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Photoswitchable Multivalent Sugar Ligands: Synthesis, Isomerization, and Lectin Binding Studies of Azobenzene−Glycopyranoside Derivatives

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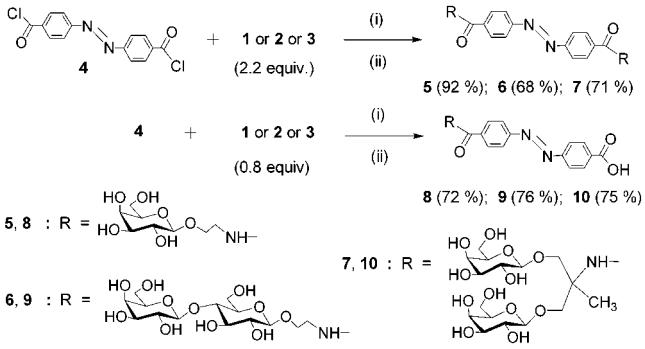
Among biological recognition processes, carbohydrate−protein recognitions are known to be rather weak ($K_a < 10^3$ M^{-1}).[1] A significant carbohydrate−protein interaction ensues in natural systems when one or both of the interacting partners is multiply presented at the binding sites.[2] In a series of pioneering contributions, Lee and co-workers have established[3] the so-called "glycoside cluster effect", wherein multivalent display of sugar residues at the binding sites of relevant lectin led to almost a logarithmic enhancement of binding potency. A number of small cluster glycosides,[4] polyvalent structures based on polymeric macromolecules,[5] and structurally homogeneous dendritic macromolecules[6] have been synthesized that have shown enhanced binding affinity to relevant lectins. In our desire toward synthesis of glycoclusters carrying a photoresponsive moiety, which will allow probing the biological recognition event, we have investigated the reversible photoisomerization properties of azobenzene-containing glycoconjugates at the interface of carbohydrate−lectin recognition processes. The facile and reversible trans ↔ cis isomerization properties of azobenzene chromophore have been studied in detail,[7] including in many biological[8] and physicochemical systems.[9] In this communication, we report the synthesis, photoisomerization, and lectin binding studies of new types of azobenzene−glycopyranoside derivatives. Most importantly, the preliminary studies described herein illustrate the existence of a cooperativity in the binding of a lactose bearing bivalent azobenzene derivative toward its high-affinity lectin, namely, *peanut agglutinin* (PNA), as judged from isothermal titration calorimetric (ITC) measurements.

The synthesis of azobenzene−glycopyranoside derivatives was accomplished by forming amide bonds between amine tethered glycopyranoside derivatives, namely, 2-aminoethyl-(2,3,4,6-tetra-*O*-benzoyl)-β-D-galactopyranoside (**1**), 2-aminoethyl-(2,3,4,6-tetra-*O*-benzoyl)-β-D-galactopyranosyl-(1 → 4)−(2,3,6-tri-*O*- benzoyl)-β-D-glucopyranoside (**2**), and 1,1′-bis-(2,3,4,6- tetra-*O*-benzoyl-β-D-galactopyranosyloxymethyl)ethylamine (**3**) and azobenzene-4,4′-dicarbonyl chloride **4**,[10] followed by removal of the *O*-benzoyl protecting groups to afford free sugar containing diamides **5**−**7** and monoamides **8**−**10** (Scheme 1), respectively.

Photoisomerization studies[11] on compounds **5**−**10** were conducted in H_2O (40−60 μM) by UV−vis spectroscopy. A decrease in absorbance (∼330 nm) of the trans-isomer of **5**−**10** was observed over a period of 5 to 30 min and the so-called photostationary state (*PS*) reached after ∼20 min. On the basis of the absorbance changes, ∼55−60% of cis isomer formation was calculated for all the compounds. Thermal reversal of the cis to trans isomer in dark conditions was monitored at ∼330 nm and the increase in absorbance, which corresponds to the evolution of the trans form, of the *PS* state was less than 5% even after 3 h. We have also

Scheme 1 [a]



[a] Conditions: (i) Et_3N, THF, 0 °C to room temperature, 24 h. (ii) 0.5 M NaOMe/MeOH, room temperature, 12 h.

Table 1. Rate Constants (k) and Activation Energies (E_a)[a] for Thermal Cis−Trans Isomerization of **6** and **9**

		70 °C	65 °C	60 °C	50 °C	E_a
6	k ($\times 10^4$ s^{-1})	1.40	1.14	0.56	0.21	20.54
9	k ($\times 10^4$ s^{-1})	1.41	1.20	0.34	0.18	22.57

[a] E_a (kcal mol^{-1}) values were obtained from the Arrhenius plots.

determined the cis:trans ratio at the *PS* state by NMR spectroscopy in D_2O (1−2 mM). In the case of symmetrical diamide derivatives **5**−**7**, the protons of the azobenzene unit having an AB type spin system in the trans isomer appear as a sharp singlet at ∼7.85 ppm. After irradiation for 2 h, a set of new peaks in the form of doublets at ∼7.50 and ∼7.00 ppm (J = 7.8 Hz) was observed, which upon integration, afforded ∼60−65% cis isomer at the *PS* state in these samples. Thermal isomerization from the cis isomer to the trans isomer was followed by the time course measurement of the absorbance changes (330 nm) for the photoirradiated solutions of **6** and **9** at different temperatures. The isomerizations followed first-order kinetics and the corresponding rate constants and the activation energies for **6** and **9** are given in Table 1.

Despite several reports on the interactions of cluster glycosides with multivalent lectins, the true cluster effect has been observed only with the asialoglycoprotein receptor (ASGPr),[3] primarily because of an exact geometric complementarity between the binding sites of the trimeric ASGPr and the triantennary oligosaccharide ligands. In most other cases, the interaction of the ligand occurs with independent, unconnected multivalent lectin molecules.[12] Consequently a true cluster effect is not seen. Also, in none of these cases has cooperativity during these interactions been observed. We explored the nature of binding by photoisomerisable bivalent lactoside derivative **6**, in which the azobenzene unit acts as the switchable scaffold. Our ITC studies pertain largely to the interaction of bivalent **6** with PNA, as lactose[13a] is known to have

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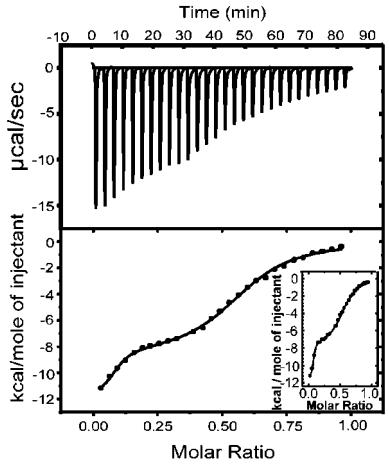

Figure 1. Binding profiles of the lactose derivative **6** in the trans and the *PS* (inset) isomeric states. The cooperativity is seen to be more in the case of the *PS* mixture than in the trans isomer.

Table 2. Thermodynamic Parameters for Binding of PNA to **6**[a]

	trans	*PS* mixture
K_b1 ($\times 10^{-4}$)	5.1 ± 0.2	4.9 ± 0.3
$\Delta H1$	-8.5 ± 0.1	-7.7 ± 0.1
$\Delta G1$	-6.4 ± 0.02	-6.4 ± 0.03
$T\Delta S1$	-2.1 ± 0.1	-1.3 ± 0.1
K_b2 ($\times 10^{-4}$)	118 ± 7.8	419 ± 15.0
$\Delta H2$	-12.5 ± 0.1	-11.8 ± 0.2
$\Delta G2$	-8.2 ± 0.04	-9.0 ± 0.02
$T\Delta S2$	-4.2 ± 0.1	-2.8 ± 0.2

[a] Values of K_b and ΔH, ΔG, and $T\Delta S$ are expressed in M^{-1} and kcal mol^{-1}, respectively. The studies were performed in 20 mM phosphate buffer, pH 7.4, and 150 mM NaCl. The titrations were done at 298 K.

higher affinity for PNA than galactose.[13b] As shown in Figure 1, the recognition of bivalent **6** by PNA exhibits pronounced cooperativity. The experimentally obtained thermodynamic parameters are given in Table 2. The distance between 4,4′-positions of the azobenzene unit is about 9 Å, while that between the anomeric oxygen at the reducing end is not expected to be greater than 17 Å. Consequently, a true cluster effect for its binding to the PNA molecule is not observed, as the least separation between these binding sites is 69 Å.[14] Even in the absence of a cluster effect, we still observe a much higher binding constant ($K_b1 = 5.1 \times 10^4$ M^{-1}) for the low-affinity part of the binding reaction, when compared to **7**, **8**, and **10** ($K_b = 3.5 \times 10^3$, 1.7×10^3, and 1.9×10^3 M^{-1}, respectively).[15]

Although the binding invokes interaction of the ligand with the separate unconnected subunits of tetrameric PNA, the geometry of the trans-azobenzene derivative **6** is such that a mere separation of 17 Å between its two lactose units promotes a cooperativity during the reaction. Further, the orientation of **6** increases the affinity of the second site in a striking manner. This can be explained on the basis that the binding of the first lactose moiety to a subunit of the first PNA tetramer aligns the second moiety in an orientation that increases the affinity for the recognition of the latter by another PNA molecule. Irrespective of the origin of the cooperativity, the affinity becomes even more pronounced in the cis-isomer (K_b2), where the distance between the two lactosides is close to 10 Å.[16]

In conclusion, the newly synthesized azobenzene−lacto- and galactopyranoside derivatives exhibit rather slow cis → trans thermal relaxation processes in aqueous solution. Lectin binding studies of bivalent derivative **6** with PNA, using ITC, have shown a biphasic binding profile, corresponding to a cooperative nature of the binding process. Although a number of multivalent glycosides have been tested previously, no report concerning the existence of cooperativity is reported so far. To the best of our knowledge, the present observation is the first report that such phenomena can exist in glycoside−lectin interactions.

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Supporting Information Available: Experimental procedures of **5**−**10** and method of ITC studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) **7**, **8**, and **10** had shown that the binding profiles were monophasic in nature, indicating the absence of any cooperativity, probably due to the fact that galactose itself exhibits low affinity for PNA. Low aqueous solubilities precluded ITC studies of **5** and **9**.
(16) It should be mentioned here that the contributions of the cis and trans forms were not taken into account separately during the ITC analysis of the *PS* mixture of bivalent derivative **6** because of practical difficulties.

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