Iron(II)-Assisted Assembly of Trivalent GalNAc Clusters and Their Interactions with GalNAc-Specific Lectins

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A novel metal-assisted assembly of mulitivalent carbohydrate ligands is described. A bipyridine-modified *N*-acetylgalactosamine (bipy-GalNAc) undergoes Fe(II)-induced self-association to form a trimeric GalNAc ligand (Fe^{II}(bipy-GalNAc)$_3$). The synthetic GalNAc cluster strongly binds to *Vicia villosa* B_4 lectin and *Glycin Max* lectin, which recognizes multiple GalNAc residues. The trimeric GalNAc ligand is formed as a mixture of four diastereomeric isomers: Δ-*fac*, Λ-*fac*, Δ-*mer*, and Λ-*mer*. These stereoisomers are in a dynamic equilibrium at room temperature. The equilibrium allows the spatial arrangement of the three GalNAc residues to change in order to fit into a multivalent carbohydrate binding site of the lectins. Detailed analysis of the kinetic and thermodynamic data for the isomerization can provide structural information of the carbohydrate binding site of the lectins.

Carbohydrates can be viewed as a storage material for biochemical information. Carbohydrates form branched structures that contain biochemical information in much more dense compared to proteins and nucleic acids that can only form a linear primary linkage. Synthetic carbohydrate ligands are a valuable tool for mechanistic studies of protein–carbohydrate interactions. They are the basis for the development of drugs to disrupt specific cell-adhesions for the treatment of infectious and inflammatory diseases. The complexity in the carbohydrate structures, however, presents synthetic problems. Synthesis of monosaccharide derivatives has been well established, and a wide array of synthetic methods are available for short oligosaccharides.[1] A combination of chemical synthesis and enzyme-catalyzed coupling reactions has been employed for the synthesis of more complicated carbohydrates.[2—4] Polymer conjugates of monosaccharides can provide an alternate route to assemble branched carbohydrate structures, although the local conformation of the conjugate polymers is not well defined.[5,6] Rigid organic templates have also been employed to display multivalent carbohydrate ligands for specific recognition of lectins.[7,8]

Complex branched carbohydrate structures can be easily assembled with metal-assisted association of individual carbohydrate components. We have demonstrated that a bipyridine-modified *N*-acetylgalactosamine (bipy-GalNAc) trimerizes to form trivalent GalNAc carbohydrate ligands in the presence of Fe(II)[9] (Scheme 1). The resulting Fe(II) complex, Fe^{II}(bipy-GalNAc)$_3$, strongly binds to *Vicia villosa* B_4 lectin, which recognizes repeating units of GalNAc-modified Ser or Thr residues (Tn antigen). The three GalNAc residues are held on the tris-bipyridine Fe(II) template in a well-defined orientation which appears to mimic the structure of the carbohydrate portions in the Tn antigen.[10]

In the metal-template system, stereochemistry at the metal center provides additional structural diversity of the carbohydrate ligands which can be used to probe carbohydrate binding sites. Octahedral complexes derived from three unsymmetrical bidentate ligands have four possible diastereomers. There are two geometrical isomers, facial (*fac*) and meridional (*mer*), each of which is enantiomeric, Δ and Λ. The

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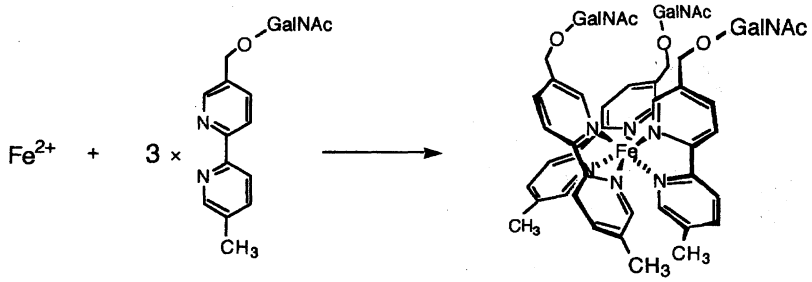


Scheme 1. Metal-assisted assembly of GalNAc cluster using Fe(II) complex.

statistical isomer ratio for such complexes is 3 : 3 : 1 : 1 for the *Δ-mer*, *Λ-mer*, *Δ-fac*, and *Λ-fac* isomers, respectively.[11] Three GalNAc residues are organized on each metal template in a significantly different way (Scheme 2). In *fac*-isomers, three GalNAc residues are placed at each corner of an equilateral triangle, and the distance between pyridyl carbons that are attached to glycosidic oxygens is 7 Å. *Mer*-isomers provides an isosceles organization of three GalNAc residues with distances between the pyridyl carbons of 7, 7 and 11 Å. Overall conformation of the trimeric GalNAc ligand should also be affected by the chirality of the metal center. Each GalNAc cluster would, therefore, exhibit considerably different affinity to a lectin that recognizes multiple GalNAc residues. These diastereomeric complexes are in dynamic equilibria at room temperature. We have reported that the equilibrium allows the three GalNAc residues to adjust their spatial orientation on the metal template to fit into the binding pocket of various GalNAc-specific lectins.[12]

We wish to report here further kinetic and thermodynamic studies of the self-adjusting binding of the trimeric GalNAc ligand to various GalNAc specific lectins.

Experimental

General. All chemicals and supplies were of high purity and available from commercial sources. 5,5′-Dimethyl-2,2′-bipyridine was synthesized according to the published procedure.[13] 5-Bromomethyl-5′-methyl-2,2′-bipyridine was synthesized according to the published procedure.[14] GalNAc was purchased from Sigma. HPLC-grade acetonitrile was purchased from Baker.

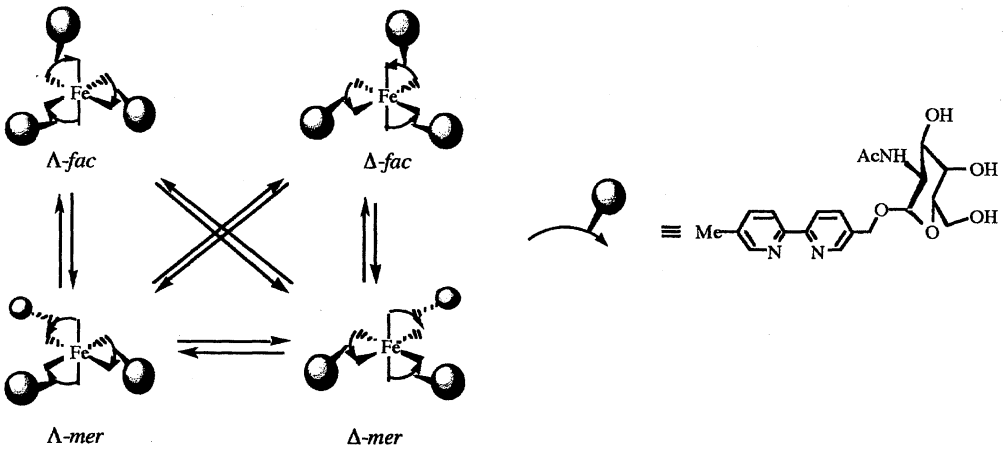
^{1}H NMR spectra were recorded on a Bruker VXR-300 at 300 MHz using TMS as an internal standard. High-resolution fast atom bombardment (FAB) mass spectra were recorded on a VG70SEQ double-focusing mass spectrometer. pH was measured with a Radiometer Copenhagen PHM84 pH meter equipped with a micro probe. UV-vis spectra were acquired on a Perkin–Elmer Lambda 3B spectrometer in 1 cm quartz semi-micro cells. HPLC was performed using a Waters 600E system, with a Perkin–Elmer LC-95 UV-vis detector, a Kipp and Zonen BD-40 chart recorder, and an HP3394A integrator. A JASCO-700 circular dichroism spectrometer with 0.1 mm pathlength cells and a nitrogen flow rate of 5 L min^{-1} was used to make circular dichroism (CD) measurements.

Synthesis of 5-[(2-Acetamido-2-deoxy-α-D-galactopyranosyl)methyl]-5′-methyl-2,2′-bipyridine (bipy-GalNAc). GalNAc (2.50 g, 11.3 mmol) was acetylated with acetic anhydride (10 mL) in pyridine (20 mL). The fully acetylated GalNAc (1.71 g, 4.40 mmol) was dissolved in cooled (0 °C) solution of ammonia-saturated acetonitrile (85 mL, prepared by bubbling ammonia gas through the solvent at 0 °C for 10 min) to selectively remove the acetyl group at the C-1 hydroxy group.[15] The resulting tri-*O*-acetyl-*N*-acetylgalactosamine (Ac_3-GalNAc) (0.4 g, 1.15 mmol) was reacted with 5-bromomethyl-5′-methyl-2,2′-bipyridine (0.53 g, 2.24 mmol) in dry DMF (0.75 mL) in the presence of Ag_2O (0.40 g, 1.72 mmol). After deacetylation with $NaOCH_3$ in methanol, bipyridine-modified *N*-acetylgalactosamine (bipy-GalNAc) was purified with C_4 reverse phase HPLC (Yield 8%, white powder) (Fig. 1). The small coupling constant of C-1 proton (δ = 5.04, J = 3.8 Hz) in ^{1}H NMR is consistent with the α-configuration of bipy-GalNAc.[16]

^{1}H NMR (300 MHz, D_2O) δ = 8.84 (brs, 1H), 8.70 (brs, 1H), 8.45 (brs, 2H), 8.31 (d, J = 8.4 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 5.04 (d, J = 3.8 Hz, 1H), 4.94 (d, J = 13.2 Hz, 1H), 4.79 (d, J = 13.2 Hz, 1H), 4.20 (dd, J = 10.5, 3.7 Hz, 1H), 4.05—3.96 (m, 3H), 3.76 (d, 2H, J = 6.0 Hz), 2.60 (s, 3H), 2.02 (s, 3H). FAB-MS m/z = 404 $(M+H)^+$.

Synthesis of Fe^{II}(bipy-GalNAc)$_3$. UV-vis titrations of bipy-GalNAc with Fe(II) confirmed the formation of a tridentate GalNAc cluster, Fe^{II}(bipy-GalNAc)$_3$. A stock solution of 1 M (1 M = 1 mol dm^{-3}), $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was freshly prepared and up to 5 equivalents of iron were added to bipy-GalNAc (0.1 mM in Tris-HCl (20 mM), pH 7.2). As iron is added, the absorption spectrum of the red iron complex Fe^{II}(bipy-GalNAc)$_3$ grows in with λ_{max} = 303, 351, 481(sh), and 516 nm. The UV-vis spectrum of Fe^{II}(bipy-GalNAc)$_3$ is similar to that of the iron(II) tris-(5,5′-dimethyl-2,2′-bipyridine) complex $Fe^{II}(Me_2bipy)_3$ (λ_{max} = 296, 348, 480(sh), and 519 nm). The CD spectra of the resulting Fe^{II}(bipy-GalNAc)$_3$ (0.33 mM) solution were measured. A cylindrical CD cell with the path length of 0.1 mm was used for the CD measurements. The CD spectrum of Fe^{II}(bipy-GalNAc)$_3$ showed only weak peaks at 294 nm ($[\theta] = 1.0\times10^5$ deg cm^2 dmol^{-1}) and 311 nm ($[\theta] = -2.0\times10^5$ deg cm^2 dmol^{-1}), in accord with the formation of four diastereomers in almost statistically random fashion.

Separation of Four Isomers of Fe^{II}(bipy-GalNAc)$_3$ and Their Stereochemical Assignments. A sample solution of Fe^{II}(bipy-GalNAc)$_3$ (0.33 mM) was prepared in 20 mM, Tris-HCl buffer (pH = 7.2). All four isomers of Fe^{II}(bipy-GalNAc)$_3$ were separated by reverse-phase HPLC, using a solvent system of water–CH_3CN



Scheme 2. Four isomeric GalNAc clusters assembled on $Fe^{II}(bipy)_3$ template.

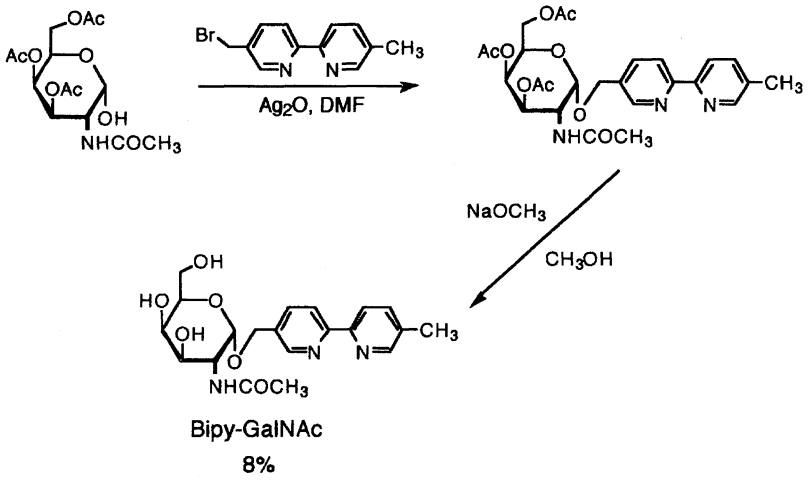


Fig. 1. Synthesis of bipy-GalNAc.

containing 0.1% TFA with a Vydac C_4 analytical column. A linear gradient of 12—24% CH_3CN over 15 min, with a flow rate of 1.0 mL min^{-1}, was employed to separate the four isomers. The chromatography was monitored at 296 nm, which is the isosbestic point for the formation of the Fe^{II}(bipy-GalNAc)$_3$ complex. Each eluted isomer was collected, and their CD spectra were immediately measured to probe enantiomeric configuration at the metal center.

Inhibition Studies. Peroxidase-labeled *Vicia villosa* B_4 lectin was used for all inhibition experiments. Binding of the peroxidase-labeled lectin to GalNAc-agarose was studied in the presence of varied concentrations of carbohydrate inhibitors, including GalNAc, benzyl-*N*-acetyl-α-D-galactosaminide (benzyl-GalNAc), bipy-GalNAc, Fe^{II}(bipy-GalNAc)$_3$, and asialoglycophorin A (MN). The concentration of unbound *Vicia villosa* B_4 lectin in solution was determined colorimetrically. Reactants were added in the following order: 30 mg of packed GalNAc-resin, 250 μL of increasing amount of inhibitors in 20 mM Tris/HCl, pH 7.2 containing 150 mM NaCl and 0.5% BSA (buffer A), and 250 μL of 1×10^{-2} mM peroxidase-labeled *Vicia villosa* B_4 lectin in buffer A. The mixture was shaken gently in a 1.5 mL Eppendorf tube at 22 °C for 2 h. After the incubation period, 100 μL of the red solution were mixed with 900 μL of water and 20 μL of a solution of 0.03% *o*-phenylenediamine and 0.09% H_2O_2 in 0.1 M citrate buffer at pH 5 (OPD-solution). After 1 min, 0.5 mL of 2 M aqueous H_2SO_4 was added to the mixture to stop the reaction. The absorbance at 490 nm was then measured to calculate the concentration of unbound lectin.[17,18] Binding constants were calculated based on the inhibition isotherm, using a non-linear curve fitting program.

Ultrafiltration. Ultrafiltration was utilized to determine the binding of Fe^{II}(bipy-GalNAc)$_3$ to various GalNAc-specific lectins; *Dolichos biflorus*, *Erythrina corallodendron*, *Glycine max*, *Helix pomatia*, *Phaseolus lirnensis*, *Vicia villosa* A_4, A_2B_2, B_4, *Vicia villosa* B_4, and *Wisteria floribund*.[19] Fe^{II}(bipy-GalNAc)$_3$, 1.67×10^{-2} mM, was incubated with lectins, 1.67×10^{-2} mM, in 20 mM Tris/HCl, 150 mM NaCl, and 1 mM $CaCl_2$, pH 7.2 for 2 h at 22 °C. The mixture was ultrafiltered through Centricon-30 (Amicon Inc.); molecular weight cut-off = 30000. The concentration of unbound Fe^{II}(bipy-GalNAc)$_3$ was determined colorimetrically at 305 nm.

Kinetic Studies of Self-Adjusting Binding of Fe^{II}(bipy-GalNAc)$_3$. Fe^{II}(bipy-GalNAc)$_3$, 1.67×10^{-2} mM, was incubated with lectins, 1.67×10^{-2} mM, in 20 mM Tris/HCl, 150 mM NaCl, and 1 mM $CaCl_2$, pH 7.2 for up to 32 h at 22 °C. An aliquot of the mixture was directly injected into C_4 reverse-phase HPLC for analysis. The acidic HPLC solvent, water–CH_3CN containing 0.1% TFA, dissociated any bound GalNAc ligands from lectins. A linear gradient of 12—24% CH_3CN over 15 min, with a flow rate of 1.0 mL min^{-1}, was employed to separate the four isomers, and the chromatography was monitored at 296 nm. The time required for analysis is significantly short relative to the time-scale of isomer interconversions.

The time-course of the self-adjusting binding was also monitored by CD. A cylindrical CD cell with the path length of 0.1 mm was used for all CD measurements. Fe^{II}(bipy-GalNAc)$_3$, 1.67×10^{-2} mM, was incubated with lectins, 1.67×10^{-2} mM, in 20 mM Tris/HCl, 150 mM NaCl, and 1 mM $CaCl_2$, pH 7.2 for up to 32 h at 22 °C. CD spectra of lectins were recorded separately in the same CD cell and subtracted from the CD spectra of Fe^{II}(bipy-GalNAc)$_3$–lectin complexes. Both lectins showed negligible CD absorption at wavelengths longer than 290 nm.

Results and Discussion

Fe(II) reacts with bipy-GalNAc to form a stable tris-complex, Fe^{II}(bipy-GalNAc)$_3$. The stoichiometry of the complex formation was determined to be 3.2 : 1 for bipy-GalNAc : Fe(II). Four isosbestic points (227, 250, 268, and 296 nm) were observed during the titration, consistent with a high third binding constant relative to the first and second ones. A trivalent GalNAc cluster is, therefore, assembled in one step on a metal template under very mild conditions. No protection groups are required for the carbohydrate moiety. This metal-assisted assembly process should be compatible with many functional groups found in natural carbohydrates because of the high specificity of Fe(II)-bipyridine interactions.

Since the bipyridine unit of bipy-GalNAc is unsymmetrical, four diastereomeric isomers of Fe^{II}(bipy-GalNAc)$_3$ complex, Δ-*fac*, Λ-*fac*, Δ-*mer*, and Λ-*mer*, could be formed with the statistical ratio of 1 : 1 : 3 : 3, respectively.[9,11] If interactions between GalNAc residues are negligible, a nearly statistical ratio should be observed at the equilibrium. HPLC analysis of Fe^{II}(bipy-GalNAc)$_3$ showed four peaks with the ratio of 29 : 46 : 10 : 15 at the retention times of 10.8, 11.1, 12.8, and 13.5 min, respectively. All four peaks were inter-

changeable as expected for a labile Fe(II)-bipy complex. The peak ratio is only slightly deviated from the statistical ratio, and the peak ratio at the equilibrium was unchanged by heat (70 °C, 24 h) and by guanidine hydrochloride (3.0 M). These data suggest negligible interactions between three GalNAc residues on a chiral Fe(II)-(bipy)$_3$ template. Also, the CD spectra of the equilibrium solution of Fe^{II}(bipy-GalNAc)$_3$ showed less than 10% diastereomeric excess at equilibrium. Together with the CD spectra of isolated peaks, the four HPLC peaks were thus assigned to *Λ-mer*, *Δ-mer*, *Δ-fac*, and *Λ-fac* in order of elution from the reverse phase column.

Table 1 shows the % binding of Fe^{II}(bipy-GalNAc)$_3$ to various lectins.[19] Among the GalNAc-specific lectins examined, *Vicia villosa* B_4 and *Glycine max* lectins showed more than 90% binding to Fe^{II}(bipy-GalNAc)$_3$. *Vicia villosa* B_4 lectin is a plant lectin whose carbohydrate binding site recognizes multiple GalNAc residues.[20,21] One of the specific ligands for the *Vicia villosa* B_4 lectin is a cancer specific antigen Tn. The Tn antigen contains α-GalNAc-serine or threonine residues and is reportedly a good marker of malignant transformation in several epithelial tissues.[22—24] The binding studies[25,26] of GalNAc-modified peptides with Tn specific antibodies showed that repeating units of at least two GalNAc-Ser or Thr residues were necessary for the epitope of the specific antigen–antibody interactions. Table 2 shows the binding constant of the synthetic GalNAc derivatives to *Vicia villosa* B_4 lectin. Data were fitted to Eq. 1;

$$(A_{obs} - A_0)/(A_\infty - A_0) = K[I]/(1 + K[I]) \quad (1)$$

where [I] and K are the concentration of GalNAc derivatives and binding constant, respectively. A_0 and A_∞ are absorbances of OPD-solution for [I] = 0 and ∞, respectively.

The calculated relative binding constants for GalNAc, benzyl-GalNAc, bipy-GalNAc, and Fe^{II}(bipy-GalNAc)$_3$ are 1.0, 1.7, 2.0, and 14.8, respectively. Asialoglycophorin A (MN) contains several repeating units of GalNAc-modified Ser or Thr residues, and has been used as a model for Tn-antigen. Asialoglycophorin A (MN) showed a similar binding constant to Fe^{II}(bipy-GalNAc)$_3$. The increased binding affinity of Fe^{II}(bipy-GalNAc)$_3$ over bipy-GalNAc toward *Vicia villosa* B_4 lectin has been attributed to the cluster formation of GalNAc residues on the metal template. The cluster effect is commonly observed in a number of carbohydrate-lectin interactions.[27,28]

Since bipyridine forms a relatively labile complex with Fe(II), Fe^{II}(bipy-GalNAc)$_3$ exists as an equilibrium mixture of four diastereomers, i.e. *Δ-mer*, *Λ-mer*, *Δ-fac*, and *Λ-fac* isomers, as discussed above. Each diastereomeric ligand should show a distinct binding affinity to the lectin. We, therefore, expect to see "dynamic" molecular recognition[29] of GalNAc specific lectins with Fe^{II}(bipy-GalNAc)$_3$ isomers. When a solution of Fe^{II}(bipy-GalNAc)$_3$ was mixed with the *Vicia villosa* B_4 lectin, the isomer ratio gradually changed to enrich the *Λ-mer* isomer up to 85% after 32 h at room temperature, as shown in Figs. 2a and 2b. The three GalNAc residues assembled on the *Λ-mer* isomer of Fe^{II}(bipy-GalNAc)$_3$ appear to provide a better complementarity to the carbohydrate binding site of the *Vicia villosa* B_4 lectin than the other isomers. The relative binding constants of the four isomers to the *Vicia villosa* B_4 lectin were calcu-

Table 1. Various GalNAc-Specific Lectins and Their Binding Affinities to Fe^{II}(bipy-GalNAc)$_3$

Lectin	Binding (%)[a]	Specificity, Blood group	MW ($\times 10^3$)	No. of subunits
Vicia villosa B_4	97	GalNAc, Tn	143	4
Glycin max	93	GalNAc	110	4
Vicia villosa A_4, A_2B_2, B_4	63	GalNAc, A_1&Tn	139	4
Helix pomatia	33	GalNAc, A	79	6
Dolichos biflorus	26	α-GalNAc, A_1	140	4
Wistaria floribunfa	28	GalNAc	68	2
Erythrina corallodendron	4	β-GalNAc(1-4)GlcNAc	60	2
Phaseolus limensis	1	GalNAc, A	124	4

a) % bound Fe^{II}(bipy-GalNAc)$_3$ determined by ultrafiltration (see Experimental section).

Table 2. Association Constants and IC_{50} of Various GalNAc Ligands for Their Binding to *Vicia villosa* B_4 Lectin

Inhibitor	K	K' (GalNAc Equivqlent)	IC_{50}
	mM^{-1}	mM^{-1}	mM
Fe^{II}(bipy-GalNAc)$_3$	97.5 ± 6.4	32.4 ± 2.1	0.010
Asialoglycophorin A	93.0 ± 4.8	31.0 ± 1.6	0.011
Bipy-GalNAc	13.2 ± 0.7	13.2 ± 0.7	0.076
Benzyl-GalNAc	11.2 ± 0.6	11.2 ± 0.6	0.090
GalNAc	6.6 ± 0.6	6.6 ± 0.6	0.153

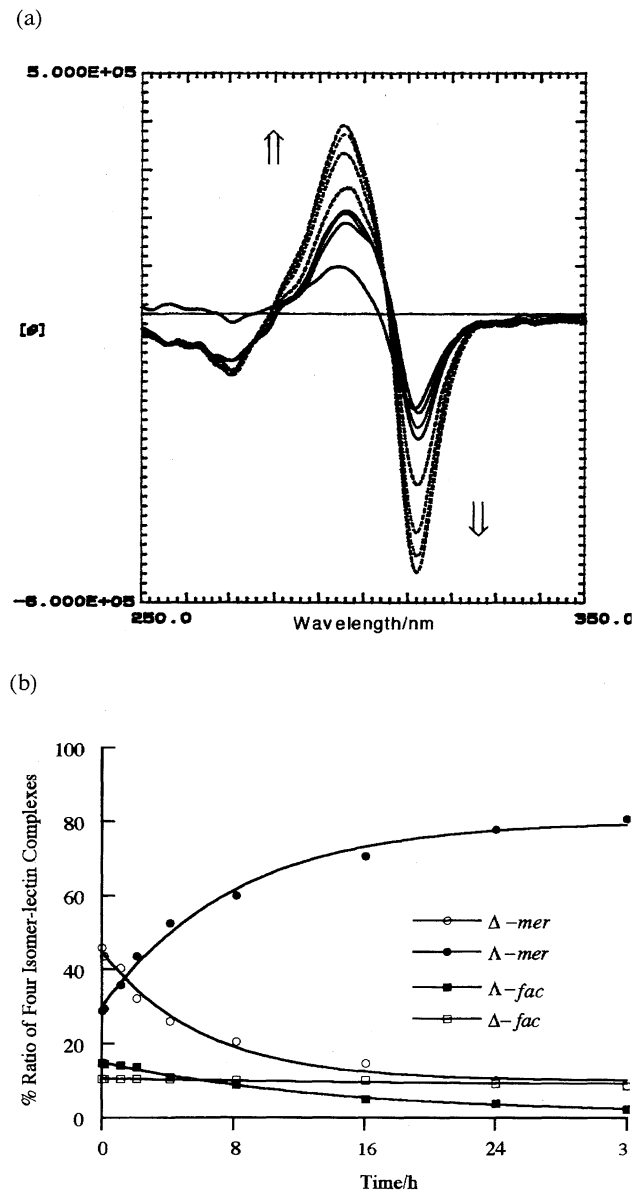


Fig. 2. Change of (a) CD spectrum and (b) isomer ratio of Fe^{II}(bipy-GalNAc)$_3$ in the presence of *Vicia villosa* B_4 lectin.

lated to be 5.4, 1.0, 1.2, and 18 for *Δ-fac*, *Λ-fac*, *Δ-mer*, and *Λ-mer*, respectively.[12] As a control experiment, excess GalNAc was added to inhibit specific carbohydrate-lectin interactions. In the presence of 0.1 M GalNAc, Fe^{II}(bipy-GalNAc)$_3$ does not bind to the *Vicia villosa* B_4 lectin[9] and the original isomer ratio remains unchanged. The binding of the GalNAc moieties of Fe^{II}(bipy-GalNAc)$_3$ to the lectin is, therefore, responsible for the shift of the isomerization equilibrium.

Glycine max lectin is specific to a terminal α-D-GalNAc residue attached to 3′-OH of galactose (A antigen);[21,30,31] its binding site is expected to be significantly different in size and shape from that of the *Vicia villosa* B_4 lectin. HPLC analysis of the isomer ratio of Fe^{II}(bipy-GalNAc)$_3$ after 32 h of equilibrium revealed the enrichment of both *Δ-fac* and *Δ-mer* isomers (Figs. 3a and 3b). The relative binding constants of the four isomers to the lectin were calculated to be 48, 2.1, 21, and 1.0 for *Δ-fac*, *Λ-fac*, *Δ-mer*, and *Λ-mer*, respectively. CD spectra of Fe^{II}(bipy-GalNAc)$_3$ also showed the shift of the isomerization equilibrium to enrich *Δ* isomer in the presence of *Glycine max* lectin.

Table 3 shows relative stabilities of the four diastereomers of Fe^{II}(bipy-GalNAc)$_3$ with and without added lectins. Weak diastereotopic interactions between GalNAc residues and chiral metal center appear to be the origin for the slight deviation of the isomer ratio of Fe^{II}(bipy-GalNAc)$_3$ from the statistical ones. The energy differences between isomers are very small; an energy difference of 0.27 kcal mol^{-1} (1 kcal mol^{-1} = 4.184 J mol^{-1}) for the most stable *Δ-mer* and the least stable *Λ-mer*. The isomer ratio, however, devi-

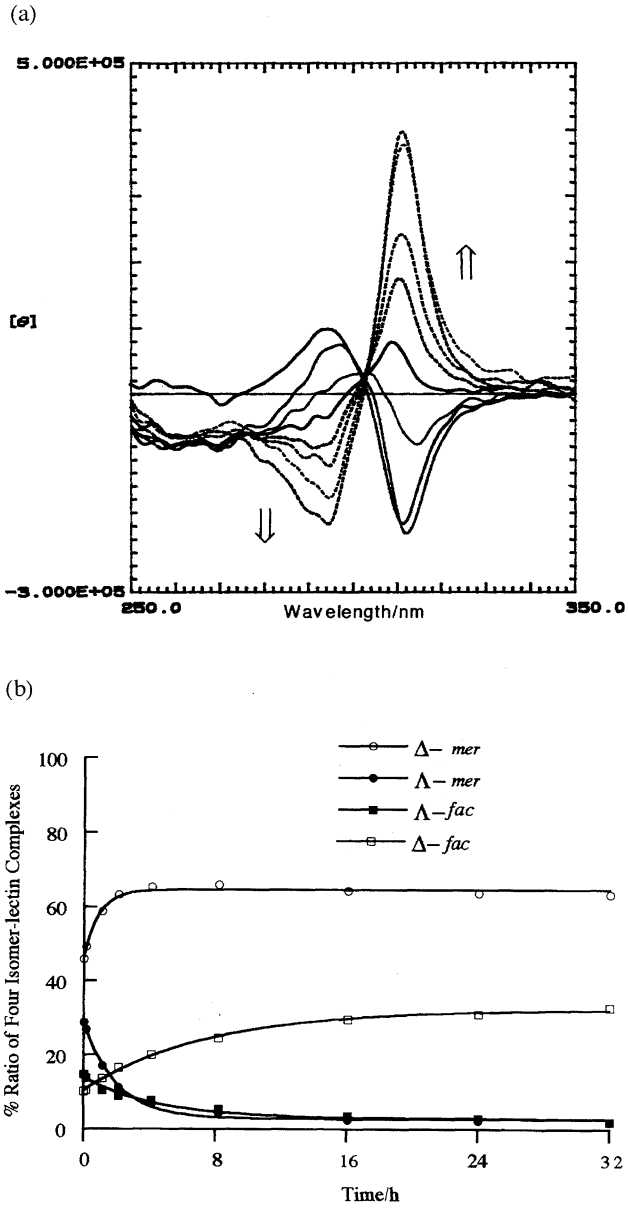


Fig. 3. Change of (a) CD spectrum and (b) isomer ratio of Fe^{II}(bipy-GalNAc)$_3$ in the presence of *Glycine max* lectin.

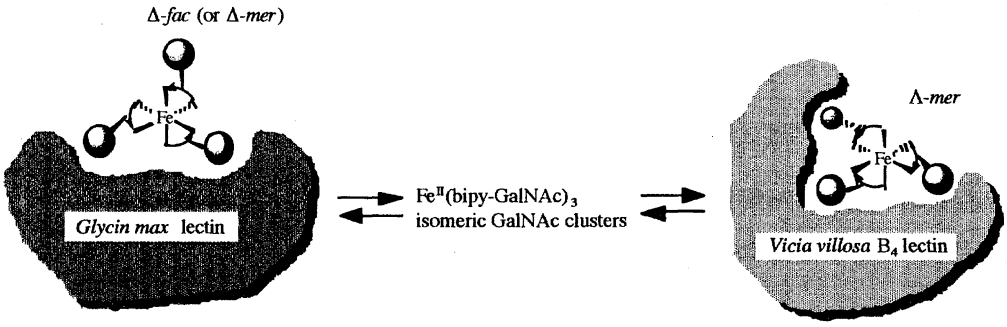
Table 3. Relative Stabilities of the Four Diasetereomers of Fe^{II}(bipy-GalNAc)$_3$ and Fe^{II}(bipy-GalNAc)$_3$-Lectin Complexes

Complex	*Δ −mer*	*Λ −mer*	*Δ −fac*	*Λ −fac*
	kcal mol^{-1}	kcal mol^{-1}	kcal mol^{-1}	kcal mol^{-1}
Fe^{II}(bipy-GalNAc)$_3$	−0.12	0.15	0.12	−0.10
Fe^{II}(bipy-GalNAc)$_3$-*V.V.* B_4 lectin	0.86	−0.45	0.22	0.99
Fe^{II}(bipy-GalNAc)$_3$-*G.M.* lectin	−0.31	1.74	−0.56	1.07

Their relative energies were calculated by an equation, $\Delta\Delta G_{iso} = -RT\ln(X_{i,exp}/X_{i,stat})$; where $X_{i,exp}$ is the experimentally determined mole fraction of isomer i and $X_{i,stat}$ is the statistically predicted mole fraction.

ates significantly from the statistical ratio in the presence of lectin, indicating strong diasterotopic interactions between Fe^{II}(bipy-GalNAc)$_3$ and the carbohydrate binding site of these lectins. The striking difference in the isomer selectivities between the *Vicia villosa* B_4 and *Glycine max* lectins must be due to the difference in the shape and functionalities of their carbohydrate binding pockets (Scheme 3). The *Vicia villosa* B_4 lectin stabilizes *Λ-mer* isomer most, and the energy difference between the most stable *Λ-mer* isomer and the least stable *Λ-fac* isomer reaches as much as 1.44 kcal mol^{-1}. The binding is also fairly specific; the energy difference between *Λ-mer* and the next stable *Δ-fac* is 0.67 kcal mol^{-1}, almost half of the maximum energy difference among diastereomers (1.44 kcal mol^{-1}). This specificity is consistent with the report[25,26] that the *Vicia villosa* B_4 lectin can recognize three GalNAc residues although the molecular basis for the observed diastereoselectivity is not clear because of the lack of structural information about the *Vicia villosa* B_4 lectin. On the other hand, *Glycin max* lectin stabilizes both *Δ-fac* and *Δ-mer* almost equally well. The chiral preference appears to reflect the handedness of the carbohydrate binding site. Two GalNAc residues on both *Δ-fac* and *Δ-mer* isomers should have the same spatial orientation. *Glycin max* lectin appears to recognize these two GalNAc residues, exhibiting similar binding affinities toward these two diastereomeric ligands. This is consistent with the carbohydrate specificity of *Glycin max* lectin. *Glycin max* lectin recognizes the A antigen (primarily one GalNAc residue) while the *Vicia villosa* B_4 lectin can bind to multiple GalNAc residues. The carbohydrate binding site of *Glycin max* lectin is, therefore, considered to be smaller than that of the *Vicia villosa* B_4 lectin.

Table 4 shows the half-life times and the initial rates of interconversions of the four diastereomers of Fe^{II}(bipy-GalNAc)$_3$ in the presence of lectins. In theory, a lectin could either increase or decrease the rates of isomerization. Scheme 4 shows the interconversion of ligand isomers **a** and **b** in the presence of lectin. If the binding site of lectin in-

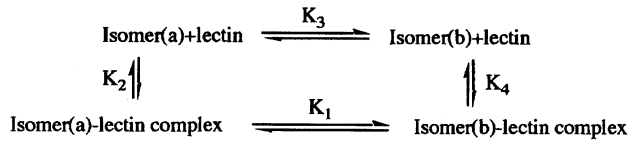


Scheme 3. Schematic diagram for self-adjusting binding of Fe^{II}(bipy-GalNAc)$_3$ to the *Vicia villosa* B_4 and *Glycin max* lectins.

Table 4. Half-Life Times (min) and Initial Rates ($\times 10^{-8}$ M min^{-1}, in Parenthesis) for the Shift in the Isomerization Equilibrium Initiated by the Addition of Lectins

Complex	*Δ −mer*	*Λ −mer*	*Δ −fac*	*Λ −fac*
Fe^{II}(bipy-GalNAc)$_3$-*V.V.* B_4 lectin	252 (−4.5)	335 (1.2)	776 (−0.04)	578 (−0.49)
Fe^{II}(bipy-GalNAc)$_3$-*G.M.* lectin	37 (6.8)	83 (−4.2)	309 (0.16)	182 (−2.1)

The half-life times of each Fe^{II}(bipy-GalNAc)$_3$ -lectin complexes were calculated using non-linear curve fitting program.



Scheme 4. Thermodynamic cycle of lectin-ligand (isomer **a** and **b**) interactions.

teracts strongly with **a** and **b**, the interconversion rate would become slower in the presence of lectin. The binding site can also interact with the transition state of the interconversion. If that is the case, the interconversion rate would increase in the presence of lectin. The observed interconversion rates are significantly slower than that of simple Fe(II)-bipy complexes such as $Fe^{II}(Me_2bipy)_3$,[35] consistent with the former mode of interaction.

The isomerization of Fe^{II}(bipy-GalNAc)$_3$ is expected to proceed in a mechanism similar to those observed in other inert d^6 metal complex,[32—34] where both rhombic twist and dangling-ligand pathway are involved. It is not clear, at this point, whether or not the isomerization occurs in the bound state of Fe^{II}(bipy-GalNAc)$_3$. If the interconversions among isomers occur in the bound state, the activation energy would have contributions both from the inorganic core and GalNAc residues-lectin interactions. It would, therefore, be possible to map out the shape and functionalities of the binding site based on a set of more detailed kinetic data of isomer interconversions.[36]

Conclusion

In conclusion, we have demonstrated a novel metal-assisted process for the assembly of tridentate GalNAc clusters. The synthetic GalNAc clusters were tested for their binding to various GalNAc-specific lectins. We also have shown that the synthetic GalNAc clusters undergoes a "self-adjusting" binding to the lectin by a dynamic interconversion of diastereomers. The isomerization equilibrium allows the carbohydrate ligand to choose an appropriate isomer that provides the maximum fitting to the binding site of various lectins. Detailed analysis of the kinetic and thermodynamic data for the isomerization can provide structural information of the carbohydrate binding site of lectins. The approach could be extended to construct heteromeric carbohydrate clusters, using a combination of various bipyridine-modified carbohydrates.

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