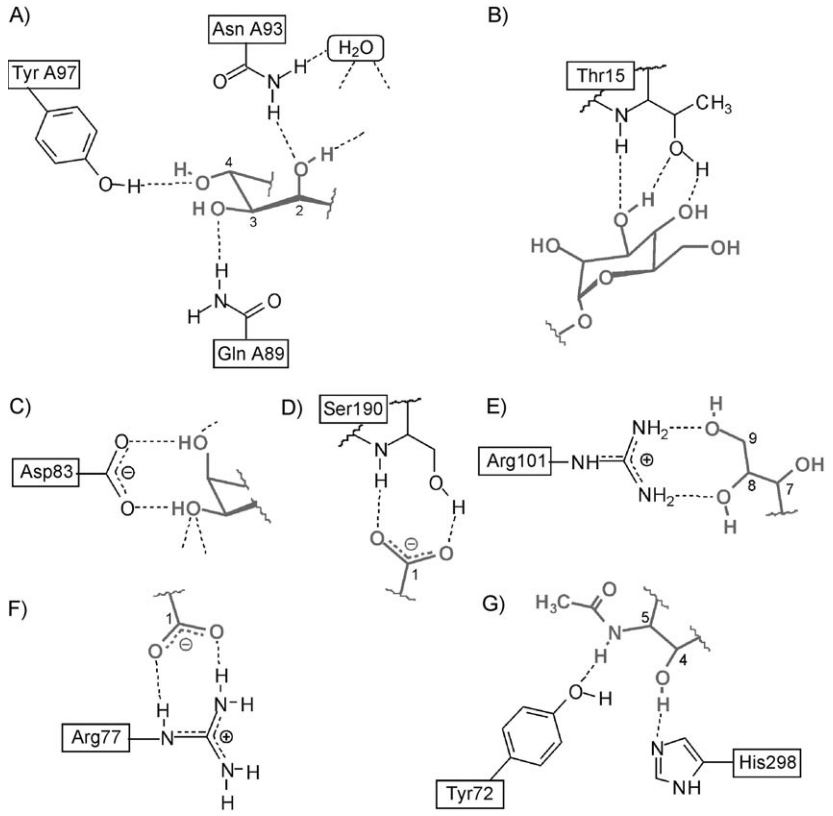
DOI: 10.1002/cbic.200800038

Design of Lectin Mimetics

Monika Mazik*[a]

The molecular recognition of carbohydrates by proteins mediates a variety of essential biological processes. The most intensively studied class of carbohydrate-binding proteins are lectins, which are widely found in nature including in plants, animals, viruses, and bacteria. As pointed out by Lis and Sharon "lectins bind mono- and oligosaccharides reversibly and with high specificity, but are devoid of catalytic activity, and in contrast to antibodies, are not products of an immune response".[1a] Lectins act as recognition determinants in diverse biological processes, such as clearance of glycoproteins from the circulatory system, adhesion of infectious agents to host cells and recruitment of leukocytes to inflammatory sites, as well as cell interactions in the immune system, in malignancy and metastasis.[1a,b] They play a key role in the control of various normal and pathological processes in living organisms. Some relatively well-characterized lectins are those utilized by pathogens as a means of attachment to eukaryotic cell surfaces. Examples of lectins involved in this process include the hemagglutinins of influenza and other viruses (see Scheme 1 D and E) as well as the toxins produced by Gram-negative bacteria.[2a] The affinity of lectins for monosaccharides is usually weak, with association constants in the millimolar range.[1a,3] However, creating extended binding regions capable of interacting with more than just a single monosaccharide residue of an oligosaccharide and/or clustering of several identical binding sites by formation of protein oligomers results in high affinities for oligosaccharides.[1a,b,2] Calorimetric studies revealed that protein–carbohydrate interactions are enthalpy driven, and, in almost all cases, the enthalpy of binding is more negative than, or equal to, the free energy of binding.[3a] The calorimetric data also showed strong linear enthalpy–entropy compensation.[3a,b]



Scheme 1. Examples of hydrogen bonds in the complexes of A) *Galanthus nivalis* lectin with mannose, B) concanavalin A with Manα6(Manα3)Man, C) peanut agglutinin with Gal(β1-3)GalNAc, D), E) rhesus rotavirus hemagglutinin with 2-α-*O*-methyl *N*-acetylneuraminic, and F), G) polyoma virus with NeuAc(α2-3)Galβ4Glc (sugar units are shown in grey).[1a,b] Tyr A97, Asn A93 and Gln A89 are the contact residues in the combining site of subdomain 1 of the lectin.

Despite the important roles that protein–carbohydrate interactions play in a wide range of biological recognition processes, the molecular details of these recognition events are generally not well understood. The structural basis for selective sugar recognition by lectins has been investigated by X-ray crystallography. According to the results of the X-ray analyses, the biological recognition processes involving neutral sugars use hydrogen-bonding (both neutral and charge-reinforced; see Scheme 1 A–C), interactions of sugar CHs with aromatic residues of the protein (often one or two aromatic residues stack on the sugar ring), oxygen–metal ion coordination, and van der Waals forces for sugar binding.[1a,b,2] Furthermore, ion pairing and ionic hydrogen-bonding are frequently observed in the complexation of proteins with ionic sugars (see Scheme 1 D–F), such as with *N*-acetylneuraminic acid (NeuAc), which is the most commonly occurring sialic acid. Quiocho et al. pointed out that "hydrogen bonds are the main factors in conferring specificity and affinity to protein–carbohydrate interac-

[a] *Prof. Dr. M. Mazik*
Institut für Organische Chemie der Technischen Universität Braunschweig
Hagenring 30, 38106 Braunschweig (Germany)
Fax: (+49) 531-3918185
E-mail: m.mazik@tu-bs.den

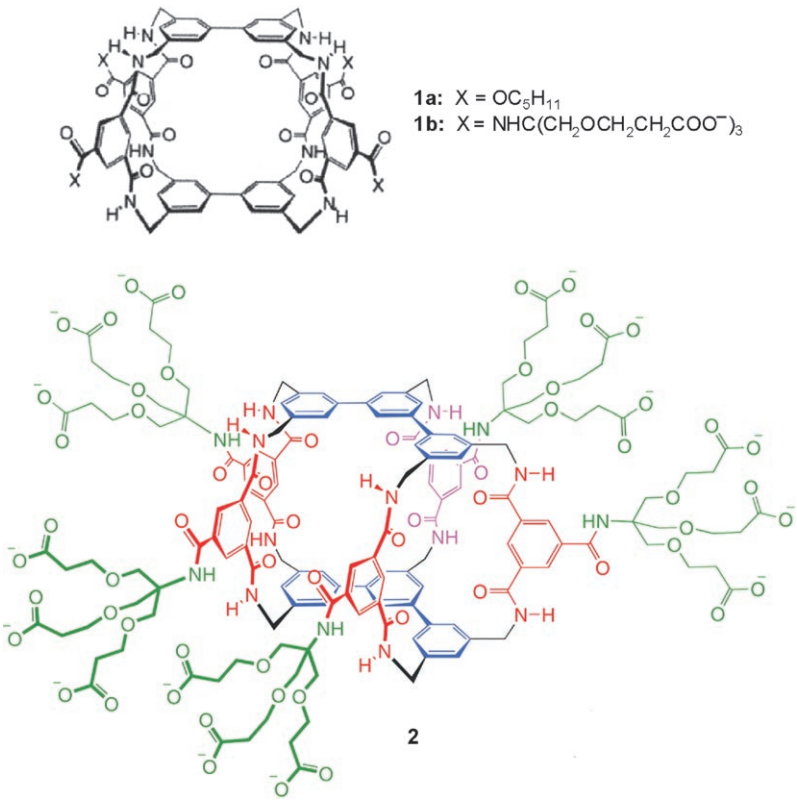
tions".[2b] The hydrogen bonds have both neutral and ionic character, and are both direct and water-mediated (see Scheme 1). The sugar OH groups usually participate in cooperative hydrogen bonds simultaneously as donors and acceptors. Carboxylate side chains play a role in anomeric- and epimeric-specific sugar recognition. Divalent cations, such as Ca^{2+} and Mn^{2+}, are involved in carbohydrate recognition either indirectly, by shaping the combining site, or through direct binding to the carbohydrate (as in the C-type lectins, which require Ca^{2+} for activity).

It should be noted that the driving force for carbohydrate binding by lectins is still uncertain. In particular, the role of water in natural carbohydrate recognition is a controversial issue.[4a] (For discussions on the role of solvent reorganization in molecular recognition of carbohydrates, see refs. [3b, 4a, b].)

On the one hand, the protein–carbohydrate interactions inspire the development of artificial receptor structures for the recognition of carbohydrates.[5–7] On the other hand, artificial carbohydrate receptors operating through noncovalent interactions provide valuable model systems for studying the basic molecular features of carbohydrate recognition. Advances in this area are likely not only to provide insight into the molecular recognition phenomenon, but also to facilitate the development of new therapeutic agents or chemosensors.

Because of subtle variations in the sugar structures and the three-dimensional arrangement of their functionality, the design of selective and effective biomimetic receptors for these ubiquitous and important biomolecules still represents a significant challenge. In particular, recognition in aqueous media through noncovalent interactions, in which solvent molecules compete significantly for the receptor binding sites, is a challenging goal of artificial receptor chemistry. It should be noted that neutral carbohydrates are especially challenging substrates to recognize.[8]

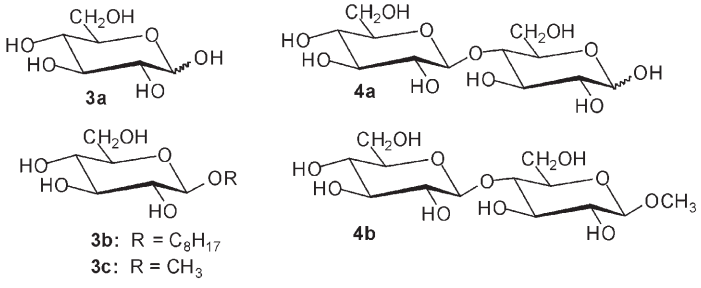
A recent interesting development from the group of Davis is the tetracyclic receptor **2**,[9] which was inspired by carbohydrate-binding proteins and represents an extended version of the biphenyl-based tricyclic polyamide receptor **1** (Scheme 2).[6j,10] The architectures **1** and **2** were designed to provide both apolar and polar contacts to a mono- or disaccharide molecule, respectively, to mimic the interactions in protein–carbohydrate complexes.



Scheme 2. Structures of receptors **1a**, **1b**, and **2**. Colour code for **2**: *meta*-terphenyl units, blue; isophthalamide units, red and magenta; water-solubilizing tricarboxylate units, green. Adapted from ref. [9] with permission. Copyright 2007, American Association for the Advancement of Science.

The tricyclic core **1** was specifically targeted at β-glucosyl derivatives **3**. It was supposed that the axial hydrogens in **3** would participate in CH–π interactions with the biphenyl groups, while the equatorial substituents would form hydrogen bonds to the isophthalamide units. Accordingly, **1a** showed high affinity for **3b** but was less effective for the octyl α-D-glucopyranoside or β-D-galactopyranoside.[10] The authors have shown that, in the form **1b**, the tricyclic cage **1** can bind carbohydrates in water with low affinities (the binding constant for **1b·3c** was found to be 32 M^{-1}), but significant selectivities.[6j] The designed preference for β-glucosyl, which was previously demonstrated in organic solvents,[10] is retained in the aqueous medium.

The *meta*-terphenyl-based tetracyclic receptor **2**[9] was developed to target all-equatorial disaccharides, such as cello-



biose.[11] The binding properties of receptor **2** were investigated by nuclear magnetic resonance, fluorescence spectroscopy, induced circular dichroism, and calorimetry in D_2O or H_2O. The receptor showed good affinities (for example, β-cellobioside **4b** was bound with $K_a \sim 900\ M^{-1}$) and remarkable selectivities for its chosen substrate in the aqueous solutions. It should be noted that the K_a value for **2·4b** (approaching $10^3\ M^{-1}$) is comparable to that for many lectin–carbohydrate interactions (see above). To assess its selectivity, receptor **2** was tested against ten disaccharides and three monosaccharides; the selectivity for cellobiose versus nontarget disaccharides was generally ~50:1. Interestingly, the cellobiose complex (**2·4a**) was formed nearly exclusively in the presence of an 18-fold excess of nontarget carbohydrate; thus, like in natural lectin, receptor **2** is able to bind its target from a complex mixture of potential substrates. Calorimetric studies showed that complex formation between **2** and **4a** is mainly enthalpically driven, and the balance between enthalpy and entropy lies within the range observed for lectins, thus supporting a lectin-like binding mode.

The binding studies with receptor **2** showed that affinities, selectivities and thermodynamic parameters all lie within the spread of values observed for lectins; thus, as mentioned by the authors, receptor **2** can be seen as a "synthetic lectin analogue".[9] Receptor **2** provides a valuable model system for studying the underlying principles of carbohydrate-based molecular recognition processes.

It should be noted that many questions remain open concerning the contribution of individual bonding interactions to selective carbohydrate recognition, the role of apolar association and the character of carbohydrate–aromatic interactions.[12,13]

The design of "lectin mimetics" is an important and exciting field of supramolecular and biomimetic chemistry. The ubiquity of lectin–carbohydrate interactions opens enormous potential for the exploitation of lectin mimetics in medicine. Such synthetic systems could be used to prevent and treat bacterial and viral infections, inflammations and perhaps even cancer.[1c] In addition, carbohydrate receptors could be used to separate carbohydrates or glycoconjugates, or as saccharide sensors. Further work is needed to develop both effective and selective biomimetic carbohydrate receptors, and to establish the potential of these systems in medicine, analytical chemistry and other areas. A lot of problems have not yet be solved and will doubtless be the subject of many rich and innovative studies in the future.

Keywords: carbohydrate-binding proteins · carbohydrates · molecular recognition · receptors · supramolecular chemistry

[1] a) H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637–674 and references therein; b) H. Lis, N. Sharon, *Lectins*, Kluwer, Dordrecht, **2003**; c) H. Lis, N. Sharon, *Sci. Am.* **1993**, *268*, 74–81.

[2] a) W. I. Weiss, K. Drickamer, *Annu. Rev. Biochem.* **1996**, *65*, 441–473; b) F. A. Quiocho, *Pure. Appl. Chem.* **1989**, *61*, 1293–1306; c) R. U. Lemieux, *Chem. Soc. Rev.* **1989**, *18*, 347–374.

[3] a) E. J. Toone, *Curr. Opin. Struct. Biol.* **1994**, *4*, 719–728; b) T. K. Dam, C. F. Brewer, *Chem. Rev.* **2002**, *102*, 387–429.

[4] a) R. U. Lemieux, *Acc. Chem. Res.* **1996**, *29*, 373–380; b) M. C. Chervenak, E. J. Toone, *J. Am. Chem. Soc.* **1994**, *116*, 10533–10539.

[5] For reviews on carbohydrate recognition with artificial receptors, see: a) A. P. Davis, T. D. James in *Functional Synthetic Receptors* (Eds.: T. Schrader, A. D. Hamilton), Wiley-VCH, Weinheim, **2005**, pp. 45–109; b) A. P. Davis, R. S. Wareham, *Angew. Chem.* **1999**, *111*, 3160–3179; *Angew. Chem. Int. Ed.* **1999**, *38*, 2978–2996.

[6] For some recent examples of carbohydrate receptors operating through noncovalent interactions, see: a) E. Klein, Y. Ferrand, E. K. Auty, A. P. Davis, *Chem. Commun.* **2007**, 2390–2392; b) M. Mazik, H. Cavga, *J. Org. Chem.* **2007**, *72*, 831–838; c) M. Mazik, A. König, *Eur. J. Org. Chem.* **2007**, 3271–3276; d) M. Mazik, H. Cavga, *Eur. J. Org. Chem.* **2007**, 3633–3638; e) O. Francesconi, A. Ienco, G. Moneti, C. Nativi, S. Roelens, *Angew. Chem.* **2006**, *118*, 6845–6848; *Angew. Chem. Int. Ed.* **2006**, *45*, 6693–6696; f) M. Mazik, A. König, *J. Org. Chem.* **2006**, *71*, 7854–7857; g) M. Mazik, H. Cavga, *J. Org. Chem.* **2006**, *71*, 2957–2963; h) M. Mazik, M. Kuschel, W. Sicking, *Org. Lett.* **2006**, *8*, 855–858; i) H. Abe, Y. Aoyagi, M. Inouye, *Org. Lett.* **2005**, *7*, 59–61; j) E. Klein, M. P. Crump, A. P. Davis, *Angew. Chem.* **2005**, *117*, 302–306; *Angew. Chem. Int. Ed.* **2005**, *44*, 298–302; k) M. G. J. ten Cate, D. N. Reinhoudt, M. Crego-Calama, *J. Org. Chem.* **2005**, *70*, 8443–8453; l) H. Abe, N. Masuda, M. Waki, M. Inouye, *J. Am. Chem. Soc.* **2005**, *127*, 16189–16196; m) M. Mazik, H. Cavga, P. G. Jones, *J. Am. Chem. Soc.* **2005**, *127*, 9045–9052; n) M. Mazik, M. Kuschel, *Chem. Eur. J.* **2008**, *14*, 2405–2419, and references therein; o) M. Mazik, M. Kuschel, *Eur. J. Org. Chem.* **2008**, 1517–1526, and references therein. Further reviews are given in refs. [5a] and [5b].

[7] Another strategy, which has been employed for the design of synthetic carbohydrate receptors, involves the exploitation of non-natural bonding interactions; this strategy relies on the reversible formation of covalent bonds from diol units and boronic acid. For reviews on boronic acid-based receptors, see: a) T. D. James, S. Shinkai, *Top. Curr. Chem.* **2002**, *218*, 159–200; b) S. Striegler, *Curr. Org. Chem.* **2003**, *7*, 81–102; c) T. D. James, K. R. A. S. Sandanayake, S. Shinkai, *Angew. Chem.* **1996**, *108*, 2038–2050; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1910–1922.

[8] Recognition of neutral sugars in aqueous solution through noncovalent interactions remains an important challenge for artificial receptor chemistry; for some examples, see: a) V. Král, O. Rusin, F. P. Schmidtchen, *Org. Lett.* **2001**, *3*, 873–876; b) R. D. Hubbard, S. R. Horner, B. L. Miller, *J. Am. Chem. Soc.* **2001**, *123*, 5810–5811; c) R. Yanagihara, Y. Aoyama, *Tetrahedron Lett.* **1994**, *35*, 9725–9728 and refs. [6f] and [6j].

[9] Y. Ferrand, M. P. Crump, A. P. Davis, *Science* **2007**, *318*, 619–622.

[10] A. P. Davis, R. S. Wareham, *Angew. Chem.* **1998**, *110*, 2397–2401; *Angew. Chem. Int. Ed.* **1998**, *37*, 2270–2273.

[11] For examples of selective disaccharide binding by receptors using noncovalent interactions, see U. Neidlein, F. Diederich, *Chem. Commun.* **1996**, 1493–1494; for other examples of a systematic variation of a macrocyclic system, see: R. Welti, F. Diederich, *Helv. Chim. Acta* **2003**, *86*, 494–503.

[12] The character of carbohydrate–aromatic interactions is still a subject of controversy; for recent discussions on the importance of these interactions, see: a) G. Terraneo, D. Potenza, A. Canales, J. Jiménez-Barbero, K. K. Baldridge, A. Bernardi, *J. Am. Chem. Soc.* **2007**, *129*, 2890–2900; b) M. I. Chávez, C. Andreu, P. Vidal, N. Aboitiz, F. Freire, P. Groves, J. L. Asensio, G. Asensio, M. Muraki, F. J. Cañada, J. Jiménez-Barbero, *Chem. Eur. J.* **2005**, *11*, 7060–7074.

[13] For examples of CH–π interactions in the crystal structures of the complexes formed between artificial receptors and carbohydrates, see ref. [6m].

Received: January 21, 2008
Published online on March 25, 2008

