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Synthesis of cluster mannosides via a Ugi four-component reaction and their inhibition against the binding of yeast mannan to concanavalin A

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Abstract—The Ugi four-component reaction (U-4CR) was utilized to prepare divalent and trivalent cluster mannosides with different scaffolds. The glycoclusters obtained were tested for their relative inhibitory potency against the binding of yeast mannan to concanavalin A by solid-phase enzyme-linked lectin assays (ELLA) using methyl α-D-mannopyranoside as a standard. Among them, a divalent mannoside containing aromatic groups showed the strongest binding affinity to concanavalin A.

Keywords: Ugi four-component reaction; Cluster mannoside; Concanavalin A; ELLA

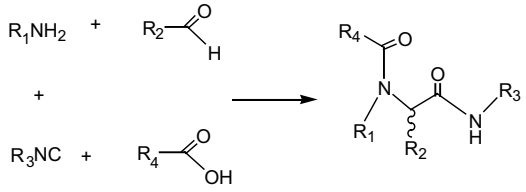
1. Introduction

Carbohydrate–protein interactions are recognized to mediate critical processes in cellular events, including neutrophil recruitment, fertilization, and immunological surveillance. It is generally recognized that the binding of proteins to monovalent carbohydrate determinants is often weak (K_d values usually ranging from mmol/L to μmol/L), yet the strength and specificity required for recognition in physiological settings is high. The simultaneous formation of multiple protein–carbohydrate interactions is one binding mode that nature employs to achieve the desired affinity.[1,2] In this context, structurally well-defined 'glycoclusters' have been introduced as mimics of the multivalent carbohydrate ligands in nature.[3]

Considering the important biological functions of mannosides, several research groups have synthesized various multivalent mannosides and studied their bioactivities.[4–7] Interestingly even low-valent cluster mannosides have enhanced affinities in comparison with monovalent ligands. As shown by Roy et al., the binding affinities of divalent and trivalent cluster mannosides to the plant lectin concanavalin A (Con A) are 24 and 62 times higher than that of methyl α-D-mannopyranoside, respectively.[5] The relationship between the structure of glycoclusters and their biological activities, however, requires further study.

To address this question, different approaches have been developed to prepare various glycoclusters with structural diversities.[8–11] Among those approaches, the Ugi four-component reaction (U-4CR, Scheme 1), a method applied more extensively to the generation of molecular diversity in combinatorial synthesis,[12–14] has also shown potential in the synthesis of glycoclusters.[15,16]

As a continuing attempt to develop synthetic methodology and to address the structure–function relationship



Scheme 1.

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of glycoclusters,[17] we described herein the synthesis of several cluster mannosides with structural diversity, using the convenient one-pot Ugi four-component reaction. The inhibitory properties of the synthesized cluster mannosides were tested by ELLA experiments using plant lectin Con A.

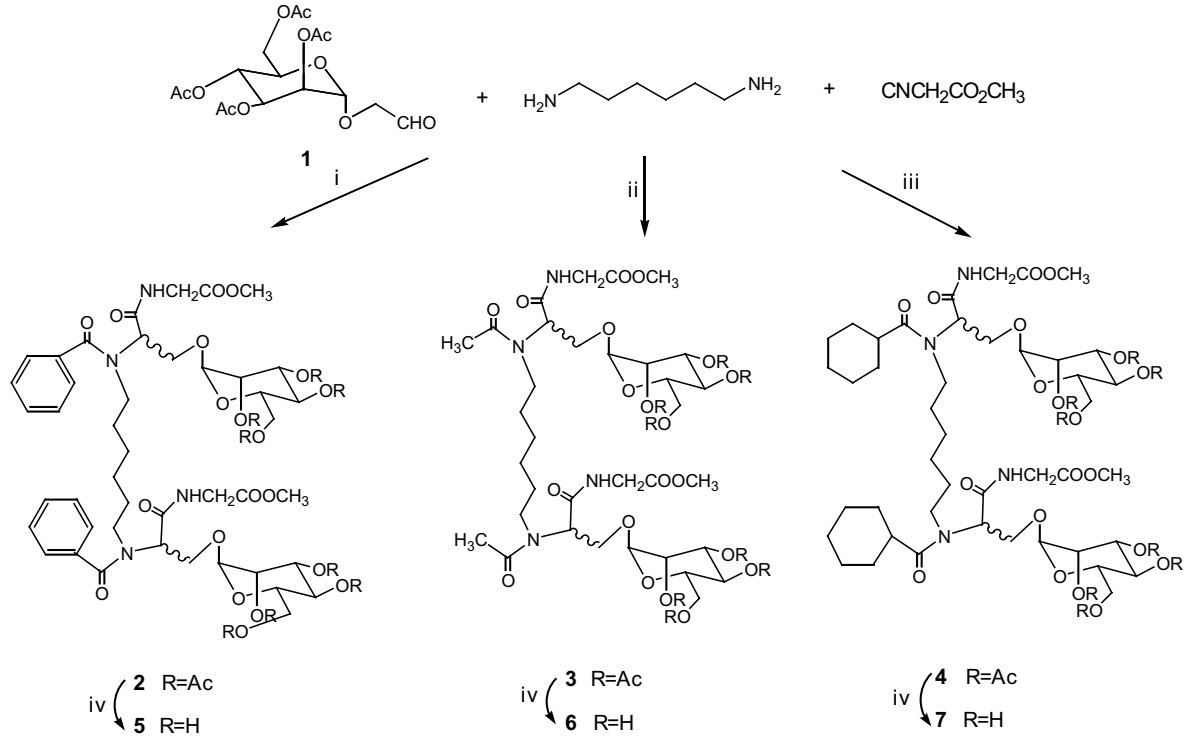
2. Results and discussion

It has been previously demonstrated that aromatic groups in carbohydrate ligands may increase their affinities toward proteins. Our preliminary design gave some cluster mannosides with aromatic groups in their structures, and some without (Scheme 2). Initially, allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside[18] was prepared as a useful precursor, which reacted with OsO_4–$NaIO_4$[19] to give glycosyl aldehyde **1**. U-4CR was then utilized to prepare divalent cluster mannosides. The reactions of glycosyl aldehyde **1**, 1,6-hexanediamine, and methyl isocyanoacetate with benzoic acid, acetic acid, or cyclohexanecarboxylic acid, gave the divalent compounds **2**, **3**, and **4**, respectively. The acetate protecting groups were removed smoothly with NaOMe–MeOH to provide the corresponding target dimers **5**, **6**, and **7**. Additional cluster mannosides of diverse structures could also be obtained readily by this method.

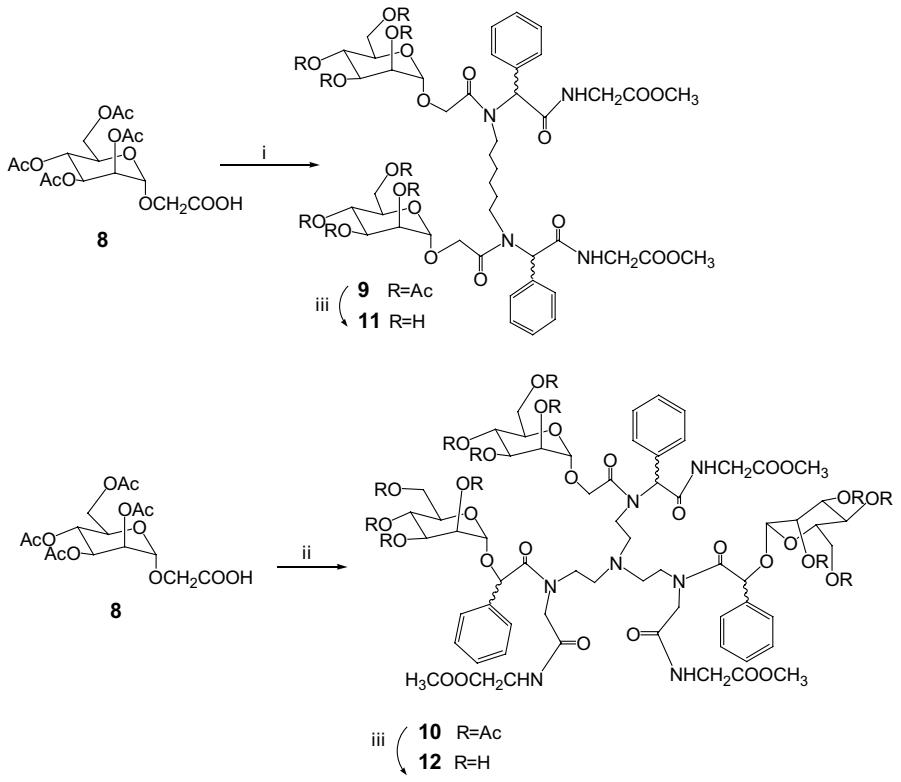
The plant lectin Con A, which can form clusters with a multivalent ligand, provides an excellent model for examining ligand-promoted oligomerization. In this work, the binding of these newly synthesized ligands to Con A was evaluated by the enzyme-linked lectin assay (ELLA)[4] (ELLA evaluates the ability of a soluble ligand to inhibit the adhesion of a multivalent lectin to a reference ligand noncovalently immobilized on the surface of a microtiter plate). Methyl α-D-mannopyranoside was used as a standard in the test. The ELLA experiment showed that compound **5** is 13-fold more potent than methyl mannoside, and still 6.5-fold more potent after valency correction (Table 1). Aromatic groups in glycocluster **5** could, as expected, increase the binding strength, which is also in agreement with the previous reports.[6,20]

Table 1. Inhibition of binding of yeast mannan to concanavalin A by the compounds methyl mannoside, **5**, **6**, **7**, **11**, and **12**

	IC_{50} (μM)	Relative potency	After valency correction
Methyl mannoside	855.0	1	1
5	66.3	13.0	6.5
6	180.5	4.7	2.4
7	110.6	7.8	3.9
11	15.4	55.5	27.7
12	30.6	27.9	9.3



Scheme 2. Reagents and conditions: (*i*) benzoic acid, MeOH, rt, 48 h, 40.8%; (*ii*) acetic acid, MeOH, rt, 48 h, 37.2%; (*iii*) cyclohexanecarboxylic acid, MeOH, rt, 48 h, 35.6%; (*iv*) NaOMe–MeOH, 98%.



Scheme 3. Reagents and conditions: (*i*) benzaldehyde, methyl isocyanoacetate, 1,6-hexanediamine, MeOH, rt, 48 h, 46.0%; (*ii*) benzaldehyde, methyl isocyanoacetate, tris(2-aminoethyl)amine, MeOH, rt, 48 h, 40.5%; (*iii*) NaOMe–MeOH, 20 min, rt, 98%.

To investigate further the influence of cluster valency on the binding affinity and the suitability of U-4CR, an additional two compounds containing aromatic residues were synthesized (Scheme 3). 2-Carboxyethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**8**) was obtained by oxidation of allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside with $RuCl_3$–$NaIO_4$.[19] Then the U-4CR of **8**, benzaldehyde, methyl isocyanoacetate, and 1,6-hexanediamine or tris(2-aminoethyl)amine afforded divalent cluster **9** and trivalent cluster **10**, respectively. Deprotection afforded cluster mannosides **11** and **12**.

According to the ELLA test results, the dimer **11** showed 55.5-fold increased binding potency in comparison with α-D-mannopyranoside, and 27.7-fold after valency correction. As for trimer **12**, the relative potency decreased to 9.3-fold (Table 1). Notably, the binding affinity did not necessarily increase with the increase in valency (Fig. 1).

The ^1H NMR spectra of the synthesized clusters were characterized by a number of overlapping peaks due to many magnetically similar CH_2 and CH groups in each molecule. The assignments were facilitated by DEPT, ^1H–^1H COSY, ^1H–^{13}C HMQC, and ^1H–^{13}C HMBC experiments. The divalent cluster mannosides (compounds **2**–**7**, **9**, and **11**) obtained here were stereoisomeric mixtures of (*R*,*R*), (*S*,*S*), (*R*,*S*), and (*S*,*R*) forms, which showed two sets of diastereomeric signals in the NMR spectra. Attempts to separate the diastereomers using routine column chromatography proved unsuccessful. For compounds **3**, **4**, **6**, and **9** the ratio of the diastereomers could be determined quantitatively from their ^1H NMR spectra. For compounds **2**, **5**, **7**, and **11**, however, the overlap of peaks in the ^1H NMR spectra made it difficult to determine the product distributions. The ^1H NMR signals of trivalent clusters **10** and **12** overlapped seriously and broad peaks were observed. The results of high-resolution MS or MALDITOF MS of the target cluster mannosides were in good agreement with the theoretical values, confirming that the product assignments were correct.

In summary, five cluster mannosides were synthesized in good yields with a convergent one-pot procedure. The Ugi four-component reaction was found convenient for the synthesis of cluster mannosides with structural diversity. The ELLA results show that: (*1*) for all cluster mannosides synthesized in this paper, the binding affinities to Con A were much higher than that of methyl α-D-mannopyranoside, even after valency correction; (*2*) cluster mannosides containing aromatic residues bound more tightly to Con A in comparison with those without aromatic residues; and (*3*) the divalent ligand **11** showed higher affinity to Con A than trivalent ligand **12**. The binding affinities of cluster mannosides to Con A, as observed in other cases,[4] did not increase necessarily

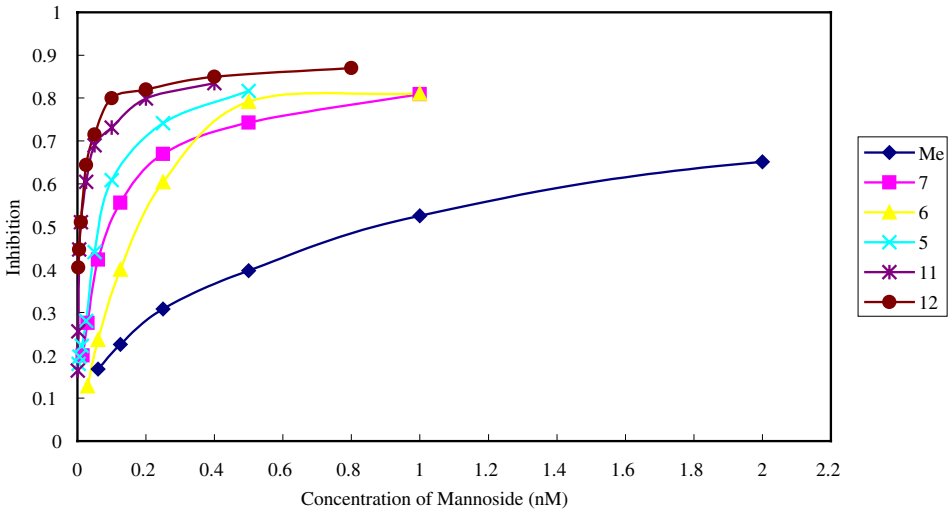


Figure 1. Inhibition of binding of yeast mannan to concanavalin A by synthesized ligands **5**, **6**, **7**, **11**, and **12**.

with increase in valency. Other factors, such as space orientation of various groups of the glycoclusters, may also have a great effect on the binding affinity.

3. Experimental

3.1. General methods

All chemicals used were reagent grade and were used as supplied, except where noted. Benzaldehyde was purified by standard methods. Thin-layer chromatography (TLC) was performed using silica gel 60 F_{254} (Merck) and column chromatography on silica Gel (100–200 mesh, Qingdao, China). The peroxidase labeled Con A was purchased from Vector. Yeast mannan from *Saccharomyces cerevisiae* was obtained from Sigma. Plates for the ELLA test were Costar 3590. Optical densities (OD) for the ELLA test were performed on Tecan Rainbow Microplate Reader.

The ^{1}H and ^{13}C NMR spectra were obtained on a Jeol 600 MHz JNM-ECP spectrometer, and assignments were based on COSY, DEPT, and HMQC experiments. Mass spectra were recorded on a Q-TOF microspectrometer and a Bruker Daltonics Inc. Apex II mass spectrometer.

3.2. General procedure for the synthesis of peracetylated divalent mannopyranosyl ligands (2, 3, 4)

The U-4CR of **1** (2.2 equiv), 1,6-hexanediamine (1.0 equiv), carboxylic acid component (2.2 equiv, benzoic acid for **2**, acetic acid for **3**, and cyclohexanecarboxylic acid for **4**, respectively) were dissolved in MeOH, and the mixture was stirred at room temperature for 1 h. Methyl isocyanoacetate (2.2 equiv) was then added and the mixture stirred at room temperature for 48 h. The mixture was concentrated, diluted with CH_2Cl_2, washed with water, dried, and concentrated. Column chromatography on silica gel of the residue afforded compounds **2**, **3**, **4**, respectively.

3.2.1. Compound 2. According to the general procedure, **1** (711 mg, 1.81 mmol), 1,6-hexanediamine (95 mg, 0.82 mmol), benzoic acid (221 mg, 1.81 mmol), and methyl isocyanoacetate (164 μL, 1.81 mmol) in MeOH (25 mL) afforded **2** (400 mg, 41%) after chromatography (40:1 to 20:1, CH_3Cl–MeOH). ^{1}H NMR ($CDCl_3$): δ 7.52–7.66 (m, 2 H, NH, *NH*), 7.44 (s, 10 H, Ph–, *Ph*–), 5.24–5.30 (m, 6 H, H-2, *H-2*, H-3, *H-3*, H-4, *H-4*), 4.87–4.93 (m, 2 H, H-1, *H-1*), 3.90–4.42 (m, 16 H, H-5, *H-5*, H-6a, *H-6a*, H-6b, *H-6b*, $NHCH_2COOCH_3$, *$NHCH_2COOCH_3$*, $ManOCH_2$, *$ManOCH_2$*, $ManOCH_2CH$–, *$ManOCH_2CH$*–), 3.75 (s, 6 H, $NHCH_2COOCH_3$, *$NHCH_2COOCH_3$*), 3.20–3.30 (m, 4 H, $NCH_2CH_2CH_2$–, *$NCH_2CH_2CH_2$*–), 1.99–2.15 (m, 24 H, $4\times CH_3CO$, *$4\times CH_3CO$*), 1.60–1.72 (m, 4 H, $NCH_2CH_2CH_2$–, *$NCH_2CH_2CH_2$*–), 1.38–1.52 (m, 4 H, $NCH_2CH_2CH_2$–, *$NCH_2CH_2CH_2$*–); ^{13}C NMR ($CDCl_3$): δ 170.7, 170.6, 170.0, 169.9, 169.8, 169.7, 169.6 (C═O), 135.5, 130.9, 128.7, 126.8 (Ph–, *Ph*–), 97.9, 97.3 (C-1, *C-1*), 69.3, 69.2, 68.9 (C-5, *C-5*, C-3, *C-3*, C-2, *C-2*), 65.8 (C-4, *C-4*), 64.8 ($ManOCH_2CH$–, *$ManOCH_2CH$*–), 62.2 (C-6, *C-6*, $ManOCH_2CH$–, *$ManOCH_2CH$*–), 52.4 ($COOCH_3$, *$COOCH_3$*), 41.1, 41.0 ($NHCH_2COOCH_3$, *$NHCH_2COOCH_3$*, $NCH_2CH_2CH_2$–, *$NCH_2CH_2CH_2$*), 28.9 ($NCH_2CH_2CH_2$–, *$NCH_2CH_2CH_2$*–), 25.9 ($NCH_2CH_2CH_2$–, *$NCH_2CH_2CH_2$*–), 20.7, 20.8 (CH_3CO, *CH_3CO*).

3.2.2. Compound 3. According to the general procedure, **1** (684 mg, 1.74 mmol), 1,6-hexanediamine (92 mg, 0.79 mmol), AcOH (99 μL, 1.74 mmol), and methyl iso-

cyanoacetate (158 μL, 1.74 mmol) in MeOH (25 mL) afforded **3** (344 mg, 37%) as a 50:50 mixture of diastereomers after chromatography (40:1 to 20:1, CH_3Cl–MeOH). ^1H NMR ($CDCl_3$): δ 7.13–7.15 (m, 1 H, NH), 7.06–7.07 (m, 1 H, *NH*), 5.19–5.30 (m, 6 H, H-2, *H-2*, H-3, *H-3*, H-4, *H-4*), 4.87 (s, 1 H, H-1), 4.82 (s, 1 H, *H-1*), 4.11–4.31 (m, 8 H, H-6a, *H-6a*, H-6b, *H-6b*, ManOC$\underline{H}_2$–, *ManOC$\underline{H}_2$*), 3.92–4.06 (m, 8 H, H-5, *H-5*, ManOCH$_2$-C$\underline{H}$–, *ManOCH$_2$C$\underline{H}$*–, NHC$\underline{H}_2$COOCH$_3$, *NHC$\underline{H}_2$COOCH$_3$*), 3.73 (s, 6 H, NHCH$_2$COOC$\underline{H}_3$, *NHCH$_2$COOC$\underline{H}_3$*), 3.31–3.34 (m, 4 H, NC$\underline{H}_2$CH$_2$CH$_2$–, *NC$\underline{H}_2$CH$_2$CH$_2$*–), 1.98, 2.05, 2.11, 2.15, 2.19 (m, 30 H, 5×CH$_3$CO, *5×CH$_3$CO*), 1.62–1.67 (m, 4 H, NCH$_2$C$\underline{H}_2$CH$_2$–, *NCH$_2$C$\underline{H}_2$CH$_2$*–), 1.36 (m, 4 H, NCH$_2$CH$_2$C$\underline{H}_2$–, *NCH$_2$CH$_2$C$\underline{H}_2$*–); ^{13}C NMR ($CDCl_3$): δ 172.4, 172.2, 170.6, 170.0, 169.8, 169.6, 169.5, 169.2 (C═O), 97.8 (C-1), 97.2 (*C-1*), 68.8, 68.9, 69.1, 69.3 (C-5, *C-5*, C-3, *C-3*, C-2, *C-2*), 65.6 (C-4), 65.7 (*C-4*), 65.2 (ManOCH$_2$C$\underline{H}$–, *ManOCH$_2$C$\underline{H}$*–), 64.6 (ManOC$\underline{H}_2$CH–, *ManOC$\underline{H}_2$CH*–), 62.1 (C-6), 62.2 (*C-6*), 52.3 (COOC$\underline{H}_3$, *COOC$\underline{H}_3$*), 48.5 (NC$\underline{H}_2$CH$_2$CH$_2$–, *NC$\underline{H}_2$CH$_2$CH$_2$*–), 40.9, 40.1 (NHC$\underline{H}_2$COOCH$_3$, *NHC$\underline{H}_2$COOCH$_3$*), 29.5 (NCH$_2$C$\underline{H}_2$CH$_2$–, *NCH$_2$C$\underline{H}_2$CH$_2$*–), 26.5 (NCH$_2$CH$_2$C$\underline{H}_2$–, *NCH$_2$CH$_2$C$\underline{H}_2$*–), 20.6, 20.7, 20.8, 21.7, 21.8 (C$\underline{H}_3$CO).

3.2.3. Compound 4. According to the general procedure, **1** (656 mg, 1.67 mmol), 1,6-hexanediamine (88 mg, 0.76 mmol), cyclohexanecarboxylic acid (214 mg, 1.67 mmol), and methyl isocyanoacetate (160 μL, 1.67 mmol) in MeOH (25 mL) afforded **4** (355 mg, 36%) as a 53:47 mixture of diastereomers after chromatography (40:1 to 20:1, CH_3Cl–MeOH). ^1H NMR ($CDCl_3$): δ 7.02–7.06 (m, 1 H, NH), 7.09–7.10 (m, 2 H, *NH*), 5.19–5.29 (m, 6 H, H-4, *H-4*, H-3, *H-3*, H-2, *H-2*), 4.87 (s, 1 H, H-1), 4.82 (s, 1 H, *H-1*), 4.21–4.31 (m, 4 H, H-6a, *H-6a*, ManOCH$_2$C$\underline{H}$, *ManOCH$_2$C$\underline{H}$*), 4.10–4.12 (m, 2 H, H-6b, *H-6b*), 4.03–4.08 [m, 2 H, NHC$\underline{H}$(H)COOCH$_3$, *NHC$\underline{H}$(H)COOCH$_3$*], 3.87–3.99 [m, 8 H, H-5, *H-5*, ManOCH$_2$, *ManOCH$_2$*, NHCH(H)COOCH$_3$, *NHCH(H)COOCH$_3$*], 3.72 (s, 3 H, COOCH$_3$), 3.73 (s, 3 H, *COOCH$_3$*), 3.33 (t, 4 H, *J* 8.1 Hz, 15.4 Hz, NCH$_2$–, *NCH$_2$*–), 2.48 (m, 22 H, cyclohexyl, *cyclohexyl*), 1.98, 2.03, 2.11, 2.14, 2.15 (m, 24 H, 4×CH$_3$CO, *4×CH$_3$CO*), 1.27–1.82 (m, 18 H, NCH$_2$C$\underline{H}_2$C$\underline{H}_2$–, *NCH$_2$C$\underline{H}_2$C$\underline{H}_2$*–, cyclohexyl, *cyclohexyl*); ^{13}C NMR ($CDCl_3$): δ 169.5, 169.6, 169.8, 169.9, 170.0, 170.7, 177.9, 178.4 (C═O), 97.2 (*C-1*), 98.1 (C-1), 68.7, 68.8, 69.0, 69.1 (C-5, *C-5*, C-3, *C-3*, C-2, *C-2*), 65.5, 65.7, 65.8 (C-4, *C-4*, ManOCH$_2$C$\underline{H}$–, *ManOCH$_2$C$\underline{H}$*–), 64.7 (ManOCH$_2$–, *ManOCH$_2$*–), 62.2 (C-6, *C-6*), 52.3 (COOC$\underline{H}_3$, *COOC$\underline{H}_3$*), 47.5 (NCH$_2$–, *NCH$_2$*–), 41.5 (cyclohexyl, *cyclohexyl*), 40.9 (–NHC$\underline{H}_2$COOCH$_3$, *–NHC$\underline{H}_2$COOCH$_3$*), 25.7, 25.8, 29.7, 30.3 (cyclohexyl, *cyclohexyl*), 29.5, 29.6 (–NCH$_2$C$\underline{H}_2$CH$_2$–, *–NCH$_2$C$\underline{H}_2$CH$_2$*–), 26.6 (–NCH$_2$CH$_2$C$\underline{H}_2$–, *–NCH$_2$CH$_2$C$\underline{H}_2$*–), 20.6, 20.7, 20.8 (C$\underline{H}_3$CO, *C$\underline{H}_3$CO*).

3.3. General procedure for the synthesis of peracetylated divalent and trivalent mannopyranosyl ligands (9 and 10)

The U-4CR of 1,6-hexanediamine (1.0 equiv) or tris(2-aminoethyl)amine (1.0 equiv) was dissolved in MeOH. Compound **8**,[16] freshly distilled benzaldehyde, and methyl isocyanoacetate (2.2 equiv of each reagent for compound **9** and 3.3 equiv of each reagent for compound **10**) were then added. The mixture was allowed to stir at room temperature for 48 h, then concentrated, diluted with CH_2Cl_2, washed with water, dried, and concentrated. Column chromatography on silica gel of the residue afforded divalent mannoside **9** and trivalent mannoside **10**, respectively.

3.3.1. Compound 9. According to the general procedure, benzaldehyde (127 μL, 1.25 mmol), 1,6-hexanediamine (66 mg, 0.57 mmol), **8** (508 mg, 1.25 mmol), and methyl isocyanoacetate (114 μL, 1.25 mmol) in MeOH (25 mL) afforded **9** (341 mg, 46%) as a 60:40 mixture of diastereomers after chromatography (1:2 to 2:9, petroleum ether–EtOAc). ^1H NMR ($CDCl_3$): δ 7.38 (m, 10 H, Ph–, *Ph*–), 6.34–6.41 (m, 2 H, –NH–, *–NH*–), 5.92 (s, H, PhCH–), 5.78 (s, 1 H, *PhCH*–), 5.48 (br s, 2 H, H-4, *H-4*), 5.25 (br s, 2 H, H-1, *H-1*), 5.17–5.19 (m, 2 H, H-3, *H-3*), 4.38–4.41 (m, 2 H, H-5, *H-5*), 4.25–4.34 (m, 4 H, ManOCH$_2$–, *ManOCH$_2$*–), 4.00–4.14 (m, 8 H, NHC$\underline{H}_2$CO, *NHC$\underline{H}_2$CO*, H-6a, *H-6a*, H-6b, *H-6b*), 3.74 (s, 6 H, CH$_3$COOC$\underline{H}_3$, *CH$_3$COOC$\underline{H}_3$*), 3.10–3.11 (m, 4 H, NC$\underline{H}_2$CH$_2$–, *NC$\underline{H}_2$CH$_2$*–), 2.15, 2.12, 2.09, 2.03, 2.02, 1.98 (m, 24 H, 4×CH$_3$CO–, *4×CH$_3$CO*–), 1.21–1.29 (m, 4 H, NCH$_2$C$\underline{H}_2$–, *NCH$_2$C$\underline{H}_2$*–), 0.83–0.89 (m, 4 H, NCH$_2$CH$_2$C$\underline{H}_2$–, *NCH$_2$CH$_2$C$\underline{H}_2$*–); ^{13}C NMR ($CDCl_3$): δ 168.9, 169.6, 169.8, 169.9, 170.2, 170.4, 170.7, 171.1 (C═O), 134.3, 129.6, 129.4, 128.9, 128.8 (Ph–, *Ph*–), 96.4 (C-1, *C-1*), 67.9 (C-4, *C-4*), 67.6 (C-3, *C-3*), 67.2 (C-2, *C-2*), 66.7 (C-5, *C-5*), 64.7 (ManOC$\underline{H}_2$–, *ManOC$\underline{H}_2$*–), 61.3 (PhC$\underline{H}$–, *PhC$\underline{H}$*–), 60.4 (C-6, *C-6*), 52.3 (–COOC$\underline{H}_3$, *–COOC$\underline{H}_3$*), 45.5 (NC$\underline{H}_2$CH$_2$–, *NC$\underline{H}_2$CH$_2$*–), 41.3 (NHC$\underline{H}_2$COOCH$_3$, *NHC$\underline{H}_2$COOCH$_3$*), 29.4 (NCH$_2$C$\underline{H}_2$–, *NCH$_2$C$\underline{H}_2$*–), 26.2 (NCH$_2$CH$_2$C$\underline{H}_2$–, *NCH$_2$CH$_2$C$\underline{H}_2$*–), 20.6, 20.7, 20.9, 21.0 (4×C$\underline{H}_3$CO, *4×C$\underline{H}_3$CO*).

3.3.2. Compound 10. According to the general procedure, benzaldehyde (185 μL, 1.82 mmol), tris(2-aminoethyl)amine (83 μL, 0.55 mmol), **8** (738 mg, 1.82 mmol), and methyl isocyanoacetate (165 μL, 1.82 mmol) in MeOH (25 mL) produced **10** (440 mg, 41%) after chromatography (1:2 to 2:9, petroleum ether–EtOAc). ^1H NMR ($CDCl_3$): δ 7.29–7.46 (Ph–), 5.68–5.73 (PhC$\underline{H}$), 5.22–5.42 (H-4, H-3, H-2), 4.90–4.92, 5.02–5.05 (H-1),

4.01–4.30 (H-5, H-6a, H-6b, ManOCH$_2$–, NHC$\underline{H}_2$CO-OCH$_3$), 3.70–3.77 (–COOC$\underline{H}_3$), 2.95–3.27 (NCH$_2$–), 2.15–2.37 (NCH$_2$C$\underline{H}_2$–), 1.97, 2.04, 2.10, 2.15 (CH$_3$CO–); ^{13}C NMR (CDCl$_3$): δ 161.1, 169.7, 169.8, 170.7 (C=O), 130.4, 129.5, 129.0, 128.4, 125.7 (Ph), 97.8 (C-1), 69.1 (C-2, C-3, C-5), 65.8 (C-4), 64.0 (PhC$\underline{H}$–), 62.2 (C-6), 60.4 (ManO$\underline{C}$H$_2$–), 52.7 (COO$\underline{C}$H$_3$), 43.8 (N$\underline{C}$H$_2$$\underline{C}H_2$–), 41.3 (NHC$\underline{H}_2$COOCH$_3$), 20.7 (CH$_3$CO).

3.4. General de-O-acetylation procedure of peracetylated mannopyranosyl ligands (5–7, 11, 12)

The acetylated cluster mannosides were dissolved in MeOH, and a catalytic amount of NaOMe was added (pH ~9). The solution was stirred at room temperature until all acetyl groups were removed, as monitored by TLC. The mixture was neutralized with Dowex-50x-8 (H^+), and then filtered and concentrated, leading to the target compounds.

Compound **2** (206 mg, 0.17 mmol) was treated with NaOMe–MeOH to give **5** (166 mg, 96%). The product was further purified using silica gel chromatography (4:1, CHCl$_3$–MeOH). ^{1}H NMR (Me$_2$SO): δ 8.40–8.46, 8.22–8.28 (2m, 2 H, NH–, *NH–*), 7.32–7.49 (m, 10 H, Ph–, *Ph–*), 4.75–4.82 (m, 4 H, OH-4, *OH-4*, OH-3, *OH-3*), 4.61–4.71 (m, 2 H, H-1, *H-1*), 4.31–4.56 (m, 6 H, OH-2, *OH-2*, OH-6, *OH-6*, ManOCH$_2$C$\underline{H}$–, *ManOCH$_2$C$\underline{H}$–*), 3.76–4.00 (m, 8 H, NHC$\underline{H}_2$COOCH$_3$, *NHC$\underline{H}_2$COOCH$_3$*, ManOC$\underline{H}_2$CH–, *ManOC$\underline{H}_2$CH–*), 3.64 (s, 6 H, COOC$\underline{H}_3$, *COOC$\underline{H}_3$*), 3.16–3.60 (m, 12 H, H-6a, *H-6a*, H-6b, *H-6b*, H-3, *H-3*, H-2, *H-2*, H-4, *H-4*, H-5, *H-5*), 3.04–3.10 (m, 4 H, NC$\underline{H}_2$CH$_2$CH$_2$–, *NC$\underline{H}_2$CH$_2$CH$_2$–*), 1.2–1.5 (m, 8 H, NCH$_2$C$\underline{H}_2$C$\underline{H}_2$–, *NCH$_2$C$\underline{H}_2$C$\underline{H}_2$–*); ^{13}C NMR (Me$_2$SO): δ 168.5, 169.0, 169.9, 170.1 (C=O), 136.7, 129.1, 128.3, 126.7, 126.2 (Ph–, *Ph–*), 100.4 (C-1), 99.8 (*C-1*), 74.0 (C-5, *C-5*), 70.9 (C-3, *C-3*), 70.1 (C-2, *C-2*), 66.7 (C-4, *C-4*), 64.3 (ManO$\underline{C}$H$_2$–, *ManO$\underline{C}$H$_2$–*), 70.1 (C-2, *C-2*), 61.1 (ManOCH$_2$$\underline{C}$H, *ManOCH$_2$$\underline{C}$H*, C-6, *C-6*), 51.8 (COO$\underline{C}$H$_3$, *COO$\underline{C}$H$_3$*), 47.8 (N$\underline{C}$H$_2$CH$_2$CH$_2$–, *N$\underline{C}$H$_2$CH$_2$CH$_2$–*), 40.8 (NH$\underline{C}$H$_2$CO-OCH$_3$, *NH$\underline{C}$H$_2$COOCH$_3$*), 24.3, 25.5, 26.7, 27.3 (NCH$_2$$\underline{C}H_2$$\underline{C}H_2$–, *NCH$_2$$\underline{C}H_2$$\underline{C}H_2$–*); MS *m/z*: [M+Na]$^+$ calcd for C$_{44}$H$_{62}$O$_{20}$N$_4$Na, 989.39; found, 989.35.

Compound **3** (210 mg, 0.18 mmol) was treated with NaOMe–MeOH to afford **6** (172 mg, 97%) as a 56:44 mixture of diastereomers after silica gel chromatography (8:4:0.5, CHCl$_3$–MeOH–H$_2$O). ^{1}H NMR (Me$_2$SO): δ 8.65–8.67 (m, 1 H, NH), 8.13–8.18 (m, 1 H, *NH*), 4.71–4.79 (m, 4 H, OH-2, *OH-2*, OH-4, *OH-4*), 4.63 (s, 1 H, H-1), 4.67 (s, 1 H, *H-1*), 4.56–4.59 (m, 2 H, OH-3, *OH-3*), 4.39–4.47 (m, 2 H, OH-6, *OH-6*), 3.83–4.01 (m, 4 H, ManOC$\underline{H}_2$–, *ManOC$\underline{H}_2$–*), 3.78–3.81 (m, 4 H, NHC$\underline{H}_2$COOCH$_3$, *NHC$\underline{H}_2$COOCH$_3$*), 3.63–3.65 (m, 2 H, H-6a, *H-6a*), 3.62 (s, 3 H, COOCH$_3$), 3.63 (s, 3 H, *2×COOCH$_3$*), 3.52–3.55 (m, 4 H, H-2, *H-2*, ManOCH$_2$C$\underline{H}$–, *ManOCH$_2$C$\underline{H}$–*), 3.41–3.48 (m, 2 H, H-6b, *H-6b*), 3.36–3.40 (m, 4 H, H-4, *H-4*, H-3, *H-3*), 3.28–3.33 (m, 2 H, H-5, *H-5*), 3.20–3.26 (m, 4 H, NC$\underline{H}_2$CH$_2$CH$_2$–, *NC$\underline{H}_2$CH$_2$CH$_2$–*), 2.05–2.07 (m, 6 H, CH$_3$CO, *CH$_3$CO*), 1.15–1.28 (m, 4 H, NCH$_2$C$\underline{H}_2$CH$_2$–, *NCH$_2$C$\underline{H}_2$CH$_2$–*), 1.11–1.15 (m, 4 H, NCH$_2$CH$_2$C$\underline{H}_2$–, *NCH$_2$CH$_2$C$\underline{H}_2$–*); ^{13}C NMR (Me$_2$SO): δ 168.7, 169.2, 169.3, 169.9, 170.1, 170.4, 170.6 (C=O), 100.1 (C-1), 100.2 (*C-1*), 73.9 (C-5), 74.1 (*C-5*), 70.8 (C-3, *C-3*), 70.1 (C-2, *C-2*), 66.7 (C-4, *C-4*), 64.7 (ManO$\underline{C}$H$_2$–, *ManO$\underline{C}$H$_2$–*), 64.3 (ManOCH$_2$$\underline{C}$H–, *ManOCH$_2$$\underline{C}$H–*), 61.1 (C-6, *C-6*), 51.7 (COO$\underline{C}$H$_3$, *COO$\underline{C}$H$_3$*), 46.8 (N$\underline{C}$H$_2$CH$_2$CH$_2$–, *N$\underline{C}$H$_2$CH$_2$CH$_2$–*), 40.7 (NH$\underline{C}$H$_2$CO-OCH$_3$, *NH$\underline{C}$H$_2$COOCH$_3$*), 29.3 (NCH$_2$$\underline{C}H_2CH_2$–, *NCH$_2$$\underline{C}H_2CH_2$–*), 26.5 (NCH$_2CH_2$$\underline{C}H_2$–, *NCH$_2CH_2$-$\underline{C}H_2$–*); MS *m/z*: [M+Na]$^+$ calcd for C$_{34}$H$_{58}$O$_{20}$N$_4$, 865.35; found, 865.31.

Compound **4** (286 mg, 0.22 mmol) was treated with NaOMe–MeOH to give **7** (209 mg, 97%). The product was further purified using silica gel chromatography (8:3:0.1, CHCl$_3$–MeOH–H$_2$O). ^{1}H NMR (Me$_2$SO): δ 8.38–8.45 (m, 1 H, NH), 7.97–8.06 (m, 1 H, NH), 4.67–4.78 (m, 4 H, OH-2, *OH-2*, OH-4, *OH-4*), 4.53–4.61 (m, 4 H, H-1, *H-1*, OH-3, *OH-3*), 4.37–4.46 (m, 2 H, OH-6, *OH-6*), 3.72–4.00 (m, 10 H, ManOC$\underline{H}_2$CH–, *ManOC$\underline{H}_2$CH–*, ManOCH$_2$C$\underline{H}$–, *ManOCH$_2$C$\underline{H}$–*, NHC$\underline{H}_2$COOCH$_3$, *NHC$\underline{H}_2$COOCH$_3$*), 3.62 (s, 6 H, NHCH$_2$COOC$\underline{H}_3$, *NHCH$_2$COOC$\underline{H}_3$*), 3.61–3.62 (m, 2 H, H-6a, *H-6a*), 3.51–3.56 (m, 2 H, H-2, *H-2*), 3.42–3.48 (m, 2 H, H-6b, *H-6b*), 3.37–3.40 (m, 4 H, H-3, *H-3*, H-4, *H-4*), 3.27–3.32 (m, 2 H, H-5, *H-5*), 3.32–3.36 (m, 4 H, NC$\underline{H}_2$CH$_2$CH$_2$–, *NC$\underline{H}_2$CH$_2$CH$_2$–*), 2.47–2.55 (m, 2 H, cyclohexyl-H, *cyclohexyl-H*), 1.60–1.77 (m, 8 H, cyclohexyl-H, *cyclohexyl-H*), 1.43–1.59 (m, 4 H, NCH$_2$C$\underline{H}_2$CH$_2$–, *NCH$_2$C$\underline{H}_2$CH$_2$–*), 1.22–1.40 (m, 12 H, cyclohexyl-H, *cyclohexyl-H*), 1.10–1.22 (m, 4 H, NCH$_2$CH$_2$C$\underline{H}_2$–, *NCH$_2$CH$_2$C$\underline{H}_2$–*); ^{13}C NMR (Me$_2$SO): δ 169.0, 169.3, 169.4, 169.9, 170.0, 175.9, 176.0 (C=O), 99.6 (C-1), 100.3 (*C-1*), 74.0 (C-5), 73.9 (*C-5*), 70.9 (C-3, *C-3*), 70.2 (C-2, *C-2*), 66.7 (C-4, *C-4*), 64.5 (ManOCH$_2$$\underline{C}$H–, *ManOCH$_2$$\underline{C}$H–*), 64.0 (ManOC$\underline{H}_2$-CH–, *ManOC$\underline{H}_2$CH–*), 61.1 (C-6, *C-6*), 51.7 (COO$\underline{C}$H$_3$, *COO$\underline{C}$H$_3$*), 45.5 (N$\underline{C}$H$_2$CH$_2$CH$_2$–, *N$\underline{C}$H$_2$CH$_2$CH$_2$–*), 40.8 (NH$\underline{C}$H$_2$COOCH$_3$, *NH$\underline{C}$H$_2$COOCH$_3$*), 40.2 (cyclohexyl, *cyclohexyl*), 29.3, 29.5, 30.2, 30.3 (cyclohexyl, *cyclohexyl*), 26.3 (NCH$_2$$\underline{C}H_2CH_2$–), 26.2 (*NCH$_2$$\underline{C}H_2$-CH$_2$–*), 25.2 (NCH$_2CH_2$$\underline{C}H_2$–), 25.5 (*NCH$_2CH_2$$\underline{C}H_2$–*); MS *m/z*: [M+Na]$^+$ calcd for C$_{44}$H$_{74}$O$_{20}$N$_4$, 1001.48; found, 1001.53.

Compound **9** (220 mg, 0.17 mmol) was treated with NaOMe–MeOH to give **11** (164 mg, 98%). The product was further purified using silica gel chromatography (4:1, CHCl$_3$–MeOH). ^{1}H NMR (Me$_2$SO): δ 8.50–8.66 (m, 2 H, NH–, *NH–*), 7.29–7.36 (m, 10 H, Ph–, *Ph–*), 6.03 (s, 1 H, PhC$\underline{H}$–), 5.58 (s, 1 H, *PhC$\underline{H}$–*), 4.71–4.79 (m, 6 H, H-1, *H-1*, OH-2, *OH-2*, OH-4, *OH-4*), 4.62 (d, 2 H, *J* 5.46 Hz, OH-3, *OH-3*), 4.43–4.49 (m, 2 H, OH-6,

OH-6), 4.29–4.37 [m, 2 H, ManOCH(H), *ManOCH(H)*], 4.18–4.22 [m, 2 H, ManOCH(H), *ManOCH(H)*], 3.79–3.99 (m, 4 H, $NHCH_2COOCH_3$, $NHCH_2COOCH_3$), 3.64–3.68 (m, 10 H, H-2, *H-2*, $COOCH_3$, $COOCH_3$, H-6a, *H-6a*), 3.42–3.47 (m, 4 H, H-3, *H-3*, H-6b, *H-6b*), 3.36–3.40 (m, 2 H, H-4, *H-4*), 3.28–3.33 (m, 2 H, H-5, *H-5*), 2.22–2.82 (m, 4 H, $NCH_2CH_2CH_2$–, $NCH_2CH_2CH_2$–), 0.41–1.06 (m, 8 H, $NCH_2CH_2CH_2$–, $NCH_2CH_2CH_2$–); ^{13}C NMR (Me_2SO): δ 168.4, 169.0, 169.7, 170.0 (C=O), 135.7, 129.5, 129.1, 128.2, 127.9 (Ph–, *Ph*–), 99.3 (C-1), 99.1 (*C-1*), 74.3 (C-5, *C-5*), 70.7 (C-3, *C-3*), 69.6 (C-2, *C-2*), 66.7 (C-4, *C-4*), 62.8 ($ManOCH_2$–, $ManOCH_2$–), 61.1 (C-6, *C-6*), 60.2 (PhCH–, *PhCH*–), 51.7 ($COOCH_3$, $COOCH_3$), 44.3 ($NCH_2CH_2CH_2$–, $NCH_2CH_2CH_2$–), 40.6 (–$NHCH_2COOCH_3$, –$NHCH_2COOCH_3$), 29.0 ($NCH_2CH_2CH_2$–, $NCH_2CH_2CH_2$–), 25.8 ($NCH_2CH_2CH_2$–, $NCH_2CH_2CH_2$–); HRMS *m/z*: $[M+Na]^+$ calcd for $C_{44}H_{62}O_{20}N_4Na$, 989.3849; found, 989.3843.

Compound **10** (241 mg, 0.12 mmol) was treated with NaOMe–MeOH to give **12** (166 mg, 97%). The product was further purified using silica gel chromatography (4:1, $CHCl_3$–MeOH); ^{1}H NMR (Me_2SO): δ 8.66 (NH), 8.46 (NH), 7.22–7.34 (Ph), 5.85–5.94, 5.51–5.57 (PhCH), 4.76–4.78 (H-1), 4.31–4.50, 4.10–4.23 ($ManOCH_2$–), 3.76–4.03 (–$NHCH_2COOCH_3$), 3.60–3.71 ($COOCH_3$, H-2), 3.23–3.59 (H-6a, H-3, H-6b, H-4, H-5), 2.57–3.23 (CH_2), 0.97–1.90 (CH_2); ^{13}C NMR (Me_2SO): δ 170.1, 169.9, 169.8 (C=O), 135.2, 129.7, 129.2, 128.4, 128.2 (3×Ph–), 99.4 (3×C-1), 74.3 (3×C-5), 70.8 (3×C-3), 70.1, 69.9 (3×C-2), 66.7 (3×C-4), 63.0 (3×$ManOCH_2$–), 61.0 (3×C-6, 3×PhCH–), 51.8 (3×$COOCH_3$), 42.7 (3×CH_2), 40.7 (3×CH_2); HRMS *m/z*: $[M+H]^+$ calcd for $C_{63}H_{88}O_{30}N_7$, 1422.5569; found, 1422.5553.

3.5. Inhibition experiments[7]

All incubations were carried out at 37 °C. Costar 3590 microtiter plates were coated overnight with yeast mannan [100 μL of a 10 μg/mL solution in phosphate-buffered saline (PBS)]. The wells were then washed three times with 300 μL/well of washing PBS buffer containing 0.05% (v/v) Tween 20. Coated wells were blocked with 1% w/v bovine serum albumin in PBS (300 μL) for 2 h. During this period, serial two-fold dilutions of ligand were also incubated with peroxidase-labeled Con A (a dilution of 800 times) for 1 h. The lectin solutions were then transferred to the blocked plated for an additional 1 h of incubation. The plates were washed as described above and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (100 μL of a 250 μg/mL solution) in citrate–phosphate buffer (pH 4.0) with 0.02% v/v hydrogen peroxide was added. Color development was stopped after 10 min by adding 100 μL/well of 1 M H_2SO_4 and the optical density (OD) was measured at 450 nm relative to that at 630 nm.

The percent inhibition was calculated as follows:

$$\%\ \text{Inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{no inhibitor})} \times 100.$$

IC_{50}s were reported as the concentration required for 50% inhibition of the coating antigen.

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