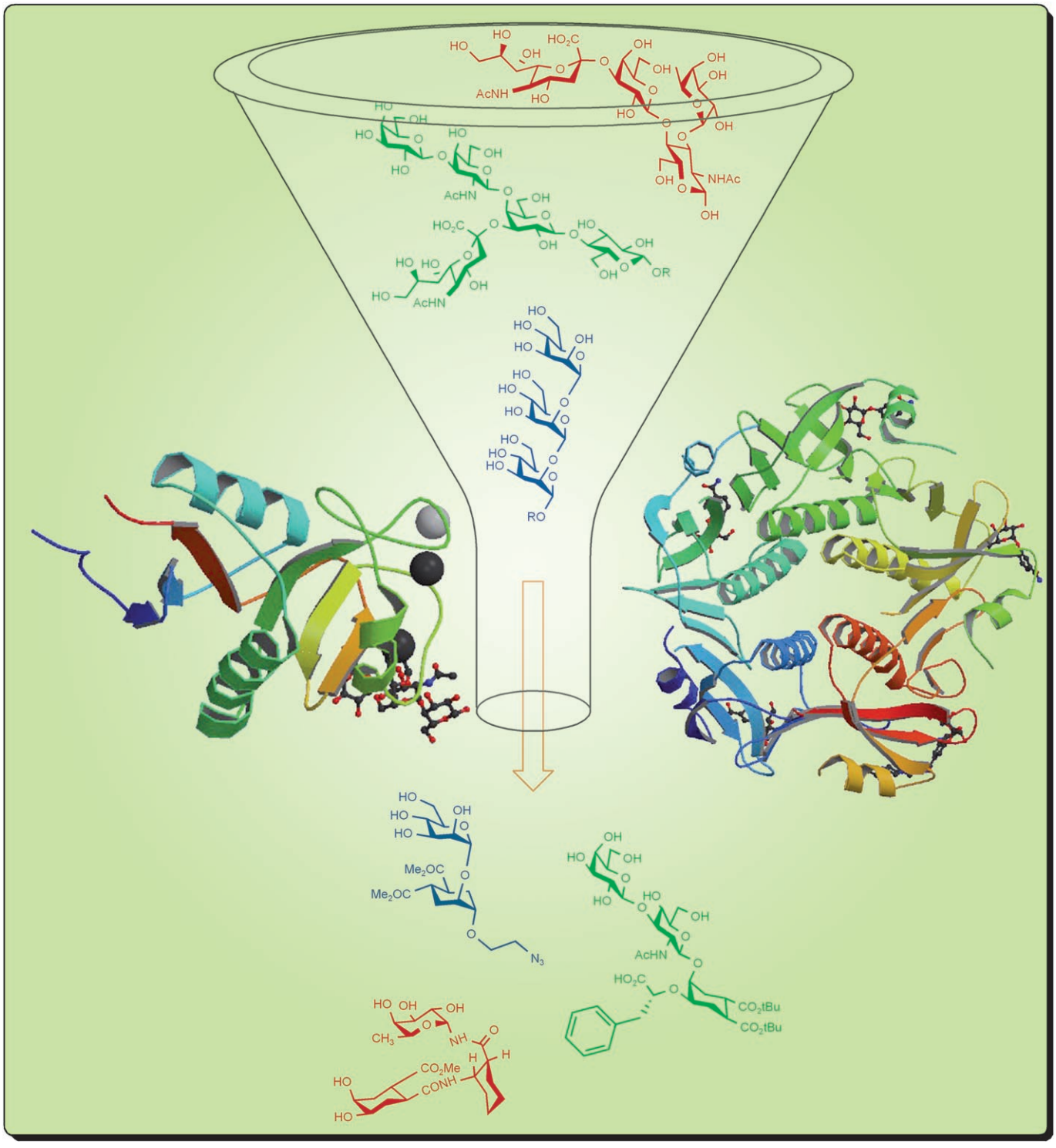
CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200800597

Interfering with the Sugar Code: Design and Synthesis of Oligosaccharide Mimics

Anna Bernardi* and Pavel Cheshev[a]

Dedicated to the memory of Claus-Wilhelm (Willi) von der Lieth



Abstract: Oligosaccharide determinants of cellular glycoconjugates interact with protein receptors triggering a variety of cellular responses within a wide range of physiological and pathological processes and with exquisitely tuned selectivity. This has led to the formulation of the hypothesis that a sugar code exists and that sugar-binding proteins (lectins) act to decipher it and translate it into biological responses. Interference with these recognition events by functional mimics of carbohydrates could thus be used to modulate or alter signal transmission, or to prevent the onset of diseases. Attempts to design and prepare glycomimetic inhibitors of well-known target lectins (cholera toxin, DC-SIGN) are reviewed in this concept paper.

Keywords: carbohydrates · inhibitors · lectins · oligosaccharides

Introduction

The ability of glycans to encode biochemical information has been brought to attention and its unraveling has been heralded as one of the most critical challenges for the post-genomic era.[1,2] The complexity of the problem has often been summarized: carbohydrates are the most abundant type of biomolecule in nature. They are widely expressed as glycolipids and glycoproteins, and glycosylation is the most widespread post-translational modification of proteins. Furthermore, glycans are way more complex and difficult to analyze and synthesize than other macromolecules. Therefore, despite numerous efforts, the extent to which the sugar code has been deciphered is still limited.

Nonetheless, it is clearly emerging that many fundamental biological processes are controlled by sugar-mediated information. Just to name a few: quality control of protein folding, intra- and extracellular trafficking of glycoconjugates, signaling, host defence pathways, modulation of cell–cell and cell–matrix adhesion, both in physiological situations (as egg-sperm interaction) and in pathological conditions (inflammation, cancer, etc)[3]—enough to justify efforts directed to understanding how chemical information is encoded in sugar structure, how this information is read out by sugar-binding proteins (lectins), and how we can control/alter this flow of information by interfering with the sugar code.

[a] Prof. Dr. A. Bernardi, Dr. P. Cheshev
Dipartimento di Chimica Organica e Industriale
Universita' degli Studi di Milano, via Venezian 21
20133 Milano (Italy)
Fax: (+39) 02-5031-4072
E-mail: anna.bernardi@unimi.it
pavel.cheshev@unimi.it

A major contribution to the understanding of the sugar code is expected to emerge from screening of glycan arrays[4] and from the use of chemoinformatic tools. Glycan-specific databases have been built[5] and data mining has begun.[6] Glycomimetic molecules that can disrupt the formation of sugar–protein complexes may be used in this context as probes of biological processes and may provide ideas for medicinal applications.

So far, most of this work has been directed towards the enzymes that tailor glycan determinants: glycosidases and glycosyltransferases. Inhibition of glycosidases has been particularly fruitful: azasugars of the nojirimycin family are well-established, general-purpose inhibitors.[7] Sialidase inhibitors have been developed in one of the first successful rational drug-design projects,[8] and are currently commercialized as anti-flu drugs under the commercial names of Relenza (Zanamivir) and Tamiflu (Oseltamivir). Inhibition of glycosyltransferases, until very recently, has proven harder, mainly due to the lack of robust non-radiometric assay strategies to detect glycosylation, but important steps forwards are being made.[9]

Less is known about the inhibition of lectin-mediated sugar recognition. As opposed to sugar-processing enzymes, lectins are proteins that recognize glycans, but do not catalyze their transformation. Initially discovered in plants and in snake venom, lectins were also identified in bacteria, viruses, vertebrates, and mammals and were recognized as the read-out machinery of carbohydrate-encoded information.[1] The lectin carbohydrate recognition domains (CRD) are often able to recognize complex oligosaccharides in a selective manner; however, the oligosaccharide recognition determinants often consist of only one or two residues that appear to act as anchors driving the entire glycoconjugate to interact with the protein.

Hydrogen bonding, association of sugar molecules with metals (for C-type lectins and related calcium-dependent proteins), ionic interactions, and hydrophobic stacking contribute to the binding energy. The energy associated with hydrogen bonding in sugar–protein interactions is significantly reduced by competition from bulk solvent and by the flexible nature of the hydroxyl groups, which result in a considerable entropic penalty when they become constrained upon binding. Sugar C–H bonds can engage in stacking interactions with protein aromatic side chains, but natural carbohydrates usually lack extended hydrophobic areas, often a dominant factor in high-affinity receptor–ligand interactions. Hence, the affinity of lectins for monovalent carbohydrates is typically weak (dissociation constants are in the mM to μM range). Most lectins, however, are multimeric and, in general, polyvalent presentation of monosaccharides acting as binding determinants for a given lectin can be used for inhibition with major affinity increases over the corresponding monovalent ligand[10] Spectacular results have been achieved through this approach, particularly for AB_5 bacterial toxins[11] and more could be achieved through the combina-

tion of judicious choice of potent monovalent inhibitors with rationally designed polyvalent scaffolds, a task that will be significantly simplified by the introduction of powerful chemoselective conjugation techniques.

The identification of unnatural inhibitors of lectin–sugar recognition has been approached mostly through rational design and synthesis of glycomimetic structures, although, more recently, non-carbohydrate lectin binders have begun to be described.[12] In this context, glycomimetics are non-carbohydrates that attempt to reproduce the 3D structure of oligosaccharides' binding determinants and thus to compete with the natural ligand for a target lectin. They are often composed of a mono- or disaccharide, working as the lectin anchor, linked to an aglycone designed to host and orient further functionalities for lectin interaction and to impart to the molecule some pharmacologically favorable properties, such as improved lipophilicity and resistance to hydrolytic enzymes.

This work is based on the following rationale:

- Despite the great structural complexity of many bioactive oligosaccharides, often only small portions of these molecules are actually recognized by their receptors. The remaining part appears to act as a scaffold that orients the binding determinants in the appropriate conformation and provides a connection to the aglycons.
- Although oligosaccharides are relatively flexible molecules, if compared to other macromolecules, "certain glycans have highly favored conformations".[13] In particular, vicinal branching appears to impart a significant conformational restriction, as seen for instance in gangliosides[14] and in the Lewis determinants.[15]
- Different lectins can select different conformations of flexible oligosaccharides.[16] Some lectins even select conformations that do not appear to be populated by more than 5–10% in the free state (ground state) of the ligand. This has clearly a consequence on the (low) affinity of such ligands for the target lectin, but it can be exploited by mimics that, by chance or design, happen to stabilize the bound conformation.

Selectins, a group of lectins that recognize the tetrasaccharides sialyl Lewis-x (sLe^X, **1**), remain the most exploited targets to date.[17] Early works in the field provided milestones for rational design of lectin antagonists. Two possible approaches towards construction of carbohydrate mimics were postulated. The first one implicates removal of non-interacting functional groups, but conservation of the original glycosidic linkages to retain the conformational properties of natural ligand. This approach reduces the polarity of the sugars, which may increase the affinity of the binding by improving hydrophobic interactions and decreasing the penalty for polar groups desolvation. Addition of a hydrophobic group or a charged group can also be beneficial. The stability of O-glycosides, which are known to have very short lifetimes in physiological conditions, may be improved by switching to C- or S-glycosidic linkages.[17]

A second approach exploits non-carbohydrate frameworks to which the required pharmacophoric sugar fragments are tethered so that they maintain the same orientation in space as they do in the natural oligosaccharide structure (bound conformation). Additional groups may also be incorporated to enhance the affinity. Replacement of the glycosidic scaffold often facilitates and accelerates the otherwise very time-consuming oligosaccharide synthesis. Following this approach, Kolb and Ernst (at that time at Novartis) developed the sialyl Lewis^X mimic **2** shown in Figure 1.[18]

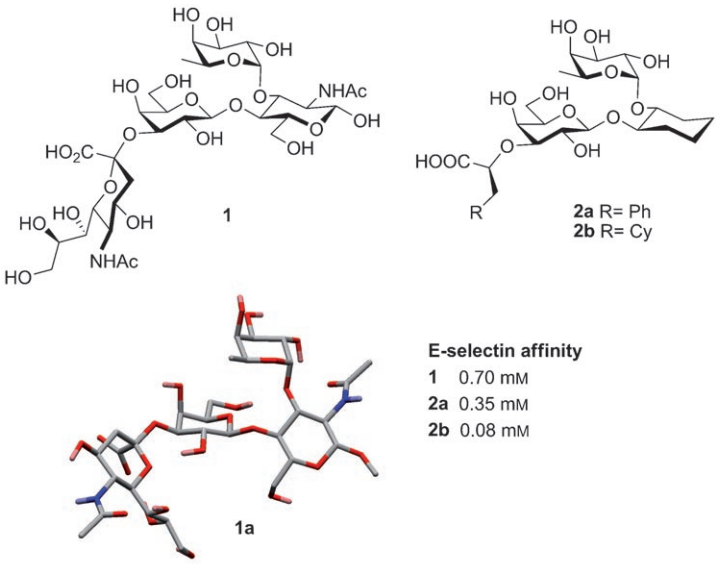


Figure 1. Sialyl Lewis^X (sLe^X, **1**), the conformation adopted by sLe^X in the selectin complex (**1a**) and the Novartis mimic **2**. E-selectin affinities from referene [18].

Two elements control the shape of this mimic: the cyclohexanediol scaffold, replacing for the 3,4-disubstituted GalNAc in sLe^X, and the *S* stereocenter of the ether fragment, which incorporates the acid used to replace the sialic acid residue of sLe^X. It was shown that this configuration favors the *gauche* orientation of the carboxy group relative to the galactose ring shown in Figure 1, which is the bioactive one for the interaction of sLe^X with E-selectin.

Building on these results we have expanded the range of tools available as non-carbohydrate scaffolds by introducing two enantiomerically pure, conformationally stable cyclohexanediols **3** and **4** (Figure 2). These molecules were designed to replicate carbohydrate branching motifs that incorporate one or more axial substituents, like the 3,4-galacto and 1,2-α-manno motifs, frequently encountered in bioactive oligosaccharides.[19] They have been used to synthesize structural and functional analogues of complex carbohydrates (pseudo-oligosaccharides) that interact with biologically relevant lectins, such as the cholera toxin and the dendritic cell receptor DC-SIGN. The carboxy groups of such diols, which act as a conformational lock on the cyclohexane ring, were

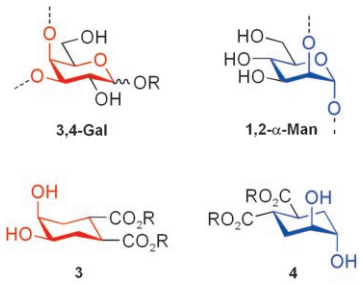


Figure 2. Dicarboxy cyclohexanediol (DCCHD) scaffolds **3** and **4** designed to mimic 3,4-disubstituted galacto and 1,2-α-manno motifs, respectively.

also exploited for conjugation to various supports, thus allowing the synthesis of polyvalent pseudo-glycoconjugates.

Ganglioside GM_1 and the Cholera Toxin (CT)

The first target we selected was the recognition pair composed by the pentavalent cholera toxin protein and its cellular receptor, the pentasaccharide head-group of ganglioside GM_1 (GM_1os, **5**; Figure 3). This is an extremely well-characterized pair in the field of sugar–protein interactions. Cholera toxin belongs to the AB_5 bacterial toxins family, which are named after their characteristic architecture comprising a single catalytically active component (A) and a nontoxic receptor-binding pentamer of B subunits that are responsible for binding to gangliosides at the cell surface.[20] The recognition function is retained even in the absence of the A subunit, but the complete AB_5 holotoxin is required for the toxic effects. There are several families of AB_5 toxins. The cholera toxin (CT) family includes enterotoxins responsible for several disorders, from the relatively mild travelers' diarrhea (from *E. coli* heat-labile toxin, LT) to the much more serious and life-threatening cholera. Given the importance of the processes that they promote, the study of the complexes formed between gangliosides and AB_5 toxins at different levels is very relevant. For basic research, they offer a paradigmatic model for studying the structural and thermodynamic basis of protein–carbohydrate interactions; for medicinal chemistry, they may provide key insights for the structure-based design of ligands that can potentially be used to treat the above-mentioned diseases.

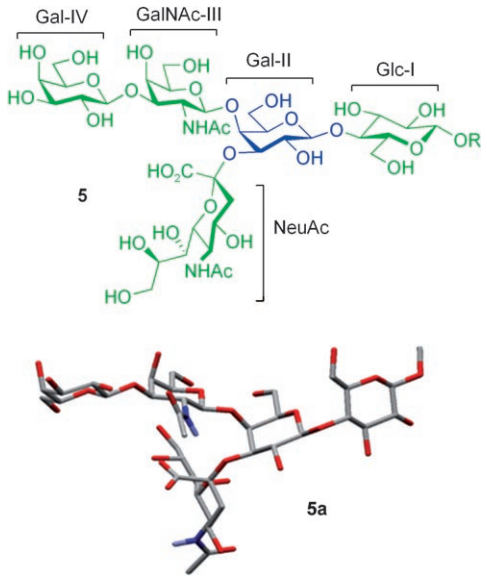


Figure 3. GM_1os **5** and its three-dimensional structure **5a**.

Rational design of galactose-based ligands for CT and LT has been reported and recently reviewed.[21] The B pentamer of CT (CTB) interacts with the soluble, monovalent oligosaccharide portion of GM_1os **5** with a strong affinity. The binding process is weakly cooperative and the dissociation constant for the monovalent interaction of one GM_1os with one pentamer binding site, is 43 nM at room temperature,[22] which places it among the highest affinity protein–carbohydrate interactions described to date. The X-ray structure of the CTB-GM_1os complex[23] shows a "two-fingered grip" of the sugar on the toxin, created by a sialic acid thumb and a Galβ(1→3)GalNAc forefinger. Most of the contacts are given by the "finger" tips. The terminal galactose residue in the "forefinger" can reach into a well-defined galactose-binding pocket, lined by the indole side chain of Trp-88 and shielded from the solvent. The rest of the toxin-binding site is shallow and exposed to solvent. The NeuAc "thumb" interacts with a carboxylate-binding region, which includes one highly conserved crystallographic water molecule. A comparison between the solution conformation of GM_1os, as determined by NMR data,[24] and the conformation observed in the toxin complex indicates that this pentasaccharide is conformationally preorganized for a near lock-and-key interaction with CTB. Such preorganization appears to be the source of the unusually high binding affinity of the CTB:GM_1os pair, as shown also by a detailed calorimetric study of the thermodynamics of CT binding by GM_1os and fragments thereof.[22] Structural data[25] converge to locate the origin of the limited conformational flexibility of GM_1os in the 3,4-branching at Gal-II (Figure 3). Thus, this residue appears to act as the scaffold holding the two terminal sugars (Gal-IV and NeuAc) at the proper place and distance for optimal interaction with CT. It is worth noting that, although both sLe^X and GM_1os include a NeuAcα2,3-Gal motif, the conformation of this fragment in the two molecules is different, resulting in a different orientation of the carboxylate binding determinant, which can be appreciated by comparing Figures 1 and 3.

From the above structure-based rationale, we have proposed a series of ganglioside mimics in which the non-interacting oligosaccharide backbone of GM_1os was replaced with the cyclohexane diol **3** (Figure 2), which was chosen to reproduce the topological features of the 3,4-disubstituted galactose residue (Gal-II) in GM_1. The pseudotetrasaccharide **6** (Figure 4) representing the first generation of mimics indeed displayed the same affinity as the natural ligand.[26] A similar affinity ($K_d = 1.8$ μM) was measured for the ana-

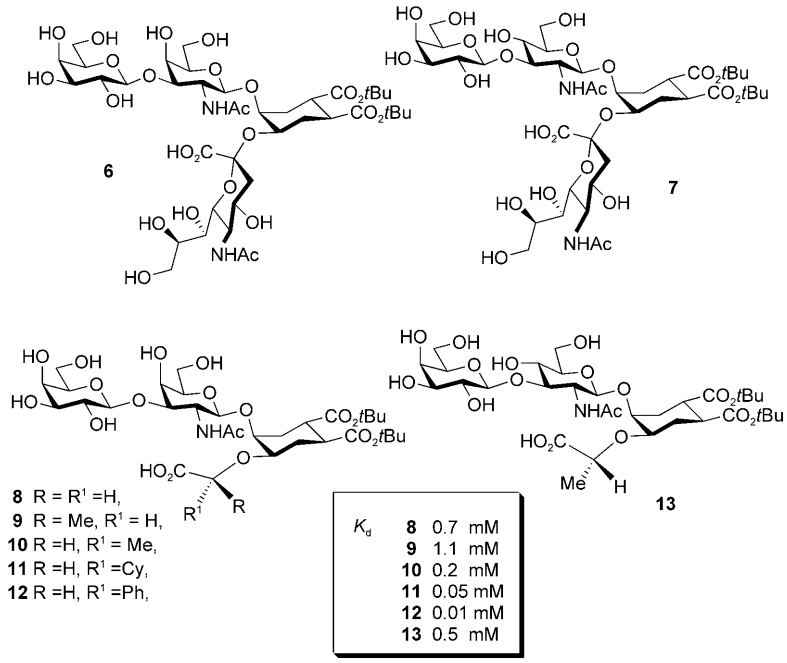


Figure 4. GM_1os mimics based on DCCHD **3**.

logue **7** in which the GalNAc residue was substituted with a GlcNAc one, a change that was shown to preserve the three-dimensional shape of the ligand.[27]

A second generation of molecules (**8**–**13**) was created by replacing sialic acid with simpler hydroxyacids (Figure 4). Comparison of the epimeric lactic acid derivatives **9** (R = Me, *S* epimer, K_d = 1.1 mM) and **10** (R = Me, *R* epimer, K_d = 0.2 mM) lead to the observation that the *R* configuration of the stereocenter on the ether side chain improves the activity of the mimic by one order of magnitude. This is in contrast with the observations described above for sLe^X mimics, which require an *S* configuration of the acid stereocenter. It also reflects both the different steric requirements for the sialic acid carboxy group in the binding site of CT compared to selectin and the existence of a lipophilic area in the CT binding region. Indeed, extensive NMR studies conducted on compounds **8**–**13** and on their CT complexes showed that a process of conformational selection takes places during binding. From the ensemble of side-chain conformations, CT selects the one that fits the galactose binding pocket, while placing the carboxy group of the hydroxy acid in the carboxylate binding region. Together with the configuration of the side-chain stereocenter, this in turn determines the orientation of the alkyl substituent, which for an *R* configuration points towards a hydrophobic area of the protein formed by Lys34 and Ile58 (Figure 5, top). The existence of such a lipophilic interaction also explains the IC_{50} increase observed passing from **10** (R = Me, K_d = 0.2 mM) to **11** (R = cyclohexyl, K_d = 0.05 mM) and **12** (R = Ph, K_d = 0.01 mM). The best results were obtained for the phenyllactic acid derivative **12**, which is preorganized in the conformation required by the existence of sugar–aromatic interactions[28] between the phenyl ring in the side chain and the GalNAc residue (Figure 5, bottom). These observations provide clear-cut evidence of the importance of such interactions in shaping three-dimensional structures of simple molecules.[29] Ligand **12** with a 10 μM dissociation constant represents a major simplification of the structure of the natural ligand GM_1os, with a relatively minor loss of affinity.

Some of the molecules discussed above display good affinity for CT and are structurally simpler than the natural ligand GM_1os. However, they all are O-glycosides and are therefore unlikely to be metabolically stable to any significant extent. Furthermore, the synthetic methods used to connect the pharmacophoric sugar moieties are those of traditional

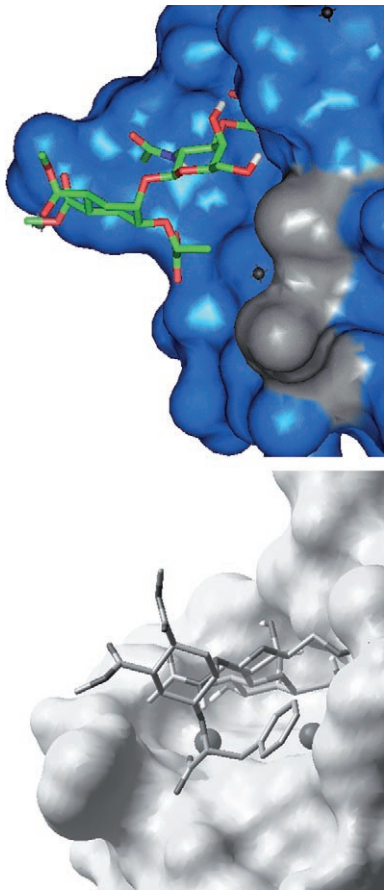


Figure 5. Top: Suggested binding mode of **10** to CT, based on modeling and NMR data. The lipophilic patch formed by Lys34 and Ile58 is shown in grey. Bottom: Suggested binding mode of **12** to CT, based on modeling and NMR data.

carbohydrate chemistry, which are often laborious and not high-yielding procedures. To circumvent these shortcomings, we are currently working toward the development of CT ligands starting from simple C-galactosides. Preliminary results have so far yielded ligands in the 0.1 mM range (Figure 6).[30]

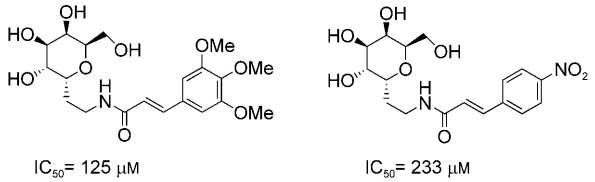


Figure 6. C-galactoside CT inhibitors. Inhibition of CT binding to an asialofetuin-coated SPR chip from ref. 30.

Since CT is a multimeric lectin, low affinity could be addressed through a cooperativity effect by switching to multivalent ligands. Polyvalent CT binders have been reported to yield strong affinity enhancements (up to 10^5 fold) starting from weak ligands, such as lactose or galactose.[11b,31] Dendrimers based on the 3,5-di-(2-aminoethoxy)benzoic acid branching unit were introduced by Pieters[31a,32] as polyvalent scaffolds for the presentation of galactose, lactose, the monovalent GM_1 mimic **10**[33] and GM_1os itself.[11d] Screening of these compounds by surface plasmon resonance (SPR) revealed a dependence of CT inhibition effect on both affinity of the monovalent ligand employed and topology of presentation. Similar results were also obtained by Bernardi, Casnati, and co-workers[11c] with a divalent ligand, prepared by tethering two units of GM_1 mimic **10** onto a functionalized calix[4]arene. A 3800-fold (1900-fold per sugar mimic) affinity enhancement was measured by fluorescence spectroscopy. The value observed is much higher than that normally measured for a divalent ligand interacting with a polyvalent receptor[10a,34] and higher than the enhancement observed for the dendrimer-based tetravalent and octavalent analogues.[33] The energetics of this interesting divalent ligand are currently under investigations.

The results described above show that multivalent presentation of designed ligands can lead to affinity levels closer to those required for practical application against AB_5 toxins. The implication is that an approach to high-affinity lectin ligands can be achieved as a combination of rational design of monovalent ligands with further enhancement by multivalent presentation using dendrimers.

DC-SIGN Inhibitors

DC-SIGN (dendritic cell-specific ICAM-3 grabbing nonintegrin) is a tetrameric C-type lectin. It is one of the dendritic cells' specific pathogen-uptake receptors that recognize highly glycosylated structures present at the surface of several pathogens, such as viruses, bacteria, yeasts, and parasites. It was brought to attention by the group of van Kooyk, who reported that HIV-1 targets DC-SIGN, but escapes degradation in lytic compartments, thus using DCs as a Trojan horse to invade the host organism.[35] Inhibition of DC-SIGN is currently considered as an interesting new target for the design of anti-infective agents.[36] Furthermore, since the detailed molecular mechanisms by which this receptor operates are not known, effective modulators of DC-SIGN are needed to help clarify the different biological processes in which it can be involved.

The main carbohydrate ligand recognized by DC-SIGN is the high mannose glycan, $(Man)_9(GlcNAc)_2$, a branched oligosaccharide that is present in multiple copies by several pathogen glycoproteins and specifically by the gp120 envelope protein of HIV. DC-SIGN can also recognize branched fucosylated structures bearing terminal galactose residues, such as the Lewis antigens. X-ray data are available both for complexes of DC-SIGN carbohydrate recognition domain (CRD) with mannose oligosaccharides and with $Lewis^X$.[37]

As a possible DC-SIGN inhibitor we have recently reported[36a] the pseudo-1,2-mannobioside **14**[38] (Figure 7), which

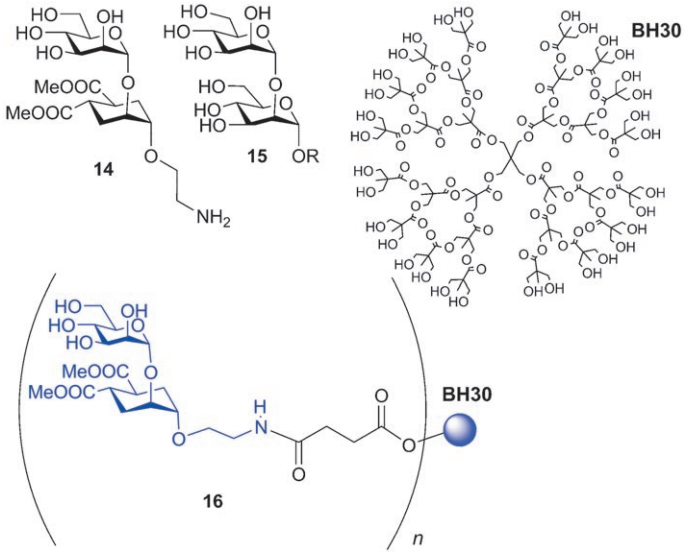


Figure 7. The monovalent pseudo-mannobioside inhibitor of DC-SIGN **14**, the polyvalent dendrimer-based version **16**, and the natural mannobioside **15**.

contains a mannose unit connected to a conformationally locked diol **4** (Figure 2). The latter acts as a mimic of a reducing end mannose residue and features a spacer-arm terminated with azido or amino functionality, useful to generate multivalent DC-SIGN ligands.

Design of mimic **14** was supported by modeling and NMR experiments. STD-NMR showed that the molecule interacts with DC-SIGN and inhibition of Ebola virus entry in DC-SIGN expressing Jurkat cells was also shown. The IC_{50} measured for **14** in this test (0.6 mM) was approximately three times lower than that of the natural disaccharide **15**, which also showed a marked cytotoxicity not exhibited by **14**. Rojo and Delgado reported that a mannosylated Boltorn dendri-

mer (Figure 6, BH30) bearing 32 mannose units (BH30-$(sucMan)_{32}$) inhibits direct DC-SIGN-mediated cell entry in an Ebola viral model with IC_{50} 337 nM.[36f] Conjugation of **14** to a dendrimer based on the Boltorn polymer through a succinic acid linker yielded the polyvalent ligand **16**, bearing an average number of 26 units of pseudo-disaccharide. In preliminary experiments, this molecule inhibits binding of DC-SIGN extra-cellular domain to a gp120-coated SPR (CM4 chip) six times better than $BH30(sucMan)_{32}$.[39]

Very recently we also introduced the first fucose-based unnatural ligand of DC-SIGN.[40] Indeed, moving from the known three-dimensional structure of the Lewis-x trisaccharide (Figure 8), we have so far identified two monovalent

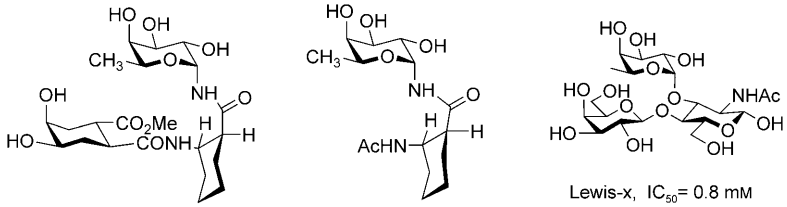


Figure 8. Fucose-based ligands of DC-SIGN. Inhibition of DC-SIGN binding to a mannosylated-BSA SPR chip from reference [40].

α-fucosylamides **17** and **18** that bind DC-SIGN with inhibitory constants 0.4–0.5 mM (by SPR) and have characterized their interaction with the protein by STD-NMR spectroscopy. The results have shown saturation transfer from DC-SIGN extra-cellular domain to the fucose residue and are in agreement with the expected binding mode of α-fucosides.[37] This work therefore establishes for the first time α-fucosylamides as functional mimics of chemically and enzymatically unstable α-fucosides. Since α-glycosyl amides are a class of virtually unexplored nonhydrolysable monosaccharide derivatives,[41] this observation may be of general relevance for the design of sugar mimics.

Like **14**, the monovalent DC-SIGN ligands **17** and **18** represent interesting candidates to prepare multivalent constructs able to block the receptor DC-SIGN with high affinity and with potential biomedical applications.

Lectin Inhibition: Carbohydrate Mimics versus "Small Molecule" Ligands

Few entirely non-carbohydrate lectin antagonists have been reported to date. Typically the selection process includes high-throughput screening (HTS) of a library, followed by SAR investigation and lead optimization. High-throughput assays for the screening of P-selectin have led a team at Wyeth to develop a quinoline salicylate class of inhibitors.[12a,b] Small-molecule DC-SIGN inhibitors have also been discovered by HTS of commercial libraries.[12c] The IC_{50} values of the most active compounds were found to range from 1.6 to 32 μM, which, compared to DC-SIGN-binding monosaccharides (8.7 mM for *N*-acetyl mannosamine and 6.7 mM for L-fucose) and oligosaccharides (0.21 mM for $Man_9GlcNAc_2$) makes them approximately 1000-fold more potent.

This is important work, because, together with the results of sugar mimics described above, it shows that despite the peculiar characteristic of their binding sites lectins can be tackled by approaches well validated for many other classes of receptors. The pharmaceutical industry, so far, has often been reluctant to embark in discovery projects in the area of carbohydrate binding proteins, which have been seen as somehow intractable entities. The above results are likely to change such perception, and developments may become as fast as the production of reliable HTS protocols. A boost in this direction should also arrive from the sizable amount of success cases obtained mostly by fragment-based approaches in another so-called intractable area, that of protein–protein interaction.[42] In particular, given the strong structural similarity between carbohydrate–lectin binding processes and protein–protein recognition events, the fragment-based method of drug discovery is likely to produce interesting results for lectins as well.

Although selectivity issues still remain to be addressed, a classical discovery approach by library screening appears to be well suited to achieve fast identification of potential lectin inhibitors. However, this type of work is not likely to produce the kind of structural information that could feedback a better understanding of how chemical and biological information is encoded in carbohydrates. In contrast, rational design of oligosaccharide mimics may not only yield lectin inhibitors, but it may also provide some of the required information and help to decipher the sugar code.

Acknowledgement

Support by COST-D34 and the CHEM-PROFARMA-NET program (RBPR05NWWC) is gratefully acknowledged. P.C. is supported by a Marie Curie International fellowship within the 6th EU Framework Programme (MIF1-CT-2006-040158).

[1] H.-J. Gabius, H.-C. Siebert, S. André, J. Jiménez-Barbero, H. Rüdiger, *ChemBioChem.* **2004**, 740–764.
[2] a) *Structural Medicine: The Importance of Glycomics for Health and Disease*, European Science Foundation, Strasbourg, **2006**; b) D. B. Werz, P. H. Seeberger in *Chemical Biology: From Small Molecules to Systems Biology and Drug Design* (Eds.: S. L. Schreiber, T. M. Kapoor, G. Wess) Wiley-VCH, Weinheim, **2007**, 668–691; c) K. T. Pilobello, L. K. Mahal, *Curr. Opin. Chem. Biol.* **2007**, *11*, 300–305; d) J. C. Paulson, O. Blixt, B. E. Collins, *Nat. Chem. Biol.* **2006**, *2*,

238–248; e) T. Luetteke, A. Bohne-Lang, A. Loss, T. Goetz, M. Frank, C.-W. von der Lieth, *Glycobiology*, **2006**, *16*, 71R–81R.
[3] J. P. Kamerling, *Comprehensive Glycoscience. From Chemistry to System Biology, Vols. 3 and 4*, Elsevier, Amsterdam, **2007**.
[4] Reviewed in: a) A. S. Culf, M. Cuperlovic-Culf, R. J. Ouellette, *OMICS* **2006**, *10*, 289–310; b) J. E. Turnbull, R. A. Field, *Nat Chem. Biol.* **2007**, *3*, 74–77.
[5] See http://www.eurocarbdb.org and links therein.
[6] D. B. Werz, R. Ranzinger, S. Herget, A. Adibekian, C.-W. von der Lieth, P. H. Seeberger *ACS Chem. Biol.* **2007**, *2*, 685–691.
[7] a) B. Winchester, G. W. J. Fleet, *Glycobiology*, **1992**, *2*, 199–210; b) M. S. M. Pearson, M. Mathé-Allainmat, V. Fargeas, J. Lebreton, *Eur. J. Org. Chem.* **2005**, 2159–2191, and references therein.
[8] M. von Itzstein, *Nat. Rev. Drug Discovery* **2007**, *6*, 967–974.
[9] B. J. Gross, J. G. Swoboda, S. Walker, *J. Am. Chem. Soc.* **2008**, *130*, 440–441.
[10] a) M. Mammen, S.-K. Choi, G. M. Whitesides *Angew. Chem.* **1998**, *110*, 2908–2953; *Angew. Chem. Int. Ed.* **1998**, 37, 2755–2794; b) C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357–2364.
[11] a) P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read, D. R. Bundle, *Nature* **2000**, *403*, 669–672; b) E. K. Fan, Z. S. Zhang, W. E. Minke, Z. Hou, C. L. M. J. Verlinde, W. G. J. Hol, *J. Am. Chem. Soc.* **2000**, *122*, 2663–2664; c) D. Arosio, M. Fontanella, L. Baldini, L. Mauri, A. Bernardi, A. Casnati, F. Sansone, R. Ungaro, *J. Am. Chem. Soc.* **2005**, *127*, 3660–3661; d) A. V. Pukin, H. M. Branderhorst, C. Sisu, C. A. G. M. Weijers, M. Gilbert, R. M. J. Liskamp, G. M. Visser, H. Zuilhof, R. J. Pieters, *ChemBioChem* **2007**, *8*, 1500–1503.
[12] a) N. Kaila, K. Janz, S. DeBernardo, P. W. Bedard, R. T. Camphausen, S. Tam, D. H. H. Tsao, J. C. Keith, C. Nickerson-Nutter, A. Shilling, R. Young-Sciame, Q. Wang, *J. Med. Chem.* **2007**, *50*, 21–39; b) N. Kaila, K. Janz, A. Huang, A. Moretto, S. DeBernardo, P. W. Bedard, S. Tam, V. Clerin, J. C. Keith, D. H. H. Tsao, N. Sushkova, G. D. Shaw, R. T. Camphausen, R. G. Schaub, Q. Wang, *J. Med. Chem.* **2007**, *50*, 40–64; c) M. J. Borrok, L. L. Kiessling, *J. Am. Chem. Soc.* **2007**, *129*, 12780–12785.
[13] S. Roseman, *J. Biol. Chem.* **2001**, *276*, 41527–41542.
[14] P. Brocca, A. Bernardi, L. Raimondi, S. Sonnino, *Glycoconjugate J.* **2000**, *17*, 283–299.
[15] For a review on structural studies on the conformation of Lewis oligosaccharides see: E. Yuriev, W. Farrugia, A. M. Scott, P. A. Ramsland, *Immunol. Cell Biol.* **2005**, *83*, 709–717.
[16] J. Jiménez-Barbero, J. L. Asensio, F. J. Cañada, A. Poveda, *Curr. Opin. Struct. Biol.* **1999**, *9*, 549 –555.
[17] E. J. Simanek, G. J. McGarvey, J. A. Jablonowski, C.-H. Wong, *Chem. Rev.* **1998**, *98*, 833–862.
[18] H. C. Kolb, B. Ernst, *Chem. Eur. J.* **1997**, *3*, 1571–1578.
[19] A. Bernardi, D. Arosio, L. Manzoni, F. Micheli, A. Pasquarello, P. Seneci, *J. Org. Chem.* **2001**, *66*, 6209–6216.
[20] E. Fan, E. A. Merritt, C. L. M. Verlinde, W. J. G. Hol, *Curr. Opin. Struct. Biol.* **2000**, *10*, 680–686.
[21] J. C. Pickens, E. A. Merritt, M. Ahn, C. L. M. J. Verlinde, W. G. J. Hol, E. K. Fan, *Chem. Biol.* **2002**, *9*, 215–224.
[22] W. B. Turnbull, B. L. Precious, S. W. Homans, *J. Am. Chem. Soc.* **2004**, *126*, 1047–1054.
[23] E. A. Merritt, P. Kuhn, S. Sarfaty, J. L. Erbe, R. K. Holmes, W. G. J. Hol, *J. Mol. Biol.* **1998**, *282*, 1043–1059.
[24] D. Acquotti, L. Poppe, J. Dabrowski, C. W. von der Lieth, S. Sonnino, G. Tettamanti, *J. Am. Chem. Soc.* **1990**, *112*, 7772–7778.
[25] Reviewed in: A. Bernardi, C. Podlipnik, J. Jiménez-Barbero in *Protein–Carbohydrate Interactions in Infectious Diseases* (Ed.: C. A. Bewley), RSC Publishing, Cambridge, **2006**, pp. 73–91.
[26] A. Bernardi, A. Checchia, P. Brocca, S. Sonnino, F. Zuccotto, *J. Am. Chem. Soc.* **1999**, *121*, 2032–2036.
[27] A. Bernardi, D. Arosio, L. Manzoni, D. Monti, H. Posteri, D. Potenza, S. Mari, J. Jiménez-Barbero, *Org. Biomol. Chem.* **2003**, *1*, 785–792.
[28] a) F. A. Quiocho, *Biochem. Soc. Trans.* **1993**, *21*, 442–448; b) S. Elgavish, B. Shaanan, *J. Mol. Biol.* **1998**, *277*, 917–932; c) M. Fernandez-Alonso, F. J. Cañada, J. Jiménez-Barbero, G. Cuevas, *J. Am. Chem. Soc.* **2005**, *127*, 7379–7386; d) M. S. Sujatha, Y. U. Sasidhar, P. V. Balaji, *Biochemistry* **2005**, *44*, 8554–8562; e) M. S. Sujatha, Y. U. Sasidhar, P. V. Balaji, *Protein Sci.* **2004**, *13*, 2502–2514.
[29] G. Terraneo, D. Potenza, A. Canales, J. Jiménez-Barbero, K. K. Baldridge, A. Bernardi, *J. Am. Chem. Soc.* **2007**, *129*, 2890–2900.
[30] C. Podlipnik, I. Velter, B. La Ferla, G. Marcou, L. Belvisi, F. Nicotra, A. Bernardi, *Carbohydr. Res.* **2007**, *342*, 1651–1660.
[31] a) I. Vrasidas, N. J. de Mol, R. M. J. Liskamp, R. J. Pieters, *Eur. J. Org. Chem.* **2001**, 4685–4692; b) E. A. Merritt, Z. S. Zhang, J. C. Pickens, M. Ahn, W. G. J. Hol, E. K. Fan, *J. Am. Chem. Soc.* **2002**, *124*, 8818–8824; c) Z. S. Zhang, E. A. Merritt, M. Ahn, C. Roach, Z. Hou, C. L. M. J. Verlinde, W. G. J. Hol, E. K. Fan, *J. Am. Chem. Soc.* **2002**, *124*, 12991–12998; d) H. M. Branderhorst, R. M. J. Liskamp, G. M. Visser, R. J. Pieters *Chem. Commun.* **2007**, 5043–5045.
[32] a) S. André, R. J. Pieters, I. Vrasidas, H. Kaltner, I. Kuwabara, F.-T. Liu, R. M. J. Liskamp , H.-J. Gabius, *ChemBioChem* **2001**, *2*, 822–830; b) I. Vrasidas, S. André, P. Valentini, C. Böck, M. Lensch, H. Kaltner, R. M. J. Liskamp, H.-J. Gabius , R. J. Pieters, *Org. Biomol. Chem.* **2003**, *1*, 803–810; c) R. Autar, A. S. Khan, M. Schad, J. Hacker, R. M. J. Liskamp, R. J. Pieters, *ChemBioChem* **2003**, *4*, 1317–1325.
[33] D. Arosio, I. Vrasidas, P. Valentini, R. M. J. Liskamp, R. J. Pieters, A. Bernardi, *Org. Biomol. Chem.* **2004**, *2*, 2113–2124.
[34] B. T. Houseman, M. Mrksich, *Top. Curr. Chem.* **2002**, *218*, 1–44.
[35] T. B. Geijtenbeek, D. S. Kwon, R. Torensma, S. J. Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor, Y. van Kooyk, *Cell*, **2000**, *100*, 587–597.
[36] a) J. J. Reina, S. Sattin, D. Invernizzi, S. Mari, L. Martínez-Prats, G. Tabarani, F. Fieschi, R. Delgado, P. M. Nieto, J. Rojo, A. Bernardi, *ChemMedChem* **2007**, *2*, 1030–1036; b) J. J. Reina, O. S. Maldonado, G. Tabarani, F. Fieschi, J. Rojo, *Bioconjugate Chem.* **2007**, *18*, 963–969; c) G. Tabarani, J. J. Reina, C. Ebel, C. Vivès, H. Lortat-Jacob, J. Rojo, F. Fieschi, *FEBS Lett.* **2006**, *580*, 2402–2408; d) M. J. Borrok, L. L. Kiessling, *J. Am. Chem. Soc.* **2007**, *129*, 12780–12785; e) N. Frison, M. E. Taylor, E. Soilleux, M.-T. Bousser, R. Mayer, M. Monsigny, K. Drickamer, A.-C. Roche, *J. Biol. Chem.* **2003**, *278*, 23922–23929; f) J. Rojo, R. Delgado, *J. Antimicrob. Chemother.* **2004**, *54*, 579–581; g) F. Lasala, E. Arce, J. R. Otero, J. Rojo, R. Delgado, *Antimicrob. Agents Chemother.* **2003**, *47*, 3970–3972; h) S. Mari, D. Serrano-Gómez, F. J. Cañada, A. L. Corbí, J. Jiménez-Barbero *Angew Chem.* **2005**, *117, 300–302; Angew Chem. Int. Ed.* **2005**, 44, 296–298; *Angew Chem. Int. Ed.* **2005**, *44*, 296–298.
[37] Y. Guo, H. Feinberg, E. Conroy, D. A. Mitchell, R. Alvarez, O. Blixt, M. E. Taylor, W. I. Weis, K. Drickamer, *Nat. Struct. Mol. Biol.* **2004**, *11*, 591–598.
[38] S. Mari, H. Posteri, G. Marcou, D. Potenza, F. Micheli, F. J. Cañada, J. Jiménez-Barbero, A. Bernardi, *Eur. J. Org. Chem.* **2004**, 5119–5125.
[39] J. Rojo, J. J. Reina, F. Fieschi, G. Tabarani, A. Bernardi, S. Sattin, unpublished results.
[40] G. Timpano, G. Tabarani, M. Anderluh, D. Invernizzi, F. Vasile, D. Potenza, P. M. Nieto, J. Rojo, F. Fieschi, A. Bernardi, *ChemBioChem* **2008**, in press.
[41] A. Bianchi, A. Bernardi *J. Org. Chem.* **2006**, *71*, 4565–4577.
[42] J. A. Wells, C. L. McClendon, *Nature*, **2007**, *450*, 1001–1009.

Published online: July 9, 2008

