New Synthesis of Mannoside-Containing Neoglycoconjugates by Reaction of Propargyl Mannosides with Polyalkylcarboxylated Scaffolds

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Abstract: A new, efficient and easy synthesis of multivalent mannoside neoglyconjugates is described by the alkynylation reaction of lithium acetylides derived from adequately protected propargyl α-D-mannoside and readily available polyalkylcarboxylate benzene derivatives as scaffolds.

Key words: glycosides, neoglycoconjugates, clusters, alkynes, polyalkylcarboxylates

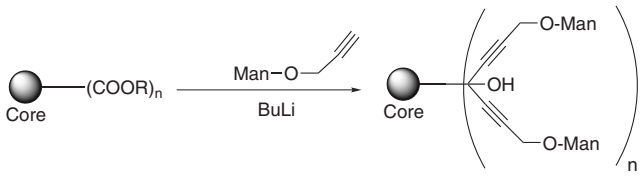
Protein-carbohydrate recognition events mediate significant biological processes including fertilization, pathogen-cell adhesion, and the inflammatory response.[1] Multivalency in ligand-receptor interactions is an important principle used by nature to increase weak interactions to biologically relevant levels.[2] Following this observation and with the expectation of understanding the molecular details of a such process and to therapeutically manipulate these interactions, synthetic glycoligands or neoglycoconjugates[3] have emerged as valuable tools. They also bear other potential advantages such as a greater flexibility for the introduction of structural modifications. Many such compounds have been constructed based on the so-called cluster glycoside effect[4] in order to mimic the multivalency and to improve their stability, specificity, and affinity.

As terminal mannoside residues have been found to interact with receptors found on macrophages,[5] hepatical sinusoidal cells[6] and different invading pathogens,[7] numerous efforts have been devoted to the synthesis of mannoside neoglyconjugates for the study of mannoside receptor interactions and their potential application as inhibitors of pathogenic infections and targeting devices.[8]

In search of new methodologies for the synthesis of neoglyconjugates we and others have recently contributed by introducing the use of propynyl glycosides as versatile and efficient synthons for the construction of multivalent systems with a well defined architecture. The particular and characteristic reactivity of the acetylenic function have found notable applications through a variety of different strategies such as the oxidative dimerization of propynyl glycopyranosides with palladium and copper-catalyzed homo- and cross-coupling reactions,[8k,9] the Sonogashira-type cross-coupling reaction of alkynyl glycosides,[8j,l,9b,10] the cyclotrimerization of terminal as well as symmetrical alkyne sugar derivatives mediated by transition metal catalysts[11] and, more recently, the alkyne-azide cycloaddition reaction catalyzed by Cu(I).[8m] In our continuing efforts towards rational design and efficient synthesis of multivalent neoglycoconjugates, we now describe that propynyl glycosides are also adequate substrates for the construction of α-D-mannoside clusters by reaction of the corresponding lithium acetylides with polyalkylcarboxylates.

The nucleophilic addition of alkynyl organomethallic reagents to unsaturated C=X systems (X = O, NR) is a very convenient and reliable strategy for carbon-carbon bond formation that has not been used as extensively as other organometallic nucleophilic addition to those systems.[12] However, to the best of our knowledge there are precedents for reactivity of metal acetylides with esters only. Hess et al.[13a] reported the first reaction of alkynyl Grignard with ethyl benzoate. By application of this reaction, geminal bis(alkynyl)carbinols has been prepared.[13b,14] Young et al.[15] have reported that the reaction of tosyl-*N*-aziridine-2-carboxylate esters with lithium trimethylsilylacetylide led to the corresponding tertiary alcohol. In addition, other work has also indicated that Grignard and non-acetylenic organolithium reagents attack the ester function in such aziridines.[16] On the other hand, the hydroalumination of bis-alkynyl alcohols has been reported[17] as a novel route for the synthesis of stereogenic tertiary alcohol centers. Finally, tetrakis-alkynyl diols have been synthesized as modules for acetylenic molecular scaffolding.[18]

Taking into consideration all this precedents, we thought that α-D-mannoside could be easily grafted onto aromatic cores by using polyalkylcarboxylate benzene derivatives readily accessible from commercial sources and acetylides derived from propargyl mannosides (see Scheme 1).



Scheme 1 Strategy for the synthesis of mannoside neoglycoconjugates

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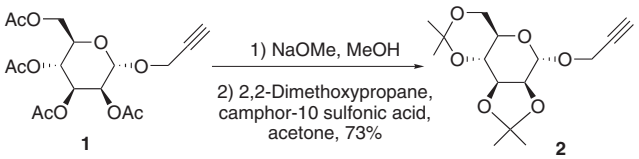
Aromatic cores are particularly attractive since the presence of an aromatic heterocyclic group next to the glycoside bond has been shown to contribute positively to the interaction of neoglycoconjugates with lectins by increasing the hydrophobicity.[8a,19] In addition, the particular topology of the resulting structures should confer a high rigidity to the neoglycoconjugates diminishing the entropic loss that is usually associated with flexible and hydrated carbohydrate ligands. Better inhibitory properties than those of their more flexible counterparts could be expected, as was previously observed.[8j,k]

For the preparation of acetylides derived from propargyl mannosides, we synthesized 2-propynyl 2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside **2** as the most appropriate synthon considering the tolerance of the O-protective group toward the alkynylation required conditions and its easy accessibility. Thus, compound **2** was readily prepared from propargyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside **1**[20] in two steps by a conventional Zemplen O-deacetylation followed by subsequent O-isopropylidination (see Scheme2).

Methyl benzoate (**3**), dimethyl 1,4- and 1,3-benzenedicarboxylate (**5** and **6**, respectively) and trimethyl 1,3,5-benzenetricarboxylate (**7**) were chosen as readily available polyalkylcarboxylate aromatic derivatives (see Table 1). The known ethyl (*p-tert*-butylphenoxy)acetate (**4**)[21] was prepared by O-alkylation of *p-tert*-butyl phenol with ethyl bromoacetate (see experimental). The alkynylation reactions were performed following standard procedures for the formation and reaction of lithium acetylides.[22] The reactions proceeded smoothly leading to the O-protected divalent (**8** and **10**), tetravalent (**12** and **14**) and hexavalent (**16**) mannoside neoglycoconjugates in good to high yields (see Table 1). O-Deprotection of these compounds was performed by acid hydrolysis using HOAc–H_2O yielding the corresponding hydroxylated derivatives **9**, **11**, **13**, **15**, and **17**, respectively.



Scheme 2 Synthesis of propargylated mannoside synthon

The various hydroxylated mannose neoglyconjugates **9**, **11**, **13**, **15**, and **17** were then evaluated for their relative binding inhibitory properties against peroxidase-labeled Concavalin A (Con A) lectin. The glycoconjugates were used to inhibit the lectin binding to *Saccharomices cerevisiae* mannan that was used as a coating material in competitive solid-phase microtiter plate assays. Triplicate results were used for the construction of the inhibition curves. The results expressed as the IC_{50} values and when compared with the low-affinity inhibitor methyl α-D-mannopyranoside (see Table 2) indicated a Con A affinity enhacement for the hexavalent mannoside **17** relative to methyl α-D-mannopyranoside (4.53-fold). For the tetravalent mannosides **13** and **15** the Con A affinities were lower as compared with compound **17**. In these compounds a higher affinity was observed for compound **13** having a 1,4-subsitution pattern for the benzene ring.

Table 1 Synthesis of Multivalent Mannoside Neoglycoconjugates

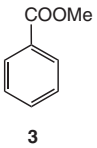
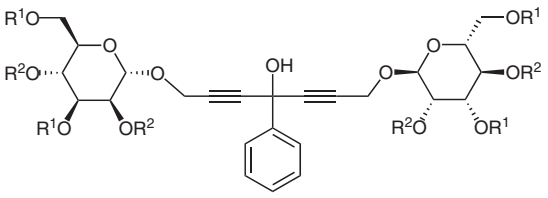
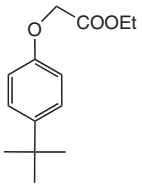
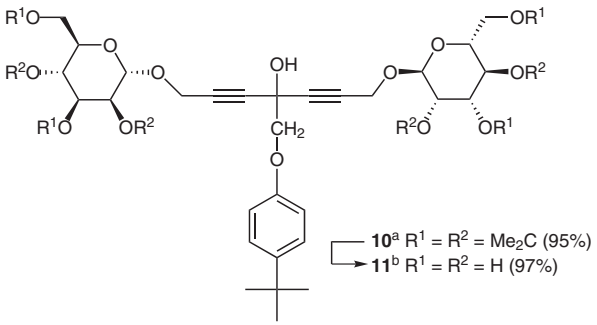
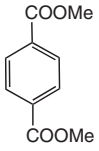
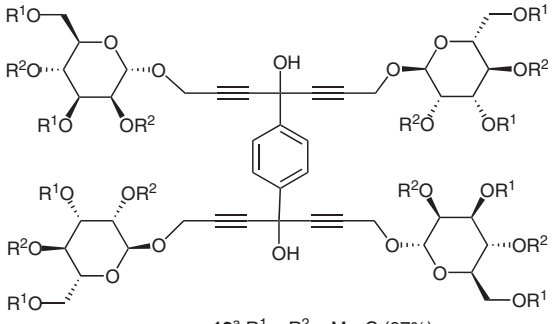
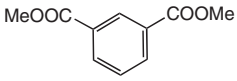
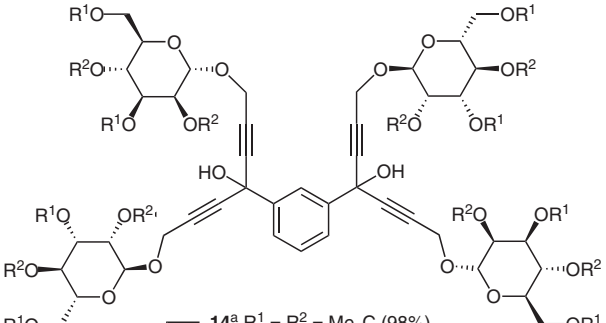
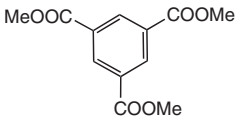
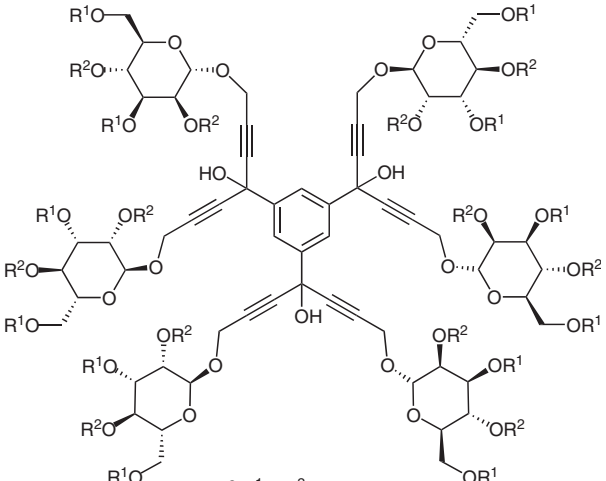
Core	Neoglycoconjugate (yield)
 **3**	 **8**[a] $R^1 = R^2 = Me_2C$ (98%) **9**[b] $R^1 = R^2 = H$ (95%)
 **4**	 **10**[a] $R^1 = R^2 = Me_2C$ (95%) **11**[b] $R^1 = R^2 = H$ (97%)

Table 1 Synthesis of Multivalent Mannoside Neoglycoconjugates (continued)

Core	Neoglycoconjugate (yield)
 **5**	 **12**[a] $R^1 = R^2 = Me_2C$ (67%) → **13**[b] $R^1 = R^2 = H$ (93%)
 **6**	 **14**[a] $R^1 = R^2 = Me_2C$ (98%) → **15**[b] $R^1 = R^2 = H$ (96%)
 **7**	 **16**[a] $R^1 = R^2 = Me_2C$ (76%) → **17**[b] $R^1 = R^2 = H$ (92%)

[a] Reagents and conditions: (i) **2**, BuLi, THF; (ii) **3**, **4**, **5**, **6**, or **7**, r.t.
[b] Reagents and conditions: HOAc–H_2O (70%), r.t.

The divalent neoglyconjugate **9** showed an IC_{50} value in the same order as that for the methyl α-D-mannopyranoside. However, the divalent mannoside **11** showed a significant decrease in relative potency when compared to methyl α-D-mannopyranoside.

In conclusion, we have demonstrated that multivalent neoglyconjugates containing mannose as sugar moieties can be easily prepared by the reaction of lithium acetylides derived from adequately protected propargyl mannosides with polyalkylcarboxylated scaffolds. The methodology is efficient and broadens the utility of propynyl glycosides as versatile synthons in the preparation of neoglycoconjugates. For the compounds prepared in the present work, aromatic scaffolds were used and the resulting neoglycoconjugates evaluated for their relative binding properties toward the natural binding plant lectin Concanavalin A showing good inhibitory properties.

TLC was performed on Merck silica gel $60F_{245}$ aluminum sheets with detection by charring with sulfuric acid and by UV light when applicable. Flash column chromatography was carried out on silica gel, Merck or Scharlau (230–400 mesh. ASTM), with the solvent systems indicated. All the concentrations were carried out under re-

Table 2 ELLA Data for Binding Inhibition of HRP-Labeled Con A by Multivalent Mannoside Neoglycoconjugates

Parameter	Compound					
	Me-O-Man	**9**	**11**	**13**	**15**	**17**
IC_{50} (μM)	0.68	0.85	1.77	0.26	0.47	0.15
Rel. Potency	1	0.80	0.45	2.62	1.44	4.53
Rel. Potency[a]	1	0.2	0.23	0.65	0.36	0.76

[a] Per mol of mannopyranosyl residue relative to methyl α-D-mannopyranoside.

duced pressure at 40 °C. Optical rotations were measured at 22 °C. Melting points are uncorrected. NMR spectra were recorded at r.t. ^{1}H NMR chemical shifts are given in ppm and referenced to internal $CHCl_3$ (δ = 7.26 ppm) for $CDCl_3$ solutions. ^{13}C NMR chemical shifts are given in ppm and referenced to $CDCl_3$ (δ = 77.0 ppm). *J* values are given in Hz. Assignments were based on COSY, HMQC, NOESY, and DEPT. FAB MS were obtained using *m*-nitrobenzyl alcohol or thioglicerol as the matrix. Anhydrous solvents were prepared according to standard procedures and were freshly distilled prior to use. Methyl α-D-mannopyranoside, peroxidase-labeled Concavalin A (Con A) lectin and *Saccharomices cerevisiae* mannan were purchased from Sigma.

Enzyme-Linked Lectin Assay (ELLA)

ELLA assays were carried out as previously described.[23] Experiments were carried out using a Metertech Σ960 instrument. Microtitration plates were coated with *S. cerevisiae* mannan at 100 μL/well of a solution containing 10 μg/mL in 10mM phosphate buffer (PBS, pH 7.4) for 2 h at 37 °C. The wells were then washed twice with 10 mM phosphate buffer containing 1% (v/v) Tween 20 (PBST) and once with PBS. This washing procedure was repeated after each incubation period. Wells were then blocked with 300 μL/well of BSA/PBS (1% w/v) for 2 h at 37 °C. Each inhibitor was added in serial dilutions (60 μL/well) to the glycoclusters **9**, **11**, **13**, **15**, and **17** or methyl α-D-mannopyranoside in PBS (pH 6.8, containing 0.1 mM $Ca^{2+}$and 0.1 mM Mn^{2+}) and the peroxidase-labeled Con A (60 μL/well of a solution of 50 μg/mL in PBS, pH 6.8, containing 0.1 mM $Ca^{2+}$and 0.1 mM Mn^{2+}) was added. The mixtures of glycoclusters or methyl α-D-mannopyranoside and the peroxidase-labeled lectin (100 μl/well) were added and the plates were incubated for 2 h at 37 °C. After that, 50μL/well of a solution of *o*-phenylenediamine dihydrochloride (20 mg/50 mL) in citrate–phosphate buffer (pH 5.0 with 0.4% H_2O_2) was added. The plates were incubated for 30 min at 37 °C. The reactions were stopped by the addition of aq H_2SO_4 (50 μL/well, 1.25 M) and the absorbance measured at 492 nm.

2-Propynyl 2,3:4,6-Di-*O*-isopropylidene-α-D-mannopyranoside (2)

A solution of 2-propynyl α-D-mannopyranoside[24] (3.33 g, 15.7 mmol) in anhyd acetone (50 mL), 2,2-dimethoxypropane (10 mL), and camphor-10-sulfonic acid (25 mg) was kept at r.t. for 24 h. Addition of Et_3N was followed by evaporation and purification of the crude product by column chromatography (hexane–Et_2O, 3:1) to give **2** (3.31 g, 73%) as a solid; mp 96–98 °C; $[\alpha]_D$ +45 (*c* 1, $CHCl_3$).

IR (KBr): 3259, 2126, 1381, 1268, 1220, 1090, 1044, 853 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.24 (s, 1 H, H-1), 4.25 (d, 2 H, *J* = 2.4 Hz, $CH_2C{\equiv}$), 4.20 (d, 1 H, *J* = 5.7 Hz, H-2), 4.15 (dd, 1 H, *J* = 7.6, 5.7 Hz, H-3), 3.89 (dd, 1 H, *J* = 10.7, 5.6 Hz, H-6), 3.76 (t, 1 H, *J* = 10.4 Hz, H-6′), 3.75 (dd, 1 H, *J* = 10.0, 7.7 Hz, H-4), 3.58 (dt, 1 H, *J* = 10.1, 5.6 Hz, H-5), 2.47 (t, 1 H, *J* = 2.4 Hz, C≡CH), 1.56, 1.52, 1.43 and 1.36 (4 s, 12 H, CMe_2).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 109.6 (CMe_2), 99.8 (CMe_2), 96.5 (C-1), 75.9 (C-2), 75.1 (*C*≡CH), 74.8 (C-3), 72.6 (C-4), 62.0 (C-6), 61.8 (C-5), 54.4 (CH_2O), 29.1, 28.2, 26.2, 18.8 [2 × C(*C*H$_3$)$_2$].

Anal. Calcd for $C_{15}H_{22}O_6$: C, 60.39, H, 7.43. Found: C, 60.77, H, 7.80.

Ethyl *p-tert*-Butylphenyloxyacetate (4)

To a solution of *p-tert*-butyl phenol (2 g, 13.3 mmol) and ethyl bromoacetate (10 mL, 40 mmol) in anhyd CH_3CN (15 mL) was added anhyd K_2CO_3 (3.7 g, 26.4 mmol). The reaction was kept at r.t. for 18 h. Filtration was followed by evaporation under vacuum of the solvent giving a residue that was purified by column chromatography ($CHCl_3$–hexane, 1:1) and gave **4** (2.74 g, 87.3%) as a liquid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31(d, 2 H, *J* = 8.8 Hz), 6.85 (d, 2 H, *J* = 8.8 Hz), 4.60 (s, 2 H), 4.27 (q, 2 H, *J* = 7.1 Hz), 1.30 (s, 9 H), 1.30 (t, 3 H, *J* = 7.1 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.1, 155.6, 144.4, 126.3, 114.1, 65.6, 61.2, 34.1, 31.5, 14.1.

Compounds 8, 10, 12, 14, and 16; General Procedure

BuLi (1.6 M in hexane, 4.5 mL, 6.4 mmol) was added to a cooled (0 °C) solution of **2** (2.0 g, 6.71 mmol) in anhyd THF (10 mL) and the reaction was stirred under argon at 0 °C for 1 h. The corresponding ester [methyl benzoate (**3**), ethyl *p-tert*-butylphenyloxyacetate (**4**), dimethyl terephthalate (**5**), dimethyl isophthalate (**6**), or 1,3,5-trimethoxycarbonylbenzene (**7**)] (0.84 mmol) in THF (4.5 mL) was then added. The reaction was allowed to warm to r.t. and left under argon for 24 h. The reaction was cooled to 0 °C and quenched by the addition of sat. aq NH_4Cl (50 mL). The layers were separated and the aqueous phase was extracted with EtOAc (2 × 50 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed in vacuo to yield a crude product that was purified by column chromatography on silica gel.

4-Phenyl-1,7-bis-*O*-[2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl]hep-2,5-diyn-1,4,7-triol (8)

Column chromatography (Et_2O–hexane, 1:1) gave **8** (0.58 g, 98.5%) as a solid: mp 96–97 °C; $[\alpha]_D$ +61 (*c* 1, $CHCl_3$).

IR (KBr): 3415, 3428, 1390, 1224, 1079, 859 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (m, 2 H), 7.37 (m, 3 H), 5.20, 5.18 (2 s, 2 H), 4.34 (s, 4 H), 4.17 (dd, 2 H, *J* = 7.3, 5.7 Hz), 4.13 (d, 2 H, *J* = 5.7 Hz), 3.92 (dd, 1 H, *J* = 10.7, 5.6 Hz), 3.90 (dd, 1 H, *J* = 10.6, 5.6 Hz), 3.74 (br t, 2 H, *J* = 10.0 Hz), 3.74 (dd, 2 H, *J* = 10.0, 7.5 Hz), 3.65–3.55 (m, 2 H), 3.13 (s, 1 H), 1.55, 1.51, 1.42, 1.35 (4 s, 24 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 141.3, 128.8, 128.6, 125.7, 109.5, 99.8, 97.0, 96.8, 86.6, 86.5, 80.3, 80.2, 75.8, 74.7, 72.8, 72.6, 64.8, 61.8, 54.8, 54.7, 29.0, 28.2, 26.2, 18.8.

HMRS (FAB): *m*/*z* calcd for $C_{37}H_{48}O_{13}Na$ (M + Na)$^+$, 723.2993; found, 723.2984.

Anal. Calcd for $C_{37}H_{48}O_{13}$: C, 63.42, H, 6.90. Found: C, 63.93, H, 7.42.

1,7-Bis-*O*-[2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl] 4-(*p*-4-*tert*-butylphenyloxymethyl)-hep-2,5-diyn-1,4,7-triol (10)
Column chromatography (Et_2O–hexane, 3:1) of the crude gave **10** (0.63 g, 95%) as a solid; mp 58–60 °C; $[\alpha]_D$ +36 (*c* 1, $CHCl_3$).

IR (KBr): 3422, 1514, 1460, 1383, 1254, 1084, 1022, 860 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.32 (d, 2 H, *J* = 8.8Hz), 6.93 (d, 2 H, *J* = 8.8Hz), 5.21, 5.20 (2 s, 2 H), 4.30 (s, 2 H), 4.21–4.12 (m, 8 H, H-2,3), 3.94 (dd, 1 H, *J* = 10.6, 5.4 Hz), 3.93 (dd, 1 H, *J* = 10.6, 5.4 Hz), 3.76 (br t, 2 H, *J* = 10.5 Hz), 3.75 (dd, 2 H, *J* = 10.0, 7.5 Hz), 3.65–3.56 (m, 2 H), 3.28 (s, 1 H), 1.56, 1.52, 1.43, 1.36 (4 s, 24 H), 1.30 (s, 9 H).

^1H NMR (400 MHz, DMSO-d_6): δ = 7.28 (d, 2 H, *J* = 8.8 Hz), 6.92 (d, 2 H, *J* = 8.8 Hz), 6.88 (s, 1 H), 5.21 (s, 2 H), 4.35 (AB system, 4 H, *J* = 16.2 Hz, Δδ = 18 Hz), 4.15 (d, 2 H, *J* = 5.6 Hz), 4.03 (br s, 2 H), 4.00 (dd, 2 H, *J* = 8.0, 5.7 Hz), 3.92 (dt, 2 H, *J* = 10.0, 5.3 Hz), 3.71–3.65 (m, 4 H), 3.40 (dt, 2 H, *J* = 10.1, 5.5 Hz), 1.45, 1.44, 1.30, 1.29 (4 s, 24 H), 1.25 (s, 9 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.9, 144.4, 126.2, 114.7, 109.5, 99.7, 96.8, 96.7, 84.0, 79.9, 79.8, 75.8, 74.7, 72.6, 62.7, 61.8, 54.6, 54.5, 34.1, 31.5, 29.0, 28.2, 26.2, 18.8.

HMRS (FAB): *m*/*z* calcd for $C_{42}H_{58}O_{14}Na$ $(M + Na)^+$, 809.3724; found, 809.3736.

Anal. Calcd for $C_{42}H_{58}O_{14}$: C, 64.11; H, 7.43. Found: C, 64.41; H, 7.53.

1,4-Bis-[1,7-di-*O*-(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)hep-2,5-diyn-1,4,7-triol-4-yl]benzene (12)
Column chromatography (Et_2O–hexane, 3:1) of the crude gave **12** (0.75 g, 67.5%) as a solid; mp 140–141 °C; $[\alpha]_D$ 34 (*c* 1, $CHCl_3$).

IR (KBr) 3417, 1460, 1383, 1221, 1076, 1043, 860 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.80 (s, 4 H), 5.17 (s, 4 H), 4.40–4.28 (m, 8 H), 4.20–4.11 (m, 8 H), 3.80–3.50 (m, 18 H), 1.55, 1.51, 1.43, 1.36 (4 s, 48 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.1, 126.2, 109.7, 99.9, 97.4, 86.1, 80.7, 75.9, 74.9, 72.7, 64.8, 61.9, 55.1 ($CH_2C{\equiv}CH$), 29.1, 28.4, 26.3, 18.9 [$C(CH_3)_2$].

HMRS (FAB): *m*/*z* calcd for $C_{68}H_{90}O_{26}Na$ $(M + Na)^+$, 1345.5618; found, 1345.564.

Anal. calcd for $C_{68}H_{90}O_{26}$: C, 61.71; H, 6.85. Found: C, 61.85; H, 6.95.

1,3-Bis-[1,7-di-*O*-(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)hep-2,5-diyn-1,4,7-triol-4-yl]benzene (14)
Column chromatography (Et_2O–hexane, 1:1) of the crude gave **14** (1.09 g, 98%) as a solid; mp 130–131 °C; $[\alpha]_D$ +30 (*c* 1, $CHCl_3$).

IR (KBr): 3421, 1383, 1221, 1089, 1043, 859 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 8.25 (t, 1 H, *J* = 1.7 Hz), 7.75 (dd, 2 H, *J* = 1.8, 7.8 Hz), 7.45 (t, 1 H, *J* = 7.8 Hz), 5.18, 5.17 (2 s, 4 H), 4.37 (AB system, 4 H, *J* = 16 Hz, Δδ = 27.0 Hz), 4.36 (AB system, 4 H, *J* = 15.9 Hz, Δδ = 19.0 Hz), 4.21 (s, 2 H), 4.19 (d, 4 H, *J* = 5.7 Hz), 4.15 (dd, 4 H, *J* = 11.8, 5.7 Hz), 3.83 (dd, 4 H, *J* = 10.6, 5.1 Hz), 3.74–3.56 (m, 12 H), 1.55, 1.51, 1.50, 1.44, 1.42, 1.36, 1.34 (7 s, 48 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.1, 129.0, 126.6, 122.6, 109.7, 99.9, 97.4, 97.1, 86.4, 86.2, 80.9, 80.5, 75.9, 74.8, 72.6, 61.9, 61.8, 64.9, 65.9, 55.3, 54.9, 29.1, 28.3, 26.2, 18.9.

HMRS (FAB): *m*/*z* calcd for $C_{68}H_{90}O_{26}Na$ $(M + Na)^+$, 1345.5618; found, 1345.5629.

Anal. Calcd for $C_{68}H_{90}O_{26}$: C, 61.71; H, 6.85. Found: C, 61.76; H 6.87.

1,3,5-Tris-[1,7-di-*O*-(2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)hep-2,5-diyn-1,4,7-triol-4-yl]benzene (16)
Column chromatography (Et_2O–hexane, 3:1) of the crude gave **16** (1.48 g, 76%) as a solid; mp 128–131 °C; $[\alpha]_D$ +36 (*c* 1, $CHCl_3$).

IR (KBr): 3422, 1373, 1220, 1116, 1079, 1043, 1023, 859 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 8.2 (s, 3 H), 5.20 (s, 6 H), 4.45–4.3 (m, 15 H), 4.20–4.14 (m, 12 H), 3.9–3.5 (m, 24 H), 1.55, 1.50, 1.43, 1.36 (4 s, 72 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.3, 123.8, 109.7, 99.9, 97.2, 97.0, 86.3, 86.1, 80.9, 80.7, 75.9, 74.8, 72.6, 61.8, 61.7, 64.7, 55.1, 54.9, 29.1, 28.3, 26.3, 18.9.

HMRS (FAB): *m*/*z* calcd for $C_{99}H_{132}O_{39}Na$ $(M + Na)^+$, 1968.828; found, 1968.831.

Anal. Calcd for $C_{99}H_{132}O_{39}$: C, 61.10; H, 6.84. Found: C, 61.17; H, 7.42.

Synthesis of the Hydroxylated Derivatives 9, 11, 13, 15, and 17; General Procedure
The corresponding glycocluster (**8**, **10**, **12**, **14**, or **16**, 0.075 mmol) was suspended in HOAc (70%, 5 mL). The reaction mixture was kept at r.t. under magnetic stirring for 12 h. After this time the solvent was removed in vacuo and the crude product was coevaporated with H_2O (3 × 5 mL). The crude product was then dissolved in H_2O (5 mL) and the solution was concentrated by liophilitation. The corresponding deprotected compounds (**9**, **11, 13**, **15**, and **17**) were isolated in almost quantitative yields.

4-Phenyl-1,7-bis-*O*-[α-D-mannopyranosyl]hep-2, 5-diyn-1,4,7-triol (9)
Syrup; $[\alpha]_D$ +51 (*c* 1, pyridine).

IR (KBr): 3399, 1069, 975, 815, 760, 697 cm^{-1}.

^{13}C NMR (75 MHz, D_2O): δ = 137.3, 130.5, 130.3, 127.2, 100.7, 88.5, 82.5, 74.7, 72.0, 71.5, 67.9, 62.1, 56.2.

MS (FAB): *m*/*z* calcd for $C_{25}H_{32}O_{13}Na$ $(M + Na)^+$, 563.1741; found, 563.1741.

1,7-Bis-*O*-[α-D-mannopyranosyl]4-(*p*-4-*tert*-butylphenyloxymethyl)hep-2,5-diyn-1,4,7-triol (11)
Syrup; $[\alpha]_D$ +135 (*c* 1, pyridine).

^{13}C NMR (75 MHz, D_2O): δ = 156.1, 144.0, 126.2, 114.9, 99.3, 98.7, 84.2, 84.1, 80.3, 73.1, 70.6, 70.4, 70.0, 69.9, 66.6, 66.4, 61.0, 62.1, 60.8, 54.7, 54.5, 54.4, 33.7, 31.3.

HRMS: *m*/*z* calcd for $C_{30}H_{42}O_{14}Na$ $(M + Na)^+$, 649.2472; found, 649.2473.

1,4-Bis-[1,7-di-*O*-α-D-mannopyranosyl)hep-2,5-diyn-1,4,7-triol-4-yl]benzene (13)
Solid; mp 158 °C; $[\alpha]_D$ +10 (*c* 1, pyridine).

IR (KBr): 3420, 1261, 1097, 1022, 801 cm^{-1}.

^{13}C NMR (75 MHz, D_2O): δ = 142.13, 126.4, 99.6, 85.9, 81.4, 73.4, 70.8, 70.3, 66.7, 64.2, 60.9, 55.1.

HRMS (MALDI-TOF): *m*/z calcd for $C_{44}H_{58}O_{26}Na$, 1025.3114; found, 1025.332 $(M + Na)^+$.

1,3-Bis-[1,7-di-*O*-α-D-mannopyranosyl)hep-2,5-diyn-1,4,7-triol-4-yl]benzene (15)
Solid; mp 200 °C (dec); $[\alpha]_D$ +52 (*c* 1, pyridine).

IR (KBr): 3390, 1561, 1423, 1060, 811, 671 cm^{-1}.

^{13}C NMR (75 MHz, D_2O): δ = 141.9, 129.9, 126.8, 123.1, 99.7, 85.9, 81.6, 73.4, 70.8, 70.3, 66.7, 64.4, 60.9, 55.1.

HRMS FAB (pos.): *m*/*z* calcd for $C_{44}H_{58}O_{26}Na$ $(M + Na)^+$, 1025.3114; found, 1025.322.

1,3,5-Tris-[1,7-di-*O*-(α-D-mannopyranosyl)hep-2,5-diyn-1,4,7-triol-4-yl]benzene (17)

Solid; mp 137 °C; $[\alpha]_D$ +5.6, $[\alpha]_{436}$ +13.0 (*c* 1, pyridine).

IR (KBr): 3410, 1439, 1379, 1246, 1070, 811 cm^{-1}.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.9, 124.0, 99.8, 85.5, 82.0, 73.4, 70.8, 70.3, 66.7, 64.3, 60.9, 55.2.

HRMS (MALDI-TOF): *m*/*z* calcd for $C_{63}H_{84}O_{39}Na$, 1487.448; found, 1487.622 $(M + Na)^+$.

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