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α-D-Mannoside ligands with a valency ranging from one to three: Synthesis and hemagglutination inhibitory properties

Hussein Al-Mughaid *, Maha Khazaaleh

Department of Chemistry, Jordan University of Science and Technology, PO Box 3030, Irbid 22110, Jordan

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ABSTRACT

Six mono-, di-, and trivalent α-D-mannopyranosyl conjugates built on aromatic scaffolds were synthesized in excellent yields by Cu(I) catalyzed azide-alkyne cycloaddition reaction (CuAAC). These conjugates were designed to have unique, flexible tails that combine a mid-tail triazole ring, to interact with the tyrosine gate, with a terminal phenyl group armed with benzylic hydroxyl groups to avoid solubility problems as well as to provide options to connect to other supports. Biological evaluation of the prepared conjugates in hemagglutination inhibition (HAI) assay revealed that potency increases with valency and the trivalent ligand **6d** (HAI = 0.005 mM) is approximately sevenfold better than the best *meta*-oriented monovalent analogues **2d** and **4d** (HAI ≈ 0.033 mM) and so may serve as a good starting point to find new lead ligands.

1. Introduction

The interactions between carbohydrates and their protein receptors play a key biological role in cell recognition and adhesion [1]. At the monosaccharide level these interactions are usually weak with dissociation constants in the millimolar to micromolar range [2]. Biological ligands present multiple copies of the monosaccharide to multiple binding sites on the protein to achieve sufficient binding strength. To displace or compete with the natural multivalent ligands [3,4], synthetic ligands need to combine better binding per unit with multivalent presentation.

Urinary tract infections (UTIs), are one of the most common bacterial infections [5–7], are mainly caused by strains of the gram negative uropathogenic bacteria, *Escherichia coli* (UPEC) [8,9]. UPEC bind to the urinary tract endothelial surface through the adhesin protein FimH at the tip of Type 1 fimbriae or pili [10], complex proteinacious hair-like structures which extend from the surfaces of the bacterial cells. FimH is a lectin that recognizes terminal α-linked D-mannopyranoside oligosaccharides of the N-linked glycoprotein Uroplakin Ia (UPIa) on the endothelial surface [11]. The mannose-binding pocket of FimH is adjacent to a hydrophobic area known as the tyrosine gate that is bordered by two tyrosines (Tyr48 and Tyr137) and one isoleucine (Ile52) [12]. The non-polar faces of the non-terminal mannopyranoses of the UPI oligosaccharides have binding interactions with the tyrosines in the tyrosine gate [13–16]. Because the mannose-binding pocket of FimH has an adjacent extended hydrophobic region, compounds designed to bind to this site are poorly recognized by the many other mannose-binding receptors in humans [17]. Initial weak binding is followed by a conformational change in FimH to a state that gives the strong binding "catch-bond binding" that is critical for attachment in the high-sheer environment of the urinary tract [18,19]. Following binding, a conformational change in the cell surfaces [20], allows the bacteria to enter and establish sub-surface colonies that are difficult to eradicate. Previous studies have shown that FimH-mediated adhesion could be inhibited by natural or synthetic ligands. Since the discovery that phenyl α-D-mannopyranosides, particularly the 2-chloro-4-nitro derivative, bound FimH effectively [21], most research in this area has focused on the synthesis of multivalent mannosides to try to obtain potent FimH antagonists [22–29]. In this strategy, mannosides are grafted in multiple copies onto common scaffolds, particularly aromatic ones [30].

During the last three decades, several families of monovalent α-D-mannoside ligands with diverse mannosyl lipophilic aglycones have been synthesized to evaluate the utility of anti-adhesion strategies against *E. coli*, such as alkyl α-D-mannoside [31], biphenyl α-D-mannosides [32–36], indolinylphenyl and (aza)indolylphenyl α-D-mannosides [37], thiazolylaminomannosides [38], branched α-D-mannosides [39], isoquinolone-based mannosides [40], squaric acid monoamide mannosides [41], mannosyl triazoles [42], and others [43–47].

Since the nature of the mannosyl aglycone which could be elongated alkyl, substituted aromatic, or extended aromatic plays a crucial role in

* Corresponding author.
E-mail address: al-mughaid@just.edu.jo (H. Al-Mughaid).

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determining the binding affinity, altering its structure will influence the inhibitory potency toward type 1 fimbriated *E. coli*. The Cu(I)-catalyzed azide-alkyne [1,3] dipolar cycloaddition (CuAAC) reaction has become a powerful tool in the regioselective formation of 1,4-disubstituted 1,2,3-triazoles. Therefore, this highly efficient reaction has emerged as an effecient conjugation strategy for the synthesis of 1,2,3- triazole-containing glycoconjugates [48].

Following our interest in using click chemistry for the synthesis of mannoside ligands [49,50], we describe the synthesis of α-D-mannoside ligands using Cu(I)-catalyzed click reaction here. These mannosides have a flexible aglycone that combines a triazole ring positioned midway between the anomeric oxygens and the terminal phenyl groups that bear benzylic hydroxyl groups to ensure water solubility, to take advantage of polar interactions (H-bonds with the hydroxyl groups of Thr51 or Tyr137), and to provide options for additional connections to other supports. FimH ligands where the linker between the anomeric oxygen and the triazole ring is two methylene groups showed 2- to 4-fold higher affinity compared to their counterparts with one methylene group, while reduction in affinity up to 8-fold was observed when the triazole ring is directly attached to the anomeric center [42]. On the basis of these findings and also because X-ray studies suggested that stronger interactions could be achieved if this linkage was longer, we investigated whether moving from two methylene groups to three in the linker between the anomeric oxygen and the triazole ring was beneficial for binding. The next aim was to explore the effect of stepwise increases in valency, from mono-to trivalency. To this end, we prepared six mannosyl conjugates (Fig. 1) with valencies ranging from one to three and with similar distances between the binding epitopes. The binding potencies of these conjugates were evaluated by a hemagglutination inhibition (HAI) assay.

2. Results and discussion

2.1. Synthesis of terminal alkynes

Two series of alkynyl-terminated benzyl alcohols were envisioned for the installation of mannose moiety. As shown in Scheme 1, the first series was prepared by $NaBH_4$ reduction of the commercially available hydroxy-aldehydes **1**, **2**, and **3** to the corresponding hydroxy-benzyl alcohols **1a**, **2a**, and **3a**. Selective *O*-alkylation at the phenolic position using 5-chloropent-1-yne, K_2CO_3, and a catalytic amount of $(Bu)_4NI$ in refluxing acetone furnished the corresponding alkynyl-terminated benzyl alcohols **1b**, **2b**, and **3b** in yields of 69%, 72%, and 77%, respectively.

In the second series, the methyl esters **4**, **5**, and **6**, prepared under conventional conditions (H_2SO_4, (cat.), MeOH, reflux) from their hydroxy-acids, were first *O*-alkylated at the phenolic position as for **1b**, **2b**, and **3b** to give the alkynyl-terminated esters **4a**, **5a**, and **6a** in good yields. Subsequent treatment with $LiAlH_4$ in refluxing THF provided the alkynyl-terminated benzyl alcohols **4b**, **5b** [51], and **6b** in yields of 93%, 88% and 93%, respectively (Scheme 2).

2.2. Synthesis of mannosyl ligands (1d-6d) via click chemistry

The efficient CuAAC click reaction [52,53] was implemented to tether alkynyl armed benzyl alcohols (**1b-4b**) to mannosyl azide **7** [54]. Thus, CuAAC conjugation of **7** and the various synthesized terminal alkynes was easily realized under the promotion of sodium ascorbate, copper (II) sulphate pentahydrate in a mixture of THF and H_2O at room temperature, affording the desired acetylated α-D-mannosides **1c-4c** in yields of 86%, 88%, 90% and 88%, respectively. Subsequent removal of acetyl groups under Zemplén conditions (MeONa, MeOH, rt) gave the acetyl free mannosides **1d-4d** in excellent yields as shown in Scheme 3.

Similarly, the dimer **5c** was also obtained by reacting terminal alkyne **5b** with two equivalents of azide **7** under the same conditions described for the synthesis of **1c-4c** in a yield of 82%. Removal of acetyl groups as above furnished dimer **5d** in 88% yield as illustrated in Scheme 4.

While compound **6c**, the trimer resulting from three simultaneous click reactions between azide **7** and terminal alkyne **6b** was then obtained in 76% yield. Removal of acetyl groups as for dimer **5c** yielded the desired acetyl free trimer **6d** in 84% yield as shown in Scheme 5.

The proposed structures of acetyl-protected mannosides **1c-6c** were confirmed by the appearance of the signals of triazolyl protons at 7.39, 7.42, 7.32, 7.42, 7.39, and 7.48 ppm, respectively, in their ^1H NMR spectra. In the ^{13}C NMR spectra, the triazolyl carbons appeared at 147.1 and 121.5 ppm for **1c**, 147.4 and 121.4 ppm for **2c,** 147.1 and 121.3 ppm for **3c**, 147.3 and 121.5 ppm for **4c,** 147.2 and 121.3 ppm for **5c**, 147.8, 147.1 ppm and 121.3 and 121.2 ppm for **6c**, further confirming their structures. While removal of the acetates from mannosides **1c-6c** to give **1d-6d** ligands was confirmed by the disappearance of methyl protons in ^1H NMR spectra and the methyl carbons as well as the carbonyl carbons (C═O) in ^{13}C NMR spectra.

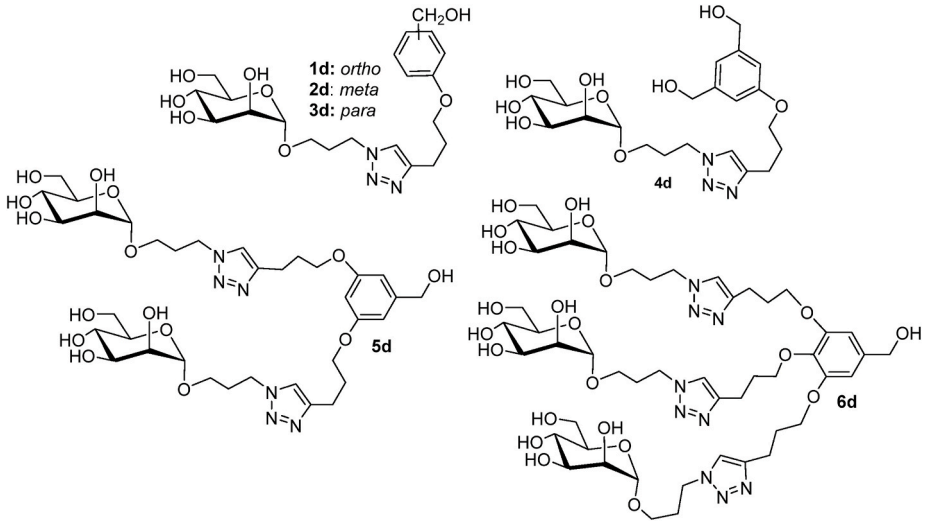
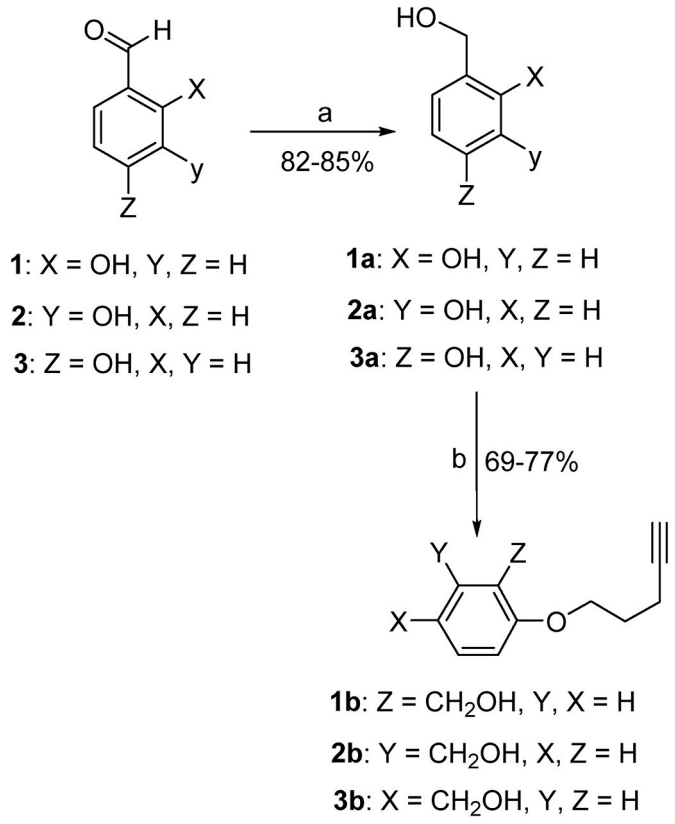
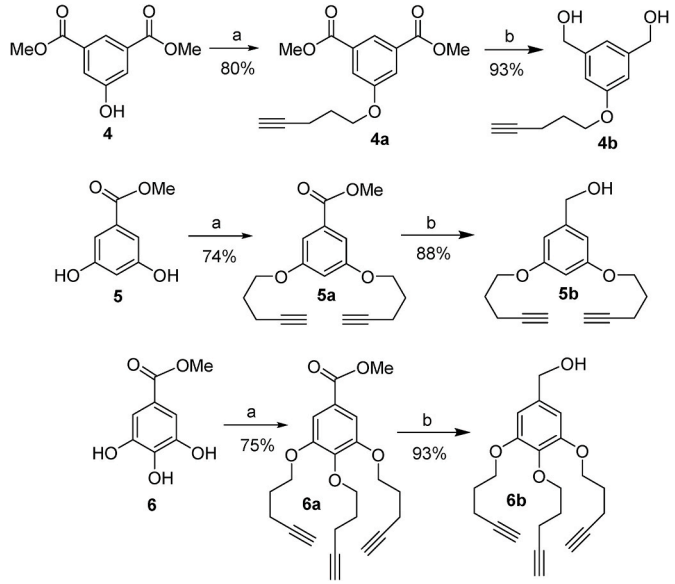


Fig. 1. Structures of mannosyl conjugates (**1d-6d**).



Scheme 1. Reaction conditions: (a) $NaBH_4$, MeOH, 0 °C-r.t.; (b) 5-chloropent-1-yne, K_2CO_3, $(Bu)_4NI$ (cat.), acetone, reflux (48 h).

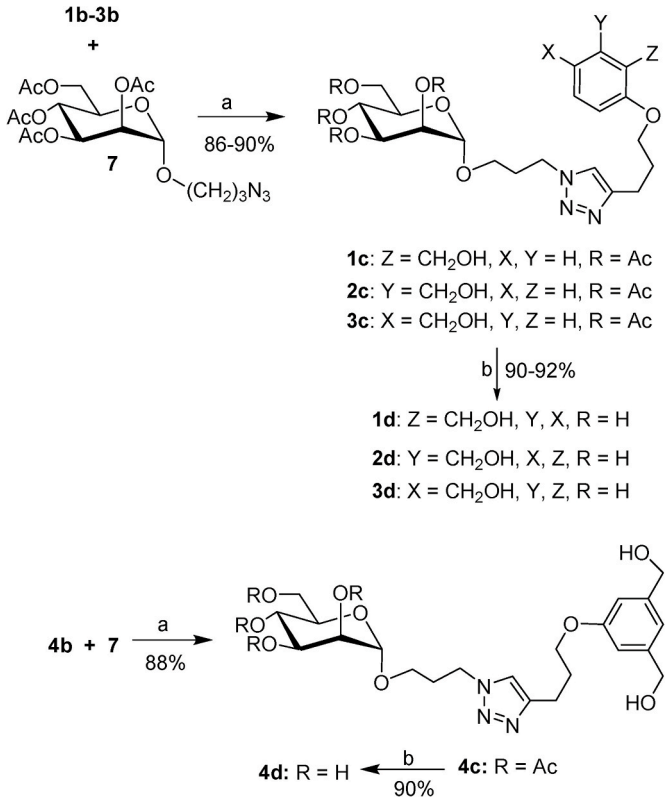


Scheme 2. Reaction conditions: (a) 5-chloropent-1-yne, K_2CO_3, $(Bu)_4NI$ (cat.), acetone, reflux (48 h), (b) $LiAlH_4$, THF, reflux (4 h).

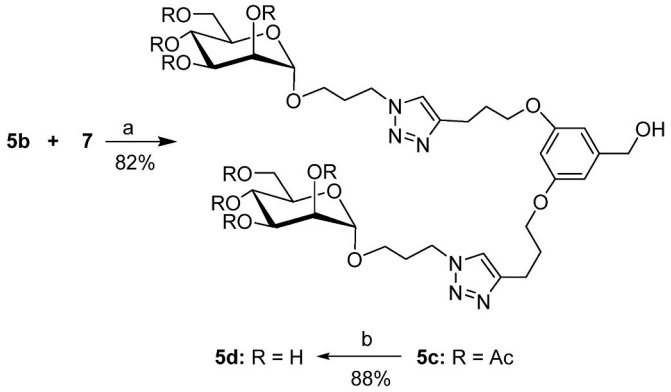
2.3. Biological assay results

Mannosylated ligands (**1d-6d**) were evaluated as inhibitors of the hemagglutination [44,50,55] of guinea pig *erythrocytes* by type 1 pilliated *E. coli* strain HB101 (pPKI4). The result of the hemagglutination (HA) test is expressed as inhibition titer (HAI) that indicates the lowest concentration required to prevent UPEC FimH from agglutinating guinea pig *erythrocytes*. The inhibition titer is then compared to methyl α-D-mannoside (MeαMan) as a reference inhibitor and therefore, leading to relative inhibition titer (RIT) as shown in Table 1.

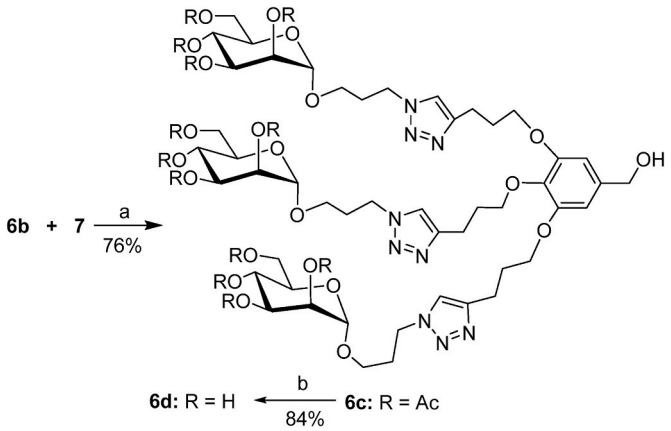
The inhibitory potency of mannosylated ligands (**1d-6d**) was found to be much higher than that of the respective MeαMan. In the series of monovalent derivatives, of the three positional isomers (**1d-3d**), the *meta* (**2d**) exhibited the highest activity and showed a 118-fold



Scheme 3. Reaction conditions: (a) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, H_2O-THF (1:1), rt, overnight, (b) MeONa, MeOH, rt, (4h).



Scheme 4. Reaction conditions: (a) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, H_2O-THF (1:1), rt, overnight, (b) MeONa, MeOH, rt, (4h).



Scheme 5. Reaction conditions: (a) $CuSO_4{\cdot}$ $5H_2O$, sodium ascorbate, H_2O-THF (1:1), rt, overnight, (b) MeONa, MeOH, rt, (4h).

improvement in activity relative to the reference ligand MeαMan. It could be hypothesized that ligand **2d** more readily interacted with the tyrosine gate. On the other hand, addition of a second methyl alcohol moiety in the other *meta* positions of ligand (**2d**) such as ligand (**4d**) displayed similar reactivity as observed for **2d**. Presumably, the aglycone of ligand **4d** adopt a conformation in which the second *meta*-oriented hydroxymethyl group does not interact with the tyrosine gate. As expected, a significant enhancement was observed for multimers **5d** and **6d** through multivalency or cluster effect. The enhancement in activity that can be achieved with appropriate synthetic multivalent ligands as compared to the corresponding monovalent ligands is known as the "glycoside cluster effect" [2]. Clearly, multivalent ligands are more advantageous than monovalent ones because of their enormous binding strength. In other words, a multivalent ligand can bind to one or a number of receptors with enhanced functional affinity and can promote receptor clustering. Thus, inhibitory potency increases with valency and the trivalent ligand **6d** is 800-fold better inhibitor than MeαMan, which means 267-fold improvement when reported on a mannose molar basis. In a direct comparison, heptyl α-D-mannopyranoside (HM), was found to be 29 times more effective than MeαMan [42]. Triazolyl mannosides with N-substituents on the triazole rings being substituted on the inner phenyl ring in the biphenyl systems have been evaluated before but none surpass the inhibitory potency of the monovalent HM. In particular, those containing a triazolyl-ethyl moiety showed 2- to 4-fold reduction in affinity when compared to HM and up to 17-fold improvement in affinity when compared to MeαMan (Fig. 2).

The best monovalent ligands prepared here **2d** and **4d** (almost identical in performance) were found to be approximately 120 times better than the reference ligand MeαMan and 4 times better than HM according to the data reported by Schwardt et al. [42] But found less inhibiting than the best *ortho*-substituted biphenyl derivatives being substituted with electronegative substituents on the remote ring or phenyl mannosides terminated with extended planar substituents [40,56]. Our results indicate that attempts should be made to determine the best position of N-substituted triazole ring in the aglycone backbone as well as the best chemical structure for the N-substituents on the triazole rings.

Table 1
HAI of MeαMan and synthetic mannosylated ligands **1d**-**6d**.

Tested ligand	Valency	HAI titer[a] (mM)	RIT[b]	RIT/Man[c]
MeαMan	1	4.0	1	1
1d	1	0.12	33	33
2d	1	0.034	118	118
3d	1	0.091	44	44
4d	1	0.033	121	121
5d	2	0.023	174	87
6d	3	0.005	800	267

[a] Based on the average value from three independent experiments.
[b] Based on the reference ligand MeαMan.
[c] Man = mannose.

3. Conclusions

In this report, we have described an efficient and experimentally simple synthesis of α-D-mannoside ligands built on aromatic platforms with a valency ranging from one to three. The flexible and unique triazole-containing linker to the α-mannosides presented on these ligands was generated through Cu(I)-catalyzed click reaction between 3-azidopropyl α-D-mannopyranoside and various alkyne-terminated benzyl alcohols. Binding potencies of the obtained mannosylated ligands (**1d**-**6d**) listed in Table 1 toward type 1 fimbriated *E. coli* were determined using a hemagglutination assay. All tested ligands showed improved affinity (HAI values of 0.12–0.005 mM) as compared to MeαMan. Unlike their monovalent analogues, the dimer **5d** (HAI = 0.023 mM) and the trimer **6d** (HAI = 0.005 mM) displayed an increase in binding potencies (cluster effect). The results obtained in this study, along with other triazolyl mannosides reported in the literature, show that the distance between the anomeric oxygen and the triazole ring was a critical factor for affinity. Work in varying positions of a triazole ring and *O*-substitution of the terminal methyl alcohol moiety with various functionalities in the aglycone backbone is currently underway in our laboratory and will be reported in the near future.

4. Experimental section

4.1. Hemagglutination tests

A recombinant type 1 fimbriated *E. coli* strain, *E. coli* HB 101 (pPKl4), used was cultured according to the protocol reported in the literature.[44,50,55] Guinea pig *erythrocytes* were isolated and used as described [44,50,55]. Hemagglutination tests were performed in V-shaped 96-well microtitre plates (Nunc). The ligands were suspended in distilled deionized water and serially diluted solutions (10 μl) were thoroughly mixed with bacteria suspension (10 μl) in wells. After 10 min, guinea pig *erythrocytes* (10 μl) were added and hemagglutination was read after approximately 10 min at room temperature.

4.2. Synthesis

4.2.1. General information

All chemicals were obtained from commercial sources and used as received without further purification, unless stated otherwise. Dichloromethane (DCM) was first dried with calcium chloride, and refluxed over calcium hydride for 1 h followed by distillation over activated 4 Å molecular sieves. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl prior to use. Melting points were determined with a SMP3 melting point apparatus and are uncorrected [1].H and[13]C NMR spectra were recorded at 300 K in 5 mm NMR tubes on Bruker Avance 400 MHz spectrometer operating at 400 MHz and 100 MHz for[1]H NMR spectra and[13]C NMR spectra, respectively. $CDCl_3$ was used as a solvent with TMS as internal reference unless otherwise indicated. The carbon and hydrogen atoms were assigned

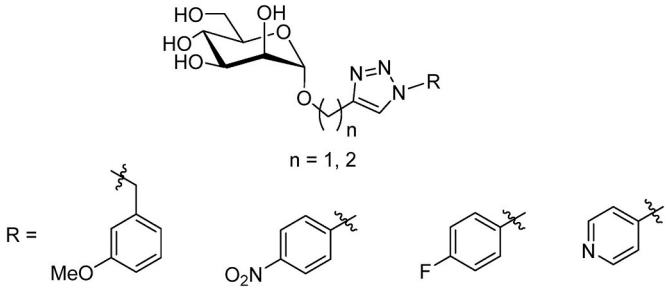


Fig. 2. Chemical structures of some previously reported mannosides containing triazolyl-methyl and ethyl moieties [42].

following analysis of their one dimensional (^{1}H, ^{13}C) and two dimensional (COSY, HSQC, and HMBC) NMR spectral data. Chemical shifts are given in parts per million (ppm) (±0.01 ppm). Data are presented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets), coupling constant (*J*) in Hertz (Hz). ESI high-resolution mass spectra were recorded on a Bruker Micro-TOF mass spectrometer using electrospray ionization. MALDI mass spectra were obtained using an α-cyano-4-hydroxycinnamic acid matrix with acquisition in the reflectron mode. Calibration was done with a peptide mixture. The reactions were monitored by thin layer chromatography (TLC) on Merk silica gel 60 F_{254} plates. Compounds were visualized by UV light (λ = 254 nm) and were located by spraying the plate with a solution of 2% ceric sulphate in 1 M H_2SO_4 followed by heating on a hot plate until color developed. Compounds were purified on silica gel (70–230 mesh) by column chromatography using specified eluents.

4.2.2. General procedure for alkylation of benzyl alcohols (**1a, 2a, and 3a**)

To a stirred solution of hydroxy-benzyl alcohols (**1a, 2a, and 3a**) in acetone (200 mL), 5-chloropent-1-yne (1 equiv.), K_2CO_3 (3 equiv.) and a catalytic amount of $(Bu)_4NI$ were added. The resulting reaction mixture was refluxed for 60 h and cooled down to room temperature, filtered and concentrated to dryness. Purification was then achieved by column chromatography (EtOAc:Hexane, 2:1).

*4.2.2.1. 2-(Pent-4-ynyloxy)phenylmethanol (**1b**).* From compound **1a** (1.00 g, 8.06 mmol), 5-chloropent-1-yne (0.85 mL, 8.06 mmol), pale yellow oil (1.05 g, 69%), R_f = 0.9 (EtOAc/Hexane, 2:1); ^{1}H NMR: δ = 7.32–6.86 (m, 4H, phenyl-**H**), 4.67 (s, 2H, CH_2OH), 4.07 (t, J = 5.9 Hz, 2H, $PhOCH_2$), 2.40 (t, J = 4.8 Hz, 2H, propargylic-CH_2), 2.04–1.99 (m, 3H, $CH_2CH_2CH_2$, acetylenic-H); ^{13}C NMR: δ = 156.2, 129.2, 128.5, 128.2, 120.5, 110.9 (phenyl-**C**), 83.3 (HC***C***), 69.1 (H***C***C), 66.0 ($PhOCH_2$), 61.0 (CH_2OH), 27.9 ($CH_2CH_2CH_2$), 15.1 (propargylic- CH_2). HRMS (ESI): *m/z* calcd for $C_{12}H_{14}NaO_2$ $[M+Na]^+$ 213.0891, found: *m/z* 213.0894.

*4.2.2.2. 3-(Pent-4-ynyloxy)phenylmethanol (**2b**).* From compound **2a** (1.00 g, 8.06 mmol), 5-chloropent-1-yne (0.85 mL, 8.06 mmol), pale brown oil, (1.11 g, 72%), R_f = 0.8 (EtOAc/Hexane, 2:1); ^{1}H NMR: δ = 7.27–6.81 (m, 4H, phenyl-**H**), 4.59 (s, 2H, CH_2OH), 4.05 (t, J = 6 Hz, 2H, $PhOCH_2$), 2.38 (t, J = 2.5 Hz, 2H, propargylic-CH_2), 2.02–1.95 (m, 3H, $CH_2CH_2CH_2$, acetylenic-H); ^{13}C NMR: δ = 159.1, 142.6, 129.6, 119.2, 113.7, 112.9 (phenyl-**C**), 83.6 (HC***C***), 69.0 (H***C***C), 66.1 ($PhOCH_2$), 65.0 (CH_2OH), 28.2 ($CH_2CH_2CH_2$), 15.2 (propargylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{12}H_{14}NaO_2$ $[M+Na]^+$ 213.0891, found: *m/z* 213.0897.

*4.2.2.3. 4-(Pent-4-ynyloxy)phenylmethanol (**3b**).* From compound **3a** (1.00 g, 8.06 mmol), 5-chloropent-1-yne (0.85 mL, 8.06 mmol), pale yellow solid (1.18 g, 77%); m.p 53–56 °C; R_f = 0.7 (EtOAc/Hexane, 2:1); ^{1}H NMR: δ = 7.26 (d, J = 8 Hz, 2H, phenyl-**H**), 6.88 (d, J = 8 Hz, 2H, phenyl-**H**), 4.57 (s, 2H, CH_2OH), 4.06 (t, J = 8 Hz, 2H, $PhOCH_2$), 2.40 (t, J = 4 Hz, 2H, propargylic-CH_2), 2.02–1.98 (m, 3H, $CH_2CH_2CH_2$, acetylenic-H); ^{13}C NMR δ = 158.5, 133.3, 128.7, 114.6 (phenyl-**C**), 83.6 (HC***C***), 69.0 (H***C***C), 66.2 ($PhOCH_2$), 65.0 (CH_2OH), 28.2 ($CH_2CH_2CH_2$), 15.2 (propargylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{12}H_{14}NaO_2$ $[M+Na]^+$ 213.0891, found: *m/z* 213.0898.

*4.2.2.4. Dimethyl 5-(pent-4-ynyloxy)isophthalate (**4a**).* According to the general procedure described above, from compound **4** (5.25 g, 25.0 mmol), 5-chloropent-1-yne (2.6 mL, 25 mmol). Column chromatography (EtOAc:Hexane, 2:1, R_f = 0.9) yielded the title compound **4a** as yellow oil (5.25 g, 80%); ^{1}H NMR: δ = 8.25, 7.74 (2s, 3H, phenyl-**H**), 4.16 (t, J = 6 Hz, 2H, $PhOCH_2$), 3.93 (s, 6H, 2 x OCH_3), 2.43 (t, J = 4 Hz, 2H, propargylic-CH_2), 2.06–2.00 (m, 3H, $CH_2CH_2CH_2$, acetylenic-H); ^{13}C NMR: δ = 166.2 (2 x C═O), 159.0, 131.8, 123.0, 119.9 (phenyl-**C**), 83.2 (HC***C***), 69.2 (H***C***C), 66.8 ($PhOCH_2$), 52.4 (2 x OCH_3), 28.1 ($CH_2CH_2CH_2$), 15.2 (propargylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{15}H_{16}NaO_5$ $[M+Na]^+$ 299.0895, found: *m/z* 299.0903.

*4.2.2.5. Methyl 3,4,5-tris(pent-4-ynyloxy)benzoate (**6a**).* According to the general procedure described above, from compound **6** (2.68 g, 14.6 mmol), K_2CO_3 (20.0 g, 10 equiv.), 5-chloropent-1-yne (4.6 mL, 43.68 mmol, 3 equiv.), and 120 mg of 18-crown-6 in 500 mL of acetone. Column chromatography (EtOAc:Hexane, 1:4, R_f = 0.33) yielded the title compound **6a** as brown oil (4.18 g, 75%); ^{1}H NMR: δ = 7.28 (s, 2H, phenyl-**H**), 4.13 (t, J = 6 Hz, 6H, 3 x $PhOCH_2$), 3.93 (s, 3H, OCH_3), 2.43 (t, J = 7 Hz, 6H, propargylic-CH_2), 2.05–1.92 (m, 9H, 3 x $CH_2CH_2CH_2$, 3 x acetylenic-H); ^{13}C NMR: δ = 166.4 C═O), 152.4, 141.6, 124.9, 107.9 (phenyl-**C**), 83.8, 83.1 (3 x HC***C***), 71.5, 69.1 (3 x H***C***C), 68.6, 67.2 (3 x $PhOCH_2$), 52.0 (OCH_3), 29.2, 28.0 (3 x $CH_2CH_2CH_2$), 15.1, 15.0 (3 x propargylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{23}H_{26}NaO_5$ $[M+Na]^+$ 405.1678, found: *m/z* 405.1695.

4.2.3. General procedure for reduction by Lithium Aluminum hydride (LAH)

To a suspension of LAH in anhydrous THF precooled to 0 °C was added a THF solution of the ester dropwise. Once the addition is complete, the solution was allowed to warm up to rt and refluxed for 4 h before being left to cool down. The reaction mixture was then acidified by the addition of 10% H_2SO_4. The solvent was evaporated under vacuum and the resulting solution was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated.

*4.2.3.1. 5-(Pent-4-ynyloxy)-1,3-benzenedimethanol (**4b**).* The reaction mixture of **4a** (3.60 g, 13 mmol) and LAH (0.39 g, 10.4 mmol) in 200 mL THF afforded the title compound **4b** as a pale yellow oil (2.76 g, 93%) after column chromatography (EtOAc, R_f = 0.7). ^{1}H NMR: δ = 6.89, 6.79 (2s, 3H, Ar–H), 4.59 (2 x CH_2OH), 4.05 (t, J = 6 Hz, 2H, $PhOCH_2$), 2.37 (t, J = 4 Hz, 2H, propargylic-CH_2), 1.99–1.95 (m, 3H, $CH_2CH_2CH_2$, acetylenic-H); ^{13}C NMR: δ = 159.4, 142.9, 117.7, 112.3 (phenyl-**C**), 83.6 (HC***C***), 69.1 (H***C***C), 66.4 ($PhOCH_2$), 65.1 (2 x CH_2OH), 28.3 ($CH_2CH_2CH_2$), 15.3 (propargylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{13}H_{16}NaO_3$ $[M+Na]^+$ 243.0997, found: *m/z* 243.1002.

*4.2.3.2. (3,4,5-Tris(pent-4-ynyloxy)phenyl)methanol (**6b**).* The reaction mixture of **6a** (6.27 g, 16.4 mmol) and LAH(0.49 g, 13 mmol) in 200 mL THF afforded the title compound **6b** as a pale yellow oil (5.39 g, 93%) after column chromatography (EtOAc/Hexane, 1:1, R_f = 0.53). ^{1}H NMR: δ = 6.59 (s, 2H, phenyl-**H**), 4.57 (s, 2H, CH_2OH), 4.08 (t, J = 4 Hz, 6H, 3 x $PhOCH_2$), 2.45 (t, J = 8 Hz, 6H, propargylic-CH_2), 2.07–1.96 (m, 9H, 3 x $CH_2CH_2CH_2$, 3 x acetylenic-H); ^{13}C NMR: δ = 152.7, 136.7, 136.5, 105.0 (phenyl-**C**), 84.1, 83.4 (3 x HC***C***), 71.6, 69.0 (3 x H***C***C), 68.5, 67.1 (3 x $PhOCH_2$), 64.9 (CH_2OH), 29.2, 28.1 (3 x $CH_2CH_2CH_2$), 15.1 (3 x propargylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{22}H_{26}NaO_4$ $[M+Na]^+$ 377.1729, found: *m/z* 377.1746.

4.2.4. General procedure for the synthesis of mannosides (**1c-6c**) by click chemistry

To a stirred solution of alkynyl-terminated benzyl alcohol and azido mannoside (1 equiv. for **1c**-**4c**, 2 equiv. for **5c**, and 3 equiv. for **6c**) in

THF (40 mL) was added sodium ascorbate (154 mg) followed by copper (II) sulphate pentahydrate (77 mg, in 40 mL of H_2O) in one portion and the resulting heterogeneous mixture was stirred vigorously for 24 h. THF was then removed under reduced pressure and CH_2Cl_2 (50 mL) was added. The organic layer was separated, dried over $MgSO_4$ and filtered. The filtrate was then concentrated and the resulting residue was purified by column chromatography.

*4.2.4.1. Mannoside (**1c**).* From compound **1b** (0.50 g, 2.63 mmol), azide **7** (1.13 g, 2.62 mmol), purification by column chromatography (EtOAc/Hexane, 2:1, $R_f = 0.2$) afforded the title mannoside **1c** as a pale yellow viscous oil (1.31 g, 86%).^{1}H NMR: $\delta = 7.39$ (s, 1H, triazole-H), 7.30–6.85 (m, 4H, phenyl-H), 5.33–5.23 (m, 3H, H-2, H-3, H-4), 4.77 (s, 1H, H-1), 4.70 (s, 2H, CH_2OH), 4.49–4.40 (m, 2H, CH_2N), 4.29 (dd, J = 12.2, 5.2 Hz, 1H, H-6a), 4.11–3.97 (m, 4H, H-5, H6b, Ph-OCH_2), 3.75–3.70 (m, 1H, OCH_2), 3.43–3.37 (m, 1H, OCH_2), 2.94 (t, $J = 7.4$ Hz, 2H, allylic-CH_2), 2.28–2.01 (m, 16H, $CH_2C\mathbf{H}_2CH_2$, $COCH_3$).^{13}C NMR: δ = 170.7, 170.3, 170.1, 169.8 (4 x C═O), 156.8 (phenyl-C), 147.1 (CH═**C**), 129.5, 129.0, 128.9 (phenyl-C), 121.5 (**C**H═C), 120.7, 111.3 (phenyl-C), 97.8 (C-1), 69.5 (C-5), 69.1 (C-2), 68.8 (C-3), 66.8 ($PhOCH_2$), 66.0 (C-4), 64.7 (CH_2OH), 62.5 (OCH_2), 61.8 (C-6), 46.9 (NCH_2), 29.9, 28.8, (2 x $CH_2\mathbf{C}H_2CH_2$), 22.2 (allylic- CH_2), 21.0, 20.8 (4 x CO**C**H_3). HRMS (ESI): *m/z* calcd for $C_{29}H_{39}N_3NaO_{12}$ $[M+Na]^+$ 644.2431, found: *m/z* 644.2434.

*4.2.4.2. Mannoside (**2c**).* From compound **2b** (0.50 g, 2.63 mmol), azide **7** (1.13 g, 2.62 mmol), purification by column chromatography (EtOAc/Hexane, 2:1, R_f = 0.3) afforded the title mannoside **2c** as a yellow viscous oil (1.34 g, 88%). ^{1}H NMR: $\delta = 7.42$ (s, 1H, triazole-H), 7.40–6.87 (m, 4H, phenyl-H), 5.38–5.31 (m, 3H, H-2, H-3, H-4), 4.84 (s, 1H, H-1), 4.73 (s, 2H, CH_2OH), 4.55–4.47 (m, 2H, CH_2N), 4.36 (dd, J = 12.1, 5.2 Hz, 1H, H-6a), 4.18–4.07 (m, 4H, H-5, H6b, Ph-OCH_2), 3.81–3.78 (m, 1H, OCH_2), 3.50–3.47 (m, 1H, OCH_2), 2.98 (t, $J = 7.5$ Hz, 2H, allylic-CH_2), 2.31–1.99 (m, 16H, $CH_2C\mathbf{H}_2CH_2$, $COCH_3$). ^{13}C NMR: δ = 170.8, 170.3, 170.2, 169.8 (4 x C═O), 159.3 (phenyl-C), 147.4 (CH═**C**), 142.9, 129.6 (phenyl-C), 121.4 (**C**H═C), 119.2, 113.8, 112.9 (phenyl-C), 97.8 (C-1), 69.5 (C-5), 69.1 (C-2), 68.8 (C-3), 66.8 ($PhOCH_2$), 66.1 (C-4), 65.2 (CH_2OH), 64.7 (OCH_2), 62.5 (C-6), 46.9 (NCH_2), 30.0, 28.8, (2 x $CH_2\mathbf{C}H_2CH_2$), 22.2 (allylic-CH_2), 21.0, 20.8 (4 x CO**C**H_3). HRMS (ESI): *m/z* calcd for $C_{29}H_{39}N_3NaO_{12}$ $[M+Na]^+$ 644.2431, found: *m/z* 644.2439.

*4.2.4.3. Mannoside (**3c**).* From compound **3b** (1.00 g, 5.20 mmol), azide **7** (2.30 g, 5.30 mmol), purification by column chromatography (EtOAc/Hexane, 2:1, $R_f = 0.3$) afforded the title mannoside **3c** as a pale brown viscous oil (1.37 g, 90%).^{1}H NMR: $\delta = 7.32$ (s, 1H, triazole-H), 7.24 (d, J = 8.2 Hz, 2H, phenyl-H), 6.83 (d, J = 8.2 Hz, 2H, phenyl-H), 5.27–5.21 (m, 3H, H-2, H-3, H-4), 4.74 (s, 1H, H-1), 4.57 (s, 2H, CH_2OH), 4.48–4.35 (m, 2H, CH_2N), 4.26 (dd, J = 12.2, 5.2 Hz, 1H, H-6a), 4.07–3.95 (m, 4H, H-5, H6b, Ph-OCH_2), 3.72–3.67 (m, 1H, OCH_2), 3.40–3.35 (m, 1H, OCH_2), 2.88 (t, J = 7.4 Hz, 2H, allylic-CH_2), 2.13–1.98 (m, 16H, $CH_2C\mathbf{H}_2CH_2$, $COCH_3$). ^{13}C NMR δ 170.6, 170.0, 169.9, 169.9 (4 x C═O), 158.2 (phenyl-C), 147.2 (CH═**C**), 133.4, 128.4 (phenyl-C), 121.3 (**C**H═C), 114.3 (phenyl-C), 97.5 (C-1), 69.3 (C-5), 68.9 (C-2), 68.5 (C-3), 66.7 ($PhOCH_2$), 65.8 (C-4), 64.5 (CH_2OH) 64.4 (OCH_2), 62.3 (C-6), 46.7 (NCH_2), 29.7, 28.7, (2 x $CH_2\mathbf{C}H_2CH_2$), 21.9 (allylic-CH_2), 20.7, 20.6, 20.5 (4 x CO**C**H_3). HRMS (ESI): *m/z* calcd for $C_{29}H_{39}N_3NaO_{12}$ $[M+Na]^+$ 644.2431, found: *m/z* 644.2448.

*4.2.4.4. Mannoside (**4c**).* From compound **4b** (0.36 g, 1.63 mmol), azide **7** (0.70 g, 1.63 mmol), purification by column chromatography (EtOAc, $R_f = 0.2$) afforded the title mannoside **4c** as colorless viscous oil (0.93 g, 88%).^{1}H NMR: $\delta = 7.42$ (s, 1H, triazole-H), 6.91 (s, 1H, phenyl-H), 6.81 (s, 2H, phenyl-H), 5.34–5.28 (m, 3H, H-2, H-3, H-4), 4.82 (s, 1H, H-1), 4.62 (s, 2H, CH_2OH), 4.50–4.45 (m, 3H, H6b, CH_2N), 4.32 (dd, J = 12.3, 5.1 Hz, 1H, H-6a), 4.12 (dd, J = 12.2, 2 Hz, 1H, H-5), 3.99 (t, J = 6 Hz, 2H, Ph-OCH_2), 3.78–3.73 (m, 1H, OCH_2), 3.47–3.42 (m, 1H, OCH_2), 2.88 (t, J = 7.5 Hz, 2H, allylic-CH_2), 2.86–2.05 (m, 16H, $CH_2C\mathbf{H}_2CH_2$, $COCH_3$). ^{13}C NMR: δ = 170.8, 170.2, 170.1, 169.8 (4 x C═O), 159.2 (phenyl-C), 147.3 (CH═**C**), 143.0 (phenyl-C), 121.5 (**C**H═C), 117.5, 111.8 (phenyl-C), 97.7 (C-1), 69.4 (C-5), 69.1 (C-2), 68.7 (C-3), 66.8 ($PhOCH_2$), 66.0 (C-4), 64.6 (OCH_2, CH_2OH), 62.4 (C-6), 46.9 (NCH_2), 29.9, 28.8, (2 x $CH_2\mathbf{C}H_2CH_2$), 22.0 (allylic-CH_2), 20.9, 20.7, 20.7 (4 x CO**C**H_3). HRMS (ESI): *m/z* calcd for $C_{30}H_{41}N_3NaO_{13}$ $[M+Na]^+$ 674.2537, found: *m/z* 674.2542.

*4.2.4.5. Dimer (**5c**).* From compound **5b** (0.30 g, 1.1 mmol), azide **7** (0.95 g, 2.2 mmol, 2 equiv.), purification by column chromatography (EtOAc/Hexane, 4:1, R_f=0.1) afforded the title mannoside **5c** as a colorless viscous oil (1.02 g, 82%).^{1}H NMR: $\delta = 7.39$ (s, 2H, triazole-H), 6.51 (s, 2H, phenyl-H), 6.35 (s, 1H, phenyl-H), 5.34–5.25 (m, 6H, H-2, H-3, H-4), 4.79 (s, 2H, H-1), 4.62 (s, 2H, CH_2OH), 4.49–4.43 (m, 4H, CH_2N), 4.29 (dd, J = 12, 8 Hz, 2H, H-6a), 4.11–3.97 (m, 10H, H-5, H-6b, Ph-OCH_2), 3.77–3.73 (m, 2H, OCH_2), 3.46–3.41 (m, 2H, OCH_2), 2.89 (t, J = 8 Hz, 4H, allylic-CH_2), 2.33–1.85 (m, 32H, $CH_2C\mathbf{H}_2CH_2$, $COCH_3$). ^{13}C NMR: δ = 170.6, 170.0, 169.9, 169.6 (8 x C═O), 160.1 (phenyl-C), 147.2 (2 x CH═**C**), 143.8 (phenyl-C), 121.3 (2 x **C**H═C), 104.9, 100.3 (phenyl-C), 97.6 (2 x C-1), 69.3 (2 x C-5), 69.0 (2 x C-2), 68.6 (2 x C-3), 66.8 (2 x $PhOCH_2$), 65.9 (2 x C-4), 64.7 (CH_2OH), 64.6 (2 x OCH_2), 62.3 (2 x C-6), 46.7 (2 x NCH_2), 29.8, 28.7, (4 x $CH_2\mathbf{C}H_2CH_2$), 22.0 (2 x allylic- CH_2), 20.8, 20.6, 20.6 (8 x CO**C**H_3). HRMS (ESI): *m/z* calcd for $C_{51}H_{70}N_6NaO_{23}$ $[M+Na]^+$ 1157.4390, found: *m/z* 1157.4406.

4.2.4.6. Trimer (6c). From compound **6b** (0.31 g, 0.87 mmol), azide **7** (1.13 g, 2.61 mmol, 3 equiv.), purification by column chromatography (EtOAc/CH_3OH, 4:1, R_f=0.74) afforded the title mannoside **6c** as a pale yellow viscous oil (1.09 g, 76%).^{1}H NMR: $\delta = 7.48$ (s, 3H, triazole-H), 6.60 (s, 2H, phenyl-H), 5.32–5.27 (m, 9H, H-2, H-3, H-4), 4.82 (s, 3H, H-1), 4.61 (s, 2H, CH_2OH), 4.52–4.43 (m, 6H, CH_2N), 4.31 (dd, $J = 12.3$, 5 Hz, 3H, H-6a), 4.13–4.04 (m, 12H, H-5, H-6b, Ph-OCH_2), 3.77–3.74 (m, 3H, OCH_2), 3.48–3.44 (m, 3H, OCH_2), 3.01 (t, $J = 7.7$ Hz, 2H, allylic-CH_2), 2.93 (t, J = 7.3 Hz, 4H, allylic-CH_2), 2.38–2.02 (m, 48H, $CH_2C\mathbf{H}_2CH_2$, $COCH_3$). ^{13}C NMR: $\delta = 170.5$, 169.9, 169.8, 169.6 (12 x C═O), 152.7 (phenyl-C), 147.8, 147.1 (3 x CH═**C**), 136.9, 136.8 (phenyl-C), 121.3, 121.2 (3 x **C**H═C), 105.1 (phenyl-C), 97.6 (3 x C-1), 72.5 ($PhOCH_2$), 69.3 (3 x C-5), 69.0 (3 x C-2), 68.6 (3 x C-3), 67.8 (2 x $PhOCH_2$), 65.9 (3 x C-4), 64.6 (CH_2OH), 64.8 (3 x OCH_2), 62.3 (3 x C-6), 46.7 (3 x NCH_2), 29.9, 29.8, 28.9, (6 x $CH_2\mathbf{C}H_2CH_2$), 22.3, 22.0 (3 x allylic- CH_2), 20.7, 20.6, 20.6, 20.5 (12 x CO**C**H_3). MALDI MS *m/z* calcd for $C_{73}H_{101}N_9O_{34}Na$ 1670.63, found: 1670.65.

*4.2.5. General procedure for De-O-acetylation of mannosides (**1c-6c**)*

To a stirred solution of a given acetylated mannoside in CH_3OH (10 mL) and THF (10 mL) was added sodium methoxide (0.5 M in CH_3OH, 1 mL), the reaction mixture was stirred overnight at room temperature and neutralized with Amberlite IR-120 (H^+), filtered and concentrated. The resulting residue was then purified by column chromatography.

*4.2.5.1. Ligand (**1d**).* From mannoside **1c** (1.5 g, 2.4 mmol), column chromatography (EtOAc/CH_3OH, 2:1, $R_f = 0.4$) afforded ligand **1d** as a pale yellow viscous oil (90%).^{1}H NMR(CD_3OD): $\delta = 7.78$ (s, 1H, triazole **H**), 7.37–6.89 (m, 4H, Phenyl **H**), 4.71 (s, 1H, H-1), 4.65 (s, 2H, CH_2OH), 4.47 (t, J = 8 Hz, 2H, NCH_2), 4.03 (t, J = 6 Hz, 2H, $PhOCH_2$), 3.84–3.58 (m, 6 H, H-2, H-3, H-4, H-5, H-6), 3.53–3.49 (m, 1H, OCH_2), 3.42–3.37 (m, 1H, OCH_2), 2.92 (t, J = 7.4 Hz, 2H, allylic CH_2), 2.19–2.14 (m, 4H, $CH_2C\mathbf{H}_2CH_2$); ^{13}C NMR(CD_3OD): $\delta = 157.6$ (phenyl-C), 148.5 (CH═**C**), 130.8, 129.5, 129.1 (phenyl-C), 123.6 (**C**H═C), 121.5, 112.2 (phenyl-C), 101.8 (C-1), 74.8 (C-5), 72.6 (C-3), 72.0 (C-2), 68.6 ($PhOCH_2$), 67.9 (C-4), 65.2 (OCH_2), 62.9 ($HOCH_2$), 60.3 (C-6), 48.5 (NCH_2), 31.2, 30.1, (2 x $CH_2\mathbf{C}H_2CH_2$), 23.0 (allylic- CH_2). HRMS (ESI): *m/z* calcd for

$C_{21}H_{31}N_3NaO_8$ $[M+Na]^+$ 476.2009, found: *m/z* 476.2016.

4.2.5.2. Ligand (2d). From mannoside **2c** (1.5 g, 2.4 mmol), column chromatography (EtOAc/CH_3OH, 2:1, $R_f = 0.4$) afforded ligand **2d** as colorless oil (90%). ^{1}H NMR(CD_3OD): δ = 7.77 (s, 1H, triazole **H**), 7.24–6.78 (m, 4H, Phenyl **H**), 4.71 (s, 1H, H-1), 4.56 (s, 2H, CH_2OH), 4.47 (t, J = 6.6 Hz, 2H, NCH_2), 3.99 (t, J = 5.8 Hz, 2H, $PhOCH_2$), 3.78–3.58 (m, 6 H, H-2, H-3, H-4, H-5, H-6), 3.52–3.41 (m, 1H, OCH_2), 3.40–3.31 (m, 1H, OCH_2), 2.89 (t, J = 7.5 Hz, 2H, allylic CH_2), 2.15–2.10 (m, 4H, $CH_2C\mathbf{H}_2CH_2$); ^{13}C NMR(CD_3OD): δ = 160.5 (phenyl-C), 148.5 (CH=**C**), 144.4, 130.4 (phenyl-C), 123.6 (**C**H=C), 120.2, 114.4, 113.9 (phenyl-C), 101.8 (C-1), 74.8 (C-5), 72.6 (C-3), 72.0 (C-2), 68.6 ($PhOCH_2$), 67.9 (C-4), 65.2 (OCH_2), 65.0 ($HOCH_2$), 62.9 (C-6), 48.5 (NCH_2), 31.2, 30.1, (2 x $CH_2\mathbf{C}H_2CH_2$), 22.9 (allylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{21}H_{31}N_3NaO_8$ $[M+Na]^+$ 476.2009, found: *m/z* 476.2012.

4.2.5.3. Ligand (3d). From mannoside **3c** (1.5 g, 2.4 mmol), column chromatography (EtOAc/CH_3OH, 2:1, $R_f = 0.4$) afforded ligand **3d** as a white solid (92%), mp 102–105 °C. ^{1}H NMR(CD_3OD): δ = 7.76 (s, 1H, triazole **H**), 7.24 (d, J = 8 Hz, 2H, Phenyl **H**), 6.87 (d, J = 8 Hz, 2H, Phenyl **H**), 4.51 (s, 1H, H-1), 4.47 (s, 2H, CH_2OH), 4.46 (t, J = 6.8 Hz, 2H, NCH_2), 3.99 (t, J = 6 Hz, 2H, $PhOCH_2$), 3.83–3.71 (m, 6 H, H-2, H-3, H-4, H-5, H-6), 3.69–3.39 (m, 2H, OCH_2), 2.88 (t, J = 7.6 Hz, 2H, allylic CH_2), 2.17–2.10 (m, 4H, $CH_2C\mathbf{H}_2CH_2$); ^{13}C NMR(CD_3OD): δ = 159.7 (phenyl-C), 148.4 (CH=**C**), 134.8, 129.6 (phenyl-C), 123.5 (**C**H=C), 115.4 (phenyl-C), 101.7 (C-1), 74.8 (C-5), 72.6 (C-3), 72.0 (C-2), 68.5 ($PhOCH_2$), 67.4(C-4), 65.2 (OCH_2), 64.9 ($HOCH_2$), 62.9 (C-6), 48.5 (NCH_2), 31.2, 30.1, (2 x $CH_2\mathbf{C}H_2CH_2$), 22.9 (allylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{21}H_{31}N_3NaO_8$ $[M+Na]^+$ 476.2009, found: *m/z* 476.2011.

4.2.5.4. Ligand (4d). From mannoside **4c** (1.00 g, 1.53 mmol), column chromatography (EtOAc/CH_3OH, 2:1, $R_f = 0.3$) afforded ligand **4d** as colorless viscous oil (90%). ^{1}H NMR(CD_3OD): δ = 7.76 (s, 1H, triazole **H**), 6.90, 6.82 (2s, 3H, Phenyl **H**), 4.71 (s, 1H, H-1), 4.57 (s, 4H, CH_2OH), 4.46 (t, J = 6.8 Hz, 2H, NCH_2), 4.01 (t, J = 6 Hz, 2H, $PhOCH_2$), 3.84–3.60 (m, 6 H, H-2, H-3, H-4, H-5, H-6), 3.53–3.50 (m, 1H, OCH_2), 3.39–3.37 (m, 1H, OCH_2), 2.88 (t, J = 7.6 Hz, 2H, allylic CH_2), 2.86–2.13 (m, 4H, $CH_2C\mathbf{H}_2CH_2$); ^{13}C NMR(CD_3OD): δ = 160.6 (phenyl-C), 148.4 (CH=**C**), 144.3 (phenyl-C), 123.5 (**C**H=C), 118.6, 112.7 (phenyl-C), 101.6 (C-1), 74.7 (C-5), 72.5 (C-3), 72.0 (C-2), 68.5 ($PhOCH_2$), 67.9 (C-4), 65.1 (OCH_2), 65.0 ($HOCH_2$), 62.8 (C-6), 48.5 (NCH_2), 31.1, 30.0, (2 x $CH_2\mathbf{C}H_2CH_2$), 22.9 (allylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{22}H_{33}N_3NaO_9$ $[M+Na]^+$ 506.2114, found: *m/z* 506.2131.

4.2.5.5. Ligand (5d). From mannoside **5c** (0.60 g, 0.53 mmol), column chromatography (EtOAc/CH_3OH, 2:1, $R_f = 0.2$) afforded ligand **5d** as colorless viscous oil (88%).^{1}H NMR(CD_3OD): δ = 7.78 (s, 2H, 2 x triazole **H**), 6.50, 6.34 (2s, 3H, Phenyl **H**), 4.71 (s, 2H, 2 x H-1), 4.52 (s, 2H, CH_2OH), 4.47 (t, J = 5.5 Hz, 4H, 2 x NCH_2), 3.98 (t, J = 5.9 Hz, 4H, 2 x $PhOCH_2$), 3.80–3.31 (m, 16 H, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 2 x H-6, 2 x OCH_2), 2.88 (t, J = 7.2 Hz, 4H, 2 x allylic CH_2), 2.18–2.10 (m, 8H, 4 x $CH_2C\mathbf{H}_2CH_2$); ^{13}C NMR(CD_3OD): δ = 161.6 (phenyl-C), 145.3 (2 x CH=**C**), 123.7 (2 x **C**H=C), 106.3, 101.3 (phenyl-C), 101.8 (2 x C-1), 74.8 (2 x C-5), 72.6 (2 x C-3), 72.0 (2 x C-2), 68.6 (2 x $PhOCH_2$), 68.0 (2 x C-4), 65.2 (2 x OCH_2), 65.1 (CH_2OH), 62.9 (2 x C-6), 48.6 (2 x NCH_2), 31.2, 30.1, (4 x $CH_2\mathbf{C}H_2CH_2$), 22.9 (2 x allylic-CH_2). HRMS (ESI): *m/z* calcd for $C_{35}H_{54}N_6NaO_{15}$ $[M+Na]^+$ 821.3545, found: *m/z* 821.3543.

4.2.5.6. Ligand (6d). From mannoside **6c** (0.25 g, 0.15 mmol), column chromatography (CH_3OH/EtOAc, 3:1, $R_f = 0.7$) afforded ligand **6d** as a brown viscous oil (84%). ^{1}H NMR(CD_3OD): δ = 7.76, 7.74 (2s, 3H, 2 x triazole **H**), 6.59 (s, 2H, Phenyl **H**), 4.76 (s, 3H, 3 x H-1), 4.48 (s, 2H, CH_2OH), 4.47 (t, J = 5.6 Hz, 6H, 3 x NCH_2), 3.99 (t, J = 6 Hz, 6H, 2 x $PhOCH_2$), 3.79–3.58 (m, 18 H, 3 x H-2, 3 x H-3, 3 x H-4, 3 x H-5, 3 x H-6), 3.50–3.45 (m, 3H, OCH_2), 3.36–3.27 (m, 3H, OCH_2), 2.92 (t, J = 7 Hz, 2H, allylic-CH_2), 2.86 (t, J = 7 Hz, 4H, allylic-CH_2), 2.13–2.02 (m, 12H, 6 x $CH_2C\mathbf{H}_2CH_2$); ^{13}C NMR(CD_3OD): δ = 154.0 (phenyl-C), 148.3 (3 x CH=**C**), 138.8, 137.7 (phenyl-C), 123.7, 123.5 (3 x **C**H=C), 106.3, 101.7 (3 x C-1), 74.7 (3 x C-5), 72.6 (3 x C-3), 72.0 (3 x C-2), 69.0 (3 x $PhOCH_2$), 68.5 (3 x C-4), 65.2 (3 x OCH_2), 65.1 (CH_2OH), 62.8 (3 x C-6), 48.5 (3 x NCH_2), 31.2, 31.0, 30.2, (6 x $CH_2\mathbf{C}H_2CH_2$), 23.2, 23.0 (3 x allylic-CH_2). MALDI MS *m/z* calcd for $C_{49}H_{77}N_9NaO_{22}$ 1143.52, found: 1143.50.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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