Noncovalent Interactions

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Supramolecular Control of Oligosaccharide–Protein Interactions: Switchable and Tunable Ligands for Concanavalin A Based on β-Cyclodextrin**

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Interactions between carbohydrate-binding proteins (lectins) and cell-surface carbohydrate ligands have been shown to be critical for a wide variety of intercellular recognition processes, including cell proliferation, signaling, and recognition.[1] The interaction of a single carbohydrate ligand and a protein molecule is, however, usually too weak to induce a useful biological response. Biological systems commonly engage in multivalent carbohydrate–protein interactions to enhance the affinity. Remarkably, the advantage gained by multiple copies of a ligand is generally more than that predicted for the sum of the individual interactions.[2] This observation was first reported by Lee and Lee and is known as the glycoside cluster effect.[3] Since then it has been speculated that nature uses carbohydrate surface density as an "on/off" switch to regulate biological events involving carbohydrate–protein interactions. However, recent results have indicated that the situation is far more complicated. Architectural and orientational factors as well as secondary interactions in a heterogeneous environment may play a decisive role in the regulation and fine tuning of the binding strength and selectivity of the interaction between a lectin and its putative ligand.[4]

A number of multivalent systems have been reported in which the loading of the saccharide binding epitopes or their overall geometry were varied to get information on the mechanisms involved in the glycoside cluster effect.[5,6] However, none of the reported examples can fully mimic the switching between the "on" and "off" states and the regulation of the binding intensity after an external stimulus. We have now developed a molecular system based on β-cyclodextrin (βCD)[7] that can reproduce this behavior by virtue of allosteric supramolecular interactions with effector/antagonist-like molecules.

Switching of carbohydrate–protein interactions has previously been achieved through chemical modification of the protein with photoisomerizable groups that induce structural perturbations at the protein binding site. In seminal work, Willner prepared chemically modified lectins with reversible photochromic properties and demonstrated the reversible photostimulated "on/off" association and dissociation of monosaccharides.[8] The present study aims at implementing the "reversible structure-dependent binding" strategy by designing conformationally switchable glycoligands that are sensitive to chemical inputs.

Our molecular design is based on the observation that the ability of βCD-centered glycoligands to bind lectins is strongly influenced by the distance between the recognition motif and the βCD scaffold.[9] We conceived that this distance could be modulated by inserting a segment that was able to undergo intramolecular inclusion into the βCD cavity in a reversible manner. Previous results showed that some amino acids grafted on the βCD core form self-inclusion complexes in aqueous solution.[10] Interestingly, this process can be shifted in the presence of a competing guest for βCDs bearing a tyrosinyl residue,[11] thus suggesting that this structural motif could act as a distance-regulating element.

To prove the above concept we have focused on concanavalin A (Con A), a mannose-specific lectin commonly used to probe protein–carbohydrate interactions,[12] and the branched trisaccharide 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyaranose (Man-tri) as the protein receptor and the sugar ligand, respectively. Con A is known to possess an extended binding site for this trisaccharide, and results in a binding affinity ($\log K = 5.4$) much higher than that encountered for trivalent or even higher-valency mannopyranosides.[1b]

According to our previous work, we attached a succinylamido substituent to the βCD prior to the incorporation of the tyrosine segment or the mannosyl ligand.[13] It was anticipated that this spacer would provide enough flexibility to allow both the self-inclusion process and glycoligand–lectin recognition events in an extended conformation. To confirm this point, compounds **1** and **2**, which differed in the absence or presence of the aromatic amino acid, were prepared. Additionally, conjugate **3**, which possesses two Man-tri units, was synthesized to check how polyvalency could affect both the lectin binding and the self-inclusion phenomena in such a system (Scheme 1).

The synthetic strategy to access compounds **1**–**3** follows a convergent approach and makes use of standard peptide-coupling techniques. The synthesis of the per-*O*-esterified Man-tri glycosylamine **4** was achieved by using a simultaneous "double-glycosylation" of 2,4-di-*O*-benzoyl-α-D-mannopyranosyl azide with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyr-

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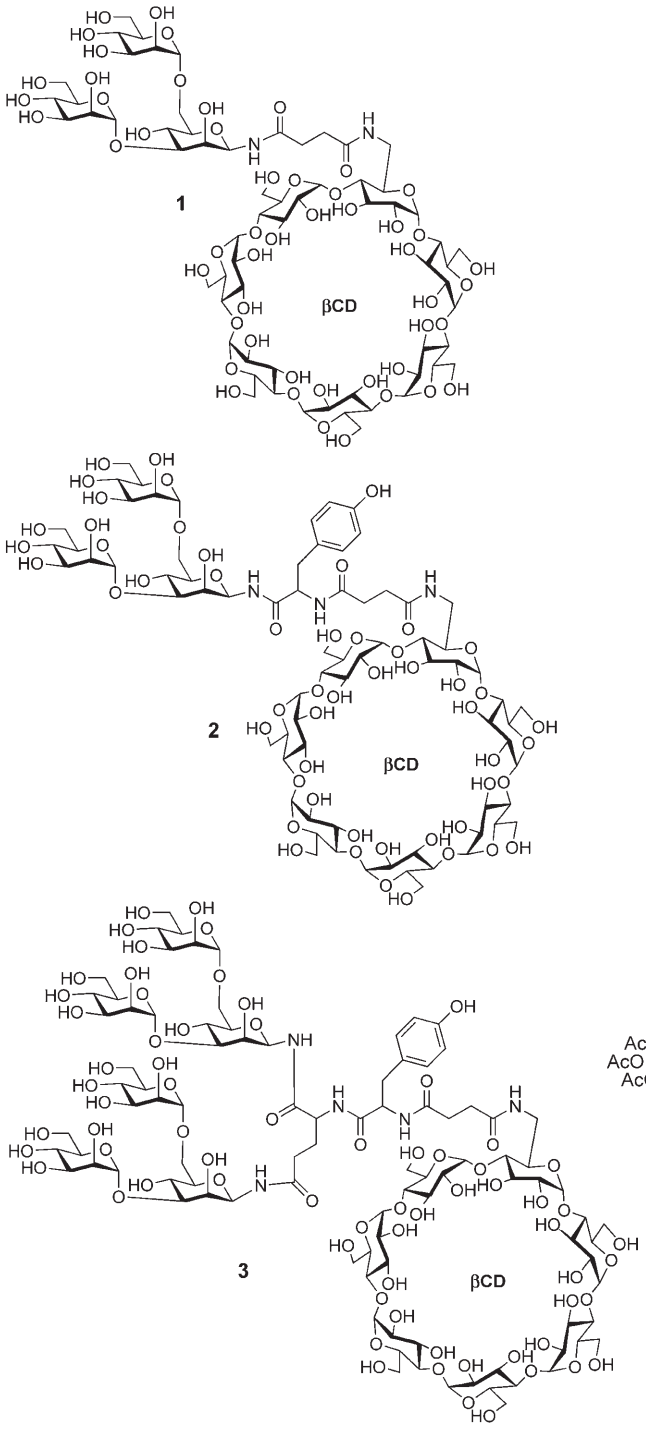
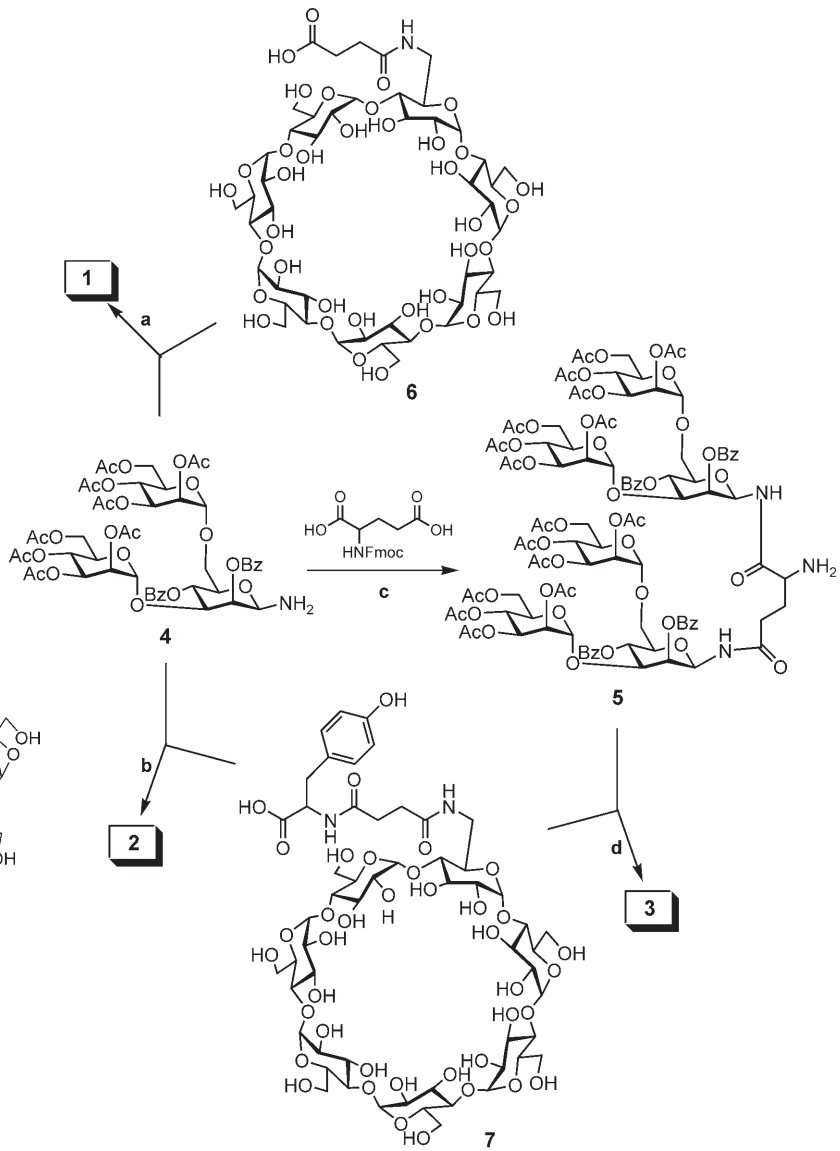


Scheme 1. Structure of the Man-tri–βCD conjugates evaluated.

anosyl trichloroacetimidate as described previously.[11,14] Reduction of the anomeric azido group to a β-amine was significantly improved by using the "catalytic transfer hydrogenation" method developed by Anwer and Spatola[15] (see the Supporting Information). Amide coupling of the known monofunctionalized βCD derivative **6**[13] and glycosylamine **4** afforded, after deprotection of the corresponding hemiacylated adduct, the succinyl-tethered conjugate **1**. An analogous coupling-deprotection sequence involving **4** and the tyrosinyl-armed precursor **7**[11] provided the elongated analogue **2**. Replacing the trisaccharide precursor by the amine-armed glycodendron **5**, accessible by double peptide coupling of **4** with N-Fmoc-protected glutamic acid and subsequent removal of the carbamate protecting group, afforded the target $(Man\text{-}tri)_2$ conjugate **3** (Scheme 2).

Comparative ^{1}H NMR and T-ROESY experiments on **1**–**3** in D_2O at different concentrations confirmed the formation of intramolecular self-inclusion complexes with the tyrosine-bearing conjugates **2** and **3**, with the aromatic ring placed inside the βCD cavity. These complexes were disrupted in $[D_6]$DMSO solution, thus indicating the reversible character of the self-inclusion process. Addition of adamantane-1-



Scheme 2. Synthesis of the βCD conjugates **1**–**3**. a) DIC, HOBt, iPr$_2$NEt, DMF, 96 h then DMF-MeOH, MeONa (1 M in MeOH), 7 days, (23%); b) Ref. [8]; c) DIC, HOBt, iPr$_2$NEt, DMF, 72 h, then piperidine, DMF, 90 min (76%); d) DIC, HOBt, iPr$_2$NEt, DMF, 72 h then MeOH, NH_3 (7 N in MeOH), 10 days (40%). DIC = diisopropylcarbodiimide, HOBt = 1-hydroxy-1*H*-benzotriazole, Fmoc = 9-fluorenylmethoxycarbonyl, Bz = benzyl.

carboxylate (AC), known to be an excellent guest for βCD (association constant (K_{ass}) $3.9\times10^4\,M^{-1}$),[16] to solutions of **2** or **3** in D_2O also caused departure of the tyrosine residue from the cavity, with formation of the 1:1 intermolecular inclusion complexes **2**:AC or **3**:AC ($K_{ass}=1.6\times10^4\,M^{-1}$), as deduced from NMR titration experiments.[17]

For evaluation of Con A binding, the enzyme-linked lectin assay (ELLA), a variation on the enzyme-linked immunosorbent assay (ELISA), was implemented.[2b] In agreement with our initial hypothesis, **2** was virtually unrecognized by the lectin at concentrations up to 1 mM, a result which is in stark contrast with data for **1** that lacks the tyrosine segment (IC_{50} 21 μM). Interestingly, a dramatic increase in Con A binding affinity was observed for **2** (IC_{50} 22 μM) in the presence of a threefold molar excess of AC, while that of **1** remained unaffected (Figure 1A). This result strongly supports the scenario depicted in Figure 1B (left-to-right). In the "off" state the epitope is not accessible to the protein binding site because of its conformation, while in the "on" state, the oligosaccharide is fully exposed to interact with the lectin. Switching between these two modes can be controlled by an allosteric supramolecular process at the CD cavity involving AC as a competing guest molecule.

Evaluation of the $(Man\text{-}tri)_2$ conjugate **3** by ELLA indicated that it had an affinity towards the protein receptor ($IC_{50}=3.2$ μM) that was more than sixfold higher than that of **1** or the **2**:AC complex. The Man-tri unit located at the α-substituent of the glutamic acid segment in **3** is probably now fully accessible for binding to Con A, the second one being only partially available to contribute to the glycoside cluster effect because of the energetic cost of disrupting the self-inclusion complex. Accordingly, a gradual increase in activity, up to a factor of three (IC_{50} 1.0 μM), was observed in the presence of increasing proportions of AC (Figure 2A). From this data, a binding isotherm consistent with formation of a 1:1 host/guest ligand was derived. Nonlinear regression analysis (Figure 2B) provided a K_{ass} value of $1.4\times10^4\,M^{-1}$ for the **3**:AC complex, which is about 2.5-fold lower than the value reported for the corresponding complex with βCD, probably because of the presence of the tyrosinyl self-inclusion element.

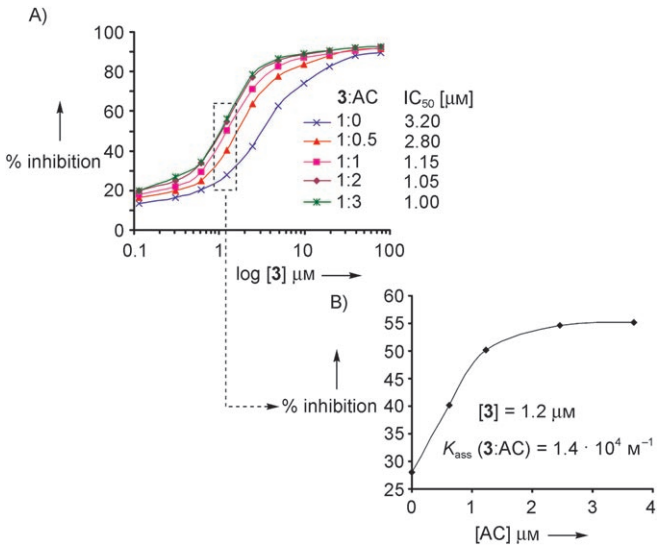


Figure 2. ELLA analysis for **3** in the presence of various concentrations of AC (A) and binding isotherm for the 1:1 **3**:AC complex (B).

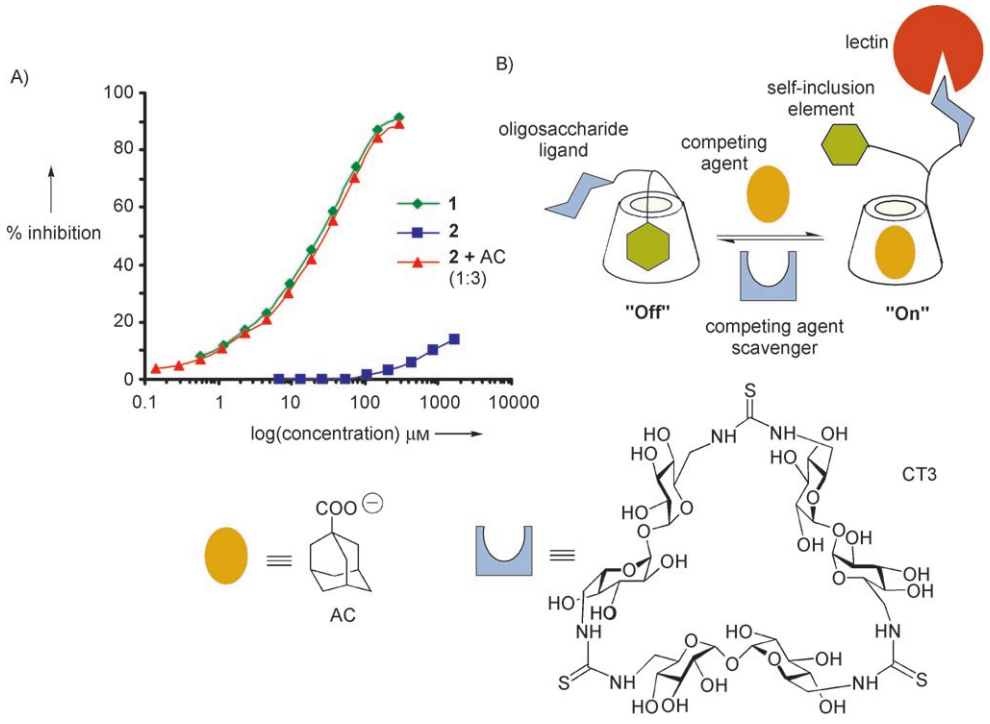


Figure 1. ELLA analysis (% inhibition of the Con A/yeast mannan binding) for **1**, **2**, and **2** + AC (A) and schematic representation of the observed "off/on" switching of carbohydrate–lectin binding through allosteric supramolecular interactions (B).

It is worth mentioning that the presence of a bulky horseradish peroxidase (HRP) label on Con A, to produce a spectrophotometric readout in the ELLA, is supposed to prevent cross-linking processes. These macromolecules likely interact with a single binding site per lectin, with proximity/statistical affinity enhancement mechanisms operating. The above result strongly suggests that both Man-tri groups become fully disposible for protein recruitment after AC stimulation, even under such conditions (microcluster effect[18,19]), which result in an increased biological response, by a biomimetic mechanism.

We hypothesized that the lectin affinity should be

depleted after addition of an appropriate AC scavenger (Figure 1 B, right-to-left). The trimeric α,α′-trehalose-based receptor CT3, which exhibits a perfect size match and symmetry complementarity with AC ($K_{ass} = 4.6 \times 10^4\ M^{-1}$)[20] was considered a good candidate for this purpose. Addition of 1.5 equivalents of CT3 per mol of effector molecule to AC-activated samples of **2** or **3** led the system back to the initial state—switch "off"—in agreement with the reversible character of the equilibria involved both at the lectin binding site and in the CD cavity.[21]

In summary, our results indicate that reversible tuning and switching of the binding affinity in a model carbohydrate–lectin system is possible through logical design. This work has included the first examples of allosteric activation/deactivation of binding and of the multivalent effect. This switchable, environment-sensitive, supramolecularly mediated carbohydrate–protein recognition offers exciting possibilities to mimic biological processes involving effector/antagonist molecules. Indeed, specific drug delivery using CD conjugates is hampered by the blocking of the protein receptors by unoccupied carriers, which might be avoided by designing switchable systems for which the drug will act as the effector (input "on"). We aim to investigate this possibility, as well as the further supramolecular modulation of multivalent interactions through variations in chemical modification in this and other systems.

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[1] a) H.-H. Gabius, H.-C. Siebert, S. André, J. Jiménez-Barbero, H. Rüdiger, *ChemBioChem* **2004**, *5*, 740 – 764; b) T. K. Dam, C. F. Brewer, *Chem. Rev.* **2002**, *102*, 387 – 429; c) C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357 – 2364.

[2] a) M. Mamen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908 – 2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754 – 2794; b) J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555 – 578.

[3] a) Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321 – 327; b) Y. C. Lee, R. T. Lee, *Glycoconjugate J.* **2000**, *17*, 543 – 551.

[4] a) N. Horan, H. Isobe, G. M. Whitesides, D. Kahne, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11782 – 11786; b) J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen, L. L. Kiessling, *J. Am. Chem. Soc.* **2002**, *124*, 14922 – 14933; c) E. K. Soller, E. D. Walter, J. R. Morgan, D. J. Singel, M. J. Cloninger, *J. Am. Chem. Soc.* **2003**, *125*, 8820 – 8826; d) M. Gómez-García, J. M. Benito, J.-X. Yu, K. Chmurski, C. Ortiz Mellet, R. Gutiérrez Gallego, A. Maestre, J. Defaye, J. M. García Fernández, *J. Am. Chem. Soc.* **2005**, *127*, 7970 – 7971; e) L. L. Kiessling, J. E. Gestwicki, L. E. Strong, *Angew. Chem.* **2006**, *118*, 2408 – 2429; *Angew. Chem. Int. Ed.* **2006**, *45*, 2348 – 2368.

[5] a) M. J. Cloninger, *Curr. Opin. Chem. Biol.* **2002**, *6*, 742 – 748; b) W. B. Turnbull, J. F. Stoddart, *Rev. Mol. Biotechnol.* **2002**, *90*, 231 – 255; c) T. K. Lindhorst, *Top. Curr. Chem.* **2002**, *218*, 201 – 235.

[6] For pioneering work on the evaluation of the influence of conformational crowding in carbohydrate–protein binding affinity using dendrimer-centred glycoligands, see: a) D. Page, R. Roy, *Bioconjugate Chem.* **1997**, *8*, 714 – 723; b) P. R. Ashton, E. F. Hounsell, N. Jayaraman, T. M. Nilsen, N. Spencer, J. F. Stoddart, M. Young, *J. Org. Chem.* **1998**, *63*, 3429 – 3437.

[7] Several glycosylated cyclodextrins have been prepared with the aim of combining both the inclusion capabilities of the hydrophobic cavity inherent in the cyclomalto-oligosaccharide structure and the biological receptor binding ability of oligosaccharides; for examples, see: a) C. Ortiz Mellet, J. Defaye, J. M. García Fernández, *Chem. Eur. J.* **2002**, *8*, 1982 – 1990; b) D. A. Fulton, J. F. Stoddart, *Bioconjugate Chem.* **2001**, *12*, 655 – 672; c) A. Vargas-Berenguel, F. Ortega-Caballero, F. Santoyo-González, J. J. García-López, J. J. Giménez-Martínez, L. García-Fuentes, E. Ortiz-Salmerón, *Chem. Eur. J.* **2002**, *8*, 812 – 827; d) T. Yamanoi, N. Yoshida, Y. Oda, E. Akaike, M. Tsutsumida, N. Kobayashi, K. Osumi, K. Yamamoto, K. Fujita, K. Takahashi, K. Hattori, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1009 – 1013; e) H. Abe, A. Kenmoku, N. Yamaguchi, K. Hattori, *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *44*, 39 – 47.

[8] I. Willner, *Acc. Chem. Res.* **1997**, *30*, 347 – 356, and references therein.

[9] a) J. M. Benito, M. Gómez-García, C. Ortiz Mellet, I. Baussanne, J. Defaye, J. M. García Fernández, *J. Am. Chem. Soc.* **2004**, *126*, 10355 – 10366; b) I. Baussanne, J. M. Benito, C. Ortiz Mellet, J. M. García Fernández, J. Defaye, *ChemBioChem* **2001**, *2*, 777 – 783.

[10] F. Djedaïni-Pilard, N. Azaroual-Bellanger, M. Gosnat, D. Vernet, B. Perly, *J. Chem. Soc. Perkin Trans. 2* **1995**, 723 – 730.

[11] D. Yockot, V. Moreau, G. Demailly, F. Djedaïni-Pilard, *Org. Biomol. Chem.* **2003**, *1*, 1810 – 1818.

[12] a) J. Bouckaert, T. Hamelryck, L. Wyns, R. Loris, *Curr. Opin. Struct. Biol.* **2001**, *11*, 635 – 643; b) H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637 – 674.

[13] R. Auzély-Velty, B. Perly, O. Taché, T. Zemb, P. Jéhan, P. Guenot, J.-P. Dalbiez, F. Djedaïni-Pilard, *Carbohydr. Res.* **1999**, *318*, 82 – 90.

[14] N. Smiljanic, S. Halila, V. Moreau, F. Djedaïni-Pilard, *Tetrahedron Lett.* **2003**, *44*, 8999 – 9002.

[15] M. K. Anwer, A. F. Spatola, *Tetrahedron Lett.* **1981**, *22*, 4369 – 4372.

[16] M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875 – 1917.

[17] J. Lin, F. Djedaïni-Pilard, P. Guénot, B. Perly, *Supramol. Chem*, **1996**, *7*, 175 – 181.

[18] The terms minicluster (microcluster) and macrocluster effect have been used previously in the literature to refer to the observed increases in lectin-binding affinities for small glycoclusters and large glycopolymers, respectively; see: a) M. S. Quesenberry, R. T. Lee, Y. C. Lee, *Biochemistry* **1997**, *36*, 2724 – 2732; b) J.-Q. Fan, M. S. Quesenberry, K. Takegawa, S. Iwahara, A. Kondo, I. Kato, Y. C. Lee, *J. Biol. Chem.* **1995**, *270*, 17730 – 17735.

[19] For recent evidence on the the existence of an aggregation-independent microcluster effect, see: M. Köhn, J. M. Benito, C. Ortiz Mellet, T. K. Lindhorst, J. M. García Fernández, *ChemBioChem* **2004**, *5*, 771 – 777.

[20] J. M. Benito, J. L. Jiménez Blanco, C. Ortiz Mellet, J. M. García Fernández, *Angew. Chem.* **2002**, *114*, 3826 – 2828; *Angew. Chem. Int. Ed.* **2002**, *41*, 3674 – 3676.

[21] Control ELLA experiments demonstrated that CT3 does not interfere in the binding of the mannosyl ligands **1**–**3** with Con A in the absence of AC. Binding of **1** to Con A was also unaffected by the presence of AC and AC:CT3 in up to threefold excesses. These results strongly support that CT3 triggers the "off" state by a mechanism involving exclusively AC trapping. Consequently, further addition of AC to a CT3-switched-off sample of **2**:AC restored the "on" state in a new cycle, thus confirming the reversibility of both input processes. NMR experiments additionally supported the absence of supramolecular interactions between CT3 and the βCD-centred glycoligands **1**–**3**.

