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Enhancement of Plant and Bacterial Lectin Binding Affinities by Three-Dimensional Organized Cluster Glycosides Constructed on Helical Poly(phenylacetylene) Backbones

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A series of poly(phenylacetylene)s bearing diverse saccharide pendants—*N*-acetyl-D-glucosamine, D-lactose, and *N*-acetyl-D-neuraminic acid—were synthesized by rhodium-mediated polymerizations of the corresponding acetyl-protected glycosylated phenylacetylenes followed by deprotection. The circular dichroism spectra of these glycosylated poly(phenylacetylene)s each displayed split-type Cotton effects in the long absorption region of the conjugated polymer backbone (260–500 nm), thus indicating predominantly one-handed helical conformations in their backbones. The binding affinities of these glycosylated poly(phenylacetylene)s, and those of previously reported phenylacetylenes bearing D-galactose, towards plant and bacterial lectins were investigated by hemagglutination inhibition assay and isothermal titration calorimetry (ITC). The stoichiometries of binding vary strongly, depending on the lectin binding sites and the accessibilities of the carbohydrate residues in the helices. The measured affinities also vary, with the maximum value observed for the interaction between poly-PA-α-Gal and lectin I from *Pseudomonas aeruginosa*, with a K_d value of 4 μM per monosaccharide representing a 200-fold increase relative to the corresponding monomer.

Introduction

Carbohydrates play important roles on cellular surfaces as recognition ligands for a variety of receptor proteins, such as pathogens, enzymes, and antibiotics in vivo.[1–3] The multivalent structure of a carbohydrate on a cellular surface compensates for weak individual protein–carbohydrate interactions. A number of multivalent or clustered glyco-ligands have been synthesized for biomimetic purposes, and their binding properties for carbohydrate-binding proteins (lectins) have been studied in vitro. Most of these artificial glyco-ligands display some enhancements in affinity for proteins, due to their multivalent carbohydrate structures: the so-called "cluster glycoside effect".[4–8] The strong affinities of these glyco-ligands for lectins are of great interest in pharmaceutical applications, such as targeting drug delivery and pathogen-sensing. Several glyco-ligands, such as glycosylated dendritic ligands, linear polymeric ligands bearing glycosyl pendants, and self-assembled nanoparticles of amphiphilic glycopolymers, have therefore been designed for these applications.[8–21] Artificial multivalent glyco-ligands have also been utilized for studying the cluster glycoside effect. It has been reported that the conformations of the backbones and the saccharide residues of the glyco-ligands, factors such as rigidity and three-dimensional organization of the polymeric framework, significantly affect the multivalency effect in carbohydrate–lectin interactions.[22–27] However, the detailed mechanism of this effect is still unclear. It is therefore of great interest to synthesize glyco-ligands with highly controlled conformations and to evaluate their affinities for lectins both from theoretical and from experimental perspectives.

Poly(phenylacetylene) is one of the most promising polymer backbones for a three-dimensional controlled framework for glyco-ligands. Poly(phenylacetylene)s feature predominantly one-handed (excess right- or left-handed) helical conformations, determined by the chiralities of the side-chain groups. The chiralities of the pendant groups transfer to the main chain, resulting in the three-dimensional organization of the pendant groups on the preferred-handed helical backbone.[28] Previously, our research group reported on three-dimensionally organized glucose (Glc) and galactose (Gal) ligands on helical poly(phenylacetylene) backbones. The phenylacetylenes bearing D-galactose pendants (poly-PA-α-Gal and poly-PA-β-Gal) showed high affinities for peanut agglutinin, due to the cluster glycoside effect, and the affinities were strongly affected by the backbone chirality based on the one-handed helical conformations.[27]

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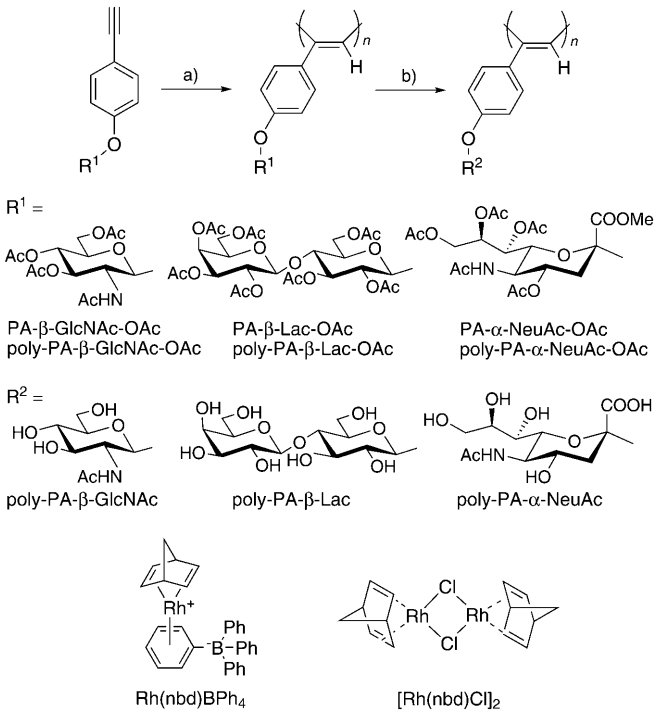
Lectins are sugar-binding proteins that specifically recognize complex carbohydrates without modifying them. They exist in most living organisms, ranging from viruses and bacteria to plants and animals.[3,29] Most of the reports on the cluster glycoside effect, however, have dealt with the interactions with plant lectins, so evaluating the interactions between multivalent glyco-ligands and lectins of other origins is of great importance. In this study, a bacterial lectin produced by *Pseudomonas aeruginosa*—PA-IL, specific for Gal—was used for the evaluation of carbohydrate–lectin interactions along with other plant lectins: that is, lectins from wheat germ (WGA) specific for *N*-acetyl-D-glucosamine (GlcNAc) and *N*-acetyl-D-neuraminic acid (NeuAc), and from *Erythrina cristagalli* (ECA) specific for lactose (Lac). *Pseudomonas aeruginosa* is an opportunistic bacterium responsible for numerous nosocomial infections in immunocompromised patients and cystic fibrosis patients.[30] PA-IL is a virulence factor and its inhibition by glyco-compounds has been demonstrated to block lung infection in murine models.[31] Glyco-ligands with high affinities for PA-IL are thus always expected to be antimicrobial agents against these diseases.

Here we report: 1) the synthesis of poly(phenylacetylene)s bearing β-GlcNAc (poly-PA-β-GlcNAc), β-Lac (poly-PA-β-Lac), and α-NeuAc (poly-PA-α-NeuAc) residues, with the aid of rhodium catalysts (Schemes 1 and 2), 2) the predominately one-handed helical conformations of these glycosylated poly(phenylacetylene)s as determined by CD analysis, and 3) the high affinities of these glycosylated poly(phenylacetylene)s and the previously reported poly-PA-α-Gal and poly-PA-β-Gal towards the lectins, characterized by hemagglutination inhibition assay, ITC, and molecular modeling.

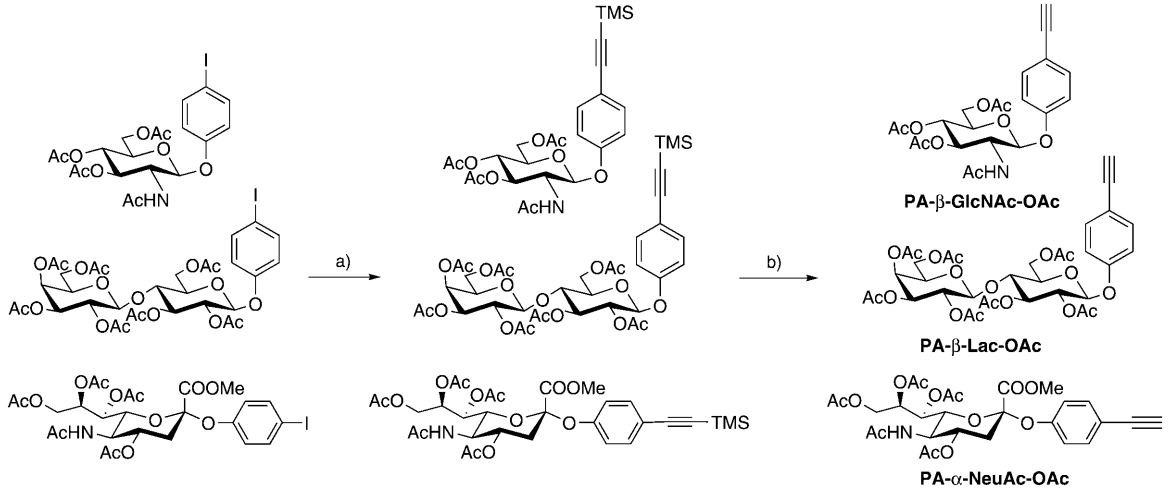


Scheme 2. Synthesis of acetyl-protected glycosylated phenylacetylene monomers. Conditions: a) trimethylsilylacetylene, CuI, $(Ph_3P)_2PdCl_2$, Et_3N, RT; b) Bu_4NF, CH_2Cl_2/THF, RT.

Results and Discussion

Synthesis of glycosylated poly(phenylacetylene)s

A series of acetyl-protected glycosylated phenylacetylene monomers (PA-β-GlcNAc-OAc, PA-β-Lac-OAc, and PA-α-NeuAc-OAc) were synthesized by Sonogashira–Hagiwara coupling reactions between trimethylsilylacetylene and the previously reported *para*-iodophenyl glycosides and subsequent removal of the TMS groups, as shown in Scheme 1. The obtained monomers



Scheme 1. Synthesis of glycosylated poly(phenylacetylene)s. Conditions: a) $Rh(nbd)BPh_4$ or $[Rh(nbd)Cl]_2$/triethylamine, THF or CH_2Cl_2, 30 °C; b) CH_3ONa, NaOH, THF or DMSO, RT.

were then polymerized through the use of $Rh(nbd)BPh_4$ or $[Rh(nbd)Cl]_2$ with triethylamine as catalysts in $CHCl_3$ or THF. All the polymerizations proceeded homogeneously to yield yellowish-brown viscous products that were purified by reprecipitation from methanol to give the acetyl-protected glycosylated poly(phenylacetylene)s (poly-PA-β-GlcNAc-OAc, poly-PA-β-Lac-OAc, and poly-PA-α-NeuAc-OAc). The results of the polymerizations are listed in Table 1. The obtained polymers had large number

Table 1. Polymerizations of acetyl-protected glycosylated phenylacetylenes.[a]

Polymer	Catalyst	Solvent	$M_n \times 10^{4}$[b]	M_w/M_n[b]
poly-PA-β-Lac-OAc	$[Rh(nbd)Cl]_2$/TEA	$CHCl_3$	7.81	3.79
poly-PA-β-GlcNAc-OAc	$Rh(nbd)BPh_4$	$CHCl_3$	13.4[c]	10.0[c]
poly-PA-α-NeuAc-OAc	$Rh(nbd)BPh_4$	THF	7.94	2.16

[a] Conditions: [monomer] = 0.1 M.; [monomer]/[Rh] = 50; temp, 30 °C; time, 20 h. [b] Determined by SEC. [c] Calculated on the $CHCl_3$-soluble part.

average molecular weights (M_n) and polydispersities (M_w/M_n) as determined by SEC analysis.[32] Figure 1 shows the ^1H NMR spectra of the obtained polymers. Broadened signals attributable to the protons due to the sugar moiety (about 3–6 ppm), the acetyl groups (about 2 ppm), and the aromatic groups (about 6–7 ppm) were observed in the spectra, so the obtained polymers were assignable as glycosylated poly(phenylacetylene)s. The acetyl-protected polymers were then treated with base for deprotection. The IR spectra of the deprotected

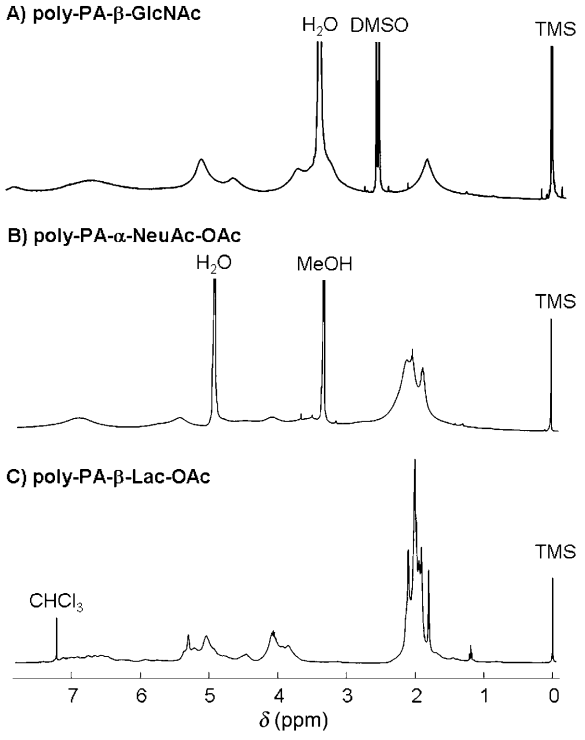


Figure 1. ^1H NMR spectra of: A) poly-PA-β-GlcNAc in $[D_6]$DMSO, B) poly-PA-α-NeuAc-OAc in CD_3OD, and C) poly-PA-β-Lac-OAc in $CDCl_3$.

polymers showed characteristic signals attributable to the hydroxy groups, due to the deacetylated sugar moieties, around 3300–3600 cm^{-1}, rather than those attributable to the acetyl groups of the acetyl-protected polymers, observed at 1748 cm^{-1} (Figure 2). The obtained products were thus assign-

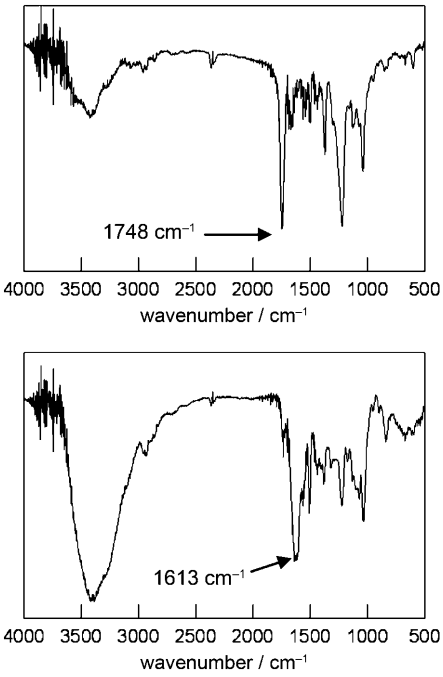


Figure 2. IR spectra of: top) poly-PA-α-NeuAc-OAc, and bottom) poly-PA-α-NeuAc.

able as poly(phenylacetylene)s with GlcNAc, Lac, and NeuAc as pendant groups (poly-PA-β-GlcNAc, poly-PA-β-Lac, and poly-PA-α-NeuAc).

Chiroptical properties of glycosylated poly(phenylacetylene)s

The structural conformations of the glycosylated poly(phenylacetylene)s were investigated through their CD and ultraviolet/visible (UV/Vis) spectra. Figure 3 shows the CD and UV/Vis spectra of poly-PA-β-GlcNAc, poly-PA-β-Lac, and poly-PA-α-NeuAc in H_2O and DMSO at room temperature. These glycosylated poly(phenylacetylene)s each displayed split-type Cotton effects in the long absorption region of the conjugated polymer backbone (260–500 nm): poly-PA-β-GlcNAc in H_2O, for example, displayed a first negative Cotton effect at 411 nm, a second positive one at 355 nm, a third negative one at 323 nm, and a fourth positive one at 281 nm. The chiroptical properties of the backbones evidently demonstrate conformational chirality and thus one-handed helical conformations of the π-conjugated double bonds in the backbones. The biased helical conformations of these glycosylated poly(phenylacetylene)s were, as we previously reported,[27] dictated by the pendant sugar functionalities and solvents, resulting in a variety of CD and UV/Vis profiles.

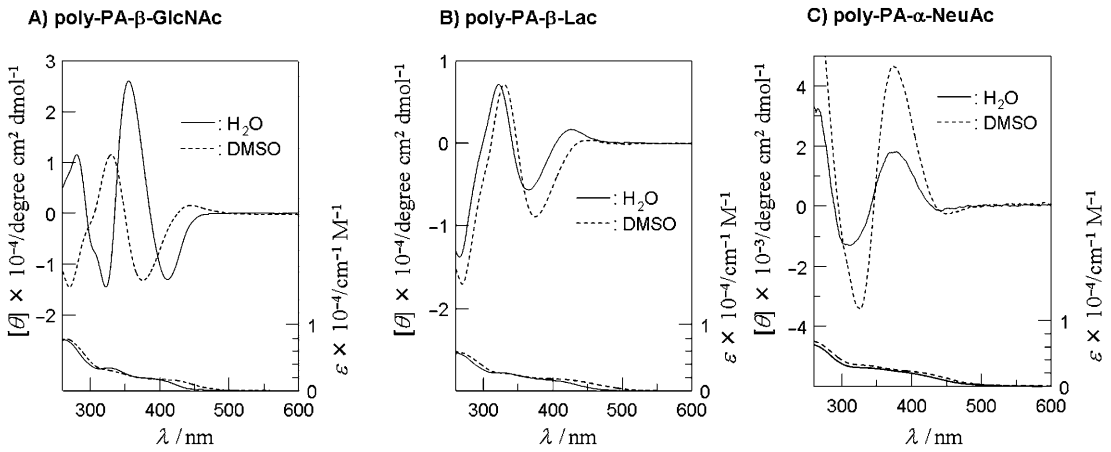


Figure 3. CD (upper) and UV/Vis (lower) spectra of: A) poly-PA-β-GlcNAc, B) poly-PA-β-Lac, and C) poly-PA-α-NeuAc in H_2O and DMSO at room temperature. [θ] is molar ellipticity.

Interactions of glycosylated poly(phenylacetylene)s with lectins: hemagglutinating inhibition assay

The binding affinities between the glycosylated poly(phenylacetylene)s and the lectins were investigated by hemagglutinating inhibition assay. The glycosylated poly(phenylacetylene)s and the corresponding monovalent glyco-ligands bind with specific lectins and thereby inhibit the agglutination between the lectins and rabbit red cells. Previously we had reported[27] that glycosylated poly(phenylacetylene)s bearing α-D-galactopyranose (poly-PA-α-Gal) and β-D-galactopyranose (poly-PA-β-Gal) derivatives showed higher affinities towards plant lectins—peanut agglutinin (PNA: specific for Gal)—than the corresponding monovalent glyco-ligands, due to multivalent carbohydrate–lectin interactions. It is therefore of great interest to compare the results obtained for the binding affinities with the plant lectin and the bacterial lectin. Binding affinities were evaluated by comparing the minimum concentrations of the different glyco-ligands needed to inhibit the agglutination (IC_{min}), as listed in Table 2 in the form of the concentrations of the glycosylated poly(phenylacetylene)s calculated on the basis of the glycosylated phenylacetylene monomer. All glyco-ligands tested demonstrated inhibition of agglutination, except for the NeuAc-containing ligands, which had no effect at the concentrations tested here. Indeed, the binding affinity between NeuAc and WGA is not strong enough to inhibit hemagglutination under these concentrations. In all other cases, the glycosylated poly(phenylacetylene)s showed lower IC_{min} values than the corresponding monovalent glyco-ligands. The IC_{min} value of poly-PA-β-GlcNAc (2×10^{-5} M) was much lower than that of β-Ph-GlcNAc (1.1×10^{-2} M), indicating an increase in affinity of three orders of magnitude for poly-PA-β-GlcNAc towards WGA in relation to β-Ph-GlcNAc. A similar effect is observed for the bacterial lectin PA-IL. Poly-PA-β-Gal ($IC_{min}=2\times10^{-5}$ M) has a much stronger affinity (two orders of magnitude) towards PA-IL than β-NPh-Gal ($IC_{min}=4.1\times10^{-3}$ M). The poly-PA-α-Gal is an even better ligand, because this compound can inhibit the hemagglutation by PA-IL at micromolar concentrations ($IC_{min}=9\times10^{-6}$ M). However, when the binding of poly-PA-β-LacNAc to ECA is analyzed, the increase is only one order of magnitude, indicating that this effect depends on the architecture of the target lectin. These multiplications of the binding affinities observed in the glycosylated poly(phenylacetylene)s in relation to the monovalent glyco-ligands should be explained by the cluster glycoside effect: that is, enhancement of the binding activities of glyco-ligands through the multivalent bindings of saccharides to lectins achieved by the highly clustered saccharide pendants along the poly(phenylacetylene) backbones.

Table 2. Hemagglutination inhibition properties of glycosylated poly(phenylacetylene)s.[a]

Inhibitor (saccharide)	Lectin	$IC_{min}\times10^3$ [M]
β-Ph-GlcNAc	WGA	11
poly-PA-β-GlcNAc	WGA	0.02
α-Ph-NeuAc	WGA	N.I.[b]
poly-PA-α-NeuAc	WGA	N.I.[b]
β-Ph-Lac	ECA	1.5
poly-PA-β-Lac	ECA	0.2
β-NPh-Gal	PA-IL	4.1
poly-PA-α-Gal	PA-IL	0.009
poly-PA-β-Gal	PA-IL	0.02

[a] [Lectins] = 4×[minimum concentration required for hemagglutination]. [b] Not inhibited by 6.5 mM for α-Ph-Neu and 6.1 mM for poly-PA-α-NeuAc.

Interactions of glycosylated poly(phenylacetylene)s with lectins: isothermal titration calorimetry (ITC)

Further insight into the binding affinities between the glycosylated poly(phenylacetylene)s and the lectins was investigated by ITC analysis. ITC is a direct method for measuring thermal changes in the binding between the glyco-ligands and the lectins, which allows determination of the association and dissociation constants (K_a and K_d), reaction stoichiometry (n), enthalpy change (ΔH), free energy change (ΔG), and entropy change (ΔS).[33] ITC measurements were performed by addition of solu-

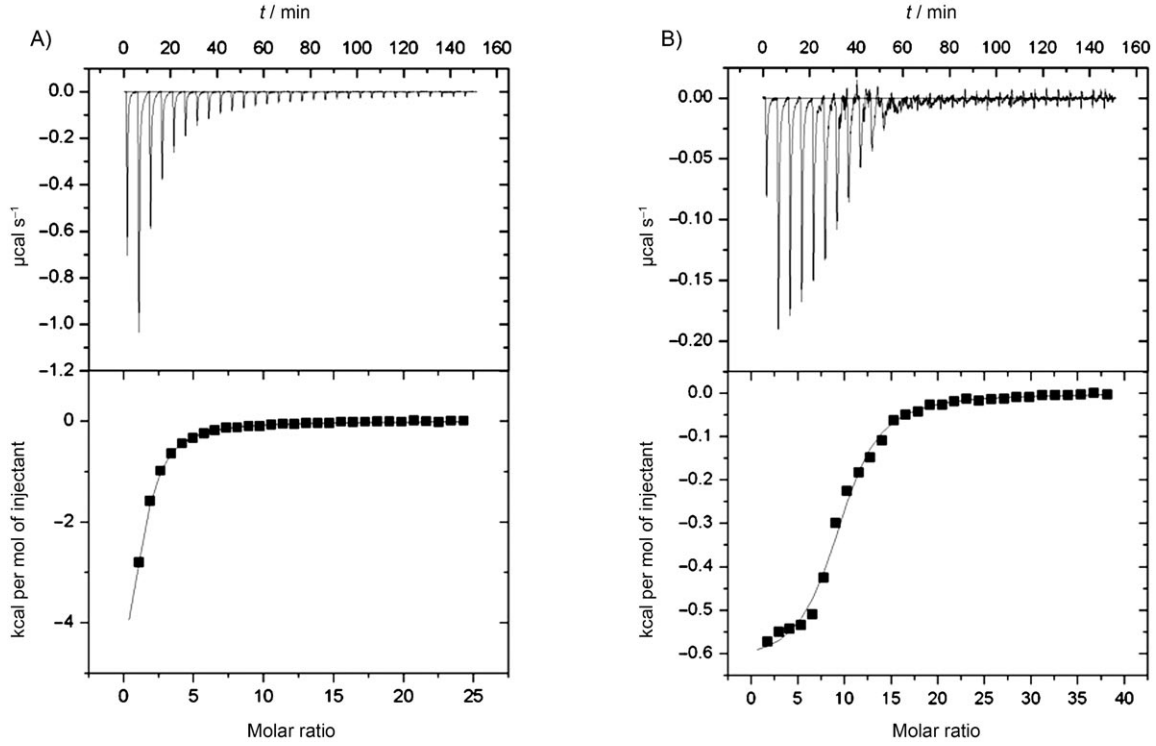


Figure 4. Binding thermogram (top) and isotherm (bottom) for: A) the addition of a solution of β-NPh-Gal (1.7 mM) to a PA-IL solution (16 μM), and B) the addition of a solution of poly-PA-α-Gal (1.7 mM) to a PA-IL solution (10 μM) at 298 K.

tions of glyco-ligands to solutions of lectins in the microcalorimeter cell. Figure 4 shows typical ITC thermograms of titrations of PA-IL by β-NPh-Gal and poly-PA-α-Gal. A strong exothermic effect due to the binding between PA-IL and the glyco-ligands was observed. The magnitude of this effect decreased along with progress of the titration, which corresponds to the occupancy of the binding sites of PA-IL. The very small exothermic signals observed at the ends of the titrations indicate that the binding of PA-IL to the glyco-ligands is complete. The thermodynamic parameters were calculated from the thermogram on the basis of the simplest model of binding ("one set of binding sites" model provided from the ORIGIN software) with the assumption that the individual glyco-ligands bind to the individual discrete binding sites of the lectins in a non-cooperative fashion.

Table 3 summarizes the thermodynamic parameters calculated from the ITC analysis of the binding reactions between the glyco-ligands and the lectins. Here, the reaction stoichiometry (*n*) is defined as the number of the monomeric glyco units per monomeric subunit of the lectin. It should be noted that the *n* values of the monovalent glyco-ligands relative to the lectins were fixed to the number of binding sites observed per monomer in the crystal structures ($n=1$ for ECA and PA-IL, $n=2$ for WGA).[34–36] Interestingly, all the glycosylated poly(phenylacety-

Table 3. Thermodynamic parameters calculated from ITC and expressed per saccharide unit[a]

Saccharide	Lectin	$K_a\times10^{-3}$ [M^{-1}]	*n* (saccharide/lectin)	ΔH [kJ mol^{-1}]	K_d [μM]	ΔG [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]
β-Ph-GlcNAc[b]	WGA[f]	1.75(±0.02)	2[j]	−117(±1)	572	−18.5	−98.8
poly-PA-β-GlcNAc[b]	WGA[f]	14.4(±1.4)	47.7(±0.8)	−2.85(±0.07)	69.4	−23.7	20.8
β-Ph-Lac[c]	ECA[g]	4.1(±1.4)	1[j]	−37.6(±0.1)	245	−20.6	−17.0
poly-PA-β-Lac[c]	ECA[g]	2.6(±0.2)	9.7(±0.6)	−2.54(±0.18)	389	−19.5	16.9
α-Ph-NeuAc[d]	WGA[h]	0.24(±0.00)	2[j]	−66.3(±0.7)	3998	−13.6	−52.6
poly-PA-α-NeuAc[d]	WGA[h]	0.51(±0.06)	77.4(±6.2)	−0.47(±0.05)	1960	−14.8	12.3
β-NPh-Gal[e]	PA-IL[i]	75.0(±1.7)	1[j]	−33.9(±0.3)	13.3	−27.8	−6.08
poly-PA-α-Gal[e]	PA-IL[i]	243(±37)	10.1(±0.2)	−2.13(±0.05)	4.12	−30.7	28.7
poly-PA-β-Gal[e]	PA-IL[i]	198(±29)	7.3(±0.1)	−2.56(±0.05)	5.05	−30.2	27.7

[a] The parameters are average values of two experiments. [b] [β-Ph-GlcNAc] and [poly-PA-β-GlcNAc] = 6.0 mM. [c] [β-Ph-Lac] and [poly-PA-β-Lac] = 12.7 mM. [d] [α-Ph-Neu] and [poly-PA-α-Neu] = 26.0 mM. [e] [β-NPh-Gal], [poly-PA-α-Gal], and [poly-PA-β-Gal] = 1.70 mM. [f] [WGA] = 0.013 mM. [g] [ECA] = 0.038 mM. [h] [WGA] = 0.024 mM. [i] [PA-IL] = 0.016 and 0.010 mM. [j] The stoichiometries of the monovalent glyco-ligands to the lectins were fixed according to the crystal structures.

lene)s showed large *n* values against the plant and bacterial lectins but with significant differences, with the *n* values varying from 7 for the interaction of poly-PA-β-Gal with PA-IL to 48 for the interaction of poly-PA-β-GlcNAc with WGA. Because the lectins are not strongly different in terms of size (tetramer of 12.75 kDa for PA-IL, dimer of 26.2 kDa for ECA, and dimer of 43.2 kDa for WGA), the differences in stoichiometry probably reflect the accessibilities of the monosaccharides, as functions both of the conformations of the glycopolymers and of the geometries of the binding sites. The poly(phenylacetylene) chain can adopt left- and right-handed conformations extended to greater or lesser degrees. The natures of the monosaccharides have some influence on the backbone conformation as demonstrated by the CD analysis above. Construction of different models, together with the observed stoichiometry, would be in favor of a "brush"-shaped conformation for the poly-PA-α-Gal, with Gal pendants easily accessible for the PA-IL binding site. The relative size of the lectin and the polymer indicate that one subunit of PA-IL can bind every eight Gal residues, in good agreement with observed stoichiometry (Figure 5). On the other hand, the flat β-GlcNAc moiety of poly-PA-β-GlcNAc has a tendency to stack with neighboring ones, favoring *transoid* helices in which the GlcNAc pendants are not accessible to WGA. The lectin can therefore bind only to monosaccharides at the extremities of the chain (or at a kink that could occur as a rare event), explaining the unusually high value of the stoichiometry (close to 50). It should also be kept in mind that functional valency might not reflect structural valency because other processes such as nonspecific binding and aggregation can affect this value.

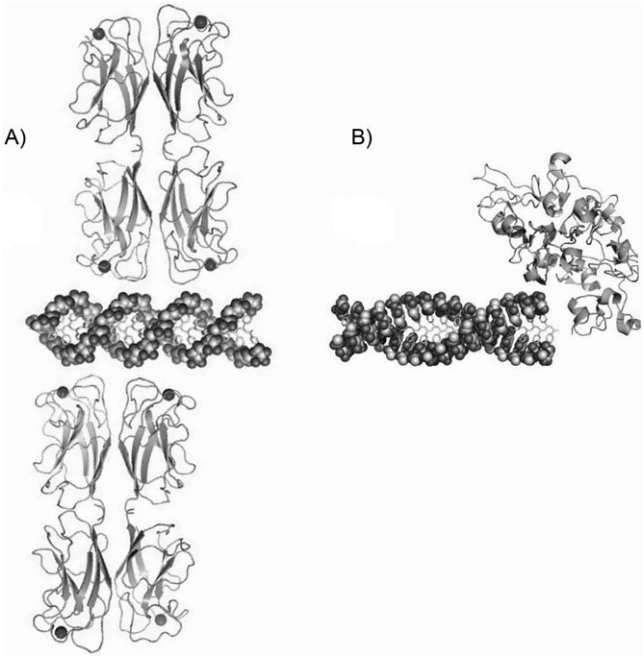


Figure 5. A) Model of poly-PA-α-Gal in the non-extended right-handed conformation and comparison with two tetramers of PA-IL, and B) model of poly-PA-β-GlcNAc in the extended right-handed conformation and comparison with a dimer of WGA.

With regard to the affinities of lectins for the glyco-ligands, the bacterial lectin PA-IL showed a much higher K_a value (order of more than $10^5\ M^{-1}$) than the other plant lectins. The plant lectins, at the same time, showed high K_a values of the order of 10^3 to $10^4\ M^{-1}$, whereas the K_a values between WGA and NeuAc-containing ligands were weaker (order of $10^2\ M^{-1}$). Interestingly, each glycosylated poly(phenylacetylene), except for poly-PA-β-Lac, showed K_a values two to eight times higher than those of the corresponding monovalent glyco-ligands: the K_a values of poly-PA-β-GlcNAc and β-Ph-GlcNAc, for example, were $1.44\times10^4\ M^{-1}$ and $1.75\times10^3\ M^{-1}$, respectively. These enhancements of the K_a values of glycosylated poly(phenylacetylene)s clearly supported the multivalency effects on the lectin affinity observed in the hemagglutination assay. In contrast, poly-PA-β-Lac did not show a higher K_a value than β-Ph-Lac, whereas it showed a 12-fold higher lectin affinity than β-Ph-Lac in the hemagglutination inhibition assay. This is probably due to precipitation by cross-linking between poly-PA-β-Lac and ECA that hampers integration of the peaks in the ITC thermogram.

Some of the observed differences in affinity can be correlated with the known structures of lectins, and more precisely with their binding sites and oligomeric architectures. Plant lectins have rather open binding sites with low affinities for monosaccharides (in the micromolar range), whereas bacterial lectins, probably as the result of evolution pressure, display higher affinities often associated with the participation of bridging cations.[37] In terms of quaternary structure, ECA is a dimeric lectin whereas PA-IL is tetrameric and WGA is dimeric but with two binding sites per monomer (tetrameric behavior). The multivalency state appears to have a crucial role on the enhancement of affinity for the polymeric compounds, because the two tetrameric systems—PA-IL and WGA—each display much stronger affinity for the polymeric compound than for the monomeric one, which is not the case for the dimeric ECA.

As classically observed for protein–carbohydrate interactions,[38] the binding reactions between the monovalent glycoligands and the lectins displayed favorable enthalpy contributions and unfavorable entropy contributions. When the binding to the glycosylated poly(phenylacetylene)s is considered and calculations per monomeric glyco unit are reported, the enthalpy of binding drops significantly. This is clearly related to the stoichiometry: because only a fraction of the carbohydrate residue is available for binding, the apparent enthalpy contribution is averaged and strongly decreased. In contrast, the entropy of binding becomes favorable for the binding of the glycosylated poly(phenylacetylene)s. For the binding between WGA and GlcNAc-containing ligands, for instance, the ΔH values varied from $-117\ kJ\,mol^{-1}$ to $-2.85\ kJ\,mol^{-1}$ and the $T\Delta S$ values varied from $-98.8\ kJ\,mol^{-1}$ to $+20.8\ kJ\,mol^{-1}$ with changing of the ligands from monomer to polymer. Similarly, for the binding between PA-IL and Gal-containing ligands, the ΔH values varied from $-33.9\ kJ\,mol^{-1}$ to $-2.13\ kJ\,mol^{-1}$ (poly-PA-α-Gal) and the $T\Delta S$ values varied from $-6.08\ kJ\,mol^{-1}$ to $+28.7\ kJ\,mol^{-1}$ (poly-PA-α-Gal).

Conclusions

Poly(phenylacetylene) derivatives bearing β-GlcNAc, β-Lac, and α-NeuAc residues were successfully synthesized by polymerizations of the corresponding acetyl-protected glycosylated phenylacetylenes, followed by deprotection. The CD spectra of these glycosylated poly(phenylacetylene)s indicated predominantly one-handed helical structures in the backbones. The high affinities of these glycosylated poly(phenylacetylene)s towards plant and bacterial lectins, based on the multivalent carbohydrate–lectin interactions, were demonstrated by the hemagglutinating inhibition assay. For all lectins except ECA, a two- to eightfold gain in affinity per saccharide is observed when the polymeric glyco-ligand is compared to the monomeric one. This could be of interest for competing with the binding of *P. aeruginosa* lectin to the host cell, because the affinity of the lectin for the natural α-galactosylated ligand is weaker (K_d ~100 μM).[39,40] The poly-PA-β-Gal glyco-ligand displays an affinity towards PA-IL of 4–5 μM (per galactose), which is close to those of the highest-affinity compounds so far described—calix[4]arene glycoconjugates bearing four β-galactose residues, with affinities of 0.2 μM (i.e., 0.8 μM per galactose).[41] The anti-infectious effects of galactosylated compounds have been demonstrated previously in a murine model,[31] but no assays were performed with polymeric material and the innocuity of such compounds towards animals has to be established before any therapeutic application.

The ITC measurements indicated the entropically favorable lectin binding of these glycosylated poly(phenylacetylene)s, with high K_a and n values. The interpretation could be hampered by aggregation processes so the thermodynamic parameters correspond to apparent ones. The n values calculated from molecular modeling, which found that the reaction stoichiometry reflects the conformation of the glycopolymer and the geometry of the binding site of the lectin, were in good agreement with the experimentally measured values. These results clearly indicated that the three-dimensional organizations of the saccharide moieties in the multivalent glycosides strongly affect the protein–carbohydrate interactions.

Experimental Section

Instruments: The ^{1}H NMR and ^{13}C NMR spectra were recorded with JEOL JNM-EX270 and JEOL JNM-A400II instruments. Size-exclusion chromatography (SEC) was performed at 40 °C in $CHCl_3$ (0.8 mL min^{-1}) with a Jasco GPC-900 system and two Shodex K-805L columns (linear, 8×300 mm; pore size, 10–1000 Å; bead size, 10 μm; exclusion limit, 4×10^6). IR spectra were recorded with a Perkin–Elmer Paragon 1000 FT-IR spectrometer. Circular dichroism (CD) and ultraviolet/visible (UV/Vis) spectra were measured in a 1 mm quartz cell with a Jasco J-720 spectropolarimeter at room temperature. Isothermal titration calorimetry (ITC) was performed with a Microcal VP-ITC titration microcalorimeter (Northampton, MA). Preparation of the polymerization solution was carried out under dry argon (H_2O, O_2 <1 ppm) in an MBRAUN stainless steel glove box with a gas purification system (molecular sieves and copper catalyst). The moisture and oxygen contents in the glove box were monitored with an MB-MO-SE 1 and an MB-OX-SE 1, respectively.

Materials: 4-Iodophenyl 3,4,6,-tri-*O*-acetyl-2-*N*-acetyl-β-D-glucopyranoside,[42] 4-iodophenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(*O*-2,3,6-tri-*O*-acetyl)-β-D-glucopyranoside,[43] methyl (4-iodophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate,[44] and Rh^+(2,5-norbornadiene)[(η^6-C_6H_5)B^-(C_6H_5)$_3$] (Rh(nbd)BPh_4)[45] were synthesized by previously reported methods. Trimethylsilylacetylene was kindly supplied by Shinetsu Chemical Co. and was used without further purification. 2,5-Norbornadiene-rhodium(I) chloride dimer ([Rh(nbd)Cl_2]$_2$) (96%) and bis(triphenylphosphine)palladium dichloride (> 98%) were purchased from Sigma–Aldrich and used as received. Copper(I) iodide (98%) was purchased from Junsei Chemical Co. and used as received. Triethylamine (>99.0%) was purchased from Kanto Chemical Co., Inc. and distilled over CaH_2 prior to use. Triphenylphosphine (>98%) was purchased from Kanto Chemical and used after being recrystallized from dichloromethane/diethyl ether. Tetrabutylammonium fluoride (1.0 M in THF), sodium methoxide (1.0 M in MeOH), dry THF (>99.5%, water content <0.005, *v*/%), and dry dichloromethane (>99.5%, water content <0.005, *v*/%) were purchased from Kanto Chemical and used as received. WGA and ECA were purchased from Seikagaku Biobusiness Co. and used as received. Recombinant PA-IL was prepared by the reported method.[39] Rabbit red cells were purchased from BioMérieux Co. and diluted to 2% with NaCl (0.15 M) prior to use.

4-(Trimethylsilylethynyl)phenyl 3,4,6,-tri-*O*-acetyl-2-*N*-acetyl-β-D-glucopyranoside (Method A): Trimethylsilylacetylene (6.0 mL, 42 mmol) was added under nitrogen at room temperature to a mixture of 4-iodophenyl 3,4,6,-tri-*O*-acetyl-2-*N*-acetyl-β-D-glucopyranoside (6.10 g, 11.2 mmol), bis(triphenylphosphine)palladium dichloride (170 mg, 240 μmol), copper(I) iodide (100 mg, 530 μmol), and triethylamine (60 mL) in dry THF (150 mL). After stirring for 18 h, the reaction mixture was diluted with $CHCl_3$ and filtered. The filtrate was washed with H_2O and dried over Na_2SO_4. After evaporation of the solvent, the product was purified by column chromatography (silica gel, CH_2Cl_2/ethyl acetate 1:1, *v*/*v*) to give 4-(trimethylsilylethynyl)phenyl 3,4,6,-tri-*O*-acetyl-2-*N*-acetyl-β-D-glucopyranoside as a white solid. Yield: 4.46 g (79%); ^{1}H NMR ($CDCl_3$): δ = 7.38, 6.91 (d, J = 8.8 Hz, 4H; Ar), 5.60 (d, J = 8.7 Hz, 1H; NH), 5.40 (dd, J = 10.4, 9.2 Hz, 1H; H-3), 5.27 (d, J = 8.2 Hz, 1H; H-1), 5.13 (dd, J = 9.5 Hz, 1H; H-4), 4.28 (dd, J = 12.2 Hz, 5.6 Hz, 1H; H-6a), 4.20–4.06 (m, 2H; H-2, H-6b), 3.88 (ddd, J = 9.8, 5.6, 2.5 Hz, 1H; H-5), 2.08, 2.07, 2.05, 1.96 (s, 12H; OAc), 0.24 ppm (s, 9H; -$SiMe_3$); ^{13}C NMR ($CDCl_3$): δ = 170.9, 170.7, 170.6 169.5 (C=O), 157.1, 133.50, 118.0, 116.7 (Ar), 104.6 (Ar-*C*≡C-$SiMe_3$), 98.6 (C-1), 93.6 (Ar-C≡*C*-$SiMe_3$), 72.1, 68.7, 62.3, 54.9 (C-2, C-3, C-4, C-5, C-6), 23.4, 20.8, 20.7 (CH_3), 0.1 ppm ($SiMe_3$); elemental analysis (%) calcd. for $C_{25}H_{33}NO_9Si$: C 57.79, H 6.40, N 2.70; found: C 57.52, H 6.42, N 2.65.

4-Ethynylphenyl 3,4,6,-tri-*O*-acetyl-2-*N*-acetyl-β-D-glucopyranoside (PA-β-GlcNAc-OAc, Method B): Tetrabutylammonium fluoride solution (1.00 M in THF, 9.12 mL) was added under nitrogen at room temperature to a solution of 4-(trimethylsilylethynyl)phenyl 3,4,6,-tri-*O*-acetyl-2-*N*-acetyl-β-D-glucopyranoside (3.96 g, 7.62 mmol) in dry CH_2Cl_2 (120 mL). After stirring for 40 min, the reaction mixture was washed with brine and the organic layer was dried over Na_2SO_4. The solvent was then evaporated and the product was purified by column chromatography (silica gel, hexane/diethyl ether 1:1, *v*/*v*) to give PA-β-GlcNAc-OAc as a white solid. Yield: 3.24 g (95%); ^{1}H NMR ($CDCl_3$): δ = 7.42, 6.93 (d, J = 8.8 Hz, 4H; Ar), 5.63 (d, J = 8.6 Hz, 1H; NH), 5.41 (dd, J = 10.4, 9.2 Hz, 1H; H-3), 5.30 (d, J = 8.2 Hz, 1H; H-1), 5.13 (dd, J = 9.5 Hz, 1H; H-4), 4.29

(dd, $J=12.2$, 5.6 Hz, 1H; H-6a), 4.20–4.05 (m, 2H; H-2, H-6b), 3.89 (ddd, $J=9.8$, 5.6, 2.5 Hz, 1H; H-5), 3.02 (s, 1H; C≡CH), 2.07, 2.06, 2.05, 1.96 ppm (s, 12H; OAc); ^{13}C NMR ($CDCl_3$): $\delta=170.9$, 170.7, 170.6, 169.5 (C=O), 157.3, 133.6, 116.9 (Ar), 98.5 (C-1), 83.2 (Ar-C≡CH), 76.8 (Ar-C≡CH), 72.1, 72.0, 68.7, 62.3, 54.8 (C-2, C-3, C-4, C-5, C-6), 23.4, 20.8, 20.7 ppm (CH_3); elemental analysis (%) calcd. for $C_{22}H_{25}NO_9$: C 59.06, H 5.63, N 3.13; found: C 58.86, H 5.63, N 2.98.

4-(Trimethylsilylethynyl)phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(*O*-2,3,6-tri-*O*-acetyl)-β-D-glucopyranoside: Method A was applied to 4-iodophenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(*O*-2,3,6-tri-*O*-acetyl)-β-D-glucopyranoside (5.09 g, 6.08 mmol), bis(triphenylphosphine)palladium dichloride (86 mg, 120 μmol), copper(I) iodide (47 mg, 250 μmol), triethylamine (21 mL), dry THF (24 mL), and trimethylsilylacetylene (1.5 mL, 42 mmol). The product was purified by column chromatography (silica gel, hexane/ethyl acetate 2:3, *v/v*) to give 4-(trimethylsilylethynyl)phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(*O*-2,3,6-tri-*O*-acetyl)-β-D-glucopyranoside as a white solid. Yield: 4.59 g (92%); ^{1}H NMR ($CDCl_3$): $\delta=7.39$, 6.89 (d, $J=$ 8.6 Hz, 4H; Ar), 5.36 (d, $J=3.2$ Hz, 1H; H-1Glc), 5.28 (m, 1H; H-1Gal), 5.20–5.05 (m, 3H; H-2Glc, H-2Gal, H-4Glc), 4.97 (m, 1H; H-3Glc), 4.52–4.47 (m, 2H; H-4Gal, H-3Gal), 4.19–4.05 (m, 3H; H-5Glc, H-6aGlc, H-6bGlc), 3.92–3.77 (m, 3H; H-5Gal, H-6aGal, H-6bGal) 2.16, 2.08, 2.07, 2.06, 2.06, 2.04, 1.97 (s, 21H; OAc) 0.24 ppm (s, 9H; $SiMe_3$); ^{13}C NMR ($CDCl_3$): $\delta=170.5$, 170.4, 170.2, 170.1, 169.8, 169.7, 169.2 (C=O), 156.8, 133.5, 118.2, 116.7 (Ar), 104.5 (Ar-C≡C-$SiMe_3$), 101.2 (C-1), 98.5 (C-1′), 93.6 (Ar-C≡*C*-$SiMe_3$), 76.3, 73.0, 72.9, 71.5, 71.0, 70.9, 69.2, 66.7, 62.1, 61.0 (C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6, C-6′), 20.8–19.8 (CH_3) 0.1 ppm ($SiMe_3$); elemental analysis (%) calcd. for $C_{38}H_{51}O_{18}Si$: C 55.40, H 6.24; found: C 55.20, H 6.08.

4-Ethynylphenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-galactopyranosyl)-(1→4)-(*O*-2,3,6-tri-*O*-acetyl)-β-D-glucopyranoside (PA-β-Lac-OAc): Method B was applied to 4-(trimethylsilylethynyl)phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(*O*-2,3,6-tri-*O*-acetyl)-β-D-glucopyranoside (4.18 g, 5.19 mmol), CH_2Cl_2 (50 mL), and tetrabutylammonium fluoride (6.22 mL; 1.00 M in THF). The product was purified by column chromatography (silica gel, hexane/ethyl acetate 2:3, *v/v*) to give PA-β-Lac-OAc as a white solid. Yield: 3.20 g (84%); ^{1}H NMR ($CDCl_3$): $\delta=7.42$, 6.92 (d, $J=8.9$ Hz, 4H; Ar), 5.36 (d, $J=2.8$ Hz, 1H; H-1Glc), 5.29 (m, 1H; H-1Gal), 5.21–5.06 (m, 3H; H-2Glc, H-2Gal, H-4Glc), 4.97 (m, 1H; H-3Glc), 4.53–4.47 (m, 2H; H-4Gal, H-3Gal), 4.19–4.05 (m, 3H; H-5Glc, H-6aGlc, H-6bGlc), 3.93–3.77 (m, 3H; H-5Gal, H-6aGal, H-6bGal), 3.03 (s, 1H; C≡CH), 2.16, 2.08, 2.07, 2.06, 2.06, 2.04, 1.97 ppm (s, 21H; OAc); ^{13}C NMR ($CDCl_3$): $\delta=170.4$, 170.3, 170.2, 170.1, 169.8, 169.6, 169.2 (C=O), 157.0, 133.7, 117.0, 116.8 (Ar), 101.2, 98.4 (C-1, C-1′), 83.1 (Ar-C≡CH), 76.8 (Ar-C≡CH), 76.3, 73.0, 72.8, 71.5, 71.0, 70.8, 69.2, 66.7, 62.1, 60.9 (C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6, C-6′), 20.7–20.6 ppm (CH_3); elemental analysis (%) calcd. for $C_{35}H_{43}O_{18}$: C 55.92, H 5.77; found: C 55.52, H 5.58.

Methyl [4-(trimethylsilylethynyl)phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid]onate: Method B was applied to methyl (4-iodophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate (3.35 g, 4.83 mmol), bis(triphenylphosphine)palladium dichloride (70.2 mg, 100 μmol), copper(I) iodide (38.1 mg, 200 μmol), triethylamine (15 mL), dry THF (15 mL), and trimethylsilylacetylene (1.4 mL, 10 mmol). The product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 30:1→20:1, *v/v*) to give methyl(4-trimethylsilylethynylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate as a white solid. Yield: 3.12 g (97%); ^{1}H NMR ($CDCl_3$): $\delta=$ 7.39, 6.98 (d, $J=8.7$ Hz, 4H; Ar), 5.35–5.39 (br, 2H; H-4, H-7), 5.25 (d, $J=10.1$ Hz, 1H; NH), 4.95 (ddd, $J=12.2$, 4.6, 4.5 Hz, 1H; H-8), 4.49 (d, $J=10.8$ Hz, 1H; H-6), 4.29–4.07 (m, 3H; H-5, H-9a, H-9b), 3.62 (s, 3H; OCH_3), 2.70 (dd, $J=13.0$, 4.7, 1H; H-3e), 2.35 (m, 1H; H-3a), 2.16, 2.13, 2.05, 1.92, 1.69 (s, 15H; OAc, NHAc), 0.23 ppm (s, 9H; TMS); ^{13}C NMR ($CDCl_3$): $\delta=171.0$, 170.7, 170.3, 170.2, 170,1 (C=O), 168.1 (C-1), 154.2, 133.3, 119.2, 118.5 (Ar), 104.6 (Ar-C≡C-TMS), 99.9 (C-2), 93.6 (Ar-C≡*C*-TMS), 73.5 (C-6), 69.0 (C-8), 68.6 (C-4), 67.2 (C-7), 62.0 (C-9), 53.0 (OCH_3), 49.4 (C-5), 38.4 (C-3), 23.3, 21.1, 20.9, 20.8 ppm (NHAc, OAc); elemental analysis (%) calcd. for $C_{31}H_{41}NO_{13}Si$: C 56.10, H 6.23, N 2.10; found: C 55.82, H 6.09, N 2.10.

Methyl (4-ethynylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate (PA-α-NeuAc-OAc): Method B was applied to methyl [4-(trimethylsilylethynyl)phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid]onate (2.93 g, 4.41 mmol), dry CH_2Cl_2 (30 mL), and tetrabutylammonium fluoride (1.00 mol L^{-1} in THF, 1.00 mL). The product was purified by column chromatography (silica gel, hexane, CH_2Cl_2/MeOH 30:1→20:1, *v/v*) to give PA-α-NeuAc-OAc as a white solid. Yield: 2.29 g (88%); ^{1}H NMR ($CDCl_3$): $\delta=7.41$, 7.00 (d, $J=8.2$ Hz, 4H; Ar), 5.35–5.38 (br, 2H; H-4, H-7), 5.24 (d, $J=10.0$ Hz, 1H; NH), 4.95 (ddd, $J=12.2$, 4.9, 4.4 Hz, 1H; H-8), 4.49 (d, $J=8.2$ Hz, 1H; H-6), 4.29–4.07 (m, 3H; H-5, H-9a, H-9b), 3.64 (s, 3H; OCH_3), 3.03 (s, 1H; C≡CH), 2.70 (dd, $J=12.9$, 4.9, 1H; H-3e), 2.24 (m, 1H; H-3a), 2.16, 2.13, 2.05, 1.92, 1.67 ppm (s, 15H; OAc, NHAc); ^{13}C NMR ($CDCl_3$): $\delta=171.0$, 170.6, 170.2, 170.1, 170,0 (C=O), 168.1 (C-1), 154.2, 133.4, 119.3, 117.4 (Ar), 99.8 (C-2) 83.1 (Ar-*C*≡CH) 76.7 (Ar-C≡*C*H), 73.5 (C-6) 69.0 (C-8), 68.6 (C-4), 67.2 (C-7), 62.0 (C-9), 53.0 (OCH_3), 49.4 (C-5), 38.3 (C-3), 23.2, 21.0, 20.8, 20.8, 20.7 ppm (NHAc, OAc); elemental analysis (%) calcd. for $C_{28}H_{33}NO_{13}$: C 56.85, H 5.62, N 2.37; found: C 56.55, H 5.54, N 2.20.

Poly(4-ethynylphenyl 3,4,6,-tri-*O*-acetyl-2-*N*-acetyl-β-D-glucopyranoside) (poly-PA-β-GlcNAc-OAc): A solution of Rh(norbornadiene)BPh_4 (12 mg, 22 μmol) in dry $CHCl_3$ (3.0 mL) was added under argon to a stirred solution of PA-β-GlcNAc-OAc (500 mg, 1.12 mmol) in dry $CHCl_3$ (8.0 mL). After 20 h at 30 °C, the reaction was terminated by addition of triphenylphosphine (39 mg, 150 μmol) and the mixture was then poured into a large amount of methanol. The precipitate was filtered off and dried in vacuo to give PA-α-Neu-OAc as a yellow powder. Yield: 490 mg; $M_{nn,\,SEC}=1.34\times10^5$; $M_w/M_{nn,\,SEC}=10.1$; IR (KBr): $\tilde{\nu}=1746$ cm^{-1} ($\nu_{C=O}$).

Poly[4-ethynylphenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-galactopyranosyl)-(1→4)-(*O*-2,3,6-tri-*O*-acetyl)-β-D-glucopyranoside] (poly-PA-β-Lac-OAc): $[Rh(nbd)Cl]_2$ (3.1 mg, 6.7 μmol) and triethylamine (950 μL, 6.8 mmol) were added under argon to a stirred solution of PA-β-Lac-OAc (500 mg, 680 μmol) in dry $CHCl_3$ (6.1 mL). After stirring for 20 h at 30 °C, the reaction was terminated by addition of an excess quantity of acetic acid, and the mixture was then poured into a large amount of methanol. The precipitate was filtered off and dried in vacuo to give poly-PA-β-Lac-OAc as a yellow powder. Yield: 330 mg; $M_{n,\,SEC}=7.81\times10^4$; $M_w/M_{nn,\,SEC}=3.79$; IR (KBr): $\tilde{\nu}=$ 1740 cm^{-1} ($\nu_{C=O}$).

Poly[methyl (4-ethynylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate] (poly-PA-α-NeuAc-OAc): A solution of Rh(norbornadiene)-BPh_4 (7.0 mg, 14 μmol) in dry THF (800 μL) was added under argon to a stirred solution of PA-α-NeuAc-OAc (400 mg, 680 μmol) in dry THF (6.0 mL). After 20 h at 30 °C, the reaction was terminated by addition of triphenylphosphine (21 mg, 80 μmol), and the mixture was then poured into a large amount of diethyl ether. The precipi-

tate was filtered off and dried in vacuo to give poly-PA-α-NeuAc-OAc as a yellow powder. Yield: 370 mg; $M_{nn,\,SEC}=7.94\times10^4$; $M_w/M_{nn,\,SEC}=2.16$; IR (KBr): $\tilde{\nu}=1748\ cm^{-1}$ ($\nu_{C=O}$).

Poly(4-ethynylphenyl 2-*N*-acetyl-β-D-glucopyranoside) (poly-PA-β-GlcNAc): CH_3ONa (1 M in MeOH, 1 mL) was added to a stirred solution of poly-PA-β-GlcNAc-OAc (300 mg) in DMSO (100 mL). After one day, water (100 mL) was added to dissolve the precipitate. The mixture was dialyzed in a cellulose tube against water for five days and then lyophilized to give poly-PA-α-GlcNAc as a yellow powder. Yield: 200 mg; IR (KBr): $\tilde{\nu}=3300–3600\ cm^{-1}$ (ν_{O-H}).

Poly(4-ethynylphenyl *O*-β-galactopyranosyl-(1→4)-β-D-glucopyranoside) (poly-PA-β-Lac): CH_3ONa (1 M in MeOH, 1 mL) was added to a stirred solution of poly-PA-β-Lac (200 mg) in THF (50 mL). After one day, water (100 mL) was added to dissolve the precipitate. The mixture was dialyzed in a cellulose tube against water for three days and then lyophilized to give poly-PA-β-Lac as a yellow powder. Yield: 110 mg; IR (KBr): $\tilde{\nu}=3300–3600\ cm^{-1}$ (ν_{O-H}).

Poly(4-ethynylphenyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranoside) (poly-PA-α-NeuAc): CH_3ONa (1 M in MeOH, 1 mL) was added to a stirred solution of poly-PA-α-NeuOAc (200 mg) in THF (40 mL). After 7 h, the mixture was evaporated and NaOH (5 mL; 0.1 M in H_2O) was added. The mixture was stirred overnight followed by treatment with Amberlite IR-120 H^+ resin for 10 min. The filtrate from the mixture was dialyzed in a cellulose tube against water for two days and then lyophilized to give poly-PA-α-NeuAc as a yellow powder. Yield: 160 mg; IR (KBr): $\tilde{\nu}=3300–3600\ cm^{-1}$ (ν_{O-H}).

Hemagglutination assay: Hemagglutination activity was assayed by the method described by Nowotny.[46] A twofold dilution series of each lectin solution (25 μL, starting at 1 mg mL^{-1}) in tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, 100 mM) buffer (pH 7.5, with 3 μM $CaCl_2$) were prepared in 96-well microtiter U-plates (Thermo Fisher Scientific, Inc.). An equal volume (25 μL) of rabbit red cell solution (2%) in aq. NaCl (150 mM) was added to each well, and the system was incubated at 37 °C for 30 min. The agglutination was then visually analyzed, and the minimum lectin concentration required for agglutination was determined.

Hemagglutinating inhibition assay: A twofold dilution series of glyco-ligand solutions in the above buffer (25 μL, starting at 2.5 mg mL^{-1}) was prepared in 96-well microtiter U-plates. An equal volume (25 μL) of lectin solution ([lectin] = 4×[minimum concentration required for hemagglutination]) was added to each well and the system was incubated at 37 °C for 30 min. Rabbit red cell solution (2%, 50 μL) in aq. NaCl (150 mM) was added to each well, and the system was incubated at 37 °C for 30 min. The agglutination was then visually analyzed and the minimum concentration of the glyco-ligand needed to inhibit agglutination was determined as IC_{min}.

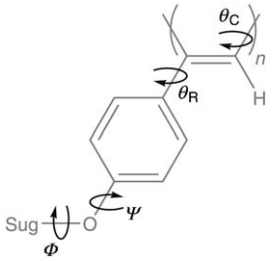
Isothermal titration calorimetry (ITC): The thermodynamics of the interaction between the glyco-ligands and the lectins were investigated by measuring the heat production from the titration experiments by ITC. A typical procedure is as follows: a solution of WGA lectin (13 μM) was placed in the reaction cell ($V=1.4478$ mL). A solution of poly-PA-β-GlcNAc (6.00 mM) was siphoned off by a computer-controlled microsyringe ($V=300$ μL) and injected into the reaction cell at 10 μL 30 times, with 5 min intervals between each injection. The reaction mixture was stirred at 300 rpm and at 25 °C during the titration. The raw experimental data were reported as the heat production versus time. The amount of heat production was calculated by integration of the areas of individual peaks with the aid of the ORIGIN 7.0 software (OriginLab Corp., Northampton, MA). The experimental data were fitted to a theoretical titration curve by use of the "one set of binding sites" model in ORIGIN 7.0 to determine the association constants (K_a), the reaction stoichiometry (n), and the enthalpy change (ΔH). Changes in free energy (ΔG) and entropy (ΔS) were calculated from Equation (1):

$$\Delta G = \Delta H - T\Delta S = RT \ln K_a \quad (1)$$

where T is the absolute temperature and $R=8.314\ J\,mol^{-1}\,K^{-1}$.

The concentrations of lectins in the reaction cells varied from 13 to 38 μM (see Footnote in Table 3). This resulted in the Wiseman "*c*" values being lower than 1 for experiments with monomeric compounds and between 1 and 50 for experiments with multimeric compounds. Measured heats of dilution of all ligands were negligible.

Molecular modeling: Glycosylated phenylacetylene monomers were built with the aid of the graphical editing menu of the Sybyl package (Tripos, Inc., St. Louis). The monosaccharide moieties were taken from the Glyco3D library (http://glyco3d.cermav.cnrs.fr/glyco3d/index.php). Atom types and atomic charges of the monosaccharides were selected according to the PIM force-field.[47] Atomic charges for the aglycon moiety were derived from MNDO calculations. The monosaccharide was oriented with Φ and Ψ torsion angles compatible with known energy minima.[48] A 32-mer



was built by using the average values for phenylacetylene bond lengths and angles taken from the literature[49] and a *cis* configuration for the double bond. Four values of the torsion angle θ_c of the single bond were considered, resulting in four helical conformations of the glycopolymer together with tilting of the θ_R angle in order to avoid steric conflicts. The four sets of torsional angles are ($\theta_c=+160°$; $\theta_R=-35°$) and ($\theta_c=+140°$; $\theta_R=-10°$) for right-handed helices and ($\theta_c=-160°$; $\theta_R=+35°$) and ($\theta_c=-140°$; $\theta_R=+10°$) for left-handed ones. Full energy minimization with the use of the TRIPOS force-field was performed and resulted in only marginal conformational adjustments.

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