.13 MHz, CD_3OD): δ = 8.06 (s, 1 H, triazole-*H*), 4.68 (br. s, 2 H, CH_{2ab}OH), 4.66–4.64 (m, 2 H, CH_2CH_{2ab} triazole), 4.52 (d, $J_{1,2}$ = 1.0 Hz, 1 H, 1-H), 4.27 (ddd, *J* = –11.3, 5.3, 4.1 Hz, 1 H, C$H_{2a}$$CH_2$ triazole), 4.00 (ddd, *J* = 6.5, 4.3 Hz, 1 H, C$H_{2b}$$CH_2$ triazole), 3.88 (dd, $J_{6a,6b}$ = –11.8, $J_{5,6a}$ = 2.3 Hz, 1 H, 6-H_a), 3.83 (dd, $J_{2,3}$ = 3.2 Hz, 1 H, 2-H), 3.70 (dd, $J_{5,6b}$ = 6.1 Hz, 1 H, 6-H_b), 3.54 (dd, $J_{3,4}$ ≈ $J_{4,5}$ ≈ 9.5 Hz, 1 H, 4-H), 3.43 (dd, 1 H, 3-H), 3.22 (ddd, 1 H, 5-H) ppm. ^{13}C NMR (150.90 MHz, CD_3OD): δ = 148.9 (C-4, triazole), 125.3 (C-5, triazole), 101.9 (C-1), 78.4 (C-5), 75.1 (C-3), 72.3 (C-2), 68.9 (C$H_2$$CH_2$ triazole), 68.5 (C-4), 62.8 (C-6), 56.5 (CH_2OH), 51.6 (CH_2CH_2 triazole) ppm. HRMS (ESI): calcd. for $C_{11}H_{19}N_3O_7Na$ [M + Na]$^+$ 328.1121; found 328.1119.

1-[(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyloxyethyl)]-4-hydroxymethyl-1,2,3-triazole (17): Employing the same method as outlined in General Procedure B, the click coupling reaction between 2-azidoethyl glycoside **12** (37 mg, 0.04 mmol) and propargyl alcohol (5 μL, 5 mg, 0.09 mmol) gave compound **17** (32 mg, 81%). $[\alpha]_D^{25}$ = –67.8 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.57–7.21 (m, 26 H, Ar-H, triazole-*H*), 5.60 (s, 1 H, PhC*H*), 5.43 (s, 1 H, PhC*H*), 5.05, 4.97 (2 d, *J* = –12.4 Hz each, 2×1 H, PhCH_2), 4.79, 4.77 (2 d, *J* = –12.5 Hz each, 2×1 H, PhCH_2), 4.71, 4.69 (2 d, *J* = –12.7 Hz each, 2×1 H, PhCH_2), 4.65 (d, $J_{CH2aOH,CH2bOH}$ = –13.3 Hz, 1 H, CH_{2a}OH), 4.62 (d, 1 H, CH_{2b}OH), 4.60 (s, 1 H, 1-H_B), 4.50 (ddd, *J* = –14.4, 4.9, 3.4 Hz, 1 H, CH_2CH_{2a} triazole), 4.47 (s, 1 H, 1-H_A), 4.35–4.24 (m, 4 H, 6-H_{aA}, 6-H_{aB}, CH_2CH_{2b} triazole, C$H_{2a}$$CH_2$ triazole), 4.23 (dd, $J_{3B,4B}$ ≈ $J_{4B,5B}$ ≈ 9.7 Hz, 1 H, 4-H_B), 4.20 (d, $J_{2A,3A}$ = 3.2 Hz, 1 H, 2-H_A), 4.02–3.97 (m, 3 H, 4-H_A, 2-H_B, C$H_{2b}$$CH_2$ triazole), 3.88 (dd, $J_{5B,6bB}$ ≈ $J_{6aB,6bB}$ ≈ 10.3 Hz, 1 H, 6-H_{bB}), 3.75 (dd, $J_{5A,6bA}$ ≈ $J_{6aA,6bA}$ ≈ 10.2 Hz, 1 H, 6-H_{bA}), 3.65 (dd, $J_{2B,3B}$ = 3.2 Hz, 1 H, 3-H_B), 3.58 (dd, $J_{3A,4A}$ = 9.9 Hz, 1 H, 3-H_A), 3.33 (ddd, $J_{4A,5A}$ = 9.8, $J_{5A,6aA}$ = 4.8 Hz, 1 H, 5-H_A), 3.25 (ddd, $J_{5B,6aB}$ = 4.9 Hz, 1 H, 5-H_B) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 148.0 (C-4, triazole), 139.0–126.0 (30 C, Ar-C), 121.9 (C-5, triazole), 103.2 (C-1_B), 101.6 (C-1_A), 101.5 (PhCH), 101.4 (PhCH), 78.7 (C-4_B), 78.0 (C-4_A), 77.7 (C-3_B), 76.0 (C-2_B), 75.7 (C-3_A), 75.6 (C-2_A), 74.5 (PhCH_2), 72.3 (PhCH_2), 71.0 (PhCH_2), 68.7 (C-6_A), 68.6 (C-6_B), 67.7 (C$H_2$$CH_2$ triazole), 67.6 (C-5_A), 67.4 (C-5_B), 56.6 (CH_2OH), 50.1 (CH_2CH_2 triazole) ppm. HRMS (ESI): calcd. for $C_{52}H_{55}N_3O_{12}Na$ [M + Na]$^+$ 936.3683; found 936.3649.

1-[(β-D-Mannopyranosyl)-(1→2)-(β-D-mannopyranosyloxyethyl)]-4-hydroxymethyl-1,2,3-triazole (2): Employing the same method as outlined in General Procedure C, hydrogenolysis of the coupling product **17** (30 mg, 0.03 mmol) gave compound **2** (12 mg, 78%). $[\alpha]_D^{25}$ = –40.6 (*c* = 1, H_2O). ^{1}H NMR (600.13 MHz, CD_3OD): δ = 7.99 (s, 1 H, triazole-*H*), 4.72 (br. s, 2 H, CH_{2ab}OH), 4.67–4.62 (m, 3 H, 1-H_A, CH_2CH_{2ab} triazole), 4.58 (d, $J_{1B,2B}$ = 0.5 Hz, 1 H, 1-H_B), 4.31 (ddd, *J* = –11.0, 5.6, 3.8 Hz, 1 H, C$H_{2a}$$CH_2$ triazole), 4.05 (d, $J_{2A,3A}$ = 3.2 Hz, 1 H, 2-H_A), 4.00 (ddd, *J* = 6.8, 3.7 Hz, 1 H, C$H_{2b}$$CH_2$ triazole), 3.89 (dd, $J_{6aA,6bA}$ = –11.8, $J_{5A,6aA}$ = 2.2 Hz, 1 H, 6-H_{aA}), 3.86 (dd, $J_{6aB,6bB}$ = –12.0, $J_{5B,6aB}$ = 2.2 Hz, 1 H, 6-H_{aB}), 3.78 (d, $J_{2B,3B}$ = 2.5 Hz, 1 H, 2-H_B), 3.70 (dd, $J_{5A,6bA}$ = 6.1 Hz, 1 H, 6-H_{bA}), 3.66 (dd, $J_{5B,6bB}$ = 6.6 Hz, 1 H, 6-H_{bB}), 3.50 (dd, $J_{3A,4A}$ ≈ $J_{4A,5A}$ ≈ 9.6 Hz, 1 H, 4-H_A), 3.49 (dd, $J_{3B,4B}$ ≈ $J_{4B,5B}$ ≈ 9.6 Hz, 1 H, 4-H_B), 3.44 (2 dd, 2 H, 3-H_A, 3-H_B), 3.22 (ddd, 1 H, 5-H_A), 3.15 (ddd, 1 H, 5-H_B) ppm. ^{13}C NMR (150.90 MHz, CD_3OD): δ = 149.2 (C-4, triazole), 124.8 (C-5, triazole), 102.3 (C-1_A), 101.9 (C-1_B), 78.7 (C-5_A), 78.6 (C-5_B), 78.5 (C-2_A), 75.1 (C-3_B), 74.5 (C-3_A), 72.2 (C-2_B), 69.0 (C-4_A), 68.9 (C-4_B), 68.7 (C$H_2$$CH_2$ triazole), 63.1 (C-6_B), 62.8 (C-6_A), 56.6 (CH_2OH), 51.9 (CH_2CH_2 triazole) ppm. HRMS (ESI): calcd. for $C_{17}H_{29}N_3O_{12}Na$ [M + Na]$^+$ 490.1649; found 490.1653.

1-[(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyloxyethyl)]-4-hydroxymethyl-1,2,3-triazole (18): The click coupling reaction was carried out starting from 2-azidoethyl glycoside **15** (72 mg, 0.08 mmol) and propargyl alcohol (10 μL, 9.4 mg, 0.17 mmol) following the same method as outlined in General Procedure B to afford the title compound **18** (66 mg, 86%). $[\alpha]_D^{25}$ = –40.1 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.50–7.24 (m, 26 H, Ar-H, triazole-*H*), 5.59 (s, 1 H, PhC*H*), 5.44 (s, 1 H, PhC*H*), 4.99, 4.93 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 4.75 (br. s, 2 H, PhCH_2), 4.69 (d, *J* = –12.5 Hz, 1 H, PhCH_2), 4.68 (br. s, 1 H, 1-H_A), 4.65 (br. s, 2 H, CH_{2ab}OH), 4.60 (d, *J* = –12.5 Hz, 1 H, PhCH_2), 4.56 (br. s, 1 H, 1-H_B), 4.50–4.46 (m, 2 H, CH_2CH_{2ab} triazole), 4.27 (dd, $J_{6aB,6bB}$ = –10.3, $J_{5B,6aB}$ = 4.8 Hz, 1 H, 6-H_{aB}), 4.24 (dd, $J_{3B,4B}$ ≈ $J_{4B,5B}$ ≈ 9.6 Hz, 1 H, 4-H_B), 4.11 (dd, $J_{1A,2A}$ = 1.6, $J_{2A,3A}$ = 3.4 Hz, 1 H, 2-H_A), 4.09 (dd, $J_{6aA,6bA}$ = –10.2, $J_{5A,6aA}$ = 4.7 Hz, 1 H, 6-H_{aA}), 4.06–4.01 (m, 2 H, 4-H_A, C$H_{2a}$$CH_2$ triazole), 3.94 (d, $J_{2B,3B}$ = 3.1 Hz, 1 H, 2-H_B), 3.87 (dd, $J_{5B,6bB}$ = 10.2 Hz, 1 H, 6-H_{bB}), 3.82–3.76 (m, 2 H, 3-H_A, C$H_{2b}$$CH_2$ triazole), 3.66 (dd, $J_{5A,6bA}$ = 10.3 Hz, 1 H, 6-H_{bA}), 3.59 (dd, 1 H, 3-H_B), 3.33 (ddd, 1 H, 5-H_B), 3.07 (ddd, 1 H, 5-H_A) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 148.3 (C-4, triazole), 138.6–125.9 (30 C, Ar-C), 122.8 (C-5, triazole), 101.5 (PhCH), 101.3 (PhCH), 100.7 (C-1_B), 98.0 (C-1_A), 78.4 (C-4_A), 78.3 (C-4_B), 77.5 (C-3_B), 75.9 (C-2_B), 75.0 (C-2_A), 74.5 (PhCH_2), 73.5 (C-3_A), 72.2 (PhCH_2), 71.5 (PhCH_2), 68.5 (C-6_A), 68.4 (C-6_B), 67.6 (C-5_B), 65.4 (C$H_2$$CH_2$ triazole), 64.2 (C-5_A), 56.4 (CH_2OH), 49.8 (CH_2CH_2 triazole) ppm.

HRMS (ESI): calcd. for $C_{52}H_{55}N_3O_{12}Na$ [M + Na]$^+$ 936.3683; found 936.3690.

1-[(β-D-Mannopyranosyl)-(1→2)-(α-D-mannopyranosyloxyethyl)]-4-hydroxymethyl-1,2,3-triazole (3): Hydrogenolysis of the coupling product **18** (55 mg, 0.06 mmol) was carried out following the same method as outlined in General Procedure C to afford title compound **3** (23 mg, 82%). $[\alpha]_D^{25}$ = –31.2 (*c* = 1, H_2O). ^{1}H NMR (600.13 MHz, CD_3OD): δ = 7.97 (s, 1 H, triazole-*H*), 4.87 (br. s, 1 H, 1-H_A), 4.69 (br. s, 2 H, CH_{2ab}OH), 4.67–4.61 (m, 3 H, CH_2CH_{2ab} triazole, 1-H_B), 4.12 (ddd, *J* = –10.9, 6.8, 3.9 Hz, 1 H, C$H_{2a}$$CH_2$ triazole), 4.00 (dd, $J_{2A,3A}$ = 2.6, $J_{1A,2A}$ = 1.8 Hz, 1 H, 2-H_A), 3.93–3.87 (m, 3 H, 2-H_B, 6-H_{aB}, C$H_{2b}$$CH_2$ triazole), 3.75 (dd, $J_{6aA,6bA}$ = –11.9, $J_{5A,6aA}$ = 2.2 Hz, 1 H, 6-H_{aA}), 3.69 (dd, $J_{6aB,6bB}$ = –12.0, $J_{5B,6bB}$ = 6.5 Hz, 1 H, 6-H_{bB}), 3.65 (dd, $J_{5A,6bA}$ = 5.7 Hz, 1 H, 6-H_{bA}), 3.63–3.58 (m, 2 H, 3-H_A, 4-H_A), 3.54 (dd, $J_{3B,4B} \approx J_{4B,5B} \approx$ 9.5 Hz, 1 H, 4-H_B), 3.46 (dd, $J_{2B,3B}$ = 3.1 Hz, 1 H, 3-H_B), 3.25 (ddd, $J_{5B,6aB}$ = 2.2 Hz, 1 H, 5-H_B), 3.13 (ddd, 1 H, 5-H_A) ppm. ^{13}C NMR (150.90 MHz, CD_3OD): δ = 149.3 (C-4, triazole), 125.2 (C-5, triazole), 100.0 (C-1_B), 99.1 (C-1_A), 78.7 (C-5_B), 78.3 (C-2_A), 75.2 (C-5_A), 75.1 (C-3_B), 72.7 (C-2_B), 72.0 (C-3_A), 68.8 (C-4_A), 68.6 (C-4_B), 66.9 (CH_2CH_2 triazole), 63.0 (C-6_B), 62.6 (C-6_A), 56.6 (CH_2OH), 51.3 (CH_2CH_2 triazole) ppm. HRMS (ESI): calcd. for $C_{17}H_{29}N_3O_{12}Na$ [M + Na]$^+$ 490.1649; found 490.1653.

Propargyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-(*p*-methoxybenzyl)-β-D-mannopyranoside (19): Employing the same method as outlined in General Procedure A, the glycosidation reaction between glycosyl donor **7** (700 mg, 1.23 mmol) and propargyl alcohol (83 mg, 86 μL, 1.48 mmol) gave compound **19** (400 mg, 63%). $[\alpha]_D^{25}$ = –82.8 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.50–7.48 (m, 2 H, Ar-H), 7.39–7.25 (m, 10 H, Ar-H), 6.85–6.83 (m, 2 H, Ar-H), 5.61 (s, 1 H, PhC*H*), 4.89, 4.79 (2 d, *J* = –11.8 Hz each, 2×1 H, PhCH_2), 4.73 (d, $J_{1,2}$ = 1.0 Hz, 1 H, 1-H), 4.66, 4.56 (2 d, *J* = –12.5 Hz each, 2×1 H, PhCH_2), 4.43 (dd, $J_{OCH2a,OCH2b}$ = –15.9, $J_{OCH2a,=CH}$ = 2.4 Hz, 1 H, OCH_{2a}), 4.40 (dd, $J_{OCH2b,=CH}$ = 2.4 Hz, 1 H, OCH_{2b}), 4.31 (dd, $J_{6a,6b}$ = –10.4, $J_{5,6a}$ = 4.8 Hz, 1 H, 6-H_a), 4.19 (dd, $J_{3,4}$ = 9.9, $J_{4,5}$ = 9.3 Hz, 1 H, 4-H), 3.95 (dd, $J_{2,3}$ = 3.1 Hz, 1 H, 2-H), 3.92 (dd, $J_{5,6b}$ = 10.1 Hz, 1 H, 6-H_b), 3.80 (s, 3 H, OCH_3), 3.61 (dd, 1 H, 3-H), 3.36 (ddd, 1 H, 5-H), 2.45 (dd, 1 H, C≡C*H*) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 159.2 (Ar-C), 138.3–126.0 (Ar-C), 113.5 (Ar-C), 101.4 (Ph*C*H), 99.3 (C-1), 78.6 (*C*≡CH), 78.5 (C-4), 77.8 (C-3), 75.3 (C-2), 75.2 (C≡*C*H), 74.5 (PhCH_2), 72.3 (PhCH_2), 68.5 (C-6), 67.6 (C-5), 55.7 (OCH_2), 55.2 (OCH_3) ppm. HRMS (ESI): calcd. for $C_{31}H_{32}O_7Na$ [M + Na]$^+$ 539.2046; found 539.2032.

Propargyl 3-*O*-Benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (20): Starting from compound **19** (400 mg, 0.77 mmol), the *p*-methoxybenzyl deprotection method was the same as that used for the preparation of compound **10** providing the title compound **20** (240 mg, 78%). $[\alpha]_D^{25}$ = –62.6 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.51–7.25 (m, 10 H, Ar-H), 5.61 (s, 1 H, PhC*H*), 4.86, 4.78 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 4.79 (d, $J_{1,2}$ = 1.2 Hz, 1 H, 1-H), 4.44 (dd, $J_{OCH2a,OCH2b}$ = –17.1, $J_{OCH2a,=CH}$ = 2.4 Hz, 1 H, OCH_{2a}), 4.43 (dd, $J_{OCH2b,=CH}$ = 2.4 Hz, 1 H, OCH_{2b}), 4.34 (dd, $J_{6a,6b}$ = –10.5, $J_{5,6a}$ = 4.9 Hz, 1 H, 6-H_a), 4.16 (ddd, $J_{2,3}$ = 3.3, $J_{2,2\text{-}OH}$ = 1.5 Hz, 1 H, 2-H), 4.14 (dd, $J_{3,4}$ = 9.5, $J_{4,5}$ = 9.4 Hz, 1 H, 4-H), 3.88 (dd, $J_{5,6b}$ = 10.1 Hz, 1 H, 6-H_b), 3.70 (dd, 1 H, 3-H), 3.39 (ddd, 1 H, 5-H), 2.56 (d, 1 H, 2-O*H*), 2.46 (dd, 1 H, C≡C*H*) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 137.8–126.0 (Ar-C), 101.6 (Ph*C*H), 97.5 (C-1), 78.3 (C-2), 78.2 (*C*≡CH), 76.7 (C-3), 75.5 (C≡*C*H), 72.6 (PhCH_2), 69.8 (C-4), 68.5 (C-6), 66.9 (C-5), 55.7 (OCH_2) ppm. HRMS (ESI): calcd. for $C_{23}H_{24}O_6Na$ [M + Na]$^+$ 419.1471; found 419.1462.

Propargyl (2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (21): Employing the same method as outlined in General Procedure A, the glycosylation reaction between glycosyl donor **11** (178 mg, 0.33 mmol) and glycosyl acceptor **20** (100 mg, 0.25 mmol) gave disaccharide **21** (190 mg, 90%). $[\alpha]_D^{25}$ = –99.7 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.56–7.24 (m, 25 H, Ar-H), 5.61 (s, 1 H, PhC*H*), 5.43 (s, 1 H, PhC*H*), 5.07, 4.98 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 4.80 (d, $J_{1B,2B}$ = 0.1 Hz, 1 H, 1-H_B), 4.79, 4.77 (2 d, *J* = –12.6 Hz, 2×1 H, PhCH_2), 4.73 (s, 1 H, 1-H_A), 4.64, 4.58 (2 d, *J* = –12.6 Hz, 2×1 H, PhCH_2), 4.37 (dd, $J_{OCH2a,OCH2b}$ = –15.8, $J_{OCH2a,=CH}$ = 2.5 Hz, 1 H, OCH_{2a}), 4.34 (dd, $J_{OCH2b,=CH}$ = 2.3 Hz, 1 H, OCH_{2b}), 4.32 (dd, $J_{6aB,6bB}$ = –10.3, $J_{5B,6aB}$ = 4.8 Hz, 1 H, 6-H_{aB}), 4.29 (dd, $J_{6aA,6bA}$ = –10.4, $J_{5A,6aA}$ = 4.8 Hz, 1 H, 6-H_{aA}), 4.26 (dd, $J_{2A,3A}$ = 3.2 Hz, 1 H, 2-H_A), 4.23 (dd, $J_{3B,4B}$ = 9.9, $J_{4B,5B}$ = 9.2 Hz, 1 H, 4-H_B), 4.16 (dd, $J_{2B,3B}$ = 3.2 Hz, 1 H, 2-H_B), 4.06 (dd, $J_{3A,4A}$ = 9.9, $J_{4A,5A}$ = 9.3 Hz, 1 H, 4-H_A), 3.91 (dd, $J_{5B,6bB}$ = 10.2 Hz, 1 H, 6-H_{bB}), 3.73 (dd, $J_{5A,6bA}$ = 10.0 Hz, 1 H, 6-H_{bA}), 3.66 (dd, 1 H, 3-H_A), 3.59 (dd, 1 H, 3-H_B), 3.38 (ddd, 1 H, 5-H_A), 3.35 (ddd, 1 H, 5-H_B), 2.43 (dd, 1 H, C≡C*H*) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 139.0–126.0 (Ar-C), 103.6 (C-1_B), 101.6 (Ph*C*H), 101.3 (Ph*C*H), 98.7 (C-1_A), 78.3 (C-4_B), 78.2 (*C*≡CH), 78.2 (C-4_A), 77.5 (C-3_B), 76.7 (C-2_A), 76.0 (C-3_A), 75.7 (C≡*C*-H), 75.5 (C-2_B), 74.7 (PhCH_2), 71.9 (PhCH_2), 71.1 (PhCH_2), 68.7 (C-6_B), 68.6 (C-6_A), 67.6 (C-5_B), 67.5 (C-5_A), 55.6 (OCH_2) ppm. HRMS (ESI): calcd. for $C_{50}H_{50}O_{11}Na$ [M + Na]$^+$ 849.3251; found 849.3237.

Propargyl 2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (22): The glycosidation reaction was carried out starting from glycosyl donor **8** (1.0 g, 2.03 mmol) and propargyl alcohol (137 mg, 142 μL, 2.44 mmol) following the same reaction procedure as described for the preparation of compound **13** providing the title compound **22** (730 mg, 82%). $[\alpha]_D^{25}$ = +23.7 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.51–7.24 (m, 10 H, Ar-H), 5.63 (s, 1 H, PhC*H*), 5.43 (dd, $J_{2,3}$ = 3.5, $J_{1,2}$ = 1.6 Hz, 1 H, 2-H), 4.99 (d, 1 H, 1-H), 4.70, 4.65 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 4.27 (dd, $J_{6a,6b}$ = –10.3, $J_{5,6a}$ = 4.8 Hz, 1 H, 6-H_a), 4.24 (dd, $J_{OCH2a,OCH2b}$ = –17.0, $J_{OCH2a,=CH}$ = 2.4 Hz, 1 H, OCH_{2a}), 4.24 (dd, $J_{OCH2b,=CH}$ = 2.4 Hz, 1 H, OCH_{2b}), 4.07 (dd, $J_{3,4}$ = 9.9, $J_{4,5}$ = 9.5 Hz, 1 H, 4-H), 4.01 (dd, 1 H, 3-H), 3.88 (ddd, $J_{5,6b}$ = 10.4 Hz, 1 H, 5-H), 3.85 (dd, 1 H, 6-H_b), 2.46 (dd, 1 H, C≡C*H*), 2.17 (s, 3 H, COCH_3) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 170.1 (*C*OCH$_3$), 137.9–126.1 (Ar-C), 101.6 (Ph*C*H), 97.5 (C-1), 78.2 (*C*≡C-H), 78.1 (C-4), 75.3 (C≡*C*-H), 73.7 (C-3), 72.1 (PhCH_2), 69.4 (C-2), 68.5 (C-6), 64.3 (C-5), 54.7 (OCH_2), 21.0 (COCH_3) ppm. HRMS (ESI): calcd. for $C_{25}H_{26}O_7Na$ [M + Na]$^+$ 461.1576; found 461.1550.

Propargyl 3-*O*-Benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (23): Compound **22** (400 mg, 0.91 mmol) was deacetylated following the same method as described for the preparation of compound **14** to give the title compound **23** (330 mg, 91%). $[\alpha]_D^{25}$ = +67.7 (*c* = 1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.50–7.28 (m, 10 H, Ar-H), 5.61 (s, 1 H, PhC*H*), 5.08 (d, $J_{1,2}$ = 1.4 Hz, 1 H, 1-H), 4.85, 4.71 (2 d, *J* = –11.8 Hz each, 2×1 H, PhCH_2), 4.27 (dd, $J_{6a,6b}$ = –10.6, $J_{5,6a}$ = 5.3 Hz, 1 H, 6-H_a), 4.25 (dd, $J_{OCH2a,OCH2b}$ = –17.0, $J_{OCH2a,=CH}$ = 2.4 Hz, 1 H, OCH_{2a}), 4.25 (dd, $J_{OCH2b,=CH}$ = 2.4 Hz, 1 H, OCH_{2b}), 4.12 (dd, $J_{3,4}$ = 9.6, $J_{4,5}$ = 9.4 Hz, 1 H, 4-H), 4.09 (ddd, $J_{2,3}$ = 3.5, $J_{2,2\text{-}OH}$ = 1.4 Hz, 1 H, 2-H), 3.93 (dd, 1 H, 3-H), 3.86 (ddd, $J_{5,6b}$ = 10.3 Hz, 1 H, 5-H), 3.85 (dd, 1 H, 6-H_b), 2.70 (d, 1 H, 2-O*H*), 2.46 (dd, 1 H, C≡C*H*) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 137.9–126.0 (Ar-C), 101.6 (Ph*C*H), 98.6 (C-1), 78.7 (C-4), 78.5 (*C*≡CH), 75.4 (C-3), 75.0 (C≡*C*H), 73.0 (PhCH_2), 69.8 (C-2), 68.7 (C-6), 63.7 (C-5), 54.5 (OCH_2) ppm.

HRMS (ESI): calcd. for $C_{23}H_{24}O_6Na$ $[M + Na]^+$ 419.1471; found 419.1460.

Propargyl (2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (24): Employing the same method as outlined in General Procedure A, the glycosylation reaction between glycosyl donor **11** (177 mg, 0.33 mmol) and glycosyl acceptor **23** (100 mg, 0.25 mmol) gave disaccharide **24** (134 mg, 64%). $[\alpha]_D^{25}$ = –34.4 (*c* = 1, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$): δ = 7.53–7.20 (m, 25 H, Ar-H), 5.60 (s, 1 H, PhC*H*), 5.50 (s, 1 H, PhC*H*), 5.05, 4.97 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 5.04 (d, $J_{1A,2A}$ = 1.4 Hz, 1 H, 1-H_A), 4.77, 4.72 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 4.70, 4.62 (2 d, *J* = –12.5 Hz each, 2×1 H, PhCH_2), 4.64 (s, 1 H, 1-H_B), 4.28 (dd, $J_{2A,3A}$ = 3.2 Hz, 1 H, 2-H_A), 4.26 (dd, $J_{6aA,6bA}$ = –10.6, $J_{5A,6aA}$ = 5.0 Hz, 1 H, 6-H_{aA}), 4.26 (dd, $J_{4B,5B}$ = 10.2, $J_{3B,4B}$ = 9.9 Hz, 1 H, 4-H_B), 4.25 (dd, $J_{6aB,6bB}$ = –10.5, $J_{5B,6aB}$ = 4.8 Hz, 1 H, 6-H_{aB}), 4.24 (dd, $J_{OCH2a,OCH2b}$ = –17.2, $J_{OCH2a,\equiv CH}$ = 2.5 Hz, 1 H, OCH_{2a}), 4.23 (dd, $J_{OCH2b,\equiv CH}$ = 2.3 Hz, 1 H, OCH_{2b}), 4.12 (dd, $J_{3A,4A}$ = 9.9, $J_{4A,5A}$ = 9.7 Hz, 1 H, 4-H_A), 4.00 (dd, $J_{2B,3B}$ = 3.2 Hz, 1 H, 2-H_B), 3.96 (dd, 1 H, 3-H_A), 3.87 (dd, $J_{5B,6bB}$ = 9.9 Hz, 1 H, 6-H_{bB}), 3.82 (ddd, $J_{5A,6bA}$ = 10.0 Hz, 1 H, 5-H_A), 3.77 (dd, 1 H, 6-H_{bA}), 3.60 (dd, 1 H, 3-H_B), 3.32 (ddd, 1 H, 5-H_B), 2.46 (dd, 1 H, C≡C*H*) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 138.7–126.0 (Ar-C), 101.6 (Ph*C*H), 101.4 (Ph*C*H), 100.7 (C-1_B), 96.8 (C-1_A), 78.4 (2 C, C-4_A, C-4_B), 78.3 (*C*≡CH), 77.6 (C-3_B), 75.9 (C-2_B), 75.2 (C-2_A), 74.7 (C≡*C*H), 74.6 (Ph*C*H_2), 74.0 (C-3_A), 72.2 (Ph*C*H_2), 71.4 (Ph*C*H_2), 68.7 (C-6_B), 68.5 (C-6_A), 67.8 (C-5_B), 64.6 (C-5_A), 54.4 (O*C*H_2) ppm. HRMS (ESI): calcd. for $C_{50}H_{54}NO_{11}$ $[M + NH_4]^+$ 844.3697; found 844.3681.

1-(3-*O*-Benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyloxyethyl)-4-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyloxymethyl)-1,2,3-triazole (25): Employing the same method as outlined in General Procedure B, the click coupling reaction between 2-azidoethyl glycoside **10** (190 mg, 0.44 mmol) and propargyl glycoside **20** (175 mg, 0.44 mmol) gave compound **25** (300 mg, 83%). $[\alpha]_D^{25}$ = –44.3 (*c* = 1, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$): δ = 7.77 (s, 1 H, triazole-*H*), 7.50–7.25 (m, 20 H, Ar-H), 5.59 (s, 1 H, PhC*H*), 5.57 (s, 1 H, PhC*H*), 4.98 (d, $J_{OCH2a\ triazole,OCH2b\ triazole}$ = –12.4 Hz, 1 H, OCH_{2a} triazole), 4.82, 4.74 (2 d, *J* = –12.3 Hz each, 2×1 H, PhCH_2), 4.81, 4.72 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 4.80 (d, 1 H, OCH_{2b} triazole), 4.68 (br. s, 1 H, 1-H_A), 4.62–4.52 (m, 2 H, CH_2CH_{2ab} triazole), 4.46 (br. s, 1 H, 1-H_B), 4.36 (dd, $J_{6aA,6bA}$ = –10.4, $J_{5A,6aA}$ = 4.9 Hz, 1 H, 6-H_{aA}), 4.29 (dd, $J_{6aB,6bB}$ = –10.4, $J_{5B,6aB}$ = 4.9 Hz, 1 H, 6-H_{aB}), 4.25 (ddd, *J* = –10.8, 3.9, 3.9 Hz, 1 H, C$H_{2a}CH_2$ triazole), 4.13 (dd, $J_{3A,4A} \approx J_{4A,5A} \approx$ 9.5 Hz, 1 H, 4-H_A), 4.09 (dd, $J_{3B,4B}$ = 9.5, $J_{4B,5B}$ = 9.4 Hz, 1 H, 4-H_B), 4.09 (ddd, $J_{2A,3A}$ = 3.2 Hz, 1 H, 2-H_A), 4.05 (ddd, $J_{2B,3B}$ = 3.3, $J_{2B,2\text{-}OH}$ = 1.3 Hz, 1 H, 2-H_B), 3.96 (ddd, *J* = 8.0, 3.8 Hz, 1 H, C$H_{2b}CH_2$ triazole), 3.89 (dd, $J_{5A,6bA}$ = 10.3 Hz, 1 H, 6-H_{bA}), 3.84 (dd, $J_{5B,6bB}$ = 10.3 Hz, 1 H, 6-H_{bB}), 3.64 (dd, 1 H, 3-H_A), 3.61 (dd, 1 H, 3-H_B), 3.40 (ddd, 1 H, 5-H_A), 3.31 (ddd, 1 H, 5-H_B), 2.79 (d, 1 H, 2-OH_B), 2.77 (br. s, 1 H, 2-OH_A) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 143.7 (C-4, triazole), 137.8–125.9 (24 C, Ar-C), 124.5 (C-5, triazole), 101.5 (Ph*C*H), 101.4 (Ph*C*H), 100.6 (C-1_B), 99.2 (C-1_A), 78.3 (C-4_A), 78.2 (C-4_B), 76.6 (C-3_A), 76.4 (C-3_B), 72.5 (Ph*C*H_2), 72.4 (Ph*C*H_2), 69.7 (C-2_A), 69.5 (C-2_B), 68.5 (C-6_A), 68.3 (C-6_B), 67.9 (O*C*H_2CH_2), 66.9 (C-5_B), 66.8 (C-5_A), 62.2 (O*C*H_2 triazole), 50.2 (CH_2*C*H_2 triazole) ppm. HRMS (ESI): calcd. for $C_{45}H_{49}N_3O_{12}Na$ $[M + Na]^+$ 846.3214; found 846.3214.

1-(β-D-Mannopyranosyloxyethyl)-4-(β-D-mannopyranosyloxymethyl)-1,2,3-triazole (4): Hydrogenolysis of the coupling product **25** (43 mg, 0.05 mmol) was carried out following the same method as outlined in General Procedure C to give compound **4** (21 mg, 86%). $[\alpha]_D^{25}$ = –43.0 (*c* = 1, H_2O). ^1H NMR (600.13 MHz, CD_3OD): δ = 8.16 (s, 1 H, triazole-*H*), 4.95 (d, $J_{OCH2a\ triazole,OCH2b\ triazole}$ = –12.5 Hz, 1 H, OCH_{2a} triazole), 4.81 (d, 1 H, OCH_{2b} triazole), 4.68–4.62 (m, 2 H, CH_2CH_{2ab} triazole), 4.61 (d, $J_{1A,2A}$ = 0.8 Hz, 1 H, 1-H_A), 4.49 (d, $J_{1B,2B}$ = 0.7 Hz, 1 H, 1-H_B), 4.26 (ddd, *J* = –11.4, 4.9, 4.9 Hz, 1 H, C$H_{2a}CH_2$ triazole), 4.00 (ddd, *J* = 6.8, 4.4 Hz, 1 H, C$H_{2b}CH_2$ triazole), 3.91 (dd, $J_{6aA.6bA}$ = –11.8, $J_{5A,6aA}$ = 2.3 Hz, 1 H, 6-H_{aA}), 3.87 (dd, $J_{6aB.6bB}$ = –11.8, $J_{5B,6aB}$ = 2.3 Hz, 1 H, 6-H_{aB}), 3.85 (dd, $J_{2A,3A}$ = 3.2 Hz, 1 H, 2-H_A), 3.82 (d, $J_{2B,3B}$ = 3.2 Hz, 1 H, 2-H_B), 3.74 (dd, $J_{5A,6bA}$ = 6.1 Hz, 1 H, 6-H_{bA}), 3.69 (dd, $J_{5B,6bB}$ = 6.1 Hz, 1 H, 6-H_{bB}), 3.56 (dd, $J_{3A,4A} \approx J_{4A,5A} \approx$ 9.5 Hz, 1 H, 4-H_A), 3.53 (dd, $J_{3B,4B} \approx J_{4B,5B} \approx$ 9.5 Hz, 1 H, 4-H_B), 3.46 (dd, 1 H, 3-H_A), 3.43 (dd, 1 H, 3-H_B), 3.26 (ddd, 1 H, 5-H_A), 3.21 (ddd, 1 H, 5-H_B) ppm. ^{13}C NMR (150.90 MHz, CD_3OD): δ = 145.3 (C-4, triazole), 126.7 (C-5, triazole), 101.9 (C-1_B), 100.5 (C-1_A), 78.5 (C-5_B), 78.4 (C-5_A), 75.2 (C-3_A), 75.1 (C-3_B), 72.4 (C-2_A), 72.3 (C-2_B), 68.8 (*C*H_2CH_2 triazole), 68.6 (C-4_A), 68.5 (C-4_B), 62.9 (C-6_A), 62.8 (C-6_B), 62.4 (O*C*H_2 triazole), 51.7 (CH_2*C*H_2 triazole) ppm. HRMS (ESI): calcd. for $C_{17}H_{28}N_3O_{12}$ $[M - H]^+$ 466.1673; found 466.1713.

1-[(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyloxyethyl)]-4-[(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyloxymethyl)]-1,2,3-triazole (26): Employing the same method as outlined in General Procedure B, the click coupling reaction between 2-azidoethyl glycoside **12** (52 mg, 0.06 mmol) and propargyl glycoside **21** (50 mg, 0.06 mmol) gave compound **26** (88 mg, 86%). $[\alpha]_D^{25}$ = –87.9 (*c* = 1, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$): δ = 7.53–7.16 (m, 50 H, Ar-H), 7.04 (s, 1 H, triazole-*H*), 5.58 (s, 1 H, PhC*H*), 5.56 (s, 1 H, PhC*H*), 5.44 (s, 1 H, PhC*H*), 5.42 (s, 1 H, PhC*H*), 5.04, 4.94 (2 d, *J* = –12.5 Hz each, 2×1 H, PhCH_2), 5.01, 4.91 (2 d, *J* = –12.6 Hz each, 2×1 H, PhCH_2), 4.82 (br. s, 1 H, 1-H_B), 4.80 (d, *J* = –12.7 Hz, 1 H, PhCH_2), 4.77–4.72 (m, 4 H, OCH_{2a} triazole, PhCH_2), 4.70, 4.57 (2 d, *J* = –12.5 Hz each, 2×1 H, PhCH_2), 4.68, 4.54 (2 d, *J* = –12.1 Hz each, 2×1 H, PhCH_2), 4.53 (br. s, 1 H, 1-H_D), 4.50 (d, $J_{OCH2a\ triazole,OCH2b\ triazole}$ = –12.7 Hz, 1 H, OCH_{2b} triazole), 4.33 (br. s, 1 H, 1-H_A), 4.29 (br. s, 1 H, 1-H_C), 4.29–4.17 (m, 9 H, 6-H_{aA}, 6-H_{aB}, 6-H_{aC}, 6-H_{aD}, 4-H_B, 4-H_D, 2-H_A, CH_2CH_{2ab} triazole), 4.16 (d, $J_{2C,3C}$ = 3.1 Hz, 1 H, 2-H_C), 4.10 (d, $J_{2B,3B}$ = 3.1 Hz, 1 H, 2-H_B), 4.07–4.02 (m, 1 H, C$H_{2a}CH_2$ triazole), 4.00 (dd, $J_{3A,4A}$ = 9.7, $J_{4A,5A}$ = 9.6 Hz, 1 H, 4-H_A), 3.95 (dd, $J_{3C,4C}$ = 9.7, $J_{4C,5C}$ = 9.6 Hz, 1 H, 4-H_C), 3.92 (d, $J_{2D,3D}$ = 3.0 Hz, 1 H, 2-H_D), 3.90–3.85 (m, 2 H, 6-H_{bB}, 6-H_{bD}), 3.82 (ddd, *J* = –11.0, 8.1, 3.9 Hz, 1 H, C$H_{2b}CH_2$ triazole), 3.73 (dd, $J_{6aA,6bA}$ = –10.1, $J_{5A,6bA}$ = 5.5 Hz, 1 H, 6-H_{bA}), 3.71 (dd, $J_{6aC,6bC}$ = –10.2, $J_{5C,6bC}$ = 5.4 Hz, 1 H, 6-H_{bC}), 3.61 (dd, $J_{3D,4D}$ = 10.0 Hz, 1 H, 3-H_D), 3.50 (dd, $J_{3B,4B}$ = 9.8 Hz, 1 H, 3-H_B), 3.49–3.45 (m, 2 H, 3-H_A, 3-H_C), 3.21–3.07 (m, 4 H, 5-H_A, 5-H_B, 5-H_C, 5-H_D) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 144.0 (C-4, triazole), 139.1–125.9 (60 C, Ar-C), 122.3 (C-5, triazole), 103.2 (C-1_B), 102.9 (C-1_D), 101.6 (Ph*C*H), 101.5 (Ph*C*H), 101.4 (C-1_C), 101.2 (Ph*C*H), 101.1 (Ph*C*H), 100.6 (C-1_A), 78.6 (C-4_B), 78.2 (C-4_D), 78.1 (C-4_A), 77.9 (C-4_C), 77.8 (C-3_B), 77.6 (C-3_D), 75.9 (C-2_D), 75.8 (C-2_A), 75.6 (C-3_C), 75.5 (C-2_B), 75.4 (C-3_A), 74.9 (C-2_C), 74.4 (Ph*C*H_2), 74.3 (Ph*C*H_2), 72.2 (Ph*C*H_2), 71.6 (Ph*C*H_2), 70.9 (Ph*C*H_2), 70.8 (Ph*C*H_2), 68.6 (2 C, C-6_A, C-6_C), 68.5 (2 C, C-6_B, C-6_D), 67.7 (C-5_A), 67.6 (C-5_C), 67.5 (C-5_B), 67.4 (C-5_D), 67.1 (*C*H_2CH_2 triazole), 62.0 (O*C*H_2 triazole), 49.9 (CH_2*C*H_2 triazole) ppm. HRMS (ESI): calcd. for $C_{99}H_{102}N_3O_{22}$ $[M + H]^+$ 1684.6955; found 1684.6948.

1-[(β-D-Mannopyranosyl)-(1→2)-(β-D-mannopyranosyloxyethyl)]-4-[(β-D-mannopyranosyl)-(1→2)-[(β-D-mannopyranosyloxymethyl)]-

1,2,3-triazole (5): Hydrogenolysis of the coupling product **26** (44 mg, 0.03 mmol) was carried out following the same method as outlined in General Procedure C to give compound **5** (20 mg, 97%). $[\alpha]_D^{25}$ = –39.9 (*c* = 1, H_2O). ^1H NMR (600.13 MHz, CD_3OD): δ = 8.10 (s, 1 H, triazole-*H*), 5.00 (d, $J_{OCH2a\ triazole,OCH2b\ triazole}$ = –12.5 Hz, 1 H, OCH_{2a} triazole), 4.83 (d, 1 H, OCH_{2b} triazole), 4.76 (br. s, 1 H, 1-H_B), 4.75 (br. s 1 H, 1-H_A), 4.69–4.63 (m, 2 H, CH_2CH_{2ab} triazole), 4.65 (br. s, 1 H, 1-H_C), 4.60 (br. s, 1 H, 1-H_D), 4.31 (ddd, *J* = –11.2, 6.2, 3.7 Hz, 1 H, C$H_{2a}CH_2$ triazole), 4.17 (d, $J_{2A,3A}$ = 3.2 Hz, 1 H, 2-H_A), 4.07 (d, $J_{2C,3C}$ = 3.3 Hz, 1 H, 2-H_C), 4.00 (ddd, *J* = 6.7, 3.5 Hz, 1 H, C$H_{2b}CH_2$ triazole), 3.96 (d, $J_{2B,3B}$ = 3.1 Hz, 1 H, 2-H_B), 3.93 (dd, $J_{6aA,6bA}$ = –11.9, $J_{5A,6aA}$ = 2.2 Hz, 1 H, 6-H_{aA}), 3.90–3.84 (m, 3 H, 6-H_{aC}, 6-H_{aB}, 6-H_{aD}), 3.77 (d, $J_{2D,3D}$ = 3.1 Hz, 1 H, 2-H_D), 3.74 (dd, $J_{5A,6bA}$ = 6.1 Hz, 1 H, 6-H_{bA}), 3.71 (dd, $J_{6aC,6bC}$ = –11.9, $J_{5C,6bC}$ = 6.1 Hz, 1 H, 6-H_{bC}), 3.69–3.66 (m, 2 H, 6-H_{bB}, 6-H_{bD}), 3.55 (dd, $J_{3A,4A}$ = 9.5, $J_{4A,5A}$ = 9.4 Hz, 1 H, 4-H_A), 3.53–3.42 (m, 6 H, 4-H_C, 4-H_D, 4-H_B, 3-H_A, 3-H_C, 3-H_D), 3.38 (dd, $J_{3B,4B}$ = 9.4 Hz, 1 H, 3-H_B), 3.33–3.27 (m, 1 H, 5-H_A), 3.24–3.14 (m, 3 H, 5-H_C, 5-H_B, 5-H_D) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 145.5 (C-4, triazole), 125.8 (C-5, triazole), 102.2 (C-1_C), 102.1 (C-1_B), 101.8 (C-1_D), 101.2 (C-1_A), 79.1 (C-2_A), 78.6 (C-5_A), 78.5 (2 C, C-5_B, C-5_C), 78.4 (C-5_D), 78.3 (C-2_C), 75.0 (C-3_B), 74.9 (C-3_D), 74.5 (C-3_A), 74.3 (C-3_C), 72.1 (C-2_B), 72.0 (C-2_D), 69.0 (*C*H_2CH_2 triazole), 68.9 (C-4_A), 68.8 (C-4_C), 68.6 (C-4_B), 68.5 (C-4_D), 63.0 (O*C*H_2 triazole), 62.9 (C-6_A), 62.8 (C-6_C), 62.7 (C-6_B), 62.6 (C-6_D), 51.9 (CH_2CH_2 triazole) ppm. HRMS (ESI): calcd. for $C_{29}H_{49}N_3O_{22}Na$ $[M + Na]^+$ 814.2705; found 814.2698.

1-[(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyloxyethyl)]-4-[(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyloxymethyl)]-1,2,3-triazole (27): Employing the same method as outlined in General Procedure B, the click coupling reaction between 2-azidoethyl glycoside **15** (52 mg, 0.06 mmol) and propargyl glycoside **24** (50 mg, 0.06 mmol) gave compound **27** (90 mg, 88%). $[\alpha]_D^{25}$ = –40.0 (*c* = 1, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$): δ = 7.52–7.20 (m, 51 H, triazole-*H*, Ar-H), 5.59 (s, 1 H, PhC*H*), 5.58 (s, 1 H, PhC*H*), 5.50 (s, 1 H, PhC*H*), 5.46 (s, 1 H, PhC*H*), 5.03 (d, *J* = –12.2 Hz, 1 H, PhCH_2), 4.99 (m, 2 H, 1-H_A, PhCH_2), 4.95, 4.93 (2 d, *J* = –12.2 Hz each, 2×1 H, PhCH_2), 4.78 (br. s, 1 H, 1-H_C), 4.77–4.58 (m, 12 H, 1-H_B, 1-H_D, OCH_{2ab} triazole, PhCH_2), 4.56–4.53 (m, 1 H, CH_2CH_{2a} triazole), 4.50–4.46 (m, 1 H, CH_2CH_{2b} triazole), 4.28–4.19 (m, 6 H, 6-H_{aB}, 6-H_{aA}, 2-H_A, 4-H_B, 4-H_D, 6-H_{aD}), 4.16 (dd, $J_{6aC,6bC}$ = –10.3, $J_{5C,6aC}$ = 4.8 Hz, 1 H, 6-H_{aC}), 4.14–4.06 (m, 4 H, 2-H_C, 4-H_A, 4-H_C, C$H_{2a}CH_2$ triazole), 3.96–3.94 (m, 2 H, 2-H_B, 2-H_D), 3.92 (dd, $J_{3A,4A}$ = 9.9, $J_{2A,3A}$ = 3.3 Hz, 1 H, 3-H_A), 3.88–3.75 (m, 6 H, C$H_{2b}CH_2$ triazole, 6-H_{bB}, 3-H_C, 5-H_A, 6-H_{bD}, 6-H_{bA}), 3.71 (dd, $J_{5C,6bC}$ = 10.3 Hz, 1 H, 6-H_{bC}), 3.60–3.57 (m, 2 H, 3-H_B, 3-H_D), 3.44 (ddd, 1 H, 5-H_C), 3.33 (ddd, $J_{6aD,6bD}$ = –9.8, $J_{5D,6bD}$ = 9.8, $J_{5D,6aD}$ = 5.0 Hz, 1 H, 5-H_D), 3.28 (ddd, $J_{6aB,6bB}$ = –9.7, $J_{5B,6bB}$ = 9.7, $J_{5B,6aB}$ = 4.9 Hz, 1 H, 5-H_B) ppm. ^{13}C NMR (150.90 MHz, $CDCl_3$): δ = 143.9 (C-4 triazole), 138.8–125.8 (60 C, Ar-C), 123.5 (C-5, triazole), 101.5 (Ph*C*H), 101.4 (Ph*C*H), 101.3 (2 C, Ph*C*H), 100.7 (C-1_D), 100.6 (C-1_B), 98.3 (C-1_C), 97.8 (C-1_A), 78.5, 78.4, 78.3 (4 C, C-4_A, C-4_C, C-4_B, C-4_D), 77.6 (2 C, C-3_B, C-3_D), 76.0 (C-2_B), 75.8 (C-2_D), 74.9 (C-2_C), 74.8 (C-2_A), 74.6 (Ph*C*H_2), 74.5 (Ph*C*H_2), 74.0 (C-3_A), 73.6 (C-3_C), 72.3 (Ph*C*H_2), 72.2 (Ph*C*H_2), 71.4 (Ph*C*H_2), 71.2 (Ph*C*H_2), 68.8, 68.6, 68.5 (4 C, C-6_A, C-6_C, C-6_B, C-6_D), 67.7 (C-5_B), 67.6 (C-5_D), 65.5 (*C*H_2CH_2 triazole), 64.6 (C-5_C), 64.4 (C-5_A), 60.3 (O*C*H_2 triazole), 49.8 (CH_2CH_2 triazole) ppm. HRMS (ESI): calcd. for $C_{99}H_{105}N_4O_{22}$ $[M + NH_4]^+$ 1701.7221; found 1701.7191.

1-[(β-D-Mannopyranosyl)-(1→2)-(α-D-mannopyranosyloxyethyl)]-4-[(β-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyloxymethyl)]-1,2,3-triazole (6): Hydrogenolysis of the coupling product **27** (70 mg, 0.04 mmol) was carried out following the same method as outlined in General Procedure C to give compound **6** (25 mg, 76%). $[\alpha]_D^{25}$ = +1.4 (*c* = 1, H_2O). ^1H NMR (600.13 MHz, CD_3OD): δ = 8.07 (s, 1 H, triazole-*H*), 5.05 (d, $J_{1A,2A}$ = 1.5 Hz, 1 H, 1-H_A), 4.87 (d, $J_{1C,2C}$ = 1.6 Hz, 1 H, 1-H_C), 4.83 (d, $J_{OCH2a\ triazole,OCH2b\ triazole}$ = –12.4 Hz, 1 H, OCH_{2a} triazole), 4.69 (br. s, 1 H, 1-H_B), 4.67 (d, 1 H, OCH_{2b} triazole), 4.68–4.64 (m, 2 H, CH_2CH_{2ab} triazole), 4.64 (br. s, 1 H, 1-H_D), 4.13 (ddd, *J* = –10.9, 6.7, 3.8 Hz, 1 H, C$H_{2a}CH_2$ triazole), 4.09 (dd, $J_{2A,3A}$ = 3.4 Hz, 1 H, 2-H_A), 4.00 (dd, $J_{2C,3C}$ = 2.9 Hz, 1 H, 2-H_C), 3.94–3.90 (m, 2 H, 2-H_B, C$H_{2b}CH_2$ triazole), 3.89–3.83 (m, 4 H, 2-H_D, 6-H_{aD}, 6-H_{aB}, 6-H_{aA}), 3.77–3.59 (m, 9 H, 6-H_{aC}, 3-H_A, 6-H_{bA}, 6-H_{bC}, 6-H_{bB}, 6-H_{bD}, 4-H_A, 3-H_C, 4-H_C), 3.58–3.56 (m, 1 H, 5-H_A), 3.55–3.51 (m, 2 H, 4-H_B, 4-H_D), 3.48 (dd, $J_{3B,4B}$ = 8.2, $J_{2B,3B}$ = 3.1 Hz, 1 H, 3-H_B), 3.47 (dd, $J_{3D,4D}$ = 8.2, $J_{2D,3D}$ = 3.1 Hz, 1 H, 3-H_D), 3.28–3.24 (m, 2 H, 5-H_B, 5-H_D), 3.20 (ddd, $J_{6aC,6bC}$ = –8.4, $J_{5C,6bC}$ = 5.6, $J_{5C,6aC}$ = 2.1 Hz, 1 H, 5-H_C) ppm. ^{13}C NMR (150.90 MHz, CD_3OD): δ = 145.4 (C-4, triazole), 126.0 (C-5, triazole), 99.8 (2 C, C-1_B, C-1_D), 98.9 (C-1_C), 98.6 (C-1_A), 78.5 (2 C, C-5_B, C-5_D), 78.3 (C-2_A), 78.1 (C-2_C), 75.0 (4 C, C-5_A, C-3_B, C-5_C, C-3_D), 75. 2 (2 C, C-2_B, C-2_D), 71.8 (2 C, C-3_A, C-3_C), 68.9 (C-4_A), 68.6 (C-4_C), 68.4 (2 C, C-4_B, C-4_D), 66.7 (*C*H_2CH_2 triazole), 62.8 (2 C, C-6_A, C-6_C), 62.6 (C-6_B), 62.5 (C-6_D), 61.1 (O*C*H_2 triazole), 51.3 (CH_2CH_2 triazole) ppm. HRMS (ESI): calcd. for $C_{29}H_{48}N_3O_{22}$ $[M - H]^+$ 790.2730; found 790.2796.

Biological Studies

Synthesis of <3 kDa *Candida albicans* Mannan: *C. albicans* mannan (20 mg/mL) was hydrolyzed under mild acidic conditions with 0.1 N HCl for up to 60 min at 100 °C. The hydrolysis products were neutralized with NaOH and analyzed by TLC using silica gel coated aluminium sheets (Merck, Darmstadt, Germany) using *n*-butanol/acetic acid/water (2:1:1, v/v/v) as eluent. The hydrolysis products were detected on TLC by using orcinol (2 g/L in 20% sulfuric acid) by heating the sheets for 10 min at 105 °C. Fractionation of the hydrolysis products by size was performed with Amicon YM3 YM1 ultrafiltration membranes (Millipore, Bedford, MA, USA) according to the manufacturer's instructions. The final fraction of <3 kDa was sterile-filtered and lyophilized for storage at 4 °C.

Competitive ELISA Inhibition: Polystyrene 96-well ELISA plates were coated with hydrolyzed low-molecular-weight (<3 kDa) *Candida albicans* Cetavlon mannan (50 µL, 0.1 mg/mL in PBS) and incubated at 4 °C overnight. The plates were then washed three times with 0.05% PBS-Tween. Blocking was achieved by using 2% BSA-PBS (Bovine albumin fraction V, ICN Biomedicals Inc., Aurora, Ohio, 810034; Tween 20 Fluka, Sigma–Aldrich Co. 93773) for 2 h at room temp. after which the plates were washed three times. The serum samples were collected from vaginal candidiasis patients and had elevated levels of IgG antibodies against *C. albicans* mannan (50 µL, diluted 1:100 in 0.5% BSA/0.05% PBS-Tween) and incubated at 37 °C for 2 h with serial ten-fold dilutions of the studied saccharides (0.0001–1 mg/mL) in a total volume of 100 µL. Thereafter the serum dilutions were applied to the coated ELISA plates and blotted and incubated at 4 °C overnight. After washing three times, 100 µL alkaline phosphatase conjugated antihuman IgG (Dako, Denmark, DO 336; 1:1000 in 0.5% BSA/PBS-Tween) was applied to the plates and they were incubated for 2 h at 37 °C. After washing three times, the color reaction was developed by using *p*-nitrophenylphosphate disodium salt (100 µL, 1 mg/mL in diethanolamine, Reagena, Finland, 180288) as substrate and terminated by the addition of 1 M NaOH (AKZO Nobel, The Netherlands)

after which the absorbance (O.D. 405 nm) was read by using an automated microplate reader. The inhibition in ELISA was expressed by using the formula (O.D. inhibition – O.D. PBS)/(O.D. no inhibition – O.D. PBS)%. Mean values of the duplicate determinations are given.

Supporting Information (see footnote on the first page of this article): Copies of ^{1}H and ^{13}C NMR spectra of the final products **1**–**6**.

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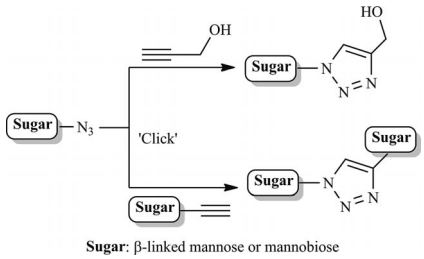
Three different β-linked divalent mannosides, along with their monovalent counterparts, have been designed and chemically synthesized by using click chemistry. The synthesized saccharides were biologically screened through a competitive inhibition enzyme-linked immunosorbent assay to determine the inhibition of specific human IgG binding to low-molecular-weight hydrolyzed *Candida albicans* mannan.



Sugar: β-linked mannose or mannobiose

C. Mukherjee, K. Ranta, J. Savolainen, R. Leino* 1–13

Synthesis and Immunological Screening of β-Linked Mono- and Divalent Mannosides

Keywords: Medicinal chemistry / Carbohydrates / Glycosylation / Click chemistry / Biological activity

