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[1] D. C. Nguyen, R. A. Keller, J. H. Jett, J. C. Martin, *Anal. Chem.* **1987**, *59*, 2158 – 2161.
[2] W. P. Ambrose, P. M. Goodwin, J. H. Jett, M. E. Johnson, J. C. Martin, B. L. Marrone, J. A. Schecker, C. W. Wilkerson, R. A. Keller, *Ber. Bunsen-Ges.* **1993**, *97*, 1535 – 1542.
[3] M. Eigen, R. Rigler, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5740 – 5747.
[4] R. F. Service, *Science* **1999**, *283*, 1668 – 1669.
[5] V. Folsom, M. J. Hunkeler, A. Haces, J. D. Harding, *Anal. Biochem.* **1989**, *182*, 309 – 314.
[6] H. Yu, J. Chao, D. Patek, R. Mujumdar, S. Mujumdar, A. S. Waggoner, *Nucleic Acids Res.* **1994**, *22*, 3226 – 3232.
[7] Z. Zhu, J. Chao, H. Yu, A. S. Waggoner, *Nucleic Acids Res.* **1994**, *22*, 3418 – 3422.
[8] *Handbook of Fluorescent Probes and Research Chemicals*, Molecular Probes, Eugene, Oregon, USA, **2000**.
[9] "Synthesis of fluorophore-labeled DNA": M. Goodman, L. Reha-Krantz (University of Southern California, Los Angeles, and University of Alberta, Edmonton), WO 97/39150 (PCT/US97/06493), **1997**.
[10] For a recent collection of reviews on this subject, see for example: "Evolutionary Protein Design": *Adv. Protein Chem.* **2001**, *55*.
[11] A. Kornberg, T. A. Baker, *DNA replication*, Freeman, New York, **1992**.
[12] D. L. Ollis, P. Brick, R. Hamlin, N. G. Xuong, T. A. Steitz, *Nature* **1985**, *313*, 762 – 766.
[13] D. Brutlag, M. R. Atkinson, P. Setlow, A. Kornberg, *Biochem. Biophys. Res. Commun.* **1969**, *37*, 982 – 989.
[14] H. Klenow, I. Henningsen, *Proc. Natl. Acad. Sci. USA* **1970**, *65*, 168 – 175.
[15] C. M. Joyce, T. A. Steitz, *Trends Biochem. Sci.* **1987**, *12*, 288 – 292.
[16] R. Rigler, J. Widengren, *BioScience* **1990**, 180 – 182.
[17] R. Rigler, *J. Biotechnol.* **1995**, *41*, 177 – 186.
[18] V. Derbyshire, P. S. Freemont, M. R. Sanderson, L. Beese, J. M. Friedman, C. M. Joyce, T. A. Steitz, *Science* **1988**, *240*, 199 – 201.
[19] R. C. Cadwell, G. F. Joyce, *PCR Methods Appl.* **1992**, *2*, 28 – 33.
[20] Z. Zhu, A. S. Waggoner, *Cytometry* **1997**, *28*, 206 – 211.
[21] M. Sauer, K. T. Han, R. Müller, S. Nord, A. Schulz, S. Seeger, J. Wolfrum, J. Arden-Jacob, G. Deltau, N. J. Marx, C. Zander, K. H. Drexhage, *J. Fluoresc.* **1995**, *5*, 247 – 262.
[22] L. Edman, Ü. Mets, R. Rigler, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6710 – 6715.
[23] K. P. Hopfner, A. Eichinger, R. A. Engh, F. Laue, W. Ankenbauer, R. Huber, B. Angerer, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3600 – 3605.
[24] P. G. Sealey, E. M. Southern, *Gel Electrophoresis of Nucleic Acids. A Practical Approach*, IRL Press, Oxford, **1982** .
[25] L. S. Beese, V. Derbyshire, T. A. Steitz, *Science* **1993**, *260*, 352 – 355.
[26] C. M. Joyce, T. A. Steitz, *Annu. Rev. Biochem.* **1994**, *63*, 777 – 822.
[27] S. Doublie, S. Tabor, A. M. Long, C. C. Richardson, T. Ellenberger, *Nature* **1998**, *391*, 251 – 258.
[28] M. M. Hingorani, M. O'Donnell, *Curr. Org. Chem.* **2000**, *4*, 887 – 913.
[29] S. Brakmann, S. Löbermann, *Angew. Chem.* **2001**, *113*, 1473 – 1476; *Angew. Chem. Int. Ed.* **2001**, *40*, 1427 – 1429.
[30] V. Picard, E. Ersdal-Badju, A. Lu, S. C. Bock, *Nucleic Acids Res.* **1994**, *22*, 2587 – 2591.
[31] P. Schwille, J. Bieschke, F. Oehlenschläger, *Biophys. Chem.* **1997**, *66*, 211 – 228.

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Dependence of Concanavalin A Binding on Anomeric Configuration, Linkage Type, and Ligand Multiplicity for Thiourea-Bridged Mannopyranosyl – β-Cyclodextrin Conjugates

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Concanavalin A (Con A), the mannose-specific lectin from *Concanavalia ensiformis*, has long been used as a model for carbohydrate – protein interactions.[1] Its commercial availability and the rather extensive structural knowledge currently available make it attractive for assessing and optimizing the functional parameters that affect its affinity for mannose neoglycoconjugates. Understanding these key elements may facilitate the development of new therapeutic strategies based on specific recognition events such as targeting of drugs.

Con A binds α-D-mannopyranosides preferentially over the corresponding β anomers.[2] The affinity for monosaccharide ligands is low; however, this is a rather common feature when considering protein – carbohydrate interactions. Carbohydrate – protein binding events usually involve several simultaneous contacts between carbohydrates that are clustered on cell surfaces and protein receptors that contain multiple carbohydrate-binding sites. Based on this concept, one could anticipate that multiplication of the saccharide epitope on the surface of the carrier may lead to a greater affinity than predicted from the sum of the constitutive one-to-one interactions[3, 4]—the so-

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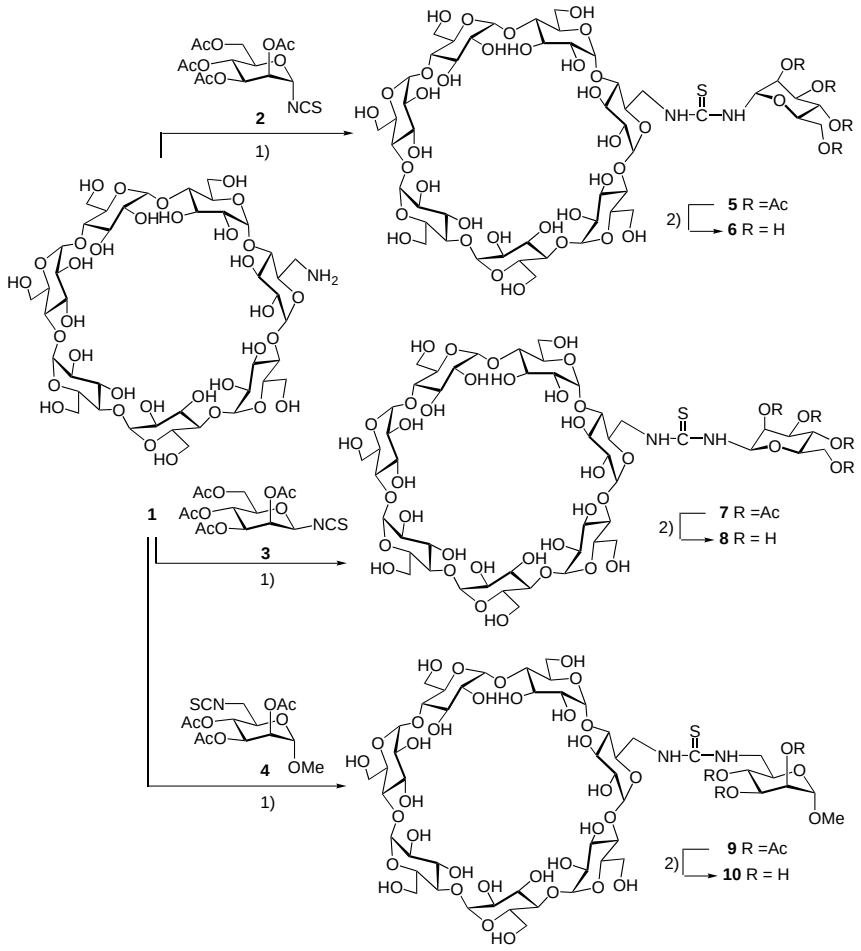
*Supporting information for this article (physical and spectroscopic data for compounds **5**, **7**, **9**, **16**, **17**) is available on the WWW under http://www.chembiochem.com or from the author.*

called cluster effect.[5] The binding efficiency and specificity may also be dependent on the nature of the aglycon as a result of secondary (hydrophobic, electronic, steric) unspecific interactions,[6] as well as on the geometrical characteristics of the multivalent assembly.[7]

We recently described an efficient preparation of β-cyclodextrin-(cyclomaltoheptaose, β-CD)-scaffolded glycoclusters from mono- as well as disaccharide glycosyl isothiocyanates and heptakis(6-amino-6-deoxy)cyclomaltoheptaose.[8] These systems offer the potential to serve as targeted drug delivery tools, taking advantage of the known drug inclusion capability of β-CD in aqueous media.[9] Moreover, the presence of the thiourea functionality as intersaccharide linkage in the CD conjugates results in improved water solubility and decreased hemolytic character as compared to the parent CDs.[10] It appeared, thus, of interest to further explore the effect of the thiourea linker and the CD core on the recognition process of the saccharide antennae by specific lectins. We now report on the preparation of several thiourea-linked D-mannose – β-CD conjugates and the evaluation of their lectin-binding ability toward Con A with respect to two pertinent issues: (i) to compare the binding efficiency for various thiourea-linked mannose – β-CD frameworks involving, eventually, changes in anomeric configuration, linkage position, and ligand multiplicity, and (ii) to pinpoint the effect of the β-CD core in the recognition process of the conjugate by the lectin.



Scheme 1. *Synthesis of the monovalent mannose – β-CD conjugates* ***6***, ***8***, *and* ***10****. 1) Acetone/water (1:1), pH 8, room temperature, 30 min; 2) NaOMe, MeOH, 0 °C.*

In addition to α- and β-D-mannopyranosylthioureido derivatives, (C-6)-thiourea-linked methyl α-D-mannopyranoside – β-CD conjugates have been included in our study. It has been recently shown that such "reverse" mannosyl ligands may represent an appealing alternative for targeting mannose-specific lectins, for example on *Escherichia coli*.[11] A comparative analysis of the binding efficiency for (C-6)-mono- and -heptasubstituted conjugates should provide information about the effect of ligand density on the interaction between the protein and the carrier.

The hemiacetylated (C-6)-monosubstituted β-CD conjugates **5**, **7**, and **9** were obtained in almost quantitative yields by nucleophilic addition of the corresponding β-CD monoamine **1** to the acetylated mannose isothiocyanates **2** – **4** in water/acetone at pH 8. The final deacetylation step, to provide the fully unprotected monovalent target derivatives **6**, **8** and **10**, was effected at 0 °C in the case of the mannopyranosylthioureido conjugates **5** and **7** to avoid anomerization at C-1′ (Scheme 1).[12]

The above protocol failed, however, in providing the pure (C-6)-heptaantennated mannopyranosylthioureido – β-CD glycoclusters. Albeit the reaction of heptakis(6-amino-6-deoxy)cyclomaltoheptaose (**11**) with the α- and β-D-mannopyranosyl isothiocyanates **2** and **3** in water/acetone at pH 8 was completed in 2 h, some deacetylation and anomerization occurred at this stage. Moreover, the anomerization proceeded in the subsequent deprotection step even at 0 °C, resulting in an anomeric mixture of heptavalent mannopyranosyl conjugates **12**. To get at least a thermodynamic distribution, the final deacetylation step was effected at room temperature for 16 h. A 1:1 relative proportion of α- versus β-D-mannopyranosyl residues was estimated from the ^{1}H NMR spectrum of the mixture in D_2O solution (H-1′ integration at 343 K). However, coupling of **11** with the acetylated deoxyisothiocyanate **4** under identical reaction conditions and further deacetylation afforded the corresponding heptavalent (6→6)-thiourea-linked methyl-α-D-mannopyranoside cluster **13** in 50% overall yield (Scheme 2).

The fast anomerization process observed at the mannopyranosyl moiety in mannopyranosylthioureido cyclodextrins points to a catalytic effect of the neighboring β-CD aglycon. This hypothesis was supported by a comparative study of the anomeric stability of the disaccharide mimetics[12] **14** and **15** and the mannopyranosylthioureido β-CD derivatives **6** and **8** (Scheme 3). Whereas the methyl α-D-glucopyranoside thioureido disaccharides were fully stable in D_2O solution at temperatures up to 70 °C (^{1}H NMR), anomerization of the mannopyr-

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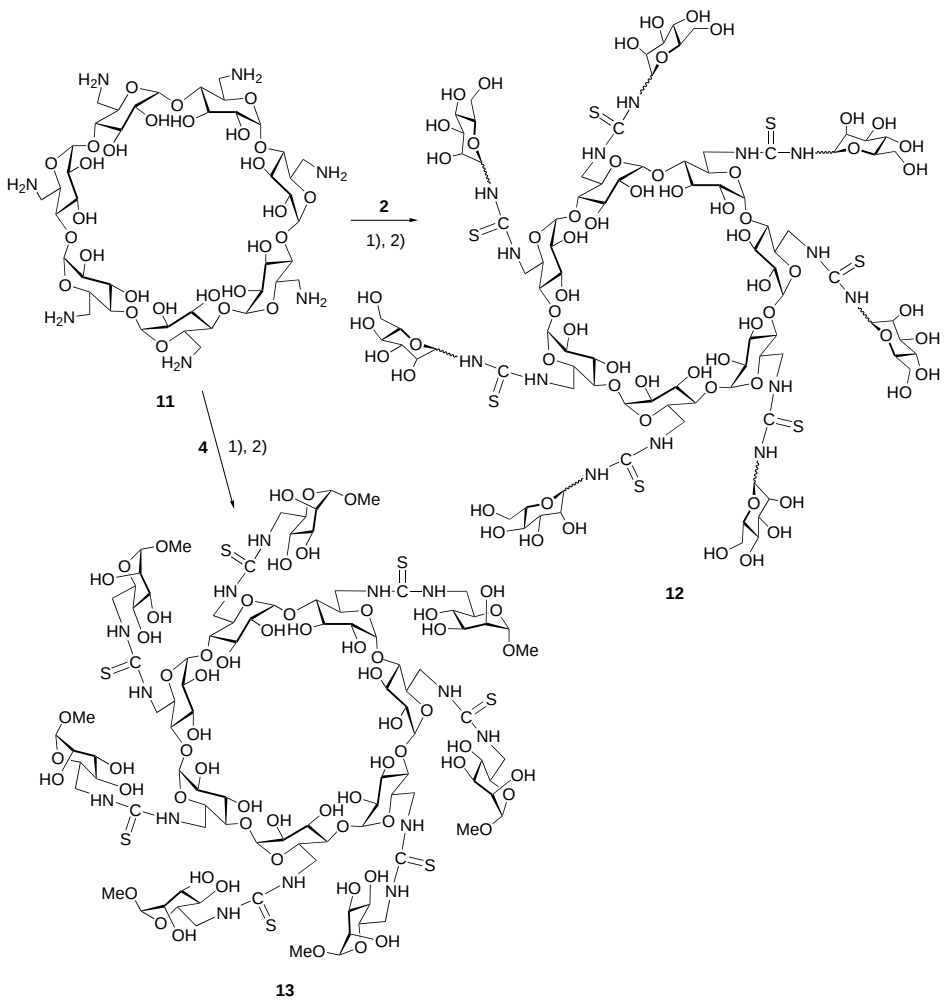
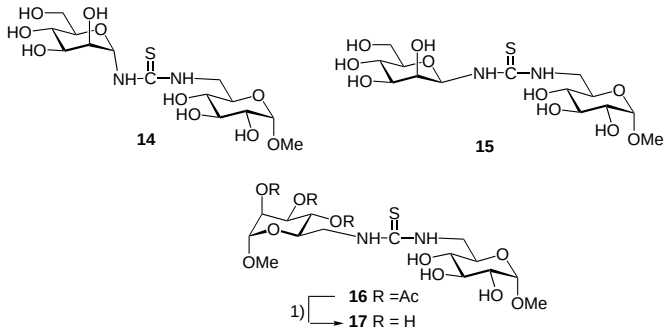


Scheme 2. *Synthesis of the heptavalent mannose – β-CD conjugates* ***12*** *and* ***13****. 1) Acetone/water (1:1), pH 8, room temperature, overnight; 2) NaOMe, MeOH.*



Scheme 3. *Structures of the thiourea-linked pseudodisaccharides* ***14****,* ***15****, and* ***17*** *that were used as reference compounds in the binding studies. 1) NaOMe, MeOH.*

anosylthioureido β-CD counterpart was already observed at 50 °C (> 10% interconversion after 3 h).

The ^{1}H and ^{13}C NMR spectra of compounds **5** – **10**, **12**, and **13**, recorded in deuterium oxide at room temperature, showed the typical line broadening associated with restricted rotation at the pseudoamide NH–C(=S) bonds.[13] Nevertheless, a satisfactory resolution was obtained at 50 – 70 °C, allowing complete structure confirmation. Thus, the ^{13}C NMR signal at $\delta_{C=S} = 184 - 183$ and the high-field signals for the carbon atoms directly linked to the thiourea group supported the (1 → 6) or (6 → 6) localization of the intersaccharide bridges. The anomeric configuration of the mannopyranosylthioureido substituents was established by $J_{C\text{-}1,H\text{-}1}$ measurements (164 – 168 Hz for α and 152 – 155 Hz for β derivatives).[14] The homogeneity of the conjugates was further confirmed by microanalytical and mass spectrometric (FAB or MALDI) data.

The affinity of the mannopyranosylthioureido ligands **6**, **8**, **10**, **12**, and **13** for Con A was evaluated by using an enzyme-linked lectin assay (ELLA).[15] In addition, the α-(1 → 6)-, β-(1 → 6)-, and (6 → 6)-thiourea-linked pseudodisaccharides **14**, **15**, and **17** (Scheme 3) were tested in the binding studies. ELLA allows to measure IC_{50} values for the inhibition of binding of horseradish-peroxidase-labeled Con A to immobilized yeast mannan.[16] Except when dealing with very long connecting units, ELLA is known to report specific protein – carbohydrate affinities, avoiding apparent affinity enhancement due to non-specific aggregation.[17]

Prior to the analysis of the pseudodisaccharide and branched β-CD ligands, it was ascertained that any isolated non-mannosyl structure could not significantly affect the lectin-binding process in solution. For example, neither β-CD nor the 6^I-deoxy-6^I-(*N*′-methylthioureido) derivative[8] did show any interaction in the Con A – yeast mannan association process. Con A is known to bind to methyl-α-D-glucopyranoside with an efficiency tenfold weaker as compared to the mannopyranosyl counterpart.[17] Yet, in the present study the corresponding 6-deoxy-6-(*N*′-methylthioureido) glucoside[18] did not interfere in the ELLA test at concentrations up to 5 mM.

Results in triplicate were used for the plotting of the inhibition curves for each individual ELLA experiment. Typically, the IC_{50} values obtained from several independently performed tests were in the range of ± 15%. Nevertheless, the relative inhibition potencies calculated from independent series of data were highly reproducible.

In contrast with the net α-anomeric specificity of Con A in the *O*-glycoside series, no significant difference in binding efficiency was found between the α- and β-D-mannopyranosylthioureas **14** and **15**. The IC_{50} values (1350 and 1365 μM, respectively) are

consistently higher as compared to data for methyl-α-D-mannopyranoside (870 μM in a parallel assay),[19] indicative of a moderate loss of lectin affinity. The thiourea-linked methyl-α-D-mannopyranoside-(6→6)-methyl-α-D-glucopyranoside pseudodisaccharide **17** behaved as a much poorer Con A ligand. At the highest concentration studied (4.2 mM), only 45% inhibition of the Con A-immobilized yeast mannan association was achieved.

The results of the inhibition studies for the mannose–β-CD conjugates are shown in Figure 1 and Table 1. The inhibition graphs for the monovalent ligands **6**, **8**, and **10** followed similar

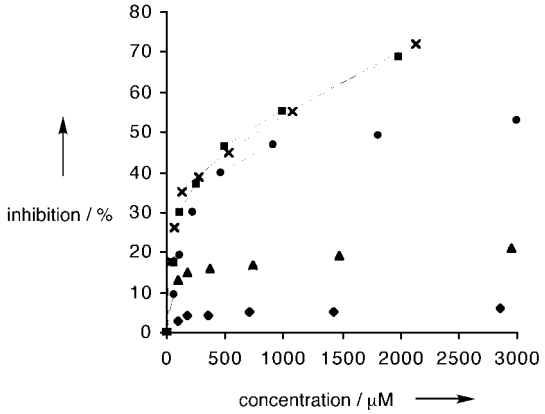


Figure 1. *Inhibition of binding of horseradish-peroxidase-labeled Con A to yeast mannan by increasing concentrations of monovalent (solid lines) and heptavalent (dotted lines) thiourea-linked mannose–β-CD conjugates.* × *:* ***6;*** ■*:* ***8;*** ●*:* ***10;*** ▲*:* ***12;*** ◆*:* ***13.***

Table 1. *ELLA data for binding inhibition of horseradish-peroxidase-labeled Con A by thiourea-linked mannose–β-CD conjugates and related mannose–glucose pseudodisaccharides.*

Parameter	Compound							
	6	**8**	**10**	**12**	**13**	**14**	**15**	**17**
IC_{50} [μM]	800	780	2000	>3200[a]	>2900[b]	1350	1365	>4200[c]
inhibition at 1 mM [%]	56	58	46	18	4	46	48	38

[a] 22% inhibition at 3.2 mM. [b] 6% inhibition at 2.9 mM. [c] 45% inhibition at 4.2 mM.

trends to that reported for the above homologous pseudodisaccharides, that is, no anomeric selectivity between the α- and β-mannopyranosylthiourea adducts was observed whereas the (6→6)-thiourea-linked derivative exhibited a strong decrease in binding efficiency. Nevertheless, the IC_{50} values (800, 780, and 2000 μM, respectively) were indicative of a binding efficiency 85–90% higher as compared with the homologous methyl-α-D-glucopyranoside derivatives **14**, **15**, and **17**. Unexpectedly, the Con A binding ability was almost totally abolished for the heptavalent glycoclusters **12** (22% inhibition at 3.2 mM) and **13** (6% inhibition at 2.9 mM).

Complete elucidation of the molecular mechanisms which account for the difference in behaviour of D-mannopyranosylthiourea compounds as compared with *O*-mannopyranosides with regard to Con A recognition will certainly need further investigation. Nevertheless, since α-(1→6)-linked mannosyl units in disaccharides exhibit a similar affinity for Con A as the methyl α-D-mannopyranoside,[20] the observed decrease in affinity and the loss of anomeric specificity can reasonably be ascribed to the presence of the thiourea functionality. Probably, the pseudoamide NH protons, with a high hydrogen bond donor character,[13, 21] affect the key hydrogen bond interaction between the vicinal OH-2 group and a bridged water molecule already found in the mannosyl ligand–Con A complex both in the solid state[22] and in solution.[23] The much more drastic decrease in binding efficiency upon replacement of the primary hydroxy group at C-6 by the thiourea linker agrees with the involvement of the former in hydrogen bonding to the residues in the binding site of the protein.

The higher Con A-binding efficiency observed for the mannopyranose–β-CD conjugates **6**, **8**, and **10** as compared with the pseudodisaccharide counterparts **14**, **15**, and **17** must be ascribed, essentially, to an additional stabilizing interaction involving the cyclic heptasaccharide aglycon. It is probable that once the mannosyl ligand has been accommodated in the recognition site of Con A, the β-CD framework is close enough to interact with residues at the protein surface. A similar situation has been found in the complex formed by a β-CD–gastrin conjugate and the human CCK-B receptor.[24] In the present case, the contribution of the β-CD–Con A interaction to the total free energy of binding can be estimated, from the comparative IC_{50} values,[16a] at about -0.35 kcal mol^{-1} under the particular conditions of the ELLA test.

The loss of binding ability for the heptaconjugates **12** and **13**, in spite of the locally enhanced ligand concentration, is noteworthy. Multivalent mannosyl analogues generally exhibit apparent affinities toward tetrameric Con A greater than those that can be rationalized solely on the basis of valency. Thus, a 3.8-fold increase (molar basis) has been reported for a nonavalent mannosylated benzenetricarboxylic-acid-centered dendrimer as compared with methyl-α-D-mannopyranoside,[16d] and up to 28- and 58-fold increases were found for octavalent and tetravalent mannosyl displays having poly(amidoamine) (PAMAM)[16c] and arylthioureido scaffolds,[16a] respectively. Similarly, the Con A-binding affinity of mannosylated glycopolymers increases by 10, 12, and 40-fold (molar basis) for degree of polymerization (DP) values of 10, 25 and 50, respectively, in comparison with the corresponding monomer.[25] Entropy-driven precipitation of three-dimensional cross-linked complexes or clustering of the lectin in solution has been shown to be responsible for this "multivalency effect".[26] The fact that Con A used in ELLA is directly labeled with horseradish peroxidase, which probably prevents formation of large ordered aggregates during ligand binding, may abolish this effect.[17] Nevertheless, assuming thermodynamic parameters equivalent to those of the monovalent counterparts for the initial mannopyranosyl–Con A interactions, a sevenfold increase in binding affinity would be expected for **12** and **13** as compared with **6**, **8**, or **10**. The observed collapse of the inhibition of mannan–Con A association likely arises from the impaired access of the convergent mannosyl cluster to the ligand-binding site.[27, 28] These results

  1439-4227/01/02/10 $ 17.50+.50/0 CHEMBIOCHEM **2001**, No. 10

are in agreement with recently reported data on carbohydrate-binding selectivity changes at high surface density for *Bauhinia purpurea* lectin,[29] supporting that expression levels of cell surface carbohydrates may modulate biological pathways in a more complex manner than just the off – on (low density – high density) model previously considered.

In the present investigation, we have demonstrated that Con A does not discriminate between α- and β-D-mannopyranosylthiourea ligands, which precludes a decrease in the binding efficiency due to possible anomerization processes. Mannoside ligands anchored through the primary C-6 position by a thiourea group exhibit a much poorer binding ability. Although the presence of the thiourea intersaccharide linker results in a slightly weaker lectin affinity as compared with normal *O*-glycosidic bonds, this effect is overcome in mono-(C-6)-mannopyranosylthioureido – β-CD conjugates by additional stabilizing interactions involving the cyclodextrin "aglycon". Persubstitution of the primary CD rim, however, precludes any recognition process. It is conceivable that the use of longer spacer arms may have beneficial effects from the biological standpoint, leading to an increase in the binding efficiency.[30, 31] Yet, heptasubstitution may also impair inclusion and stabilization of potential guests.[32, 33] An improvement of the biorecognition properties of these molecular hosts might then be achieved by monoconjugation of the β-CD core with multivalent saccharide markers. Progress toward this goal, exploiting the powerful high-yielding thiourea-based synthetic strategy here illustrated, is currently in progress in our laboratories.[34]

Experimental Section

Materials and methods: 6^{I}-Amino-6^{I}-deoxycyclomaltoheptaose[35] (**1**) was synthesized from the (C-6)-monotosyl derivative[34, 36] by replacement of the toluene-4-sulfonyl (tosyl) group by the azide anion and final reduction. The per-(C-6)-amino-β-CD derivative[37] **11** was obtained from the corresponding heptakis(6-deoxy-6-iodo)-β-CD[38] following a similar reaction sequence. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl isothiocyanate (**2**) was obtained from the corresponding α-D-mannopyranosyl bromide by reaction with KNCS following the procedure of Camarasa et al.[39] The β anomer **3** and the deoxyisothiocyanate derivative **4** were prepared by isothiocyanation of the amine precursors with thiophosgene as previously reported.[11, 40] Horseradish-peroxidase-labeled concanavalin A (Sigma), mannan from *Saccharomyces cerevisiae* (Sigma) and all other chemicals were of highest commercial purity and used as supplied. Optical rotations were measured at 20 °C in 1-cm or 1-dm tubes on a Perkin – Elmer 141 MC polarimeter. ^{1}H (and ^{13}C) NMR spectra were recorded at 300 (75.5) and 500 (125.7) MHz with Bruker 300 AMX, 500 AMX, and 500 DRX spectrometers. 1D TOCSY, 2D COSY, HMQC, and HSQC experiments were used to assist on NMR assignments. Thin-layer chromatography (TLC) was carried out on aluminium sheets coated with Kieselgel 60 F_{254} (Merck), with visualization by UV light and by charring with 10% H_2SO_4. Column chromatography was carried out with Silica Gel 60 (Merck, 230 – 400 mesh). FAB mass spectra were obtained with a Kratos MS-80 RFA instrument by using the following conditions: The primary beam consisted of Xe atoms with a maximum energy of 8 keV; the samples were dissolved in thioglycerol, and the positive ions were separated and accelerated over a potential of 7 keV; NaI was added as cationizing agent. MALDI-TOF mass spectra were recorded on a GSG System spectrometer operating in the positive-ion mode with an accelerating potential of 28 keV. Samples were dissolved in water at millimolar concentration and mixed with a standard solution of 2,5-dihydroxybenzoic acid (DHB, 10 mg mL^{-1} in 10% (v/v) aq EtOH) in 1:1 (v/v) relative proportions; 1 μL of the mixture was loaded onto the target plate and dried under vacuum immediately before acquisition. Elemental analyses were performed at the Instituto de Investigaciones Químicas (Sevilla, Spain).

Chemical syntheses: The monovalent thiourea-linked mannose – β-CD adducts were prepared by a coupling reaction of β-CD monoamine **1** (160 mg, 0.14 mmol) with isothiocyanates **2** – **4** (0.16 mmol, 1.1 equiv) in water/acetone (1:1; 2 mL) at pH 8 (aq $NaHCO_3$) at room temperature for 30 min. The corresponding partially acetylated compounds were purified by column chromatography (MeCN/water, 3:1) and deacetylated (26 μmol in 4 mL MeOH) by treatment with methanolic NaOMe (1 M, 52 μL) at 0 °C. After 5 min, a white precipitate appeared and the resulting suspension was stirred for 10 min. Water (1 mL) was then added and the clear solution was stirred for 10 min at 0 °C, then neutralized with Amberlite IR 120 (H^+) ion-exchange resin and further demineralized with Duolite MB 6113 (H^+, OH^-) mixed ion-exchange resin. The fully unprotected target conjugates were isolated as white lyophilisates.

6^{I}-Deoxy-6^{I}-(*N*′-α-D-mannopyranosylthioureido)cyclomaltoheptaose (6): Yield 99%; $[\alpha]_D = +107.1$ ($c = 0.7$, water); $R_f = 0.22$ (MeCN/water/NH_4OH, 6:3:1); ^{1}H NMR (500 MHz, D_2O, 343 K): $\delta = 6.03$ (br s, 1H, H-1′), 5.47 (d, $J_{1,2} = 3.1$ Hz, 1H, H-1^{II}), 5.44 – 5.42 (m, 6H, H-1^{I}, H-1$^{III–VII}$), 4.64 (m, 1H, H-6a^{I}), 4.52 (td, $J_{5,6a} = 2.3$ Hz, $J_{5,6b} = 9.8$ Hz, 1H, H-5^{I}), 4.42 (m, 1H, H-5^{II}), 4.36 (dd, $J_{1',2'} = 2.0$ Hz, $J_{2',3'} = 2.8$ Hz, 1H, H-2′), 4.33 – 4.15 (m, 27H, H-3′, H-6a′, H-6b′, H-3$^{I–VII}$, H-5$^{III–VII}$, H-6a$^{II–VII}$, H-6b$^{II–VII}$), 4.09 (t, $J_{3',4'} = J_{4',5'} = 9.7$ Hz, 1H, H-4′), 4.03 – 3.90 (m, 14H, H-5′, H-2$^{I–VII}$, H-4$^{II–VII}$), 3.86 (dd, $J_{6a,6b} = 14.8$ Hz, 1H, H-6b^{I}), 3.78 (t, $J_{3,4} = J_{4,5} = 9.8$ Hz, 1H, H-4^{I}); ^{13}C NMR (125.7 MHz, D_2O, 333 K): $\delta = 182.8$ (CS), 102.5 – 101.9 (C-1$^{I–VII}$), 83.8 (C-1′), 81.7 – 81.2 (C-4$^{I–VII}$), 78.2 (C-3′), 74.7 (C-5′), 73.8 – 73.5 (C-3$^{I–VII}$), 72.8 – 72.0 (C-2$^{I–VII}$, C-5$^{II–VII}$), 71.4 (C-4′), 70.2 (C-5^{I}), 67.7 (C-2′), 61.5 (C-6′), 61.0 – 60.6 (C-6$^{II–VII}$), 46.3 (C-6^{I}); MS (FAB, positive mode): *m/z*: 1376 $[M+Na]^+$; elemental analysis (%) calcd for $C_{49}H_{82}N_2O_{39}S$: C 43.42, H 6.10, N 2.07; found: C 43.24, H 5.99, N 2.10.

6^{I}-Deoxy-6^{I}-(*N*′-β-D-mannopyranosylthioureido)cyclomaltoheptaose (8): Yield 99%; $[\alpha]_D = +160.0$ ($c = 0.8$, water); $R_f = 0.23$ (MeCN/water/NH_4OH, 6:3:1); ^{1}H NMR (500 MHz, D_2O, 343 K): $\delta = 5.88$ (br s, 1H, H-1′), 5.44 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1^{II}), 5.42 – 5.39 (m, 6H, H-1$^{II–VII}$), 4.69 (m, 1H, H-6a^{I}), 4.42 (br t, 1H, $J_{4,5} = J_{5,6b} = 9.0$ Hz, H-5^{I}), 4.60 (m, 1H, H-5^{II}), 4.34 (d, 1H, $J_{2',3'} = 2.5$ Hz, H-2′), 4.30 – 4.15 (m, 25H, H-6a′, H-3$^{I–VII}$, H-5$^{III–VII}$, H-6a$^{II–VII}$, H-6b$^{II–VII}$), 4.08 (dd, $J_{5',6b'} = 5.4$ Hz, $J_{6a',6b'} = 12.0$ Hz, 1H, H-6b′), 4.06 (dd, $J_{3',4'} = 8.5$ Hz, 1H, H-3′), 4.00 – 3.90 (m, 14H, H-4′, H-2$^{I–VII}$, H-4$^{II–VII}$), 3.80 (m, 2H, H-5′, H-6b^{I}), 3.76 (t, $J_{3,4} = J_{4,5} = 9.0$ Hz, 1H, H-4^{I}); ^{13}C NMR (125.7 MHz, D_2O, 303 K): $\delta = 183.0$ (CS), 102.2 – 101.6 (C-1$^{I–VII}$), 83.4 (C-4^{I}), 82.1 (C-1′), 81.1 – 80.3 (C-4$^{II–VII}$), 73.5 (C-5′), 73.5 – 72.9 (C-3$^{I–VII}$), 72.2 – 71.7 (C-2$^{I–VII}$, C-5$^{II–VII}$), 72.1 (C-3′), 70.7 (C-2′), 70.6 (C-5^{I}), 66.4 (C-4′), 61.0 (C-6′), 60.9 – 59.9 (C-6$^{II–VII}$), 45.8 (C-6^{I}); MS (FAB, positive mode): *m/z*: 1377 $[M+Na]^+$; elemental analysis (%) calcd for $C_{49}H_{82}N_2O_{39}S$: C 43.42, H 6.10, N 2.07; found: C 43.08, H 6.00, N 2.01.

6^{I}-Deoxy-6^{I}-[*N*′-(methyl-6-deoxy-α-D-mannopyranosid-6-yl)thioureido]cyclomaltoheptaose (10): Yield 84%; $[\alpha]_D = +98.0$ ($c = 1.0$, water); $R_f = 0.24$ (BuOH/AcOH/H_2O, 2:1:1); ^{1}H NMR (500 MHz, D_2O, 343 K): $\delta = 5.45$ (d, $J_{1,2} = 3.7$ Hz, 1H, H-1^{II}), 5.43 – 5.41 (m, 6H, H-1^{II}, H-1$^{III–VII}$), 5.08 (d, $J_{1',2'} = 1.6$ Hz, 1H, H-1′), 4.61 (m, 1H, H-6a^{I}), 4.45 (m, 1H, H-5^{II}), 4.42 (br t, $J_{5,6b} = J_{4,5} = 9.5$ Hz, 1H, H-5^{I}), 4.32 (dd, 1H, $J_{6a',6b'} = 14.4$ Hz, $J_{5',6a'} = 2.5$ Hz, H-6a′), 4.27 (dd, $J_{2',3'} = 3.2$ Hz, 1H, H-2′), 4.26 (t, $J_{3,4} = J_{2,3} = 9.4$ Hz, 1H, H-3^{I}), 4.35 – 4.13 (m, 26H, H-2′, H-6a′, H-3$^{II–VII}$,

H-5$^{II–VII}$, H-6a$^{II–VII}$, H-6b$^{II–VII}$), 4.09 (dd, $J_{2',3'}=3.2$ Hz, $J_{3',4'}=9.3$ Hz, 1H, H-3′), 4.06 (ddd, $J_{4',5'}=9.3$ Hz, $J_{5',6a'}=2.5$ Hz, $J_{5',6b'}=7.2$ Hz, 1H, H-5′), 4.00–3.95 (m, 13H, H-2$^{I–VII}$, H-4$^{II–VII}$), 3.93 (t, 1H, H-4′), 3.88 (dd, $J_{5',6b'}=7.2$ Hz, $J_{6a',6b'}=14.4$ Hz, 1H, H-6b′), 3.78–3.75 (m, 2H, H-4^{I}, H-6b^{I}), 3.73 (s, 3H, OMe); ^{13}C NMR (125.7 MHz, D_2O, 343 K): $\delta=182.2$ (CS), 102.5–101.6 (C-1$^{I–VII}$), 101.6 (C-1′), 83.7 (C-4^{I}), 81.7–81.3 (C-4$^{II–VII}$), 73.7–73.5 (C-3$^{I–VII}$), 72.8–72.3 (C-2$^{I–VII}$, C-5$^{II–VII}$), 71.1 (C-3′), 70.6 (C-5′), 70.5 (C-2′), 70.3 (C-5^{I}), 69.0 (C-4′), 61.2–60.8 (C-6$^{II–VII}$), 55.4 (OMe), 45.9 (C-6^{I}), 45.7 (C-6′); MS (FAB, positive mode): *m/z*: 1369 $[M+H]^+$; elemental analysis (%) calcd for $C_{50}H_{84}N_2O_{39}S$: C 43.86, H 6.18, N 2.05; found: C 43.52, H 6.34, N 1.90.

Preparation of heptavalent glycoclusters

The heptavalent thiourea-linked mannose–β-CD glycoclusters were similarly obtained from the heptaamine **11** (as its heptahydrochloride, 29 μmol) and the mannose isothiocyanates **2** and **4** (0.20 mmol). The reaction time was increased to 16 h and the reaction mixture was subjected to column chromatography (MeCN/water, 5:1). The resulting product, containing partially deacetylated adducts, was further deacetylated by treatment with NaOMe/MeOH at room temperature for 16 h and demineralized as described above.

Heptakis[6-deoxy-6-(*N*′-D-mannopyranosylthioureido)]cyclomaltoheptaose (12): A mixture of heptamannosylated clusters, having a statistic distribution of α- and β-mannopyranosylthioureido substituents in a relative proportion close to 1:1 (H-1′ integration), was obtained in 40% yield. ^{1}H NMR (500 MHz, D_2O, 353 K): $\delta=6.2$ (br s, 3H, H-1′α), 5.9 (br s, 3H, H-1′β), 5.62–5.5 (m, 7H, H-1); ^{13}C NMR (125.7 MHz, D_2O, 353 K): $\delta=183.6$ (CS), 103.0–101.5 (C-1), 84.2–82.0 (C-1′), 83.5–83.0 (C-4), 74.6–74.0 (C-5′), 73.9–73.2 (C-3), 72.0–71.2 (C-2, C-5), 70.9–70.2 (C-2′, C-3′), 67.5–67.1 (C-4′), 61.6–61.4 (C-6′), 46.5–45.0 (C-6); MS (MALDI-TOF, positive mode): *m/z*: 2676 $[M+H]^+$, 2698 $[M+Na]^+$, 2724 $[M+K]^+$; elemental analysis (%) calcd for $C_{91}H_{154}N_{14}O_{63}S_7$: C 40.83, H 5.80, N 7.33; found: C 40.50, H 5.62, N 7.12.

Heptakis[6-deoxy-6-(*N*′-(methyl-6-deoxy-α-D-mannopyranosid-6-yl)thioureido)]cyclomaltoheptaose (13): Yield 50%; $[\alpha]_D=+49.9$ ($c=1.0$, water); $R_f=0.74$ (MeCN/water/NH_4OH, 5:3:5); ^{1}H NMR (500 MHz, D_2O, 343 K): $\delta=5.41$ (d, $J_{1,2}=4.0$ Hz, 7H, H-1), 5.08 (d, $J_{1',2'}=2.0$ Hz, 7H, H-1′), 4.45 (m, 14H, H-5, H-6a), 4.35 (m, 7H, H-6a′), 4.27 (t, $J_{2,3}=J_{3,4}=10.0$ Hz, 7H, H-3), 4.26 (dd, $J_{2',3'}=3.5$ Hz, 7H, H-2′), 4.09 (dd, $J_{3',4'}=10.5$ Hz, 7H, H-3′), 4.05 (m, 7H, H-5′), 3.97 (dd, 7H, H-2), 3.96 (m, 7H, H-6b′), 3.93 (t, $J_{4',5'}=10.5$ Hz, 7H, H-4′), 3.80 (m, 2H, H-4, H-6b), 3.71 (s, 3H, OMe); ^{13}C NMR (125.7 MHz, D_2O, 343 K): $\delta=$ 182.7 (CS), 102.2 (C-1), 101.6 (C-1′), 83.2 (C-4), 73.3 (C-3), 72.6 (C-2), 71.6 (C-5′), 71.1 (C-3′), 70.9 (C-5), 70.5 (C-2′), 69.0 (C-4′), 55.4 (OMe), 46.1 (C-6), 45.5 (C-6′); MS (MALDI-TOF): *m/z*: 2676 $[M+H]^+$, 2698 $[M+Na]^+$, 2724 $[M+K]^+$; elemental analysis (%) calcd for $C_{98}H_{168}N_{14}O_{63}S_7$: C 42.42, H 6.10, N 7.07; found: C 42.24, H 6.03, N 7.54.

Preparation of pseudodisaccharide ligands: The (1→6)-thiourea-linked pseudodisaccharide ligands **14** and **15**, used as reference compounds for binding studies, were prepared by reaction of the mannopyranosyl isothiocyanates **2** and **3**, respectively, with methyl 6-amino-6-deoxy-α-D-glucopyranoside[39] in pyridine at room temperature for 2 h and further deacetylation at 0 °C (0.5 equiv of NaOMe per mole of acetylated compound in MeOH) as reported.[12] Analogously, nucleophilic addition of methyl 6-amino-6-deoxy-α-D-glucopyranoside (53 mg, 0.33 mmol) to the deoxyisothiocyanate **4** (110 mg, 0.3 mmol) in pyridine (5 mL) and further deacetylation of the hemiacetate **16** afforded the new (6→6)-thiourea-bridged mannose–glucose pseudodisaccharide **17**.

Enzyme-linked lectin assay (ELLA): Nunc-Immuno plates (MaxiSorp) were coated overnight with yeast mannan at 100 μL per well, diluted from a stock solution of 10 μg mL^{-1} in 0.01 M phosphate-buffered saline (PBS, pH 7.3, containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+}) at room temperature. The wells were then washed three times with 300 μL of washing buffer (containing 0.05% (v/v) Tween 20) (PBST). The washing procedure was repeated after each incubation throughout the assay. The wells were then blocked with 150 μL per well of 1% bovine serum albumin (BSA)/PBS for 1 h at 37 °C. After washing, the wells were filled with 100 μL of a serial dilution of horseradish-peroxidase-labeled concanavalin A (Con A–HRP) from 10^{-1} to 10^{-5} mg mL^{-1} in PBS and incubated at 37 °C for 1 h. The plates were washed and 50 μL per well of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (1 mg per 4 mL^{-1}) in citrate buffer (0.2 M, pH 4.0, with 0.015% H_2O_2) was added. The reaction was stopped after 20 min by adding 50 μL per well of 1 M H_2SO_4, and the absorbances were measured at 415 nm. Blank wells contained citrate–phosphate buffer. The concentration of lectin–enzyme conjugate that resulted in an absorbance between 0.8 and 1.0 was used for the inhibition experiments.

Inhibition experiments: The microtiter plates were coated overnight at room temperature with yeast mannan (100 μL per well of 10 μg mL^{-1} solution). The wells were then washed and blocked with BSA as described. The inhibitors were used as stock solutions with concentrations varying from 5 to 7 mg mL^{-1} in PBS. Each inhibitor was added in a serial of twofold dilutions (60 μL per well) in PBS with 60 μL of the desired Con A–peroxidase conjugate concentration on Nunclon (Delta) microtiter plates and incubated for 1 h at 37 °C. The above solutions (100 μL) were then transferred to the mannan-coated microplates, which were incubated for another hour at 37 °C. The plates were washed and the ABTS substrate was added (50 μL per well). Color development was stopped after 20 min and the absorbances were measured. The amount of inhibition was calculated as follows:

$$\text{inhibition (\%)} = \frac{A_{\text{no inhibitor}} - A_{\text{with inhibitor}}}{A_{\text{no inhibitor}}} \times 100$$

IC_{50} values were reported as the concentration required for 50% inhibition of the Con A–coating mannan association.

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[1] For leading reviews see: a) H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637–674; b) R. Loris, T. Hamelryck, J. Bouckaert, L. Wyns, *Biochim. Biophys. Acta* **1998**, *1383*, 9–36; c) J. Bouckaert, T. Hamelryck, L. Wyns, R. Loris, *Curr. Opin. Struct. Biol.* **1999**, *9*, 572–577; d) M. E. Etzler in *Carbohydrates in Chemical Biology, Part II, Vol. 4* (Eds.: B. Ernst, G. W. Hart, P. Sinaÿ), Wiley-VCH, Weinheim, **2000**, pp. 535–548; for a recent example of Con A–mannosyl ligand binding screening see: e) O. Ramström, J.-M. Lehn, *ChemBioChem* **2000**, *1*, 41–48.

[2] a) I. J. Goldstein, R. D. Poretz in *The Lectins: Properties, Functions and Applications in Biology and Medicine* (Eds.: N. Sharon, I. J. Goldstein), Academic Press, Orlando, **1986** pp. 33–247; b) R. D. Poretz, I. J. Goldstein, *Biochemistry* **1970**, *9*, 2890–2896.

[3] a) K. G. Rice in *Glycosciences, Status and Perspectives* (Eds.: H.-J. Gabius, S. Gabius), Chapman & Hall, Weinheim, **1997**, pp. 471–483; b) K. J. Yarema, C. Bertozzi, *Curr. Opin. Chem. Biol.* **1998**, *2*, 49–61; c) C. A. Lingwood, *Curr. Opin. Chem. Biol.* **1998**, *2*, 695–700; d) T. Ouchi, Y. Ohya in *Neoglycoconjugates: Preparation and Applications* (Eds.: Y. C. Lee, R. T. Lee), Academic Press, San Diego, **1994**, pp. 465–499; e) B. G. Davies, *J. Chem. Soc. Perkin Trans. 1* **1999**, 3215–3237; f) H. Lis, N. Sharon, *Curr. Opin. Struct. Biol.* **1991**, *1*, 741–749; g) J. M. Rini, Y. D. Lobsanov, *Curr. Opin. Struct. Biol.* **1999**, *9*, 578–584; h) K. Drickamer, M. E. Taylor, *Annu. Rev. Cell Biol.* **1993**,

9, 237 – 264; i) H. D. Ward in *Glycosciences, Status and Prespectives* (Eds.: H. J. Gabius, S. Gabius), Chapman & Hall, Weinheim, **1997**, pp. 399 – 413.

[4] For reviews see: a) T. K. Lindhorst in *Bioorganic Chemistry. Highligths and Perspectives* (Eds.: U. Diederichsen, T. K. Lindhorst, B. Westermann, L. A. Wessjohann), Wiley-VCH, Weinheim, **1999**, pp. 133 – 150; b) R. Roy, *Curr. Opin. Struct. Biol.* **1996**, *6*, 692 – 702; c) N. Jayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem. Eur. J.* **1997**, *3*, 1193 – 1199; d) Y. Kim, S. Zimmerman, *Curr. Opin. Chem. Biol.* **1998**, *2*, 733 – 742; e) R. Roy, *Top. Curr. Chem.* **1997**, *187*, 241 – 274; for selected examples on polymannosyl displays see: f) T. K. Lindhorst, C. Kieburg, *Angew. Chem.* **1996**, *108*, 2083 – 2086; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1953 – 1956; g) C. Kieburg, M. Dubber, T. K. Lindhorst, *Synlett* **1997**, 1447 – 1449; h) B. König, T. Fricke, A. Wassmann, U. Krallmann-Wenzel, T. K. Lindhorst, *Tetrahedron Lett.* **1998**, *39*, 2307 – 2310; i) C. Saitz-Barría, A. Torres-Pinedo, F. Santoyo-González, *Synlett* **1999**, 1891 – 1894.

[5] a) M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908 – 2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754 – 2794; b) Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321 – 327.

[6] a) N. Sharon, H. Lis, *FASEB J.* **1990**, *4*, 3198 – 3208; b) F. G. Loontiens, J. P. Van Wauwe, R. De Gussem, C. K. De Bruyne, *Carbohydr. Res.* **1973**, *30*, 51 – 62; c) R. D. Poretz, I. J. Goldstein, *Biochem. Pharmacol.* **1971**, *20*, 2727 – 2739.

[7] L. L. Kiessling, N. L. Phol, *Chem. Biol.* **1996**, *3*, 71 – 77.

[8] C. Ortiz Mellet, J. M. Benito, J. M. García Fernández, H. Law, K. Chmurski, J. Defaye, M. L. O'Sullivan, H. N. Caro, *Chem. Eur. J.* **1998**, *4*, 2523 – 2531.

[9] a) K.-H. Frömming in *Comprehensive Supramolecular Chemistry*, *Vol. 3* (Eds.: J. L. Atwood, J. E. D. Davies, D. C. MacNicol, F. Vögtle), Pergamon, Oxford, **1996**, pp. 57 – 188; b) K. Uekama, F. Hirayama, T. Irie, *Chem. Rev.* **1998**, *98*, 1741 – 2076; c) T. Loftsson, M. E. Brewster, *J. Pharm. Sci.* **1996**, *85*, 1017 – 1025; d) J. Szejtli, *Med. Res. Rev.* **1994**, *14*, 353 – 386.

[10] J. Defaye, C. Ortiz Mellet, J. M. García Fernández (CNRS), WO 9733919, FR 96-3221. [*Chem. Abstr.* **1997**, *127*, 264489].

[11] S. Kötter, U. Krallmann-Wenzel, S. Ehlers, T. K. Lindhorst, *J. Chem. Soc. Perkin Trans. 1* **1998**, 2193 – 2200.

[12] J. M. Benito, C. Ortiz Mellet, K. Sadalapure, T. K. Lindhorst, J. Defaye, J. M. García Fernández, *Carbohydr. Res.* **1999**, *320*, 37 – 48.

[13] J. M. García Fernández, C. Ortiz Mellet, *Adv. Carbohydr. Chem. Biochem.* **2000**, *55*, 35 – 135.

[14] I. Tvaroska, T. Bleha, *Adv. Carbohydr. Chem. Biochem.* **1989**, *47*, 45 – 124.

[15] I. Ofek, *Methods Enzymol.* **1995**, *253*, 528 – 537.

[16] For selected examples of applications of the ELLA protocol to the evaluation of Con A binding see: a) R. Roy, D. Pagé, S. Figueroa Pérez, V. Vérez-Bencomo, *Glycoconjugate J.* **1998**, *15*, 251 – 263; b) D. Pagé, D. Zanini, R. Roy, *Bioorg. Med. Chem.* **1996**, *4*, 1949 – 1961; c) D. Pagé, R. Roy, *Bioconjugate Chem.* **1997**, *8*, 714 – 723; d) 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.

[17] J. B. Corbell, J. J. Lundquist, E. Toone, *Tetrahedron: Asymmetry* **2000**, *11*, 95 – 111.

[18] J. M. García Fernández, C. Ortiz Mellet, V. M. Díaz Pérez, J. L. Jiménez - Blanco, *Tetrahedron* **1996**, *52*, 12947 – 12970.

[19] Other values reported in the literature are 924 and 530 μM (cf. refs. [16a] and [17], respectively).

[20] H. Kaku, I. J. Goldstein, S. Oscarson, *Carbohydr. Res.* **1991**, *213*, 109 – 116.

[21] J. L. Jiménez Blanco, J. M. Benito, C. Ortiz Mellet, J. M. García Fernández, *Org. Lett.* **1999**, *1*, 1217 – 1220 and references therein.

[22] a) Z. Derewenda, J. Yariv, J. R. Helliwell, A. J. Kalb, E. Dodson, M. Z. Papiz, T. Wan, J. Campbell, *EMBO J.* **1989**, *8*, 2189 – 2193; b) J. H. Naismith, C. Emmerich, J. Habash, S. J. Harrop, J. R. Helliwell, W. N. Hunter, J. Raferty, A. J. Kalb, J. Yariv, *Acta Crystallogr. Sect. D* **1994**, *50*, 847 – 858; c) J. H. Naismith, R. A. Field, *J. Biol. Chem.* **1996**, *271*, 972 – 976; d) R. Loris, D. Maes, F. Poortmans, L. Wyns, J. Bouckaert, *J. Biol. Chem.* **1996**, *271*, 30614 – 30618.

[23] C. P. Swaminathan, N. Surolia, A. Surolia, *J. Am. Chem. Soc.* **1998**, *120*, 5153 – 5159.

[24] N. Schaschke, S. Fiori, E. Wehyher, C. Escrient, D. Fourmy, G. Müller, L. Moroder, *J. Am. Chem. Soc.* **1998**, *120*, 7030 – 7038.

[25] a) M. Kanai, K. H. Mortell, L. L. Kiessling, *J. Am. Chem. Soc.* **1997**, *119*, 9931 – 9932; b) D. A. Mann, M. Kanai, D. J. Maly, L. L. Kiessling, *J. Am. Chem. Soc.* **1998**, *120*, 10575 – 10582.

[26] a) T. K. Dam, R. Roy, S. K. Das, S. Oscarson, C. F. Brewer, *J. Biol. Chem.* **2000**, *275*, 14223 – 14230; b) S. D. Burke, Q. Zhao, M. C. Schuster, L. L. Kiessling, *J. Am. Chem. Soc.* **2000**, *122*, 4518 – 4519.

[27] For a discussion of the role of ligand density in the accessibility to a protein receptor see: a) B. T. Houseman, M. Mrksich, *Angew. Chem.* **1999**, *111*, 876 – 880; *Angew. Chem. Int. Ed.* **1999**, *38*, 782 – 785; for examples of a decrease in binding affinity of mannosyl ligands upon clustering at high density on liposome surfaces see: b) J. D. Baldeschwieler, *Ann. N.Y. Acad. Sci.* **1985**, *446*, 349 – 367; c) K. Tagawa, N. Sendai, K. Ohno, T. Kawaguchi, H. Kitano, *Bioconjugate Chem.* **1999**, *10*, 354 – 360.

[28] The high ligand density alone does not provide a satisfactory explanation for the observed complete loss of binding affinity. Increased binding avidity to Con A has been reported for mannosyl clusters having as few as three C–C bonds between the monosaccharide epitopes (see ref. [16d]). The location of the mannosyl subunits in the convergent primary positions of the β-CD scaffold through semirigid thiourea linkers likely results in a jelly-fish-like compact arrangement (see ref. [8]) that impairs lectin approaching.

[29] N. Horan, L. Yan, H. Isobe, G. M. Whitesides, D. Kahne, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11782 – 11786.

[30] To confirm this point, and following the suggestion of one of the referees, an analogue of **12** having a five-carbon spacer between the mannopyranosylthioureido ligands and the 6-deoxy-6-thioureido groups in the β-CD template, namely heptakis[6-deoxy-6-[*N*′-[5-(*N*′-D-mannopyranosyl-thioureido)pentyl]thioureido]cyclomaltoheptaose, was prepared. Evaluation of its Con A affinity by the ELLA protocol gave an IC_{50} value of 70 μM, more than ten times lower as compared with data for the monovalent compounds **6** and **8** (Table 1). This result strongly supports that a spatial organization, and not an intrinsic structural effect associated to either the mannopyranosylthiourea or the 6-deoxy-6-thioureido β-CD moieties, is responsible for the lack of binding affinity in the case of **12**.

[31] For further examples of per-(C-6)-substituted β-CD glycoclusters exhibiting enhanced lectin binding ability see: a) J. J. García-López, F. Hernández-Mateo, J. Isac-García, J. M. Kim, R. Roy, F. Santoyo-González, A. Vargas-Berenguel, *J. Org. Chem.* **1999**, *64*, 522 – 531; b) N. Yasuda, N. Aoki, H. Abe, K. Hattori, *Chem. Lett.* **2000**, 706 – 707; c) T. Furuike, S. Aiba, S.-I. Nishimura, *Tetrahedron* **2000**, *56*, 9909 – 9915.

[32] Our own results indicated a 10 – 20% decrease in the water solubilization ability of branched β-CDs, measured for taxane anticancer drugs, upon heptafunctionalization.

[33] V. Lainé, A. Coste-Sarguet, A. Gadelle, J. Defaye, B. Perly, F. Djedaïni-Pilard, *J. Chem. Soc. Perkin Trans. 2* **1995**, 1479 – 1487.

[34] I. Baussanne, J. M. Benito, C. Ortiz Mellet, J. M. García Fernández, H. Law, J. Defaye, *Chem. Commun.* **2000**, 1489 – 1490.

[35] R. C. Peter, C. T. Salec, G. Sikorski, G. Kumaravel, F.-T. Lin, *J. Am. Chem. Soc.* **1990**, *112*, 3860.

[36] J. Defaye, S. Crouzy, N. Evrard, H. Law, WO (99) 61483, FR (98) 06605. [*Chem. Abstr.* **2000**, *132*, 24077a].

[37] J. M. García Fernández, C. Ortiz Mellet, J. L. Jiménez Blanco, J. Fuentes - Mota, A. Gadelle, A. Coste-Sarguet, J. Defaye, *Carbohydr. Res.* **1995**, *268*, 57 – 71.

[38] A. Gadelle, J. Defaye, *Angew. Chem.* **1991**, *101*, 94 – 96; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 78 – 80.

[39] M. J. Camarasa, P. Fernández-Resa, M. T. García-López, F. G. de las Heras, P. P. Méndez-Castrillón, A. San Félix, *Synthesis* **1984**, 509 – 510.

[40] J. M. García Fernández, C. Ortiz Mellet, J. Fuentes, *J. Org. Chem.* **1993**, *58*, 5192 – 5199.

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