White Rose Research Online

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Leeds.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/79773/

Version: Published Version

Article:
Bernardi, A, Jiménez-Barbero, J, Casnati, A et al. (2013) Multivalent glycoconjugates as anti-pathogenic agents. Chemical Society Reviews, 42 (11). 4709 - 4727. ISSN: 0306-0012

https://doi.org/10.1039/c2cs35408j

Reuse
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

Chem Soc Rev

RSC Publishing

REVIEW ARTICLE

View Article Online
View Journal | View Issue

Multivalent glycoconjugates as anti-pathogenic agents†

Cite this: *Chem. Soc. Rev.*, 2013, **42**, 4709

Anna Bernardi,[a] Jesus Jiménez-Barbero,[b] Alessandro Casnati,[c] Cristina De Castro,[d] Tamis Darbre,[e] Franck Fieschi,[f] Jukka Finne,[g] Horst Funken,[h] Karl-Erich Jaeger,[h] Martina Lahmann,[i] Thisbe K. Lindhorst,[j] Marco Marradi,[k] Paul Messner,[l] Antonio Molinaro,[d] Paul V. Murphy,[m] Cristina Nativi,[n] Stefan Oscarson,[o] Soledad Penadés,[k] Francesco Peri,[p] Roland J. Pieters,[q] Olivier Renaudet,[r] Jean-Louis Reymond,[e] Barbara Richichi,[n] Javier Rojo,[s] Francesco Sansone,[c] Christina Schäffer,[l] W. Bruce Turnbull,[t] Trinidad Velasco-Torrijos,[u] Sébastien Vidal,[v] Stéphane Vincent,[w] Tom Wennekes,[x] Han Zuilhof[xy] and Anne Imberty*[z]

Multivalency plays a major role in biological processes and particularly in the relationship between pathogenic microorganisms and their host that involves protein–glycan recognition. These interactions occur during the first steps of infection, for specific recognition between host and bacteria, but also at different stages of the immune response. The search for high-affinity ligands for studying such interactions involves the combination of carbohydrate head groups with different scaffolds and linkers generating multivalent glycocompounds with controlled spatial and topology parameters. By interfering with pathogen adhesion, such glycocompounds including glycopolymers, glycoclusters, glycodendrimers and glyconanoparticles have the potential to improve or replace antibiotic treatments that are now subverted by resistance. Multivalent glycoconjugates have also been used for stimulating the innate and adaptive immune systems, for example with carbohydrate-based vaccines. Bacteria present on their surfaces natural multivalent glycoconjugates such as lipopolysaccharides and S-layers that can also be exploited or targeted in anti-infectious strategies.

Received 4th October 2012

DOI: 10.1039/c2cs35408j

www.rsc.org/csr

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

[a] *Università di Milano, Dipartimento di Chimica Organica e Industriale and Centro di Eccellenza CISI, via Venezian 21, 20133 Milano, Italy*
[b] *Centro de Investigaciones Biológicas, CSIC, 28040 Madrid, Spain*
[c] *Università degli Studi di Parma, Dipartimento di Chimica, Parco Area delle Scienze 17/a, 43100 Parma, Italy*
[d] *Department of Chemical Sciences, Università di Napoli Federico II, Complesso Universitario Monte Santangelo, Via Cintia 4, I-80126 Napoli, Italy*
[e] *Department of Chemistry and Biochemistry, University of Berne, Freiestrasse 3, CH-3012, Berne, Switzerland*
[f] *Institut de Biologie Structurale, 41 rue Jules Horowitz, 38027 Grenoble Cedex 1, France*
[g] *Department of Biosciences, University of Helsinki, P. O. Box 56, FI-00014 Helsinki, Finland*
[h] *Institute of Molecular Enzyme Technology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, D-42425 Jülich, Germany*
[i] *School of Chemistry, Bangor University, Deiniol Road Bangor, Gwynedd LL57 2UW, UK*
[j] *Otto Diels Institute of Organic Chemistry, Christiana Albertina University of Kiel, Otto-Hahn-Platz 3-4, D-24098 Kiel, Germany*
[k] *Laboratory of GlycoNanotechnology, CIC biomaGUNE and CIBER-BBN, P° de Miramón 182, 20009 San Sebastián, Spain*
[l] *Department of NanoBiotechnology, NanoGlycobiology Unit, University of Natural Resources and Life Sciences, Muthgasse 11, A-1190 Vienna, Austria*
[m] *School of Chemistry, National University of Ireland, Galway, University Road, Galway, Ireland*
[n] *Dipartimento di Chimica, Universitá degli Studi di Firenze, Via della Lastruccia, 13, I-50019 Sesto Fiorentino – Firenze, Italy*
[o] *Centre for Synthesis and Chemical Biology, UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland*
[p] *Organic and Medicinal Chemistry, University of Milano-Bicocca, Piazza della Scienza, 2, 20126 Milano, Italy*
[q] *Department of Medicinal Chemistry and Chemical Biology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands*
[r] *Département de Chimie Moléculaire, UMR-CNRS 5250 & ICMG FR 2607, Université Joseph Fourier, BP53, 38041 Grenoble Cedex 9, France*
[s] *Glycosystems Laboratory, Instituto de Investigaciones Químicas, CSIC – Universidad de Sevilla, Av. Américo Vespucio, 49, Seville 41092, Spain*
[t] *School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, LS2 9JT, UK*
[u] *Department of Chemistry, National University of Ireland, Maynooth, Maynooth Co. Kildare, Ireland*
[v] *Institut de Chimie et Biochimie Moléculaires et Supramoléculaires UMR 5246, CNRS, Université Claude Bernard Lyon 1, 43 Boulevard du 11 Novembre 1918, F-69622 Villeurbanne, France*
[w] *University of Namur (FUNDP), Département de Chimie, Laboratoire de Chimie Bio-Organique, rue de Bruxelles 61, B-5000 Namur, Belgium*
[x] *Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands*
[y] *Department of Chemical and Materials Engineering, King Abdulaziz University, Jeddah, Saudi Arabia*
[z] *Centre de Recherche sur les Macromolécules Végétales (CERMAV – CNRS), affiliated with Grenoble-Université and ICMG, F-38041 Grenoble, France.*
E-mail: Imberty@cermav.cnrs.fr

† Part of the Multivalent Scaffolds in Glycosciences themed issue.

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

1. Introduction

Carbohydrates constitute the most abundant class of biomolecules on Earth. They play roles in the natural world as diverse as energy storage, molecular recognition for intracellular trafficking or interactions between pathogenic microorganisms and viruses and the surfaces of mammalian cells.[1] The carbohydrates present inside and at the surface of cells mediate many biological processes that are fundamentally important for both the healthy and diseased states of living organisms.[2,3] Usually carbohydrates are covalently linked to other biomolecules such as proteins or lipids. The molecular and supramolecular scaffolds of such glycoconjugates provide them with a very important property: multivalency.[4–7] Individual interactions between sugars and proteins are very weak, however, multiple simultaneous interactions between carbohydrate ligands and their receptors reinforce one-another like molecular velcro to achieve functionally useful avidities. Recent advances in carbohydrate microarray technologies[8–14] combined with the utilization of freely accessible resources of the Consortium for Functional Glycomics (http://www.functionalglycomics.org/) have greatly expanded our knowledge of carbohydrate-binding proteins, and reinforced the importance of multivalent interactions.[15–21] It has become apparent that the avidities and selectivities of specific interactions are very dependent on the density of the sugar groups and also the chemical structure of the linker group to the underlying multivalent scaffold. Translating this knowledge for the design and preparation of multivalent glycoconjugates remains a significant challenge to diagnose and treat diseases.[22–25]

Protein–carbohydrate interactions frequently mediate the first step of the infection process for many pathogens including viruses, fungi, bacteria, and bacterial toxins.[26–29] Therefore, a vast array of unnatural glycoconjugates (neoglycoconjugates) with various valencies and spatial arrangement of the ligands have been constructed to prevent or treat diseases caused by pathogens. Scaffolds based on proteins,[30–32] polymers,[33–36] calixarenes,[37–40] dendrimers,[41–45] cyclodextrins,[46,47] cyclopeptides,[48–50] fullerenes,[51,52] gold nanoparticles,[53,54] and quantum dots[55–57] provide nanoscale materials with anti-adhesive and cell targeting properties. Such structures that can competitively interfere with the recognition processes between host cells and pathogens have the potential to prevent colonisation or even reverse the formation of biofilms. Another alternative to fight pathogens relies on the utilization of glycoconjugates that can act as vaccines and immunomodulators. Vaccines have long relied on attenuated strains of microorganisms as a means of delivering the extracellular carbohydrate antigens. As the cell surface of pathogenic bacteria and viruses is often covered with unusual carbohydrates, structurally defined glycoconjugates displaying these structures are starting to emerge as the vaccines for the future.[58,59] Following a lectin-mediated cellular uptake mechanism, such carbohydrate-based vaccines can prepare the immune defense mechanisms in advance of an infection, or to stimulate the body to protect itself against an existing chronic infection.

This review will give an overview of synthetic or natural multivalent glycoconjugates that can be used to inhibit the adhesion of viruses, bacterial toxins, and bacteria to host cells or to stimulate the innate and adaptive immune systems against these pathogens. In addition to the pioneering work of several groups mainly from North America,[7,17,25,60,61] this field is currently flourishing in Europe, and this review is aimed at giving an overview of this very active domain.

Anne Imberty

Dr. Imberty is Research Director at the Centre de Recherches sur les Macromolécules Végétales (CERMAV), affiliated to the Centre National de la Recherche Scientifique (CNRS) based at Grenoble, France. She graduated in biology from Ecole Normale Supérieure in Paris. In 1984, *she joined the CNRS in Grenoble and did her PhD on starch structure. She started studying protein-carbohydrate interaction during her post-doc in Toronto. Since* 1999 *she has a senior research position in CNRS-Grenoble and is the head of the Molecular Glycobiology group. Her research interests are in the field of structural glycobiology, with main interest on biologically active oligosaccharides and their interaction with lectins and glycosyltransferases. She now focuses on characterization of the molecular basis of recognition between lectins from pathogens and human glycoconjugates and design of glycocompounds with anti-infectious properties.*

2. Multivalent glycoconjugates against viral infection

2.1 Targeting DC-SIGN uptake by dendritic cells

Among pathogens, human immunodeficiency virus (HIV) represents one of the big challenges of current basic and medical research. Attempts to generate effective microbicides have failed, so that integrated systems and new strategies are needed in order to prevent or block the viral infection. Multivalent glycoconjugates can be utilized in novel vaccine approaches (see Section 6.2.1) or as inhibitors of first step of infection. One of the main pathways of HIV infection is mediated by antigen-presenting cells (APC) at the mucosal endothelium. The high-mannose glycans of envelope glycoprotein gp120 promote HIV infection by interaction with the C-type lectin DC-SIGN (dendritic cell-specific ICAM 3-grabbing non-integrin) expressed on dendritic cells.[62] Dendritic cells, a main group of APC, migrate to the lymph nodes where they efficiently transfer the virus by so-called *trans*-infection to T lymphocytes where viral replication occurs. Mimicking the surface of the virus is a strategy for designing carbohydrate-based antiviral agents against HIV and Ebola infections.

Glycodendritic compounds have been used as tools to study and to interfere with infectious processes in which DC-SIGN is

 This journal is © The Royal Society of Chemistry 2013

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

involved with the aim to develop new antiviral drugs and immune modulators.[63] The conjugation of glycomimetics to dendritic compounds has provided multivalent compounds with interesting antiviral activity. IC_{50}s in the low nanomolar range have been obtained in biological assays using pseudotyped Ebola viral particles.[64] Also, these compounds present very good activity as inhibitors of HIV trans-infection of T-cells, a more relevant infection model where DC-SIGN is implicated.[65,66]

Glycodendritic structures have also been prepared recently using a convergent approach with extensive use of the Cu(I) catalyzed azide–alkyne cycloaddition (CuAAC) reaction also known as "click" chemistry (Fig. 1). In this new strategy, first and second generation glycodendrons have been prepared conveniently functionalized for further conjugation on different scaffolds including a fullerene molecule or a virus-like particle protein.[52,67]

The new glycodendritic compounds display a wide variety of valencies and spatial presentation of carbohydrate ligands. The glycodendrofullerenes (*e.g.*, **1**) prepared using this strategy are soluble under physiological conditions and present a very low cellular toxicity. The globular disposition of carbohydrates on this spherical scaffold provides an interesting multivalent system which allows the carbohydrates to be recognized by lectins in a multivalent manner. Antiviral activity of these compounds using pseudotyped Ebola viral particles is in the micromolar range.[52,67]

In another attempt to mimic the cluster presentation of high-mannose-type glycans on the HIV envelope, gold nanoparticles biofunctionalized with oligomannosides (*manno*-GNPs, **3a–3d**, Fig. 2) of gp120 high-mannose type glycans have been prepared and tested as anti-HIV agents. These *manno*-GNPs inhibited the DC-SIGN/gp120 binding in the micro- to nanomolar range, while the corresponding monovalent oligomannosides required millimolar concentrations, as measured by surface plasmon resonance (SPR) experiments.[68] Furthermore, *manno*-GNPs were able to inhibit the DC-SIGN-mediated HIV *trans*-infection of human activated peripheral blood mononuclear cells at nanomolar concentrations in an experimental setting, which mimics the natural route of virus transmission from dendritic cells to T lymphocytes.[69]

2.2 Interfering with galectin attachment in lymph nodes

Galectins are lectins with affinity for β-galactosides which are involved in self/nonself recognition. Galectins from both invertebrates and vertebrates recognize a variety of viral and bacterial pathogens and protozoan parasites.[70] Galectin-1 is abundant in thymus and lymph nodes and promotes HIV-1 infection by facilitating virus attachment to the host cell surface glycan.[71] Given that this lectin is directly involved in pathogen recognition, it could provide a strategy for disrupting the galectins in virus–host invasion. Host galectins also recognize endogenous glycans on the host cell surface, which are important for certain necessary developmental and immunological processes.

Structurally defined bivalent lactose-containing clusters have been designed for optimal binding to galectins.[41,72–74] These compounds were evaluated for binding to the entire set of adhesion/growth-regulatory galectins from chicken. Differential sensitivities were detected between distinct galectin forms within the chicken series. Two of the bivalent glycoclusters, **4** and **5** (Fig. 3), were identified as sensors for different galectin subtypes. Most pronounced were the selectivities of these two glycoclusters for the chimera-type galectin (galectin-3).

3. Multivalent glycoconjugates against bacterial toxins

Cholera, still a life-threatening disease in many parts of the world, is caused by the cholera toxin (CT) produced by *Vibrio cholerae.* This toxin consists of a single disease-causing A-subunit that is surrounded by five lectin-like B-subunits (CTB). The B-subunits are responsible for attachment of the toxin to the intestinal surface by binding to exposed GM1-oligosaccharide (GM1os) moieties.[75] The five B-subunits represent a well-defined multivalent protein target with binding sites for the GM1os units spaced *ca.* 30 Å apart. Polyvalent CT inhibitors were developed starting from weak ligands, such as galactose. Huge potency increases (up to 10^6-fold with IC_{50} = 40 nM) compared to that of D-galactose were reported by Fan and co-workers, who prepared and screened a series of penta- and decavalent ligands with linkers of various lengths.[76]

The advent of 'click' chemistry in combination with chemoenzymatic synthesis of the complex oligosaccharide enabled the assembly of multivalent versions of the GM1os[77] into glycodendrimers (Fig. 4).[78] Divalent compound **7a** was almost 10 000 times more potent as an inhibitor of CTB binding to GM1os than monovalent compound **6**. This enhancement in inhibitory potency is related to the multivalent interactions between CTB and the divalent compound **7a**, as the latter does not show an enhancement in binding to antibodies that do not allow multiple interactions.[79]

An additional increase was observed for tetravalent **8a** (83 000 fold). The most complex glycodendrimer in this study, octavalent **9a**, was 380 000 fold more potent (IC_{50} = 50 pM and relative inhibitory potency of 47 500 per GM1os).[78] A detailed study of the mode of action revealed that complex aggregates between the inhibitor and toxin are formed. These are possible because of the mismatch between the valencies of the toxin (five) *versus* those of the inhibitors (two, four, eight).[80]

The galactose dendrimers **7b, 8b** and **9b** are a simplified glycomimetic version of the multivalent GM1 derivatives. The inhibitory potency did suffer due to this modification since the relatively large binding site of the B-subunit remains partly unoccupied resulting in a lower binding affinity. Nevertheless, multivalency effects were able to counteract the lower binding affinity of galactose and the inhibitory potencies of compounds **8b** and **9b** were shown to be competitive with the natural GM1os ligand.[81]

A series of ganglioside mimics, in which the non-interacting oligosaccharide backbone of GM1os was replaced by an

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

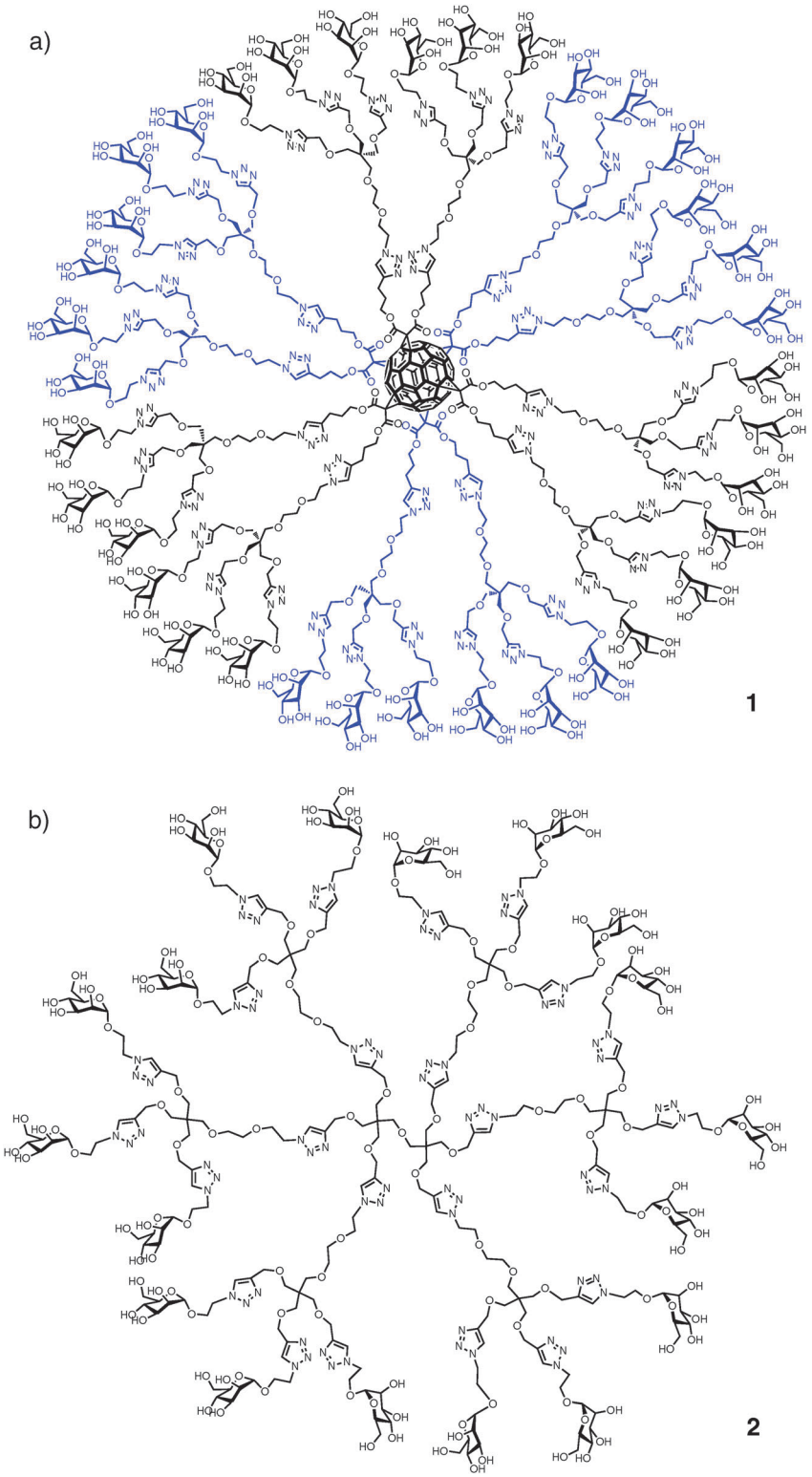
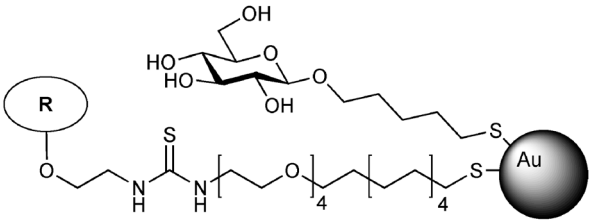


Fig. 1 (a) Glycodendrofullerene **1** with 36 mannoses; (b) glycodendrimer **2** with 18 mannoses prepared using a CuAAC click reaction.

appropriate cyclohexanediol, was chosen to reproduce the topological features of the 3,4-disubstituted galactose residue (Gal-II) in GM1os.[82,83] The divalent presentation of a structurally simplified second generation mimic **10** on a functionalized calix[4]arene scaffold (Fig. 5) led to a 3800-fold (1900-fold per sugar mimic) enhancement of CTB affinity, thus reaching the potency of GM1os itself.[84] Although computational studies show that the divalent ligand **11** could easily span two binding

 This journal is © The Royal Society of Chemistry 2013



3a R = Man(α1-2)Man(α1-

3b R = Man(α1-2)Man(α1-2)Man(α1-

3c R = Man(α1-2)Man(α1-2)Man(α1-3)Man(α1-

3d R = Man(α1-2)Man(α1-3)[Man(α1-2)Man(α1-6)]Man(α1-

Fig. 2 Gold nanoparticles **3a–3d** bearing high-mannose type glycans (*manno*-GNPs) present on HIV envelope glycoprotein gp120 as anti-HIV synthetic glycoconjugates.

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

sites on cholera toxin, NMR data indicate that the action of this divalent ligand is likely to involve additional interactions between the linker and the protein.

4. Multivalent glycoconjugates against bacterial adhesion to human cells

4.1 Uropathogenic *E. coli*

4.1.1 FimH. Type 1 fimbriae, or pili, are the most abundant surface structures both in pathogenic and non-pathogenic Gram-negative bacteria. Type 1 fimbriae are widely expressed by *E. coli* and are used by uropathogenic strains to mediate attachment to specific niches in the urinary tract.[85] Type 1 fimbriae mediate attachment to glycosylated surfaces through FimH, a mannose-specific lectin located at the tip of pili. FimH is a two-domain lectin, whose crystal structure has been solved.[85] Since the FimH binding site can accommodate only one α-mannoside, multivalency effects which have been observed in the inhibition of type 1 fimbriae-mediated bacterial adhesion[42,86–90] cannot be explained on the basis of the structure of mannose-specific lectin FimH.

Nevertheless, avidity effects have been frequently observed with a variety of multivalent mannose-containing glycomimetics, like **12–14** (Fig. 6a–c). Such avidity can originate from statistical effects arising from (i) a higher concentration of mannose in the proximity of the carbohydrate binding site, (ii) existence of additional carbohydrate binding sites on the lectin FimH, or (iii) occurrence of the natural multivalent process, since fimbriae occur on the bacterial surface in several hundreds of copies. Thus, mannose-terminated multivalent glycocompounds have become important to test mannose-specific bacterial adhesion in a supramolecular context.[88,91] Meanwhile, testing of type 1 fimbriae-mediated bacterial adhesion has been greatly facilitated by employing GFP-transfected strains.[92] Interestingly, adhesion on multivalent glycomaterials can be utilized for aggregating *E. coli* and removing them from solution with the use of appropriate filters. Glyconanodiamonds decorated with mannose (Fig. 6d) have been shown to be able to clean bacteria-polluted water.[93]

Fullerene *hexakis*-adducts bearing 12 peripheral mannose moieties (**15–20**) have been prepared by grafting sugar derivatives onto the fullerene core[52] and assayed as inhibitors of FimH (Fig. 7).[88] Dissociation constants in the range of 12 to 95 nM were measured using isothermal titration calorimetry (ITC), surface plasmon resonance (SPR) and hemagglutination assays. Most importantly, the number of possible interactions between the multimers and the lectin and the average binding strength per functional mannose unit could be measured. Thus, this study demonstrated for the first time that a globular C_{60} structure can accommodate up to seven FimH molecules.

4.1.2 PapG. While type 1 fimbriae terminated with mannose specific FimH are involved in lower urinary tract infection, *E. coli* presenting P-fimbriae cause bladder infections resulting in acute pyelonephritis.[94] P-fimbriae are terminated by PapG, a tip-associated adhesin that recognizes the Galα(1-4)Gal (galabiose) moiety of the globo-series of glycolipids.[95] For the study of the inhibition of this adhesion by oligovalent galabiose derivatives, a live bacteria-SPR assay was established, in order to mimic the flow conditions of natural infections.[96]

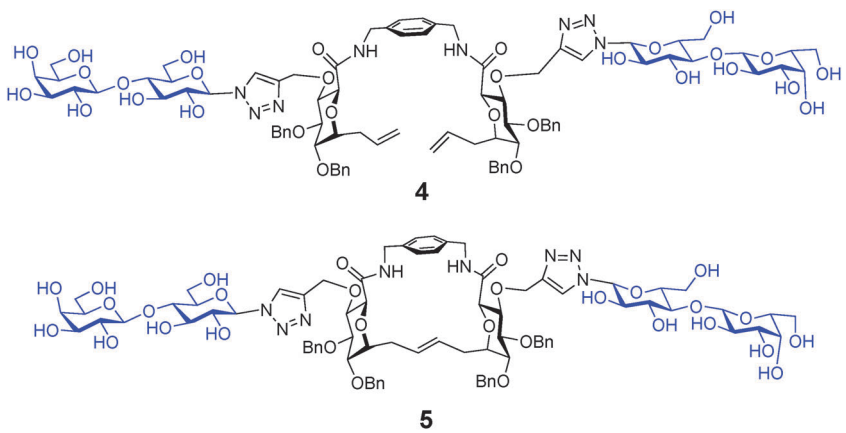


Fig. 3 Bivalent glycoclusters **4** (acyclic) and **5** (macrocyclic), identified as sensors for different galectin subtypes.

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

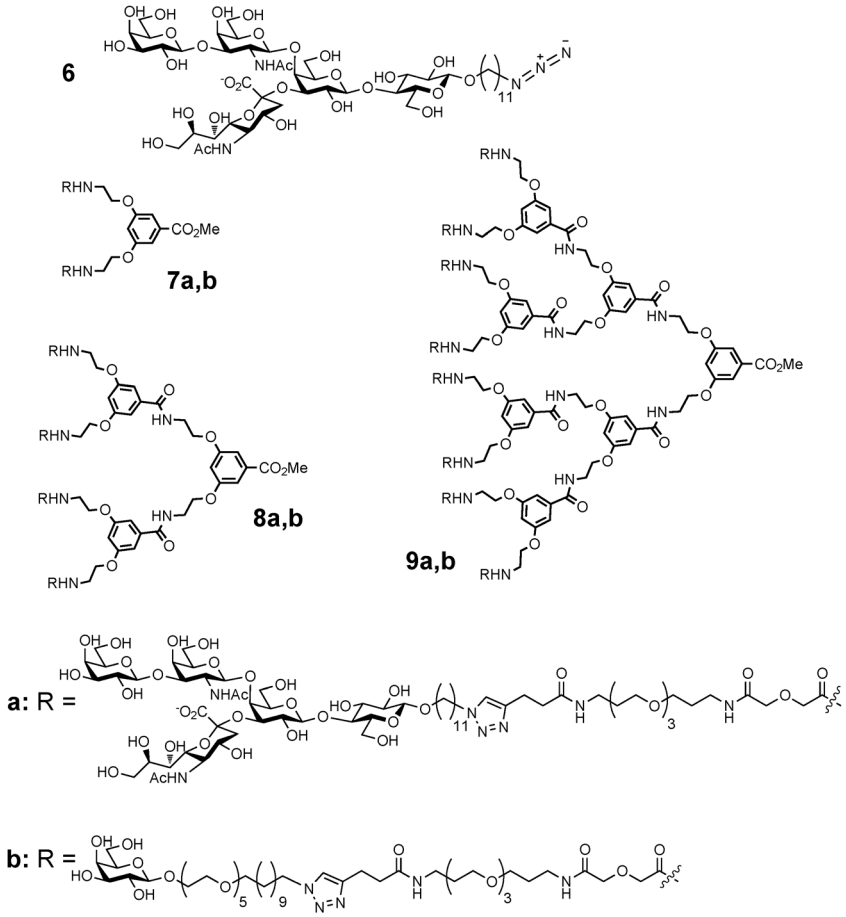


Fig. 4 Structures of GM1os- (**7a, 8a** and **9a**) and galactose (**7b, 8b** and **9b**)-based inhibitors of cholera toxin binding.

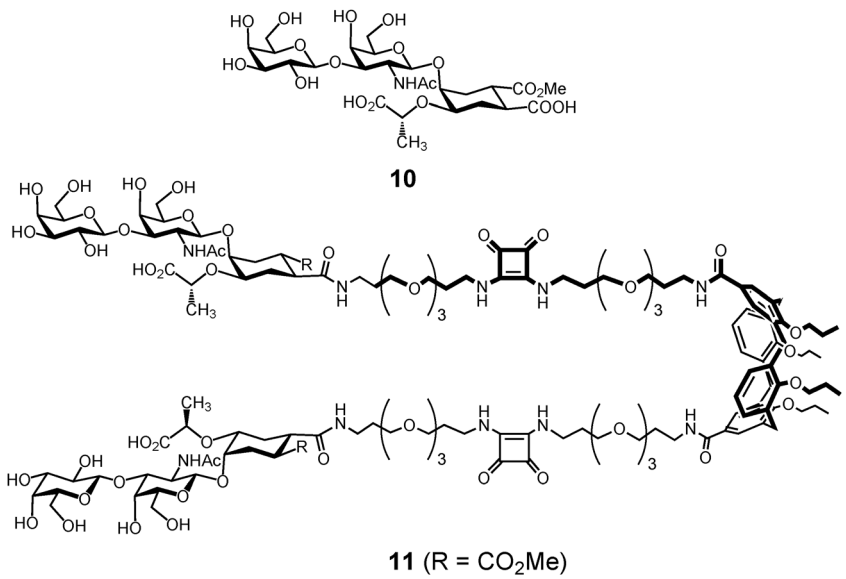


Fig. 5 Structurally simplified GM1os mimic **10** grafted onto a functionalized calix[4]arene scaffold to give divalent ligand **11**.

Synthetic mono- and multivalent galabiose derivatives **21a–d** (Fig. 8) inhibited bacterial adhesion to the coated chip surfaces in a dose-dependent manner (Table 1). An octavalent galabiose compound **21d** was superior to the tetravalent derivative **21c**, which in turn was a better inhibitor than the monovalent galabiose derivative **21a.** However, the multivalency effect was

 This journal is © The Royal Society of Chemistry 2013

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

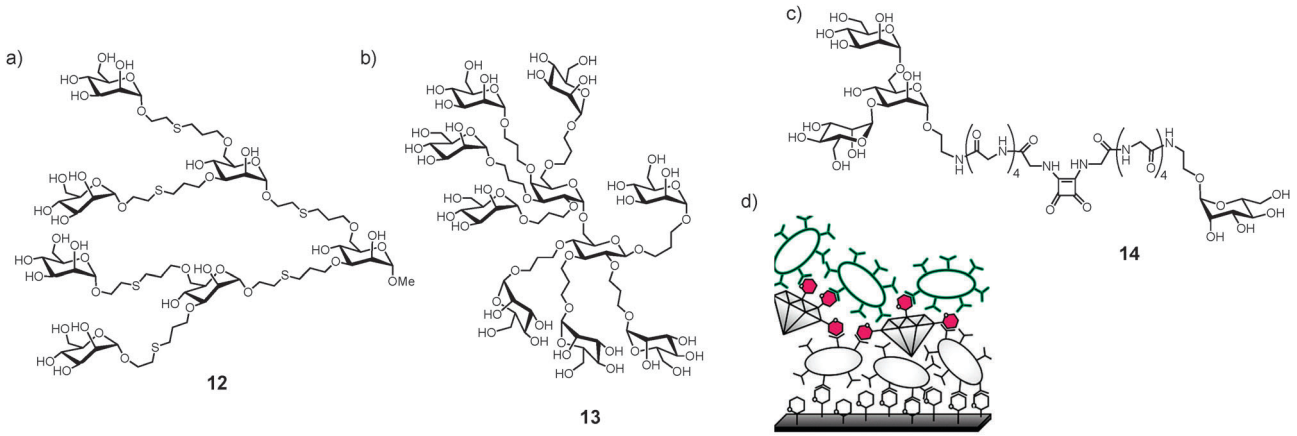


Fig. 6 Examples of various multivalent glycoconjugates inhibiting type 1 fimbriae-mediated bacterial adhesion. (a) Octopus glycosides **12**; (b) glycodendrimer **13**; (c) bifunctional ligand **14** to test multiple binding sites on FimH; (d) glyconanodiamonds to remove pathogenic bacteria from polluted water sources, a sandwich assay is displayed, utilizing two different bacterial strains.

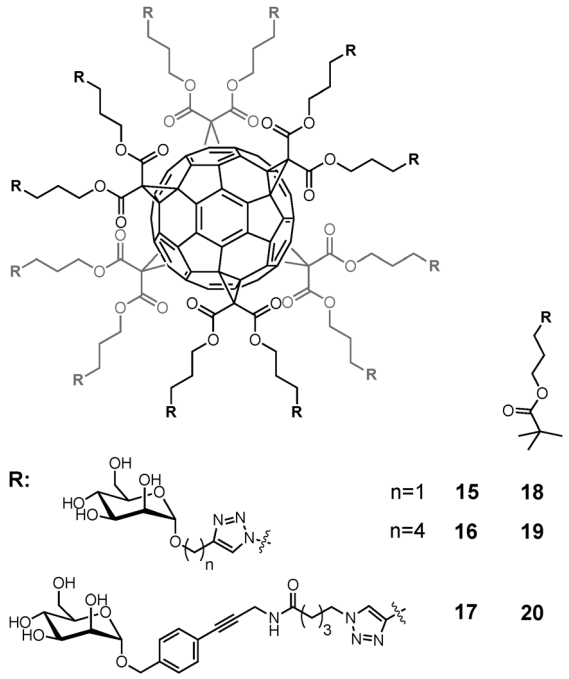


Fig. 7 Dodecavalent mannofullerenes **15–20** as FimH inhibitors.

much more pronounced in the case of the *Streptococcus suis* adhesion when compared to *E. coli PapG*. On the other hand, a more significant multivalency effect was observed in the inhibition of the mannose-specific type-1-fimbriated *E. coli* with similar multivalent mannose molecules.[97] It would appear that multivalent inhibitors do not reach multiple *E. coli* adhesin molecules as effectively as in the case of other bacteria such as *S. suis* (Table 1), and therefore the spacing of the binding sites in the adhesins may differ.

4.2 Lung pathogens

4.2.1 *Pseudomonas aeruginosa* soluble lectins. *Pseudomonas aeruginosa* is an opportunistic pathogen associated with chronic airway infections, especially in cystic fibrosis patients. This strain synthesizes two lectins, LecA and LecB,[98] which play a prominent role in human infections. It was demonstrated that solutions of galactose and fucose, that bind to LecA and LecB, respectively, have a therapeutic effect against *P. aeruginosa* pneumonia in mice models[99] and cystic fibrosis patients.[100] Recently, glycomimetic peptides were identified that had a high affinity for binding to both LecA and LecB and could inhibit their ciliotoxic activity.[101]

LecA (also called PA-IL) is a tetrameric cytotoxic lectin consisting of four subunits of 121 amino acids (12.75 kDa)[98,102] with specificity for α-D-galactose and binding preferentially to Galα-(1-4)Gal containing globotriaosylceramide Gb3 sphingolipid. The LecA crystal structure demonstrated the structural basis of the affinity for galactose monosaccharides with the participation of a calcium ion in the binding site.[103] In addition to its cytotoxicity, it has been suggested that this lectin contributes to the formation of bacterial microcolonies and the formation of biofilms.[104]

Many of multivalent glycoconjugates have been synthesized for inhibiting the binding of LecA to galactosylated surfaces (Table 2 and Fig. 9). High-valency compounds such as galactosylated helical poly(phenylacetylene) polymer **29**,[34] fullerenes **23**,[51] glyconanoparticles **31**,[105] or glycodendrimers **32**[106] are efficient ligands for inhibition, but their aggregative properties and the strong resulting precipitation create difficulties for measuring affinity constants. Excellent results were obtained with calix[4]arenes **28**,[38,107] calix[6]arenes **26**, β-peptoids **25**, porphyrins **27**[108] and resorcin[4]arenes **22**.[109] Among these molecules, the 1,3-alternate conformer of calix[4]arene demonstrated the most efficient and dramatic increase in affinity. A chelate-binding mode with two galactose residues interacting with two neighbouring binding sites in a single LecA tetramer could be confirmed by the observation of well-defined nanometric fibers of lectin–glycocluster complexes through atomic force microscopy (AFM) study.[110]

A recent study demonstrated that a divalent ligand **30** with an appropriate linker was sufficient to induce a chelation effect

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

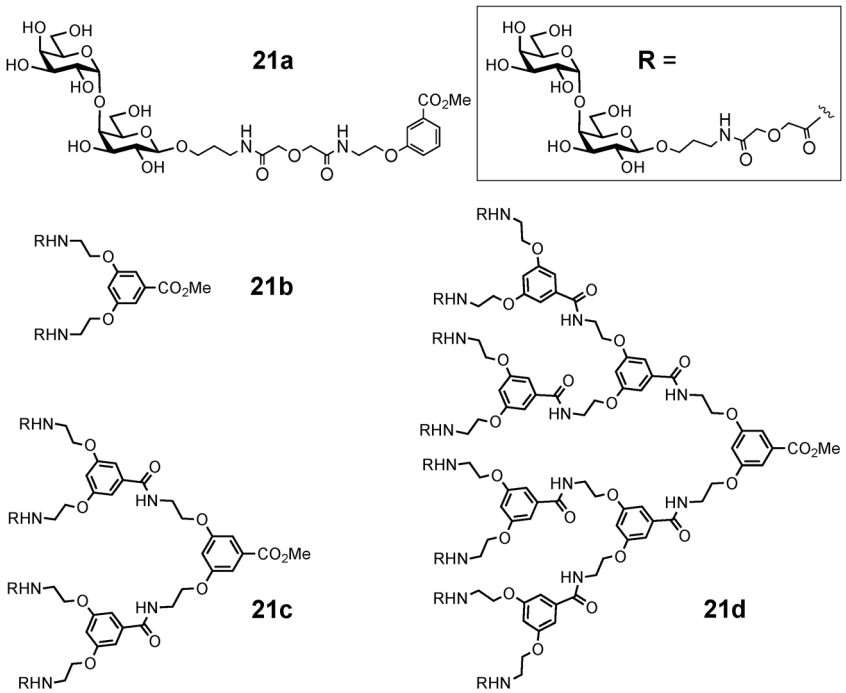


Fig. 8 Oligovalent galabiose derivatives.

Table 1 Relative potencies per sugar unit of galabiose derivatives (Fig. 8) in surface plasmon resonance (SPR) or hemagglutination inhibition (HAI) assays of *E. coli* and *S. suis* adhesion

	E. coli (SPR)	*E. coli* (HAI)	*S. suis* (SPR)
Monovalent (**21a**)	1.0	1.0	1.0
Divalent (**21b**)	1.3	1.1	6.7
Tetravalent (**21c**)	1.9	2.0	63
Octavalent (**21d**)	5.3	4.4	39

with LecA.[111] A rigid spacer was designed based on the alternation of glucose moieties linked at the 1 and 4 positions by a 1,2,3-triazole unit *via* "click" chemistry. The resulting spacer was relatively rigid and straight and was linked to galactose units at both ends. The number of building blocks was varied, as well as the linker between the spacer and galactose ligand. This linker was shown to be of great importance and only compound **30** (Fig. 9) has the appropriate linker-length for achieving an inhibitory potency increase of 545-fold over a relevant reference compound.

Potent ligands for lectin LecA have been also obtained by synthesis of glycopeptide dendrimers **GalAG2 33** and **GalBG2 34** (Fig. 10).[112] Multivalency strongly influenced binding, with the monovalent and divalent analogs showing much weaker interactions with the lectin. The much stronger binding of the phenyl galactosides to LecA compared to the thiopropyl-galactosides was explained by crystallographic analysis of the lectin–glycopeptide complexes, which revealed a specific interaction between a histidine residue on the lectin and the phenyl group

Table 2 Multivalent compounds targeted against LecA. β values are calculated *versus* the monomeric compounds with linkers, unless otherwise stated

Comp.	Valency	HIA (MIC)	ELLA (IC_{50})	SPR (K_d)	ITC (K_d)	Ref.
αMeGal	1		150 μM		50 μM	106
βMeGal	1		190 μM		94 μM	106
22	4	Haemolysis	0.7 μM, β = 315	Not soluble	—/—	109
23	12	0.78 μM, β = 12820	0.040 μM, β = 458	0.367 μM, β = 173	—/—	51
24	4	> 2000 μM	—/—	3.5 μM, β = 18	1.8 μM, β = 83	108
25	4	> 2000 μM	—/—	2.5 μM, β = 25	0.3 μM, β = 500	108
26	6	63 μM, β = 159	—/—	0.8 μM, β = 80	0.14 μM, β = 1071	108
27	4	63 μM, β = 159	—/—	1.4 μM, β = 45	0.33 μM, β = 454	108
28	4	500 μM, β = 20	—/—	0.5 μM, β = 144	0.176 μM, β = 852	38
29	Polymer	9 μM	—/—	—/—	4.12 μM	34
30	2	—/—	0.22 μM, β = 545	—/—	—/—	111
31	67	0.45 μM, β = 100	—/—	33 μM	0.05 μM, β = 2824	105
32	9	—/—	—/—	—/—	230 nM, β = 409	106
33	4	0.78 μM, β = 4000[a]	—/—	—/—	0.1 μM, β = 875	112

[a] β value calculated with galactose as reference.

 This journal is © The Royal Society of Chemistry 2013

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

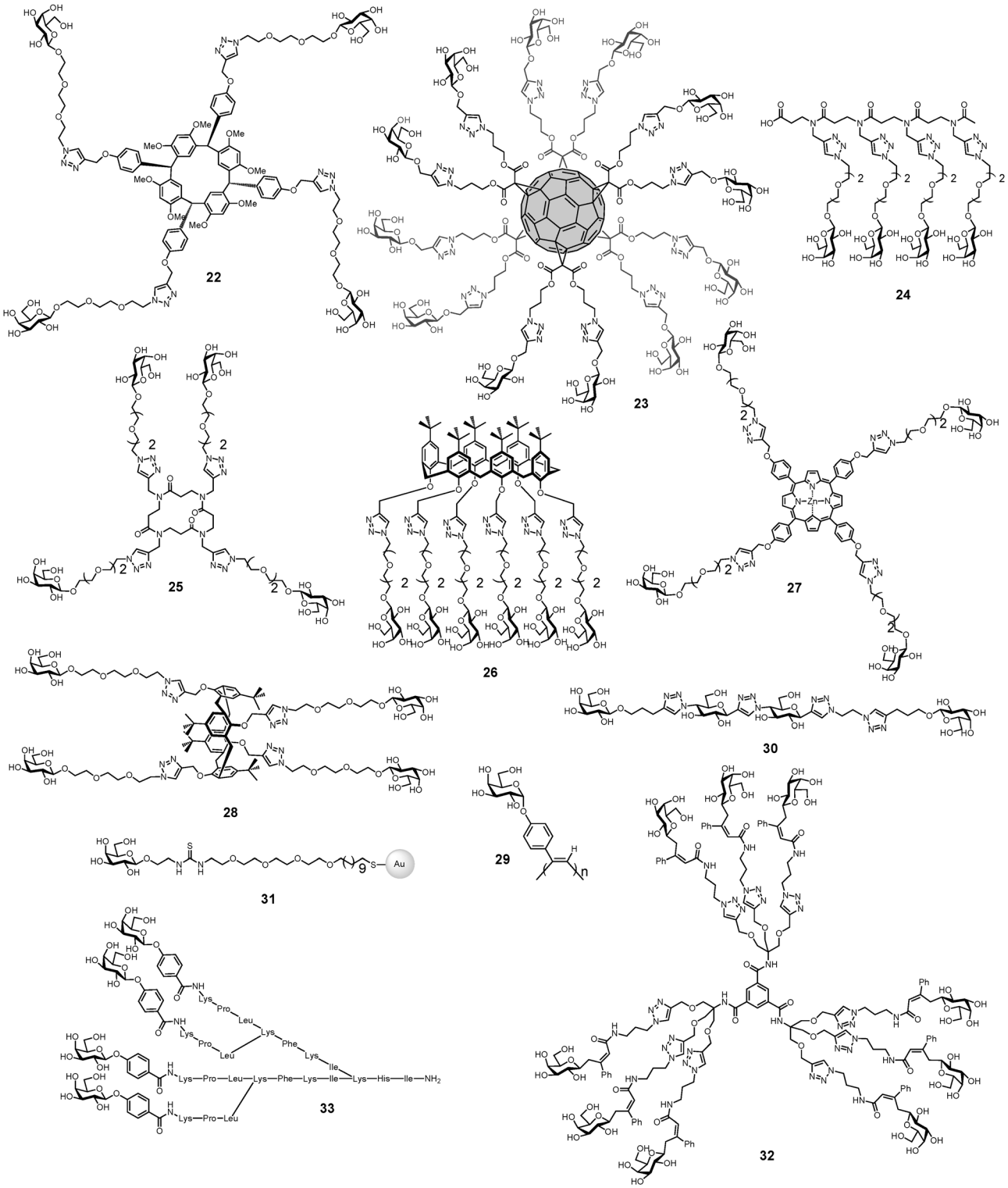


Fig. 9 Multivalent glycoconjugates **22–33** as LecA high affinity ligands.

in the ligands, while the thiopropyl side-chain was more disordered.[112]

LecB (also called PA-IIL) is a tetramer consisting of four 11.73 kDa subunits with high specificity for L-fucose and a weaker one for D-mannose.[98,102] The LecB crystal structure revealed the occurrence of two bridging calcium ions in the binding site. This unique mode of binding is not observed in other lectins,[113] but explains the very high affinity for fucosides and Lewis a. Although most of the LecB is cytoplasmic, it could also be detected in the outer membrane, including on the

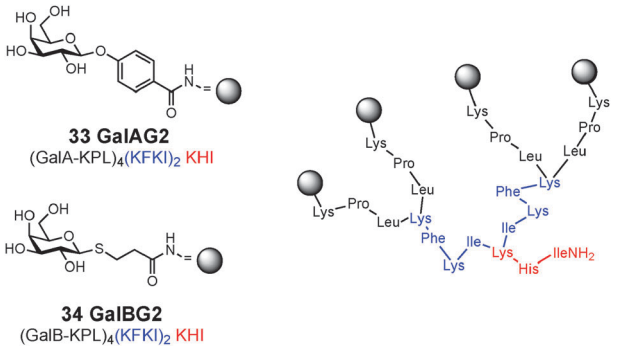


Fig. 10 Structure of glycopeptide dendrimer inhibitors of *P. aeruginosa* biofilms.

surface of biofilm cells, from which it can be released by application of L-fucose.[114] It has recently been hypothesized that LecB undergoes transient *N*-glycosylation that could play a role in the secretion mechanism.[115] The search for a putative binding partner led to the proposal of outer membrane protein OprF which is a nonspecific, weakly cation-selective porin channel protein. LecB may mediate the adhesion of *P. aeruginosa* cells to receptors that are located on its surface and facilitate biofilm formation, thereby promoting colonization of host tissues.

The search for high affinity ligands for LecB initiated the synthesis of several classes of fucose-containing compounds (Table 3 and Fig. 11) based on calixarene **35**,[116] pentaerythritol **39**[117] or peptide dendrimer **36–38** scaffolds. Compounds have also been designed for bivalent presentation of αFuc(1 → 4)GlcNAc **40**,[118] and *N*-fucosyl amides **41**.[119]

Glycopeptide dendrimer ligands for LecB were identified by screening combinatorial libraries of peptide dendrimers[120–122] functionalized with *N*-terminal *C*-fucoside residues at the end of the dendrimer branches. **FD2 36** and **PA8 37** (Fig. 11) turned out to be potent ligands for LecB.[123,124]

Structure–activity relationship studies showed that multivalency was important for activity, in particular divalent and linear peptide analogs of the dendrimers showed strongly reduced binding at the level of monosaccharides (Table 4). These studies led to the identification of dendrimer **2G3 38** with 8 fucosyl endgroups as the most potent glycopeptide dendrimer ligand to LecB. The diastereoisomer D-**36** prepared from D-amino acids was also demonstrated to be a similarly potent ligand to LecB.[125]

Table 3 Multivalent compounds targeted to LecB. β values are calculated *versus* fucose

Comp.	Val.	ELLA	ITC	Ref.
αMeFuc	1			167
			0.47 μM	
35	4	Test biofilm 80% inhibition at 200 μM		116
36	4	0.14 μM, β = 79	—/—	125
37	4	0.11 μM, β = 100	—/—	126
38	8	0.025 μM, β = 440	—/—	168
39	10	0.25 μM, β = 22	—/—	117
40	2	—/—	0.09 μM	118
41	2	—/—	0.62 μM, β = 3	119

4.2.2 Inhibition of *P. aeruginosa* biofilms. One of the key factors responsible for the virulence and antibiotic resistance of this pathogen is the formation of biofilms. The development of therapeutic agents acting on the bacterial biofilm is of utmost importance. The *C*-fucosylpeptide dendrimers **36** and D-**36** inhibit biofilm formation and efficiently disperse established biofilms of wild type and hospital strains of *P. aeruginosa* (Table 4).[125,126] Control experiments with LecB-deletion mutants and dendrimers that are not ligands of LecB showed that biofilm inhibition required both a potent ligand and LecB expression, suggesting that the effect was indeed caused by binding of the ligand to the lectin. The galactosylated peptide dendrimer such as **GalAG0/1/2 33** and **GalBG0/1/2 34** (Fig. 10 and Table 4) had strong affinity for LecA and demonstrated biofilm inhibition and dispersion activity.[112] This anti-biofilm effect mediated by glycopeptide dendrimers was unprecedented and suggests a new therapeutic approach to control *P. aeruginosa* infections.

4.2.3 *Burkholderia cenocepacia* soluble lectins. *Burkholderia cenocepacia* is another opportunistic bacterium causing infections in patients suffering from chronic granulomatous disease and cystic fibrosis with significant morbidity and mortality. A family of four soluble lectins has been identified in *B. cenocepacia*, each containing at least one domain with strong sequence similarity with LecB from *P. aeruginosa* described above.[127] BC2L-A is a small lectin consisting of one dimer of LecB-like domain that binds mannose and oligomannose-type *N*-glycans.[127,128]

BC2L-A has a strong affinity for α-D-mannosides (K_d of 2 μM for methyl α-D-mannopyranoside) and mannobioses. Bridging interaction with the branched trimannoside Manα1-3(Manα1-6)-Man resulted in the formation of molecular strings as detected by protein crystallography and AFM. Oligomannose analogs presenting two mannosides separated by either rigid (**42**) or flexible (**43**) spacer arms were also tested (Fig. 12). Only the rigid linker yielded high affinity with a fast kinetics of clustering, while the flexible analog and the trimannoside displayed moderate affinities and no clustering.[128]

Micelles formed from mannosylated poly(ethylene oxide)-*b*-poly(ε-caprolactone) (PEO-*b*-PCL) diblock copolymer and nanoparticles of poly(D,L-lactic acid) functionalized with the same copolymer have also been demonstrated to bind efficiently to BC2L-A.[36,129]

4.3 Zoonotic bacteria

Streptococcus suis is an important emerging worldwide pig pathogen and zoonotic agent, which causes meningitis, pneumonia and sepsis in pigs and also meningitis in humans.[130] An adhesion activity based on the recognition of glycoconjugates containing the disaccharide galabiose (*i.e.* Galα(1-4)Gal) has been characterized in *S. suis*. The hydroxyl groups identified to be involved in the interaction indicate that *S. suis* adhesin has a different binding mode to galabiose than PapG from uropathogenic *E. coli*. These two adhesins do not display sequence similarity neither, indicating that they have evolved through convergent evolution.[131]

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

This journal is © The Royal Society of Chemistry 2013

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

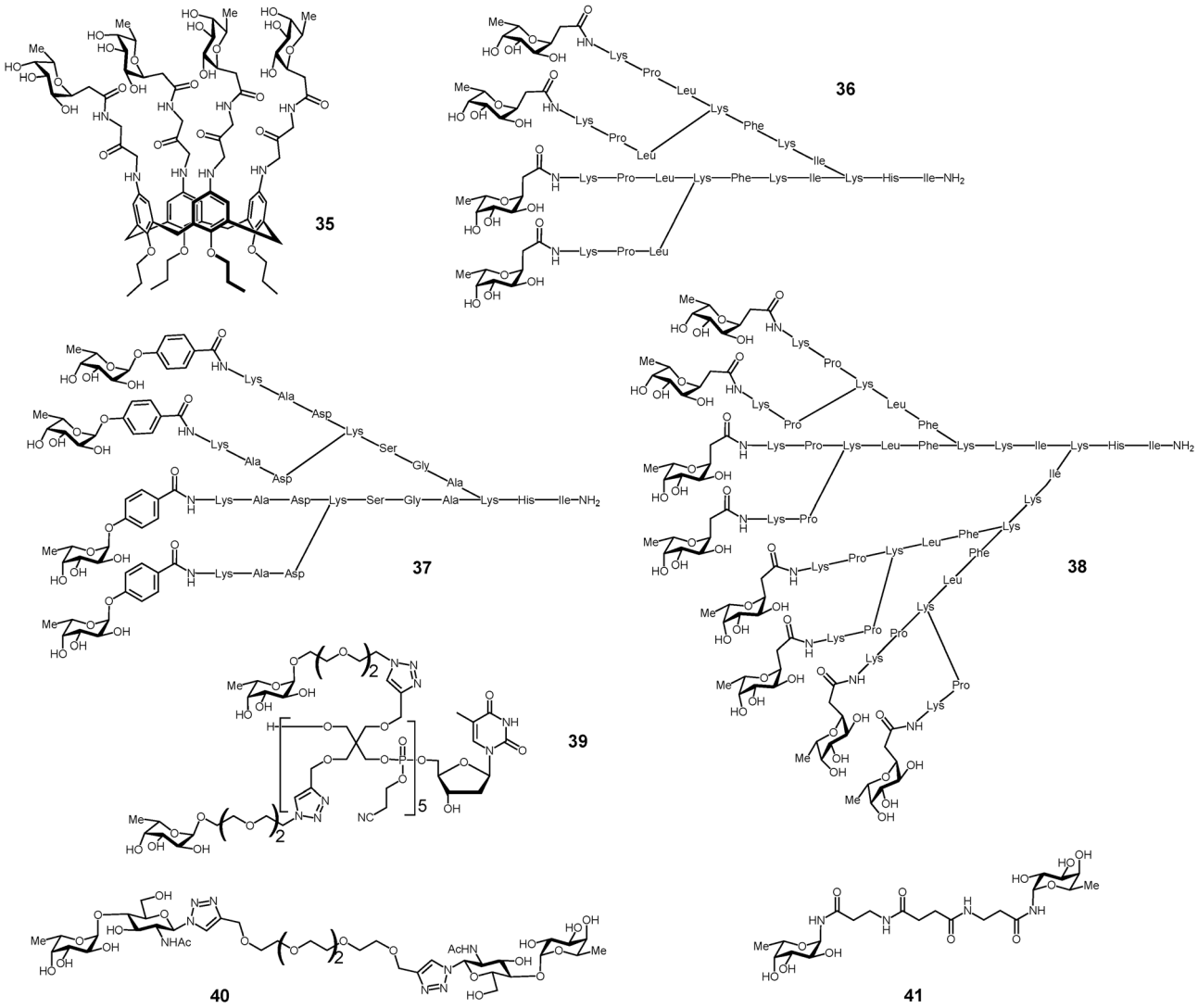


Fig. 11 Multivalent glycoconjugates **35–41** as LecB high affinity ligands.

Synthetic derivatives of the receptor disaccharide galabiose on one hand, or polyvalent dendrimers of galabiose on the other hand (Fig. 8), have turned out to be exceptionally efficient inhibitors of *S. suis* adhesion, both at nanomolar concentrations.[132,133] Inhibitors of the adhesion of *S. suis* to cells have also been found in natural sources, berries and juices, but their chemical nature has not yet been identified.[134] In the characterization of the adhesion specificities and comparison of various mono- or oligovalent inhibitors, a live-bacteria application of surface plasmon resonance has turned out to be very useful.[96]

Due to phase variation, the expression of bacterial adhesins is not uniform even within a single bacterial strain. Therefore it may become important to be able to detect specifically bacteria that express a specific adhesin of potential clinical impact. Magnetic glyconanoparticles may represent ideal tools for this purpose. Magnetic beads containing derivatives of galabiose (Fig. 13) were capable of selecting bacteria in a mixture and determining their amounts in a luminescence assay.[135] Optimisation of the carbohydrate ligand and its multivalent presentation in appropriate carriers are predicted to further improve the efficiency of the ligand in bacterial adhesion inhibition and detection.

5. Bacterial cell surface as a target for anti-infectious agents

5.1 Bacterial LPS: a natural multivalent glycoconjugate

In the cell wall of Gram-negative bacteria, carbohyrates are mostly conjugated to lipids in the outer membrane, where they are present in a very high concentration. The bacterial outer membrane is asymmetric.[136] Its external leaflet is almost entirely constituted of lipopolysaccharides (LPSs) which cover approximately 75% of the surface giving rise to a multivalent glycoconjugate surface with very low fluidity and a highly ordered structure. LPSs are necessary for bacterial viability and this natural multivalent assembly plays many roles in

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

Table 4 *P. aeruginosa* lectin binding and biofilm inhibition by glycopeptide dendrimers

No	Structure[a]	Lectin	IC_{50}, μM (ELLA)[b]	K_D, μM (ITC)[c]	r.p./n[d]	Biofilm inhibition[e]
α-NPF	α-(*p*-Nitrophenyl)-L-fucoside	LecB	5.27 ± 0.55		2.1	—
T1	$(cFuc\text{-}RL)_2$*B*RIFV	LecB	5 ± 0.45		1.7	n.d.
KT1 37	$(cFuc)_4$(*K*RL)$_2$*B*RIFV	LecB	0.59 ± 0.059		7.2	n.d.
2G0	cFuc-KPL	LecB	5.94 ± 1.24		1.9	n.d.
2G1	$(cFuc\text{-}KPL)_2$*K*FKI	LecB	2.7 ± 0.56		2.0	n.d.
37	$(cFuc\text{-}KPL)_4$(*K*FKI)$_2$ *K*HI	LecB	0.14 ± 0.035		20	++++
D-36	$(cFuc\text{-}kpl)_4$(*K*fkl)$_2$ *K*hl	LecB	0.66 ± 0.12		4.2	+++
2G3	$(cFuc\text{-}KP)_8$(*K*LF)$_4$(*K*KI)$_2$*K*HI	LecB	0.025 ± 0.005		55	n.d.
NPG	*p*-Nitrophenyl β-D-galactopyranoside	LecA		14.1 ± 0.2	6.2	—
GalAG0	GalA-KPL	LecA		4.2 ± 0.9	21	+
GalAG1	$(GalA\text{-}KPL)_2$*K*FKI	LecA		0.5 ± 0.2	91	++
GalAG2 33	$(GalA\text{-}KPL)_4$(*K*FKI)$_2$*K*HI	LecA		0.1 ± 0.01	220	+++
GalBG0	GalB-KPL	LecA		51.5 ± 6.7	1.7	+
GalBG1	$(GalB\text{-}KPL)_2$*K*FKI	LecA		2.1 ± 1.0	21	++
GalBG2 34	$(GalB\text{-}KPL)_4$(*K*FKI)$_2$*K*HI	LecA		0.4 ± 0.1	60	+++

[a] Standard peptide notation with N-terminus at left and C-terminus at right. Amino acids are given in one-letter codes, italics indicate branching diaminoacids, *B* is L-2,3-diaminopropionic acid, the C-terminus (at right) is carboxamide ($CONH_2$) in all cases. See also Fig. 10 for exemplification of the topology and the structure of the glycoside groups *C*-Fuc, GalA and GalB. [b] Enzyme-linked lectin assay. [c] Isothermal titration calorimetry. [d] r.p./*n* is the relative potency compared to the free sugar (L-fucose or D-galactose) per glycoside group. [e] Biofilm inhibition measured with the steel coupon assay at 50 μM. n.d. = not determined, — = no inhibition, + = less than 20% inhibition, ++ = 30 to 50% inhibition, +++ = up to 90% inhibition, ++++ = 100% inhibition.

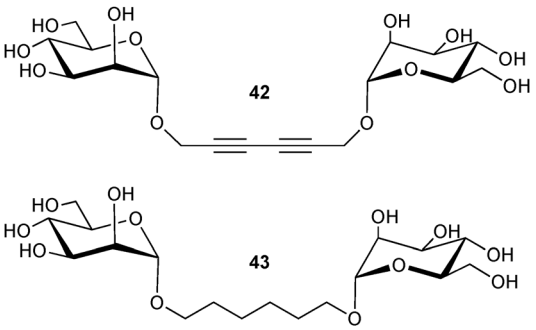


Fig. 12 Divalent mannosylated compounds as ligands of BC2L.

host–bacterium interactions.[136] LPSs are classified as MAMPs (Microbe-Associated Molecular Patterns) because they are recognized by the innate immune system. This recognition takes place through the involvement of specific PRRs (Pathogen Related Receptors) finely tuned on a MAMP motif, the lipid A domain.

LPSs isolated or extracted from their natural environment aggregate in solution due to their amphiphilic nature, resulting in a supramolecular aggregate which still displays multivalent features that can be of direct use. Indeed, it has recently been shown that the HIV protective antibody 2G12 is able to recognize the LPS produced from *Rhizobium radiobacter* Rv3.[137] This particular LPS possesses a carbohydrate structure (Fig. 14) which resembles the epitope on the surface of HIV for which the antibody is specific. It has been observed that 2G12 interacted with lipooligosaccharides (LOS) only when the lipid moiety was present, so as to allow formation of a supramolecular aggregate in water (C. De Castro, personal communication).

Another interesting example of LPS multivalency relates to the stabilizing effect on the protein conformation embedded in

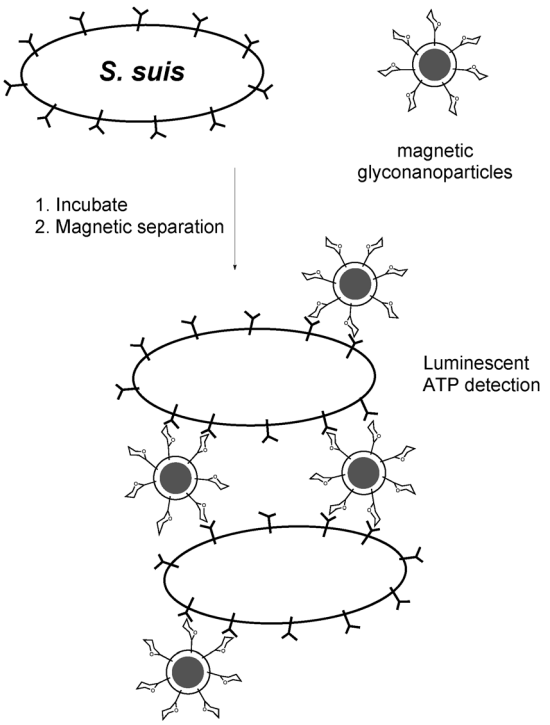


Fig. 13 Use of galabiose-functionalized magnetic beads for identifying and isolating *S. suis* bacteria.

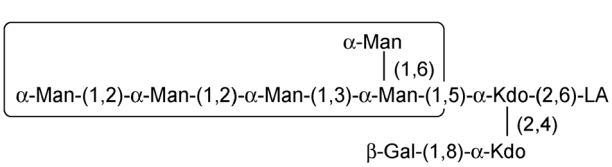


Fig. 14 Structure of the LPS from *Rhizobium radiobacter* Rv3. The area in the box displays strong structural similarity with the epitope recognized from the mAb 2G12. LA stands for lipid A.

 This journal is © The Royal Society of Chemistry 2013

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

the membrane. This concept guided the formulation of the new generation vaccine for *Neisseria meningitidis* group B (MenB),[138] which is the causative agent of meningitis. The strain belonging to group B is the only member within its species for which the synthesis of a synthetic glycoprotein vaccine has not been successful so far as its capsular polysaccharide is not immunogenic. The current MenB vaccine is a vesicle prepared from fragments of the bacterial membrane; the antigenicity mainly arises from a selected pool of *Neisseria* proteins embedded in this artificial membrane, in which LPSs are thought to work as a multivalent glycoconjugate that stabilises the vesicle.

Given the high biological importance of LPSs as natural multivalent glycoconjugates in the elicitation/suppression of eukaryotic immunity, the structural and supramolecular study of such molecules plays an important role. In an attempt to create a ''non-natural'' LPS multivalent surface, these molecules have been extracted from different microbial sources and reconstituted in liposomes. The physico-chemical investigation of these systems has been performed by a combined experimental strategy, which has allowed characterisation at different observation scales, from the morphological to the micro-structural level.[139,140] The next step will be the study of the elicitation of eukaryotic immunity of such an ''artificial'' bacterial surface.

5.2 Inhibition of LPS synthesis by multivalent glycoconjugates

L-Heptosides (L-*glycero*-D-*manno*-heptopyranoses) are components of the core region of LPS. Since LPS is essential for bacterial viability, its biosynthesis can be targeted for the development of novel antibacterial agents. A series of fullerene *hexakis*-adducts bearing twelve copies of peripheral sugars displaying the *manno*pyranose core structure of bacterial L-heptosides have been synthesized (Fig. 15). The multimers were assembled through an efficient CuAAC reaction as the final step. The final fullerene sugar balls were assayed as inhibitors of heptosyltransferase WaaC, the glycosyltransferase catalyzing the incorporation of the first L-heptose into LPS. Interestingly, the inhibition of the final molecules was found in the low micromolar range (IC_{50} value of 7 to 45 μM) while the corresponding monomeric glycosides displayed high micromolar to low millimolar inhibition levels (IC_{50} always above 400 μM). When evaluated on a ''per-sugar'' basis, these inhibition data showed that, in each case, the average affinity of a single glycoside of the fullerenes towards WaaC was significantly enhanced when displayed as a multimer, thus demonstrating an unexpected multivalent effect.[141] To date, such a multivalent mode of inhibition had never been evidenced with glycosyltransferases.

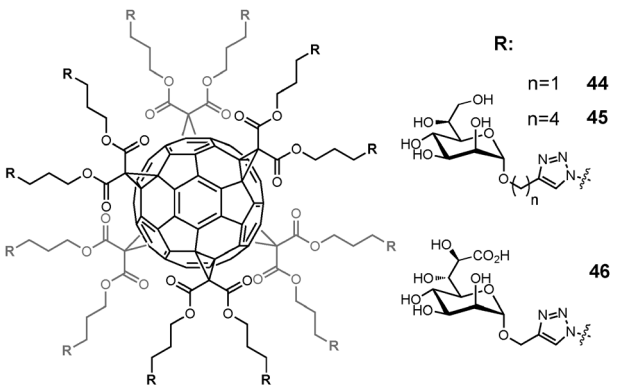


Fig. 15 Multivalent glycosylated fullerenes for inhibition of LPS heptosyltransferase WaaC.

5.3 Exploitation of bacterial S-layers as a natural multivalent glycan display system

Natural molecular self-assembly systems are prime candidates for use in nanobiotechnology. Crystalline-cell surface (S-) layers of prokaryotic organisms are very potent self-assembly systems, which can be used in bottom-up processes as a patterning element for the multivalent display of biofunctional epitopes such as glycans.[142]

The S-layer system is being exploited for multivalent glycan display based on the groundbreaking demonstrations that proteins can be recombinantly equipped with tailor-made glycosylation in an easily tractable bacterial system such as *Escherichia coli*[143] and that glycosylation modules from different bacterial sources, including glycoproteins, lipopolysaccharides or exopolysaccharides, can be combined to achieve functional glycosylation.[144] Using a combination of protein- and glycosylation-engineering approaches to produce self-assembling S-layer *neo*glycoconjugates, the feasibility of this system could be proven *in vitro* as well as *in vivo*, with the latter approach presenting interesting possibilities for live glycoconjugate delivery in future antipathogenic therapy.[145]

In a proof-of-concept study, the S-layer protein SgsE from *Geobacillus stearothermophilus* NRS 2004/3a (AF328862) was used as a matrix for the display of a branched heptasaccharide from the *Campylobacter jejuni* protein AcrA as well as for the *E. coli* O7 antigen.[146] SgsE is a 903-amino acid protein which aligns in a 2D lattice with oblique (p2) symmetry and which is naturally *O*-glycosylated at multiple sites. The SgsE protein was engineered by including the signal peptide of PelB (pectate lyase from *Erwinia carotovora*) for periplasmic targeting. Furthermore, one of the natural protein *O*-glycosylation sites was engineered into an *N*-glycosylation site to be recognized by the heterologous oligosaccharyltransferase PglB. In this way, S-layer *neo*-glycoproteins could be produced based on plasmid-encoded glycosylation information for either of the model glycan structures. The degree of glycosylation of the S-layer *neo*glycoproteins after purification from the periplasmic fraction of the *E. coli* cell factory reached up to 100%. Electron microscopy revealed that recombinant glycosylation is fully compatible with the S-layer protein self-assembly system (Fig. 16). Thus, the S-layer system is a promising strategy for multivalent glycan display approaches, where strict (''nanometer-scale'') control over position and orientation of the glycan epitopes is desired.

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

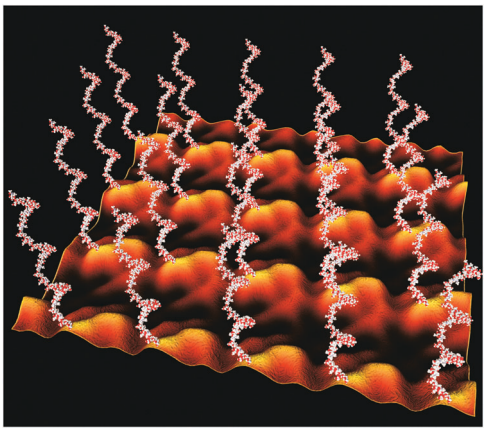


Fig. 16 Model of a self-assembled SgsE-neoglycoprotein monolayer periodically displaying recombinant *E. coli* O7 antigens with nanometer-scale precision. Image reconstruction using Cinema 4 is based on a negatively stained preparation of the S-layer protein self-assembled in solution and on the pdb data of the glycans generated with Sweet at http://www.glycosciences.de/ (adapted from ref. 146, Wiley-VCH Weinheim).

6. Glycoconjugate analogs and multivalent glycoconjugates for immune system stimulation and vaccine approach

6.1 Glycoconjugate analogs to trigger the innate immunity

Early recognition of invading bacteria by the innate immune system has a crucial function in antibacterial defense by triggering inflammatory responses that prevent the spread of infection and suppress bacterial growth. Toll-like receptor 4 (TLR4), the innate immunity receptor of bacterial endotoxins, plays a pivotal role in the induction of inflammatory responses. TLR4 activation by bacterial lipopolysaccharide (LPS) is achieved by the coordinate and sequential action of three other proteins, LBP, CD14 and MD-2 receptors, that bind lipopolysaccharide (LPS) and present it to TLR4 by forming the activated $(TLR4\text{-}MD\text{-}2\text{-}LPS)_2$ complex (Fig. 17).[147]

TLR4 trigger can be remarkably sensitive and robust, stimulating prompt and powerful host defence responses to different species of invading bacteria. However, an excessively potent host response generates life-threatening syndromes such as acute sepsis and septic shock. Non-toxic LPSs or lipid A obtained from non-pathogenic bacteria such as *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* are potent LPS antagonists *in vitro*[148] although no molecules usable for pharmacological treatment for sepsis are not available yet.

Several synthetic molecules capable of modulating TLR4 activity have been developed.[149,150] IAXO compounds **47–52**, which include lipidated monosaccharides with an amino group on the C-6 position of the pyranose ring of D-glucose, were active in blocking the TLR4 signal in cells and *in vivo* models (Fig. 17).[151] Their antagonist effect was due to the capacity of these molecules to displace from CD14 and TLR4-MD-2 complexes.[152] These molecules are now commercially available with the proprietary name of IAXO (Innaxon) as selective small-molecule inhibitors of the TLR4 signal pathway, and lead

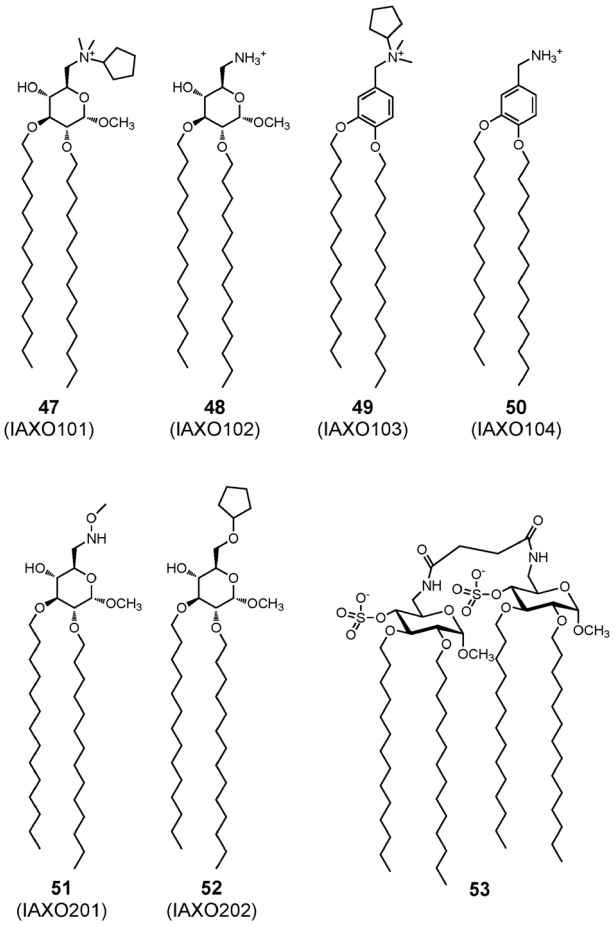


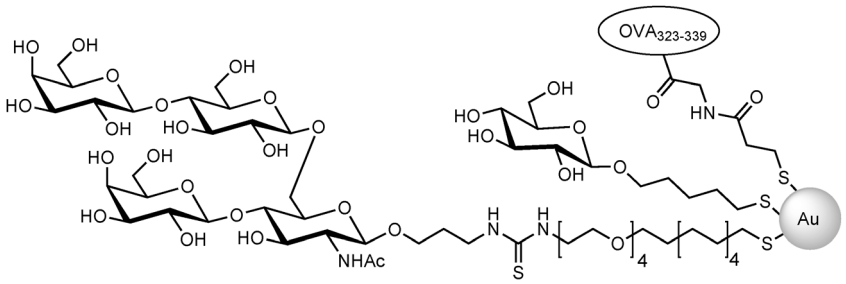
Fig. 17 Synthetic lipids with TLR4-modulating activity.

compound IAXO-102 **48** is in a preclinical phase as an anti-sepsis agent. More recently, lipid A analogues such as **53** were developed with a structure composed of two glucoside units connected by a linker both units bearing on C-4 an anionic sulfate group (Fig. 17).[153] These compounds have an antagonist effect if administered together with endotoxin, and a mild agonist effect if administered alone. In the context of vaccine adjuvants acting on innate immunity receptors, natural LPS immobilized on nanoparticles was also demonstrated to have interesting TLR4 activating properties.[154]

6.2 Multivalent glycoconjugates in vaccine development

6.2.1 HIV vaccine. An important aspect of HIV glycans is their role in protecting the underlying protein epitopes from exposure to the immune response of the host. Nevertheless, the broadly neutralizing monoclonal antibody 2G12 can target a cluster of high-mannose oligosaccharides of HIV gp120.[155] Moreover, new broadly neutralizing antibodies that are also carbohydrate-directed have been isolated recently from patients.[156] Mimicking the cluster presentation of oligomannosides expressed at the HIV-1 surface is a strategy for eliciting a vaccine response. Several groups, including those of Wang,[157,158] Wong,[159,160] Danishefsky,[48,161] and Rappuoli,[162] have multimerized high mannose-type oligosaccharides on synthetic

 This journal is © The Royal Society of Chemistry 2013



54

Fig. 18 Gold nanoparticles bearing Galβ(1-4)Glcβ(1-6)[Galβ(1-4)]GlcNAcβ(1- (repeating units of *S. pneumoniae* type 14 capsular polysaccharide), T-helper ovalbumin (OVA) peptide $OVA_{323-239}$ and D-glucose as carriers for synthetic carbohydrate-based vaccines.

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

scaffolds in order to mimic the carbohydrate epitope of 2G12. Although most of these systems were able to induce a carbohydrate-specific immune response in animals, the IgG antibodies were unable to bind to gp120 or neutralize the virus. The difficulty of eliciting high titers of antibodies against the 2G12 epitope remains a still open challenge in search for synthetic carbohydrate-based vaccines against HIV.

Together with new studies on the molecular basis of the interactions between glycans and anti-HIV antibodies, gold nanoparticles functionalized with oligomannosides (*manno*-GNPs, Fig. 2) can offer an alternative in this direction. In order to gain deeper insights into the interactions between 2G12 and selected oligomannosides at the molecular level, the structural and affinity details of the 2G12/oligomannosides interactions have been studied by saturation transfer difference NMR spectroscopy (STD-NMR) and transferred NOE in isotropic solution.[163] It was found that linear oligomannosides show a single binding mode to 2G12, with the non-reducing terminal disaccharide Man(α1-2)Man(α1-, making the closest antibody/oligosaccharide contacts in the bound state. In contrast, a branched pentamannoside showed two alternate binding modes involving both ligand arms, contrary to previous X-ray studies.[164] Among the analysed series of ligands, the strongest 2G12 binders were the linear tri- and tetramannosides. This information is of key importance for the design of synthetic multivalent gp120 high-mannose mimics for HIV vaccine development. Indeed *manno*-GNPs were able to bind with high affinity and to interfere with the 2G12/gp120 binding as determined by SPR-based biosensors and STD-NMR.[165] Cellular neutralization assays with *manno*-GNPs also demonstrated that GNPs coated with a linear tetramannoside could block the 2G12-mediated neutralization of a replication-competent HIV-1 under conditions that resemble those in which normal serum prevents infection of the target cell. All these results prove that selected *manno*-GNPs could function as an anti-adhesive barrier at an early stage of HIV infection, but also as synthetic mimics of the 2G12 epitope in the route of a carbohydrate-based vaccine against HIV.

6.2.2 *Streptococcus pneumoniae* vaccine. The feasibility of using gold nanoparticles as carriers has been tested for the production of a carbohydrate-based vaccine candidate. Gold GNP **54** coated with a conjugate of the synthetic tetrasaccharide Galβ(1-4)Glcβ(1-6)[Galβ(1-4)]GlcNAcβ(1-, the repeating units of *Streptococcus pneumoniae* capsular polysaccharide, and a T-helper peptide (Fig. 18) were able to induce a carbohydrate-specific immune response in mice, which resulted in the generation of functional IgG antibodies against this bacterium.[166]

7. Conclusions

The recent advances in the synthesis of complex glycomolecules with controlled topology and valency coupled with the progress in understanding the importance of protein–carbohydrate interactions have driven forward the domain of multivalent glycomolecules. Indeed, these molecules have proven to be very potent frameworks for binding to a range of glycan receptors, and therefore have the ability to stimulate, mediate or inhibit a variety of biological or pathological processes. The different applications of glycocompounds in anti-infectious strategies should be further developed in the future, with knowledge-based approaches that take into account the structure of the protein receptors. Designing multivalent glycoconjugates that perfectly fit the topology of binding sites will increase their affinity toward the target, and help in fine-tuning their specificity. Based on the reports summarized in this review, it appears that multivalent glycoconjugates are close to be utilised in anti-adhesive therapies against viral and bacterial infection, but can are be used to mimic bacterial cell surfaces in order to stimulate the immune systems.

Acknowledgements

The authors acknowledge the support from EU COST Actions CM1102 and COST BM1003. In addition, the work reviewed has been supported by the sources listed in the original publications cited and by institutions mentioned in the affiliation list.

References

1 A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart and M. E. Etzler, *Essentials of Glycobiology*, Cold Spring Harbour, New York, 2nd edn, 2009.
2 H. J. Gabius, S. Andre, J. Jimenez-Barbero, A. Romero and D. Solis, *Trends Biochem. Sci.*, 2012, **36**, 298–313.

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

3 H. Lis and N. Sharon, *Chem. Rev.*, 1998, **98**, 637–674.
4 L. L. Kiessling, J. E. Gestwicki and L. E. Strong, *Curr. Opin. Chem. Biol.*, 2000, **4**, 696–703.
5 Y. C. Lee and R. T. Lee, *Acc. Chem. Res.*, 1995, **28**, 321–327.
6 J. J. Lundquist and E. J. Toone, *Chem. Rev.*, 2002, **102**, 555–578.
7 M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754–2794.
8 V. I. Dyukova, N. V. Shilova, O. E. Galanina, A. Y. Rubina and N. V. Bovin, *Biochim. Biophys. Acta*, 2006, **1760**, 603–609.
9 T. Feizi, F. Fazio, W. Chai and C. H. Wong, *Curr. Opin. Struct. Biol.*, 2003, **13**, 637–645.
10 T. Horlacher and P. H. Seeberger, *Chem. Soc. Rev.*, 2008, **37**, 1414–1422.
11 O. Oyelaran and J. C. Gildersleeve, *Curr. Opin. Chem. Biol.*, 2009, **13**, 406–413.
12 J. C. Paulson, O. Blixt and B. E. Collins, *Nat. Chem. Biol.*, 2006, **2**, 238–248.
13 E. H. Song and N. L. Pohl, *Curr. Opin. Chem. Biol.*, 2009, **13**, 626–632.
14 C. D. Rillahan and J. C. Paulson, *Annu. Rev. Biochem.*, 2011, **80**, 797–823.
15 T. K. Dam and C. F. Brewer, *Adv. Carbohydr. Chem. Biochem.*, 2010, **63**, 139–164.
16 T. K. Dam, T. A. Gerken and C. F. Brewer, *Biochemistry*, 2009, **48**, 3822–3827.
17 J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen and L. L. Kiessling, *J. Am. Chem. Soc.*, 2002, **124**, 14922–14933.
18 M. Reynolds and S. Perez, *C. R. Chim.*, 2011, **14**, 74–95.
19 Y. M. Chabre and R. Roy, *Adv. Carbohydr. Chem. Biochem.*, 2010, **63**, 165–393.
20 D. Deniaud, K. Julienne and S. G. Gouin, *Org. Biomol. Chem.*, 2011, **9**, 966–979.
21 R. J. Pieters, *Org. Biomol. Chem.*, 2009, **7**, 2013–2025.
22 T. Darbre and J. L. Reymond, *Curr. Top. Med. Chem.*, 2008, **8**, 1286–1293.
23 K. J. Doores, D. P. Gamblin and B. G. Davis, *Chem.–Eur. J.*, 2006, **12**, 656–665.
24 A. Imberty, Y. M. Chabre and R. Roy, *Chem.–Eur. J.*, 2008, **14**, 7490–7499.
25 P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read and D. R. Bundle, *Nature*, 2000, **403**, 669–672.
26 A. Imberty and A. Varrot, *Curr. Opin. Struct. Biol.*, 2008, **18**, 567–576.
27 K. A. Karlsson, *Adv. Exp. Med. Biol.*, 2001, **491**, 431–443.
28 R. J. Pieters, *Adv. Exp. Med. Biol.*, 2011, **715**, 227–240.
29 N. Sharon, *Adv. Exp. Med. Biol.*, 1996, **408**, 1–8.
30 B. G. Davis, *Chem. Rev.*, 2002, **102**, 579–602.
31 R. J. Payne and C. H. Wong, *Chem. Commun.*, 2010, **46**, 21–43.
32 P. M. Rendle, A. Seger, J. Rodrigues, N. J. Oldham, R. R. Bott, J. B. Jones, M. M. Cowan and B. G. Davis, *J. Am. Chem. Soc.*, 2004, **126**, 4750–4751.
33 L. Bes, S. Angot, A. Limer and D. M. Haddleton, *Macromolecules*, 2003, **36**, 2493–2499.
34 I. Otsuka, B. Blanchard, R. Borsali, A. Imberty and T. Kakuchi, *ChemBioChem*, 2010, **11**, 2399–2408.
35 D. Ponader, F. Wojcik, F. Beceren-Braun, J. Dernedde and L. Hartmann, *Biomacromolecules*, 2012, **13**, 1845–1852.
36 J. Rieger, F. Stoffelbach, D. Cui, A. Imberty, E. Lameignere, J. L. Putaux, R. Jérôme, C. Jérôme and R. Auzély-Velty, *Biomacromolecules*, 2007, **8**, 2717–2725.
37 L. Baldini, A. Casnati, F. Sansone and R. Ungaro, *Chem. Soc. Rev.*, 2007, **36**, 254–266.
38 S. Cecioni, R. Lalor, B. Blanchard, J.-P. Praly, A. Imberty, S. E. Matthews and S. Vidal, *Chem.–Eur. J.*, 2009, **15**, 13232–13240.
39 A. Dondoni and A. Marra, *Chem. Rev.*, 2010, **110**, 4949–4977.
40 S. Andre, C. Grandjean, F. M. Gautier, S. Bernardi, F. Sansone, H. J. Gabius and R. Ungaro, *Chem. Commun.*, 2011, **47**, 6126–6128.
41 S. Andre, R. J. Pieters, I. Vrasidas, H. Kaltner, I. Kuwabara, F. T. Liu, R. M. Liskamp and H. J. Gabius, *ChemBioChem*, 2001, **2**, 822–830.
42 C. Heidecke and T. K. Lindhorst, *Chem.–Eur. J.*, 2007, **13**, 9056–9067.
43 M. A. Mintzer, E. L. Dane, G. A. O'Toole and M. W. Grinstaff, *Mol. Pharm.*, 2012, **9**, 342–354.
44 M. Touaibia and R. Roy, *Mini–Rev. Med. Chem.*, 2007, **7**, 1270–1283.
45 W. B. Turnbull and J. F. Stoddart, *J. Biotechnol.*, 2002, **90**, 231–255.
46 D. A. Fulton and J. F. Stoddart, *Bioconjugate Chem.*, 2001, **12**, 655–672.
47 M. Gomez-Garcia, J. M. Benito, A. P. Butera, C. Ortiz Mellet, J. M. Garcia Fernandez and J. L. Jimenez Blanco, *J. Org. Chem.*, 2012, **77**, 1273–1288.
48 I. J. Krauss, J. G. Joyce, A. C. Finnefrock, H. C. Song, V. Y. Dudkin, X. Geng, J. D. Warren, M. Chastain, J. W. Shiver and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2007, **129**, 11042–11044.
49 T. Ohta, N. Miura, N. Fujitani, F. Nakajima, K. Niikura, R. Sadamoto, C. T. Guo, T. Suzuki, Y. Suzuki, K. Monde and S. Nishimura, *Angew. Chem., Int. Ed.*, 2003, **42**, 5186–5189.
50 A. M. Pujol, M. Cuillel, O. Renaudet, C. Lebrun, P. Charbonnier, D. Cassio, C. Gateau, P. Dumy, E. Mintz and P. Delangle, *J. Am. Chem. Soc.*, 2011, **133**, 286–296.
51 S. Cecioni, V. Oerthel, J. Iehl, M. Holler, D. Goyard, J.-P. Praly, A. Imberty, J.-F. Nierengarten and S. Vidal, *Chem.–Eur. J.*, 2011, **17**, 3252–3261.
52 J.-F. Nierengarten, J. Iehl, V. Oerthel, M. Holler, B. M. Illescas, A. Munoz, N. Martin, J. Rojo, M. Sanchez-Navarro, S. Cecioni, S. Vidal, K. Buffet, M. Durka and S. P. Vincent, *Chem. Commun.*, 2010, **46**, 3860–3862.
53 M. Marradi, M. Martin-Lomas and S. Penades, *Adv. Carbohydr. Chem. Biochem.*, 2010, **64**, 211–290.
54 X. Wang, E. Matei, L. Deng, O. Ramstrom, A. M. Gronenborn and M. Yan, *Chem. Commun.*, 2011, **47**, 8620–8622.

 This journal is © The Royal Society of Chemistry 2013

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

55 R. Kikkeri, P. Laurino, A. Odedra and P. H. Seeberger, *Angew. Chem., Int. Ed.*, 2010, **49**, 2054–2057.
56 A. Robinson, J. M. Fang, P. T. Chou, K. W. Liao, R. M. Chu and S. J. Lee, *ChemBioChem*, 2005, **6**, 1899–1905.
57 Y. Yang, M. Yu, T. T. Yan, Z. H. Zhao, Y. L. Sha and Z. J. Li, *Bioorg. Med. Chem.*, 2010, **18**, 5234–5240.
58 R. D. Astronomo and D. R. Burton, *Nat. Rev. Drug Discovery*, 2010, **9**, 308–324.
59 V. Verez-Bencomo, V. Fernandez-Santana, E. Hardy, M. E. Toledo, M. C. Rodriguez, L. Heynngnezz, A. Rodriguez, A. Baly, L. Herrera, M. Izquierdo, A. Villar, Y. Valdes, K. Cosme, M. L. Deler, M. Montane, E. Garcia, A. Ramos, A. Aguilar, E. Medina, G. Torano, I. Sosa, I. Hernandez, R. Martinez, A. Muzachio, A. Carmenates, L. Costa, F. Cardoso, C. Campa, M. Diaz and R. Roy, *Science*, 2004, **305**, 522–525.
60 N. Nagahori, R. T. Lee, S. Nishimura, D. Page, R. Roy and Y. C. Lee, *ChemBioChem*, 2002, **3**, 836–844.
61 A. Nelson, J. M. Belitsky, S. Vidal, C. S. Joiner, L. G. Baum and J. F. Stoddart, *J. Am. Chem. Soc.*, 2004, **126**, 11914–11922.
62 Y. van Kooyk and T. B. Geijtenbeek, *Nat. Rev. Immunol.*, 2003, **3**, 697–709.
63 M. Sánchez-Navarro and J. Rojo, *Drug News Perspect.*, 2010, **23**, 557–572.
64 J. Luczkowiak, S. Sattin, I. Sitkeviciute, J. Reina, M. Sánchez-Navarro, M. Thépaut, L. Martínez-Prats, A. Daghetti, F. Fieschi, R. Delgado, A. Bernardi and J. Rojo, *Bioconjugate Chem.*, 2011, **22**, 1354–1365.
65 A. Berzi, J. Reina, R. Ottria, I. Sutkeviciute, P. Antonazzo, M. Sánchez-Navarro, E. M. Chabrol, M. Biasin, D. Trabattoni, I. Cetin, J. Rojo, F. Fieschi, A. Bernardi and M. Clerici, *AIDS*, 2012, **26**, 127–137.
66 S. Sattin, A. Daghetti, M. Thépaut, A. Berzi, M. Sánchez-Navarro, G. Tabarani, J. Rojo, F. Fieschi, M. Clerici and A. Bernardi, *ACS Chem. Biol.*, 2010, 301–312.
67 M. Sánchez-Navarro, A. Muñoz, B. Illescas, J. Rojo and N. Martín, *Chem.–Eur. J.*, 2011, **17**, 766–769.
68 O. Martinez-Avila, K. Hijazi, M. Marradi, C. Clavel, C. Campion, C. Kelly and S. Penades, *Chem.–Eur. J.*, 2009, **15**, 9874–9888.
69 O. Martinez-Avila, L. M. Bedoya, M. Marradi, C. Clavel, J. Alcami and S. Penades, *ChemBioChem*, 2009, **10**, 1806–1809.
70 G. R. Vasta, *Nat. Rev. Microbiol.*, 2009, **7**, 424–438.
71 M. Ouellet, S. Mercier, I. Pelletier, S. Bounou, J. Roy, J. Hirabayashi, S. Sato and M. J. Tremblay, *J. Immunol.*, 2005, **174**, 4120–4126.
72 S. Andre, D. V. Jarikote, D. Yan, L. Vincenz, G. N. Wang, H. Kaltner, P. V. Murphy and H. J. Gabius, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 313–318.
73 R. Leyden, T. Velasco-Torrijos, S. Andre, S. Gouin, H. J. Gabius and P. V. Murphy, *J. Org. Chem.*, 2009, **74**, 9010–9026.
74 G. N. Wang, S. Andre, H. J. Gabius and P. V. Murphy, *Org. Biomol. Chem.*, 2012, **10**, 6893–6907.
75 E. Fan, E. A. Merritt, C. L. Verlinde and W. G. Hol, *Curr. Opin. Struct. Biol.*, 2000, **10**, 680–686.
76 Z. Zhang, E. A. Merritt, M. Ahn, C. Roach, Z. Hou, C. L. Verlinde, W. G. Hol and E. Fan, *J. Am. Chem. Soc.*, 2002, **124**, 12991–12998.
77 A. V. Pukin, C. A. Weijers, B. van Lagen, R. Wechselberger, B. Sun, M. Gilbert, M. F. Karwaski, D. E. Florack, B. C. Jacobs, A. P. Tio-Gillen, A. van Belkum, H. P. Endtz, G. M. Visser and H. Zuilhof, *Carbohydr. Res.*, 2008, **343**, 636–650.
78 A. V. Pukin, H. M. Branderhorst, C. Sisu, C. A. Weijers, M. Gilbert, R. M. Liskamp, G. M. Visser, H. Zuilhof and R. J. Pieters, *ChemBioChem*, 2007, **8**, 1500–1503.
79 A. V. Pukin, B. C. Jacobs, A. P. Tio-Gillen, M. Gilbert, H. P. Endtz, A. van Belkum, G. M. Visser and H. Zuilhof, *Glycobiology*, 2011, **21**, 1642–1650.
80 C. Sisu, A. J. Baron, H. M. Branderhorst, S. D. Connell, C. A. Weijers, R. de Vries, E. D. Hayes, A. V. Pukin, M. Gilbert, R. J. Pieters, H. Zuilhof, G. M. Visser and W. B. Turnbull, *ChemBioChem*, 2009, **10**, 329–337.
81 H. M. Branderhorst, R. M. Liskamp, G. M. Visser and R. J. Pieters, *Chem. Commun.*, 2007, 5043–5045.
82 A. Bernardi, A. Checchia, P. Brocca, S. Sonnino and F. Zuccotto, *J. Am. Chem. Soc.*, 1999, **121**, 2032–2036.
83 A. Bernardi and P. Cheshev, *Chem.–Eur. J.*, 2008, **14**, 7434–7441.
84 D. Arosio, M. Fontanella, L. Baldini, L. Mauri, A. Bernardi, A. Casnati, F. Sansone and R. Ungaro, *J. Am. Chem. Soc.*, 2005, **127**, 3660–3661.
85 S. D. Knight and J. Bouckaert, *Top. Curr. Chem.*, 2009, **288**, 67–107.
86 M. Almant, V. Moreau, J. Kovensky, J. Bouckaert and S. G. Gouin, *Chem.–Eur. J.*, 2011, **17**, 10029–10038.
87 M. Dubber, O. Sperling and T. K. Lindhorst, *Org. Biomol. Chem.*, 2006, **4**, 3901–3912.
88 M. Durka, K. Buffet, J. Iehl, M. Holler, J. F. Nierengarten, J. Taganna, J. Bouckaert and S. P. Vincent, *Chem. Commun.*, 2011, **47**, 1321–1323.
89 M. Hartmann and T. K. Lindhorst, *Eur. J. Org. Chem.*, 2011, 3583–3609.
90 M. Touaibia, A. Wellens, T. C. Shiao, Q. Wang, S. Sirois, J. Bouckaert and R. Roy, *ChemMedChem*, 2007, **2**, 1190–1201.
91 M. J. Weissenborn, R. Castangia, J. W. Wehner, R. Sardzik, T. K. Lindhorst and S. L. Flitsch, *Chem. Commun.*, 2012, **48**, 4444–4446.
92 M. Hartmann, A. K. Horst, P. Klemm and T. K. Lindhorst, *Chem. Commun.*, 2010, **46**, 330–332.
93 M. Hartmann, P. Betz, Y. Sun, S. N. Gorb, T. K. Lindhorst and A. Krueger, *Chem.–Eur. J.*, 2012, **18**, 6485–6492.
94 J. R. Johnson, *Clin. Microbiol. Rev.*, 1991, **4**, 80–128.
95 M. C. Lane and H. L. Mobley, *Kidney Int.*, 2007, **72**, 19–25.
96 A. Salminen, V. Loimaranta, J. A. Joosten, A. S. Khan, J. Hacker, R. J. Pieters and J. Finne, *J. Antimicrob. Chemother.*, 2007, **60**, 495–501.
97 C. Appeldoorn, J. Joosten, F. el Maate, U. Dobrindt, J. Hacker, R. Liskamp, A. Khan and R. Pieters, *Tetrahedron: Asymmetry*, 2005, **16**, 361–372.

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

98 N. Gilboa-Garber, *Methods Enzymol.*, 1982, **83**, 378–385.
99 C. Chemani, A. Imberty, S. de Bentzmann, M. Pierre, M. Wimmerova, B. P. Guery and K. Faure, *Infect. Immun.*, 2009, **77**, 2065–2075.
100 H. P. Hauber, M. Schulz, A. Pforte, D. Mack, P. Zabel and U. Schumacher, *Int. J. Med. Sci.*, 2008, **5**, 371–376.
101 H. Gustke, R. Kleene, G. Loers, N. Nehmann, M. Jaehne, K. M. Bartels, K. E. Jaeger, M. Schachner and U. Schumacher, *Eur. J. Clin. Microbiol. Infect. Dis.*, 2012, **31**, 207–215.
102 A. Imberty, M. Wimmerova, E. P. Mitchell and N. Gilboa-Garber, *Microbes Infect.*, 2004, **6**, 222–229.
103 G. Cioci, E. P. Mitchell, C. Gautier, M. Wimmerova, D. Sudakevitz, S. Pérez, N. Gilboa-Garber and A. Imberty, *FEBS Lett.*, 2003, **555**, 297–301.
104 S. P. Diggle, R. E. Stacey, C. Dodd, M. Camara, P. Williams and K. Winzer, *Environ. Microbiol.*, 2006, **8**, 1095–1104.
105 M. Reynolds, M. Marradi, A. Imberty, S. Penadés and S. Pérez, *Chem.–Eur. J.*, 2012, **18**, 4264–4273.
106 Y. M. Chabre, D. Giguère, B. Blanchard, J. Rodrigue, S. Rocheleau, M. Neault, S. Rauthu, A. Papadopoulos, A. A. Arnold, A. Imberty and R. Roy, *Chem.–Eur. J.*, 2011, **17**, 6545–6562.
107 S. Cecioni, J. P. Praly, S. E. Matthews, M. Wimmerová, A. Imberty and S. Vidal, *Chem.–Eur. J.*, 2012, **18**, 6250–6263.
108 S. Cecioni, S. Faure, U. Darbost, I. Bonnamour, H. Parrot-Lopez, O. Roy, C. Taillefumier, M. Wimmerová, J.-P. Praly, A. Imberty and S. Vidal, *Chem.–Eur. J.*, 2011, **17**, 2146–2159.
109 Z. H. Soomro, S. Cecioni, H. Blanchard, J.-P. Praly, A. Imberty, S. Vidal and S. E. Matthews, *Org. Biomol. Chem.*, 2011, **9**, 6587–6597.
110 D. Sicard, S. Cecioni, M. Iazykov, Y. Chevolot, S. E. Matthews, J.-P. Praly, E. Souteyrand, A. Imberty, S. Vidal and M. Phaner-Goutorbe, *Chem. Commun.*, 2011, **47**, 9483–9485.
111 F. Pertici and R. J. Pieters, *Chem. Commun.*, 2012, **48**, 4008–4010.
112 R. U. Kadam, M. Bergmann, M. Hurley, D. Garg, M. Cacciarini, M. A. Swiderska, C. Nativi, M. Sattler, A. R. Smyth, P. Williams, M. Cámara, A. Stocker, T. Darbre and J.-L. Reymond, *Angew. Chem., Int. Ed.*, 2011, **50**, 10631–10635.
113 E. Mitchell, C. Houles, D. Sudakevitz, M. Wimmerova, C. Gautier, S. Perez, A. M. Wu, N. Gilboa-Garber and A. Imberty, *Nat. Struct. Biol.*, 2002, **9**, 918–921.
114 D. Tielker, S. Hacker, R. Loris, M. Strathmann, J. Wingender, S. Wilhelm, F. Rosenau and K.-E. Jaeger, *Microbiology*, 2005, **151**, 1313–1323.
115 K. M. Bartels, H. Funken, A. Knapp, M. Brocker, M. Bott, S. Wilhelm, K. E. Jaeger and F. Rosenau, *J. Bacteriol.*, 2011, **193**, 1107–1113.
116 G. M. L. Consoli, G. Granata, V. Cafiso, S. Stefani and C. Geraci, *Tetrahedron Lett.*, 2011, **52**, 5831–5834.
117 F. Morvan, A. Meyer, A. Jochum, C. Sabin, Y. Chevolot, A. Imberty, J.-P. Praly, J.-J. Vasseur, E. Souteyrand and S. Vidal, *Bioconjugate Chem.*, 2007, **18**, 1637–1643.
118 K. Marotte, C. Preville, C. Sabin, M. Moume-Pymbock, A. Imberty and R. Roy, *Org. Biomol. Chem.*, 2007, **5**, 2953–2961.
119 M. Andreini, M. Anderluh, A. Audfray, A. Bernardi and A. Imberty, *Carbohydr. Res.*, 2010, **345**, 1400–1407.
120 A. Clouet, T. Darbre and J. L. Reymond, *Angew. Chem., Int. Ed.*, 2004, **43**, 4612–4615.
121 N. Maillard, A. Clouet, T. Darbre and J. L. Reymond, *Nat. Protocols*, 2009, **4**, 132–142.
122 J.-L. Reymond and T. Darbre, *Org. Biomol. Chem.*, 2012, **10**, 1483–1492.
123 E. M. V. Johansson, E. Kolomiets, F. Rosenau, K.-E. Jaeger, T. Darbre and J.-L. Reymond, *New J. Chem.*, 2007, **31**, 1291–1299.
124 E. Kolomiets, E. M. Johansson, O. Renaudet, T. Darbre and J. L. Reymond, *Org. Lett.*, 2007, **9**, 1465–1468.
125 E. M. V. Johansson, R. U. Kadam, G. Rispoli, S. A. Crusz, K.-M. Bartels, S. P. Diggle, M. Camara, P. Williams, K.-E. Jaeger, T. Darbre and J.-L. Reymond, *MedChemComm*, 2011, **2**, 418–420.
126 E. M. V. Johansson, S. A. Crusz, E. Kolomiets, L. Buts, R. U. Kadam, M. Cacciarini, K.-M. Bartels, S. P. Diggle, M. Cámara, P. Williams, R. Loris, C. Nativi, F. Rosenau, K.-E. Jaeger, T. Darbre and J.-L. Reymond, *Chem. Biol.*, 2008, **15**, 1249–1257.
127 E. Lameignere, L. Malinovská, M. Sláviková, E. Duchaud, E. P. Mitchell, A. Varrot, O. Šedo, A. Imberty and M. Wimmerová, *Biochem. J.*, 2008, **411**, 307–318.
128 E. Lameignere, T. C. Shiao, R. Roy, M. Wimmerová, F. Dubreuil, A. Varrot and A. Imberty, *Glycobiology*, 2010, **20**, 87–98.
129 J. Rieger, H. Freichels, A. Imberty, J. L. Putaux, T. Delair, C. Jérôme and R. Auzély-Velty, *Biomacromolecules*, 2009, **10**, 651–657.
130 Z.-H. Lun, Q.-P. Wang, X.-G. Chen, A.-X. Li and X.-Q. Zhu, *Lancet Infect. Dis.*, 2007, **7**, 201–209.
131 A. Kouki, S. Haataja, V. Loimaranta, A. T. Pulliainen, U. J. Nilsson and J. Finne, *J. Biol. Chem.*, 2011, **286**, 38854–38864.
132 H. M. Branderhorst, R. Kooij, A. Salminen, L. H. Jongeneel, C. J. Arnusch, R. M. Liskamp, J. Finne and R. J. Pieters, *Org. Biomol. Chem.*, 2008, **6**, 1425–1434.
133 J. Ohlsson, A. Larsson, S. Haataja, J. Alajaaski, P. Stenlund, J. S. Pinkner, S. J. Hultgren, J. Finne, J. Kihlberg and U. J. Nilsson, *Org. Biomol. Chem.*, 2005, **3**, 886–900.
134 M. Toivanen, S. Huttunen, J. Duricova, P. Soininen, R. Laatikainen, V. Loimaranta, S. Haataja, J. Finne, S. Lapinjoki and C. Tikkanen-Kaukanen, *Phytother. Res.*, 2010, **24**, S95–S101.
135 N. Parera Pera, A. Kouki, S. Haataja, H. M. Branderhorst, R. M. Liskamp, G. M. Visser, J. Finne and R. J. Pieters, *Org. Biomol. Chem.*, 2010, **8**, 2425–2429.
136 C. Raetz and C. Whitfield, *Annu. Rev. Biochem.*, 2002, **71**, 635–700.
137 B. E. Clark, K. Auyeung, E. Fregolino, M. Parrilli, R. Lanzetta, C. De Castro and R. Pantophlet, *Chem. Biol.*, 2012, **19**, 254–263.

 This journal is © The Royal Society of Chemistry 2013

Published on 19 December 2012 on http://pubs.rsc.org | doi:10.1039/C2CS35408J
Downloaded on 13/05/2013 19:08:26. Open Access Article.

138 X. Bai, J. Findlow and R. Borrow, *Expert Opin. Biol. Ther.*, 2011, **11**, 969–985.
139 G. D'Errico, A. Silip, G. Mangiapia, A. Molinaro, L. Paduano and R. Lanzetta, *Phys. Chem. Chem. Phys.*, 2009, **11**, 2314–2322.
140 G. D'Errico, A. Silipo, G. Mangiapia, G. Vitiello, A. Radulescu, A. Molinaro, R. Lanzetta and L. Paduano, *Phys. Chem. Chem. Phys.*, 2010, **12**, 13574–13585.
141 M. Durka, K. Buffet, J. Iehl, M. Holler, J. F. Nierengarten and S. P. Vincent, *Chem.–Eur. J.*, 2012, **18**, 641–651.
142 R. Ristl, K. Steiner, K. Zarschler, S. Zayni, P. Messner and C. Schaffer, *Int. J. Microbiol.*, 2011, **2011**, 127870.
143 M. F. Feldman, M. Wacker, M. Hernandez, P. G. Hitchen, C. L. Marolda, M. Kowarik, H. R. Morris, A. Dell, M. A. Valvano and M. Aebi, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 3016–3021.
144 I. Hug and M. F. Feldman, *Glycobiology*, 2011, **21**, 138–151.
145 K. Zarschler, B. Janesch, B. Kainz, R. Ristl, P. Messner and C. Schaffer, *Carbohydr. Res.*, 2010, **345**, 1422–1431.
146 K. Steiner, A. Hanreich, B. Kainz, P. G. Hitchen, A. Dell, P. Messner and C. Schaffer, *Small*, 2008, **4**, 1728–1740.
147 R. Jerala, *Int. J. Med. Microbiol.*, 2007, **297**, 353–363.
148 H. Loppnow, P. Libby, M. Freudenberg, J. H. Krauss, J. Weckesser and H. Mayer, *Infect. Immun.*, 1990, **58**, 3743–3750.
149 F. Peri and M. Piazza, *Biotechnol. Adv.*, 2012, **30**, 251–260.
150 F. Peri, M. Piazza, V. Calabrese, G. Damore and R. Cighetti, *Biochem. Soc. Trans.*, 2010, **38**, 1390–1395.
151 M. Piazza, C. Rossini, S. Della Fiorentina, C. Pozzi, F. Comelli, I. Bettoni, P. Fusi, B. Costa and F. Peri, *J. Med. Chem.*, 2009, **52**, 1209–1213.
152 M. Piazza, L. Yu, A. Teghanemt, T. Gioannini, J. Weiss and F. Peri, *Biochemistry*, 2009, **48**, 12337–12344.
153 M. Piazza, V. Calabrese, G. Damore, R. Cighetti, T. Gioannini, J. Weiss and F. Peri, *ChemMedChem*, 2012, **7**, 213–217.
154 M. Piazza, M. Colombo, I. Zanoni, F. Granucci, P. Tortora, J. Weiss, T. Gioannini, D. Prosperi and F. Peri, *Angew. Chem., Int. Ed.*, 2011, **50**, 622–626.
155 C. N. Scanlan, R. Pantophlet, M. R. Wormald, E. Ollmann Saphire, R. Stanfield, I. A. Wilson, H. Katinger, R. A. Dwek, P. M. Rudd and D. R. Burton, *J. Virol.*, 2002, **76**, 7306–7321.
156 R. Pejchal, K. J. Doores, L. M. Walker, R. Khayat, P. S. Huang, S. K. Wang, R. L. Stanfield, J. P. Julien, A. Ramos, M. Crispin, R. Depetris, U. Katpally, A. Marozsan, A. Cupo, S. Maloveste, Y. Liu, R. McBride, Y. Ito, R. W. Sanders, C. Ogohara, J. C. Paulson, T. Feizi, C. N. Scanlan, C. H. Wong, J. P. Moore, W. C. Olson, A. B. Ward, P. Poignard, W. R. Schief, D. R. Burton and I. A. Wilson, *Science*, 2011, **334**, 1097–1103.
157 J. Ni, H. Song, Y. Wang, N. M. Stamatos and L. X. Wang, *Bioconjugate Chem.*, 2006, **17**, 493–500.
158 J. Wang, H. Li, G. Zou and L.-X. Wang, *Org. Biomol. Chem.*, 2007, **5**, 1529–1540.
159 R. D. Astronomo, E. Kaltgrad, A. K. Udit, S. K. Wang, K. J. Doores, C. Y. Huang, R. Pantophlet, J. C. Paulson, C. H. Wong, M. G. Finn and D. R. Burton, *Chem. Biol.*, 2010, **17**, 357–370.
160 R. D. Astronomo, H. K. Lee, C. N. Scanlan, R. Pantophlet, C. Y. Huang, I. A. Wilson, O. Blixt, R. A. Dwek, C. H. Wong and D. R. Burton, *J. Virol.*, 2008, **82**, 6359–6368.
161 J. G. Joyce, I. J. Krauss, H. C. Song, D. W. Opalka, K. M. Grimm, D. D. Nahas, M. T. Esser, R. Hrin, M. Feng, V. Y. Dudkin, M. Chastain, J. W. Shiver and S. J. Danishefsky, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 15684–15689.
162 A. Kabanova, R. Adamo, D. Proietti, F. Berti, M. Tontini, R. Rappuoli and P. Costantino, *Glycoconjugate J.*, 2010, **27**, 501–513.
163 P. M. Enriquez-Navas, M. Marradi, D. Padro, J. Angulo and S. Penades, *Chem.–Eur. J.*, 2011, **17**, 1547–1560.
164 D. A. Calarese, C. N. Scanlan, M. B. Zwick, S. Deechongkit, Y. Mimura, R. Kunert, P. Zhu, M. R. Wormald, R. L. Stanfield, K. H. Roux, J. W. Kelly, P. M. Rudd, R. A. Dwek, H. Katinger, D. R. Burton and I. A. Wilson, *Science*, 2003, **300**, 2065–2071.
165 M. Marradi, P. Di Gianvincenzo, P. M. Enriquez-Navas, O. M. Martinez-Avila, F. Chiodo, E. Yuste, J. Angulo and S. Penades, *J. Mol. Biol.*, 2011, **410**, 798–810.
166 D. Safari, M. Marradi, F. Chiodo, H. A. T. Dekker, Y. Shan, R. Adamo, S. Oscarson, G. T. Rijkers, M. Lahmann, J. P. Kamerling, S. Penadés and H. Snippe, *Nanomedicine*, 2012, **7**, 651–662.
167 C. Sabin, E. P. Mitchell, M. Pokorná, C. Gautier, J.-P. Utille, M. Wimmerová and A. Imberty, *FEBS Lett.*, 2006, **580**, 982–987.
168 E. Kolomiets, M. A. Swiderska, R. U. Kadam, E. M. V. Johansson, K.-E. Jaeger, T. Darbre and J.-L. Reymond, *ChemMedChem*, 2009, **4**, 562–569.