

Cite this: *New J. Chem.*, 2012, **36**, 324–339

www.rsc.org/njc

PERSPECTIVE

Glycodendrimers as functional antigens and antitumor vaccines†

Tze Chieh Shiao and René Roy*

Received (in Montpellier, France) 11th October 2011, Accepted 29th November 2011

DOI: 10.1039/c2nj20873c

Glycodendrimers have advantageous characteristics of providing potent immunostimulating and adjuvant properties in vaccine preparation due to their molecularly defined multivalent scaffolds together with their abundant architectural variations. Their versatile syntheses allow the production of highly defined conjugates with small antigens. An overview of the use of dendrimers as carriers of carbohydrate antigens including constructs that have built-in adjuvant properties and as stand-alone adjuvants that can be mixed with carbohydrate antigens to provide efficient vaccine formulations is provided. A brief description of the innate and adaptive immune responses toward glycoconjugates is provided to allow better understanding and basic constituent requirements for future design. The necessary distinction between antigens and immunogens (vaccines) is also discussed. To counterbalance the poor immunogenicity and T-cell independent characteristics of carbohydrate antigens, chemists have developed original hybrid molecules aimed at targeting specific pattern-recognition receptors to trigger competent immune cell proliferations and protective antibody production. Although early glycodendrimers were found to lack immunogenicity, these architecturally impressive nanomolecules were shown to bind avidly to various carbohydrate binding proteins, including antibodies. More recently, the hitherto non-immunogenic dendritic components have been successfully used as adjuvants and carriers to direct other molecules to immunocompetent cells. Commendable examples against tumor associated carbohydrate antigens will illustrate the immunochemical strategies engaged in the development of potent and exclusively synthetic carbohydrate-based anticancer vaccines.

1. Introduction: carbohydrate immunity

Carbohydrates, as opposed to proteins and peptides, are *T-cell independent antigens*,^{1–8} and as such they are not properly equipped to trigger the participation of T-helper cells, and hence could not induce immune cell proliferation, antibody class switch and affinity/specification maturation. The major successful advances initially encountered with carbohydrate-based vaccines have been supported by the discovery that, when properly conjugated to protein carriers, serving as *T-cell dependent epitopes*, bacterial capsular polysaccharides became capable of acquiring the requisite immunochemical ability. A brief overview of the four known carbohydrate antigen uptake mechanisms by antigen presenting cells (APCs) represented by the families of dendritic cells, B-cells, and macrophages is presented in Fig. 1.^{9,10} As implied above, initial commercial glycoconjugate vaccines were those made of bacterial capsular polysaccharides that were covalently bound to strong immunogenic protein carriers

such as keyhole limpet hemocyanin (KLH), tetanus toxoid (TT) and diphtheria toxoid (DT), all of which expressing several key peptide sequences containing ~15-amino acids (*T cell epitopes*) known to bind strongly to a Major Histocompatibility Complex (MHC). B-cells possess surface immunoglobulins that bind to multivalent antigens such as polymers and polysaccharides. Hence, carbohydrate-bearing dendrimers (glycodendrimers)¹¹ can also bind to these immunoglobulins and trigger signaling events leading to the production of low affinity and low specificity IgM antibodies. Moreover, some APCs express mannopyranoside binding receptors known as DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin) also known as CD209 (Cluster of Differentiation 209).¹² DC-SIGN is a C-type lectin receptor present on both macrophages and dendritic cells. DC-SIGN on macrophages and dendritic cells recognizes and binds to mannose type carbohydrates, a class of pathogen associated pattern recognition receptors (PRRs) commonly found on viruses, bacteria and fungi. These binding interactions activate endocytosis and phagocytosis. Consequently, mannoside-bearing glycodendrimers become “cargo molecules” to deliver other antigens.¹³

An additional recently discovered APCs uptake mechanism was based on the early observations that Toll-like receptors (TLRs) are capable of internalizing lipid bound antigens and

PharmaQAM – Department of Chemistry, Université du Québec à Montréal, P.O. Box 8888, Succ. Centre-ville, Montreal, Quebec, Canada H3C 3P8. E-mail: roy.rene@uqam.ca;
Fax: +1 514 987 4054; Tel: +1 514 987 3000 x 2546

† This article is part of the themed issue Dendrimers II, guest-edited by Jean-Pierre Majoral.

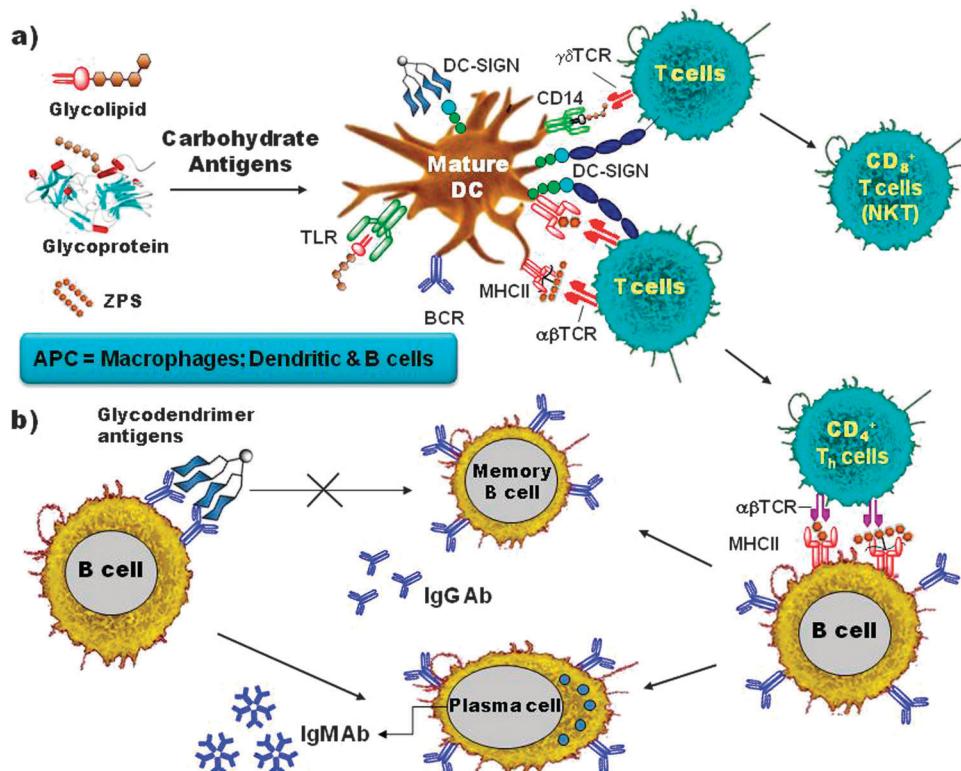
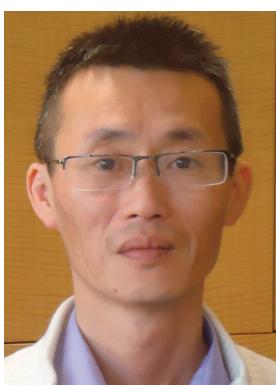


Fig. 1 The four known carbohydrate antigen uptake mechanisms by Antigen Presenting Cells (APCs: dendritic cells, B-cells, and macrophages) of the innate/adaptive immune systems. (a) neoglycoproteins (conjugated vaccines) and zwitterionic polysaccharides are first captured by pattern recognition receptors of APCs, internalized, and then presented, after initial catabolism and breakdown processes, to $\alpha\beta$ -TCR molecules on CD4 $^{+}$ T-helper cells by MHC class II proteins in association with co-receptors that trigger signaling pathways leading to B cell proliferation, differentiation, and ultimately to high affinity/selectivity IgG-type antibody production and memory B cell activation through diverse chemokines. Alternatively, glycolipids are internalized through Toll-like receptors (TLRs) followed by presentation to $\gamma\delta$ -TCR on T cells by Clusters of Differentiation (CDs) leading to CD8 $^{+}$ T cells (Natural Killer Cells) activation. In addition, glycodendrimers can be captured by the mannose receptor DC-SIGN of dendritic cells where they can deliver other immunogens. (b) multivalent carbohydrate antigens such as polysaccharides, glycopolymers, and glycodendrimers are first captured by cell surface immunoglobulins of B-cells. On their own, they do not stimulate memory B cells and hence, no antibody class switch is observed. They can however, through signaling events, stimulate low affinity/specification IgM antibody production.



Tze Chieh Shiao

the supervision of Prof. René Roy. He then became research assistant in Prof. Roy laboratory. His main research interests are carbohydrate-based synthetic vaccines and glycochemistry. He has 24 publications to date.



René Roy

René Roy was born in Québec (Canada). He holds a Canadian Research Chair in Therapeutic Chemistry in the Department of Chemistry of the Université du Québec à Montréal (Qc, Canada) since 2004. After getting his PhD from the Université de Montréal, he joined the National Research Council of Canada in Ottawa from 1980–1985. He was then professor in the Department of Chemistry at the University of Ottawa from 1985–2002. He has been the recipient of the 2003 Melville L. Wolfson Award from the ACS Division of Carbohydrate Chemistry for his contributions in the design of vaccines and glycodendrimers. He has published over 270 publications and has contributed to the development of two commercial carbohydrate-based vaccines. He is actually the founder and Director of the Université du Québec research center Pharmaqam, devoted to the accelerated development of new drugs.

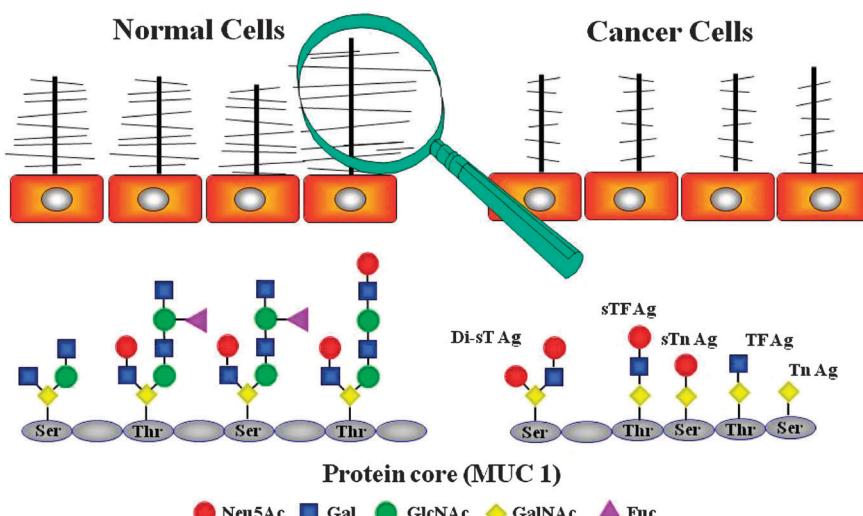


Fig. 2 Differences between healthy and cancer cells related to cell surface mucin glycoproteins.²⁵ On healthy tissues, *O*-glycans are complex and constitute more than 50% of the weight of the glycoproteins while on cancer cells, the complexity is greatly reduced due to aberrant glycosyltransferase dysfunctions. The major consequence is that certain TACAs are exposed while they were cryptic on the healthy cells.

other bacterial components.¹⁴ TLRs are also recognized as “pattern recognition” receptors. Common carbohydrate antigens harboring such lipophilic moieties are members of Lipid A, the inner most of the three regions of Gram-negative lipopolysaccharides (LPS), glycolipids, and gangliosides. Known human Toll-like receptors constitute a family of ten protein members recognizing a wide variety of microbial lipophilic molecules,¹⁵ one of which has particularly retained the attention of glycochemists, the TLR2 receptor.¹⁶ TLR2 has been shown to initiate potent immune responses by initially recognizing simple diacylated and triacylated lipopeptides such as tripalmitoyl-*S*-glyceryl-cysteinylserine **Pam**₃**CysSerLys**₄-bound (**Pam**₃**CSK**₄) peptides. Therefore, these well characterized TLR ligands have been utilized in conjugation to specific carbohydrate antigens to create a new family of hybrid glycodendrimers fully equipped with the necessary adjuvanted molecules to trigger the required immune responses.

Another entry mechanism was also recently identified wherein zwitterionic polysaccharides (ZPS) could invoke, on their own, MHC class II-mediated CD4⁺ T-cell responses in the absence of protein carriers.¹⁷ Several bacterial polysaccharides have zwitterionic elements, and among these, *Bacteroides fragilis* PSA1 and PSA2, and *Streptococcus pneumoniae* Sp1 harbor multiple charges. Hence, recent reports are now emerging describing the covalent attachment of carbohydrate epitopes to such carriers, including one of the tumor associated carbohydrate antigens presented herein.¹⁸

At this stage, a clear distinction should also be given between carbohydrate antigens and immunogens. As such, carbohydrate antigens can bind to antibodies or related carbohydrate binding proteins of non-immune origins such as lectins and the like but they cannot induce a memory immune response leading to antibody class switch from IgM to high affinity/specification antibodies to the IgG subtypes. Thus, multivalent antigens are still fully equipped to provoke protein and receptor cross-linking.

In this review, various “glycodendrimer” (in a wide sense) constructs will be described concentrating on the most actively pursued vaccine candidates against tumor associated carbohydrate

antigens (TACAs). A brief historical perspective will follow to allow the readers toward a better appreciation of the fundamental differences between carbohydrate antigenicity *versus* immunogenicity.

2. Tumor associated cancer vaccines: historical perspective

As briefly discussed above, malignant cells overexpress a range of *O*-glycan patterns of either glycoproteins or glycolipids found on the outer cell membranes.¹⁹ The glycoproteins constitute a family of proteins collectively known as mucins (MUC) with MUC1 representing the most widely investigated.²⁰ These glycoproteins are heavily glycosylated on normal cells while limited *O*-glycosylations on cancer cells have been clearly demonstrated (Fig. 2). This is attributed to the underexpression of key glycosyltransferases and overexpression of sialyltransferases. Gangliosides (GM2, GD2, GD3) and neutral glycolipids (globo-H and Lewis^y) on epithelial tumor cells of breast, lung, colon, bladder, and prostate are also considerably altered both in number and in structure.^{2-8,21} In the tumor-associated carbohydrate antigens (TACAs) of mucins, a down-regulation in the amount of β -1,6-GlcNAc transferase triggers an accumulation of shorter glycan chains such as α -GalNAc-*O*-Ser/Thr and of β -Gal-(1 → 3)- α -GalNAc-*O*-Ser/Thr. These antigens are referred to as the T_N- and the TF-antigens (TF = Thomsen-Friedenreich), respectively. In healthy tissues, the active glycosyltransferase enzymes are responsible for a much more complex pattern of glycosylation, amounting to 50% of the glycoprotein masses. The consequences of these modified glycosylation profiles are profound as they contribute to MUC1, and to a lesser extent MUC4, conformational changes, better exposition of peptide backbones, and obviously to an over accumulation of the above carbohydrate-based cancer markers which are otherwise cryptic (masked) on healthy tissues, although detectable on normal cells. MUC1 is a membrane-bound mucin and is found in more than 90% of breast carcinomas.²² Furthermore, a large amount of the α -GalNAc-*O*-Ser/Thr (T_N-antigen)

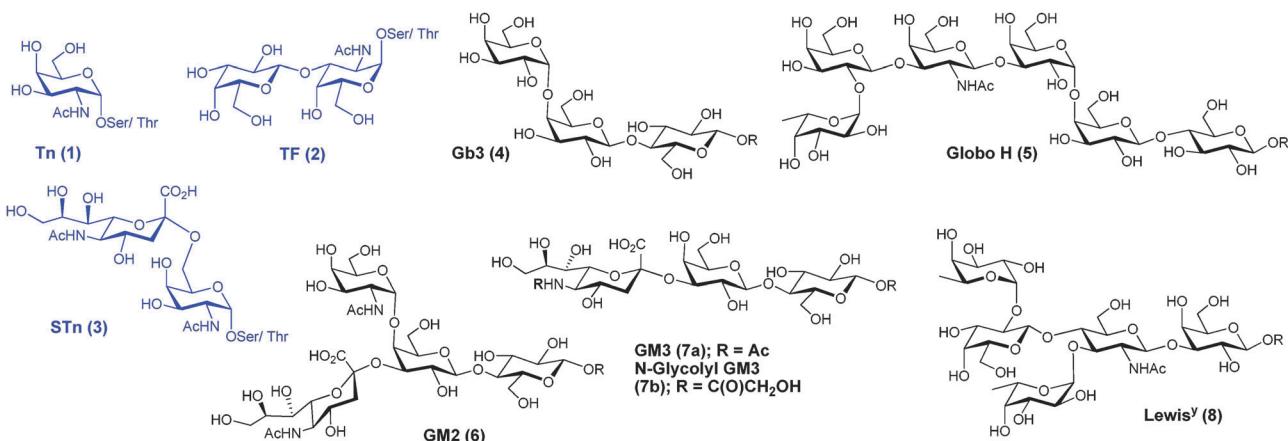


Fig. 3 Typical structures of representative TACAs from glycoproteins (blue) and glycolipids accumulated on cancer cells.

precursor is also accumulated together with the corresponding sialylated counterparts (sialyl TF- or sialyl-T_N antigens (STF and ST_N)) due to an 8–10 fold increase in the activity of sialyltransferases in tumor cells. Consequently, most efforts to provide classical carbohydrate-based vaccines have been devoted to the above antigens and the related ones (Fig. 3).

Patients with higher levels of anti-tumor associated carbohydrate antigen antibodies have a better prognosis than patients with lower levels. The presence of these circulating antibodies in the patient sera formed the basis of clinical markers.²³ Additionally, circulating antibodies are responsible for the elimination of tumor cells.^{19b,24} Hence, the immediate target in prophylactic vaccine preparations was to trigger humoral immunity, *i.e.* stimulation of long lasting, high affinity antibodies, preferably of the IgG isotypes. The presence of these isotypes following vaccination is a good indicator of T cell help through activation of MHCII CD4⁺ receptors and B cell maturation.

Several groups have developed systematic research projects aimed at the synthesis of various vaccine compositions.^{12–18,21} In spite of the limited success encountered with traditional vaccine formulations based on neoglycoproteins harboring the above carbohydrate antigens,²⁶ considerable efforts have been devoted to the chemical design of novel dendritic architectures. Most of the initial failures were principally due to the complexity and the heterogeneity of the resulting neoglycoconjugates, particularly when KLH was chosen as a carrier. Even though the highly immunogenic protein carrier KLH has been used exhaustively, the potential commercial limitations of this carrier have rapidly emerged as the chemical analyses of the resulting neoglycoproteins are extremely complex given that KLH is a very large and heterogeneous glycoprotein composed of several 350 kDa subunits affording a material of molecular weight ranging from 4.5–13 MDa. These facets further contribute to the need of well defined chemical constructs.

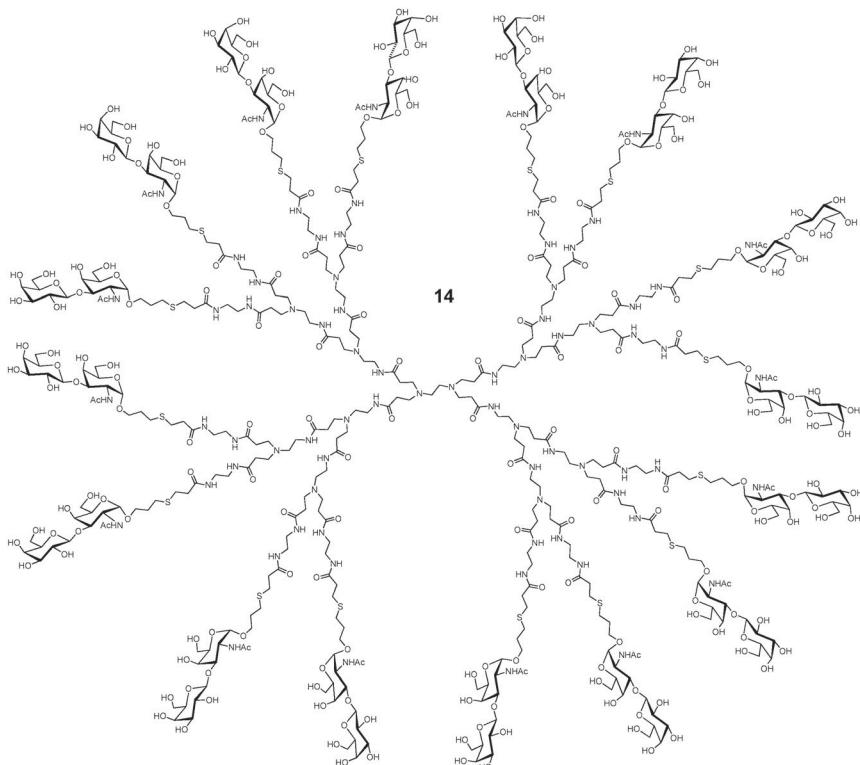
As stated above, membrane bound mucins exhibit large domains of tandemly repeated peptides having high density of *O*-linked (threonine/serine) residues serving as scaffolds for other antigenic oligosaccharides. The mucins' glycoforms harbour several copies of *O*-linked *N*-acetylgalactosamine (T_N, GalNAc) and β-D-Gal-(1 → 3)-α-D-GalNAc (TF) antigens (**1–3**). The T_N (**1**) and TF (**2**) antigens are often capped by sialic acid residues (sialyl-T_N (**3**) and sialyl-TF) (Fig. 3). These anomalies are also

responsible for the metastatic behaviour of tumor cells due to the antiadhesive shielding effects responsible for the lack of cell-cell or cell-matrix contacts mediated by E cadherin or integrins, respectively. The overexpression of mucins by carcinoma cells is considered as the principal factor in the resistance to natural killer and cytotoxic cells. Thus, MUC1 TACAs constitute primary targets for tumor defence and vaccination strategies.

3. Antigenic but not immunogenic glycodendrimers

Glycodendrimer-based antigens and immunogens are molecularly well defined synthetic biomacromolecules with surface exposed biologically relevant carbohydrate ligands (epitopes) built on a wide range of highly functionalized and somewhat repetitive scaffolds.¹¹ They were initially designed as bioisosteres of cell surface multiantennary glycans found on most glycoproteins.²⁷ Additionally, they have been shown to play critical roles in signal transduction and in receptor cross-linking. This section will highlight initial investigations toward the syntheses and antigenic evaluations of a range of glycodendrimers bearing the immuno-dominant TF-antigen disaccharide β-D-Gal-(1 → 3)-α-D-GalNAc found on malignant cells of carcinomas, particularly related to breast cancer. This antigen, usually cryptic on healthy tissues (see Fig. 2 and 3 and discussion above), is heavily expressed on cancer cells as a result of aberrant glycosylation. It is considered to be a critical component of TACAs.^{4–8,28} The high incidence of carcinomas to invade other tissues such as lymph nodes, lung, and liver by metastasis was one of the early arguments raised to generate TF-antigen dendrimers that might have the potential to block the receptor sites following surgery. This was later confirmed when the phenomena have been associated with galectins.²⁹

In order to generate mouse monoclonal antibodies (MAb) against the TF-Ag to be used in evaluating the relative antigenicity of the glycodendrimers, it was necessary to first prepare a vaccine conjugate.^{25,30–36} The conjugate vaccine was prepared from an *N*-acrylamido derivative of an amino-ending TF derivative derived from **9**³⁴ using 1,4-conjugate addition from the ε-amino groups of the lysine residues of either bovine serum albumin (BSA) or the more immunogenic tetanus toxoid (TT) (Scheme 1).³¹ Interestingly, conjugates containing only 3–4 TF-Ag residues/protein were deemed sufficient to



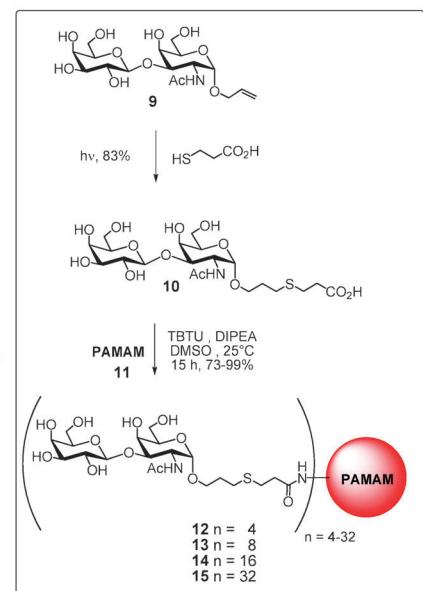
Scheme 1 Representative TF-antigens built around PAMAM dendrimers.^{25,30}

stimulate high antibody responses. This observation has not been particularly noticed by the community since it implied that successful anti-TF vaccines may not necessarily require the presence of the generally associated mucin peptides (MUC, see below).

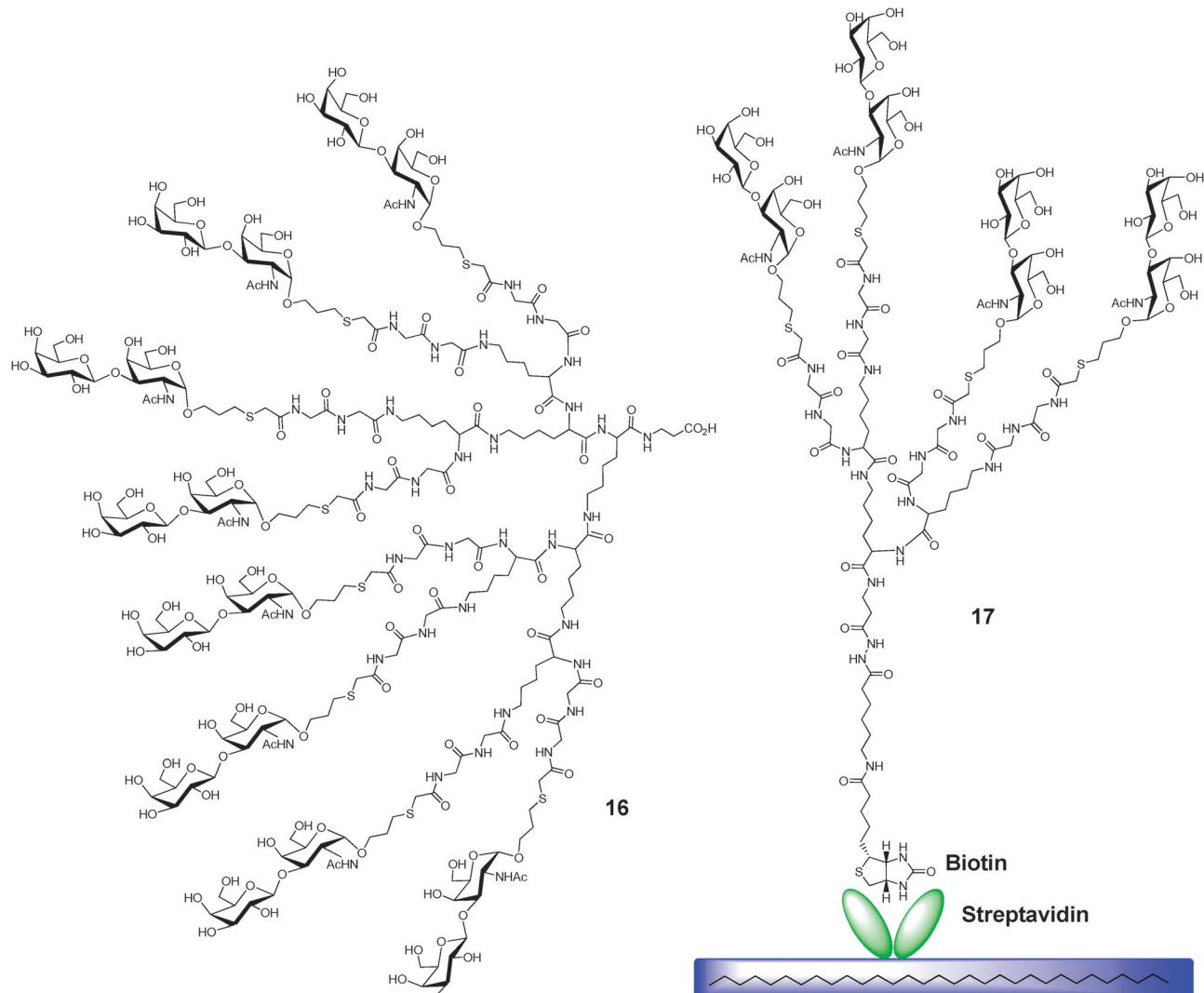
A synthetic glycopolymer constructed on a polyacrylamide backbone was also deemed necessary to screen the above MAb and to serve as model cell surface mucin for solid-phase competitive immunoassays.^{32,33} In this way, two monoclonal antibodies were selected for binding specificity and affinity studies. One was an IgM, while the selected IgG was of the IgG3 subfamily (JAA-F11).³¹ Alternatively, the screening antigen was a glycopolymer which was synthesized by an addition/elimination of an amino derivative of the TF-Ag onto preformed poly(*N*-hydroxysuccinate)³² followed by quenching with ammonia or by direct copolymerization of the above *N*-acryloylated derivative with acrylamide.

Dendritic scaffolds made of poly(amidoamine) (PAMAM) (11) (Scheme 1), poly(propylene imine), *N,N*-bis(acrylamidoacetic acid), and finally hyperbranched L-lysine (Scheme 2) were used to construct relatively small glycodendrimers bearing TF-antigen moieties (16).^{25,30} Few glycodendrimers were also linked to fluorescein and biotin probes (17) to generate ligands that can be used to detect TF-Ag receptor sites (Scheme 2).

With these tools in hand, the required GlycoPAMAM dendrimers bearing the TF-Ag (12–15) were next prepared by amide bond formation between TF-Ag acid (10) and PAMAM dendritic cores (11) to generate G0 to G3 ($n = 4$ to 32) glycodendrimers (12–15) (Scheme 1).^{25,30,34} Analogously, several other TF-glycopolymers were constructed, including those built on hyperbranched L-lysine (16) (Scheme 2).³⁵



The relative potencies of the TF-glycodendrimer families (12–15) to inhibit the binding of a mouse monoclonal IgG antibody to an ELISA plate coated with TF-copolymer were determined using goat anti-mouse monoclonal IgG and the results are shown in Table 1. For the glycoPAMAMs, the degree of inhibition was proportional to the conjugate valencies and showed maximum inhibition when the 32-mer G3 was used (Table 1). The concentrations of TF-PAMAMs to give 50% inhibition (IC_{50}) of the antibody-binding to the coated TF-copolymer were 5.0, 2.4, 1.4, and 0.6 nM for conjugates G0–G3, respectively, where the monomeric TF-Ag (9) required 2.3 μ M. These values represent 460-, 960-, 1700- and 3800-fold enhancement of inhibitory potencies over that of the TF-monomer. Yet, when expressed on a per TF-Ag epitope corrected basis, they were still on average 115-fold more efficient than the monomer, irrespective of the dendrimer valency. When compared to another *N,N*-bis(acrylamidoacetic acid)-based dendrimer series (not shown),³⁶ the dimer showed the poorest inhibitory value (IC_{50} 174 nM), while the two homologous tetramers were approximately equipotent with an IC_{50} of 18 nM and the corresponding hexamer showed a noticeable decrease in affinity with an IC_{50} value of 48 nM. For that series and on a per TF-Ag basis, the tetramers were the most potent with ~31-fold enhancement over that of the monomer. When compared together, the glycoPAMAM series G0–G3 ($n = 4$ –32) (12–15, Scheme 1) showed the best value with overall 3.7-fold better binding ability over the *N,N'*-bis(acrylamidoacetic acid)-based dendrimer series. The explanation for these observations is not straightforward but could partly be due to a slightly longer distance between the aglyconic oxygen and the branching fifteen atoms for PAMAMs in comparison to the nine atom linkers of the later, which may



Scheme 2 Antigenic TF-poly-L-lysine dendron and an analogous biotinylated probe used as an indirect coating antigen in ELISA assays precoated with streptavidin.³⁵

Table 1 Relative inhibitory potencies (IC_{50} 's) of various TF-antigen dendrimer scaffolds to mouse monoclonal antibody (IgG3) binding to coated TF-copolymer

Compound	IC_{50}^a/nM	Relative potency ^a
Monomer	2300	1
12 PAMAM (G0) (4-mer)	5.0 (20.0)	460 (115)
13 PAMAM (G1) (8-mer)	2.4 (19.2)	960 (120)
14 PAMAM (G2) (16-mer)	1.4 (22.4)	1700 (106)
15 PAMAM (G3) (32-mer)	0.6 (19.2)	3800 (119)
Dimer ^b	174 (347)	13.3 (6.6)
4-mer ^b	19 (76)	120.5 (30.1)
4-mer ^b	18 (72)	128.1 (32.2)
6-mer ^b	48 (288)	47.8 (8.0)

^a Values in parentheses are based on per TF-antigen basis. ^b *N,N'*-Bis(acrylamidoacetic acid)-based dendrimer series (not shown).³⁴

provide better accessibility of the TF-Ag for the receptor sites within the antibody combining sites. Interestingly, these results confirmed earlier findings³⁶ illustrating that tetrameric TF-Ag clusters may represent the optimum size for an antibody-glycocluster inhibition.

In fact (see below), successful TF-based synthetic vaccine candidates contain four residues built on various scaffolds and on RAFT in particular. The protein binding properties of these glycodendrimers were also evaluated using the plant lectin from *Arachis hypogaea* (peanut lectin) which constitutes a perfect antibody mimic, albeit tetravalent.³⁰

It is also worth mentioning that while these TF-dendrimers were highly *antigenic*, that is, they could strongly bind to antibodies, they all failed to elicit any immune response hence, they were shown to be *non-immunogenic*.³⁷ In other words, potent synthetic carbohydrate antigens may represent poor immunogens. The lack of immunogenicity was also confirmed when hyperbranched L-lysine were used as scaffolding dendrimers (**16**) (Scheme 2).³⁵ These observations can now be readily rationalized on the basis that, even when TACAs are presented as multivalent entities, they lack immunocompetent molecular entities, pattern recognition receptor ligands, as described above (see discussion below).

Another noteworthy application was found with a TF-linked poly-L-lysine tetrameric dendron onto which was anchored a

biotin probe (**17**) (Scheme 2). Dendron **17** was found to be very useful in enzyme linked immunosorbent assays (ELISA) as it could be anchored to the solid-phase through streptavidin binding for antibody capture.^{25,35} Curiously, despite their apparent hydrophilicity, the TF-PAMAM dendrimers were also capable of directly coating the surface of hydrophobic ELISA plates.³⁰

However, when conjugated to effective immunogenic protein carriers, the TF-antigen together with its shorter T_N-antigen (α -GalNAc-*O*-Ser/Thr) precursor as well as their sialylated counterparts (see Fig. 3) were strongly immunogenic.³⁷ For instance, natural asialo ovine submaxillary mucin (A-OSM), known to contain almost exclusively T_N antigens, provided protection against challenge with a highly invasive mouse mammary carcinoma. The vaccine also induced *in vitro* proliferation of CD4⁺ T lymphocytes, thus indicating cellular immunity and protection. In addition, recent findings also suggested that a linear trimeric version of the T_N-antigen was more antigenic. Thus, analogous artificial vaccines composed of mono-, di- and tri-meric T_N-antigens coupled to Ovine Serum Albumin (OSA) successfully provided antibody responses, although the trimeric antigen was more potent.

Over the past decade, Jung *et al.*^{14,38} have developed totally synthetic peptide vaccines using the lipopeptide tripalmitoyl-S-glycerylcysteinylserine (**Pam**₃**CSK**₄) as a combined carrier, and an adjuvant system targeting the Toll-like receptors 2 (TLR-2). **Pam**₃**CSK**₄ is non-immunogenic and has no toxic side effects, nor does it cause tissue damage in animals. In addition, its adjuvanticity is comparable to that of the classical Freund's adjuvant (human toxic). Added together, these observations triggered the interest toward the syntheses of better chemically defined entities harbouring multivalent carbohydrate antigens as well as effective T helper cell (Th) epitopic peptides and lipids. The ground was thus set for effective glycodendrimer vaccine syntheses.

4. Second generation TF-vaccine

As mentioned, the Thomsen–Friedenreich (TF)-antigen over-expression on the cell surface of several types of tumor cells contributes to cancer cell adhesion and severe metastasis to sites containing TF-Ag binding lectins (lungs, liver, lymph nodes). Our group showed that a highly specific immunoglobulin IgG3 monoclonal antibody (MAb) developed against the TF-Ag (JAA-F11)³¹ impeded TF-Ag binding to vascular endothelium, blocking a primary metastatic step and providing a survival advantage.³⁹ In addition, in patients, even low levels of antibodies to TF-Ag seem to improve prognosis; thus, it is expected that vaccines generating antibodies toward TF-Ag would be clinically valuable. Unfortunately, vaccinations with protein conjugates of TACAs have induced clinically inadequate humoral immune responses. However, immunization using peptides that mimic carbohydrate Ags has resulted in both Ab and T-cell responses. We hypothesized that vaccinations with unique TF-Ag peptide mimics might generate immune responses to TF-Ag epitopes on tumor cells, useful for active immunotherapy against relevant cancers. A fifteen-mer peptide mimic (**18**) (Fig. 4) of the TF-Ag (H-I-H-G-W-K-S-P-L-S-S-L-G-G-G) was selected by phage display biopanning using our IgG3 antibody (JAA-F11) and rabbit anti-TF-Ab and was analyzed *in vitro* to confirm TF-Ag peptide mimicry (Fig. 4).⁴⁰

In vitro, TF-Ag peptide mimics bound to TF-Ag-specific peanut agglutinin and blocked TF-Ag-mediated rolling and stable adhesion of cancer cells to vascular endothelium. *In vivo*, the immunization with TF-Ag-mimicking multiple antigenic peptides induced TF-Ag reactive Ab production. This novel active immunotherapy approach will hopefully decrease tumor burden in cancer patients by specifically targeting TF-Ag positive cancer cells and blocking metastasis. This represented, to the best of our knowledge, the first case of a dendritic glycomimetics of a carbohydrate related antigen.

5. Tumor associated cancer vaccines: recent perspective

Kunz and co-workers further pioneered the field by designing numerous vaccine compositions.^{7,41–45} They concluded that, even when conjugated to BSA, mostly IgM murine isotypes were triggered from TACAs.⁴⁶ They demonstrated that fully synthetic glycoconjugates consisting of TACAs' glycopeptides linked to Th-cell epitopes from ovalbumin could raise selective IgGs in only a third of transgenic mice.⁴⁴ Moreover, they showed that STn-MUC1 peptide–tetanus toxoid conjugates elicited strong immune responses,⁴⁷ while the corresponding and fully synthetic construct bearing a TLR-2 lipopeptide ligand (**Pam**₃**CSK**₄) could also behave likewise,⁴⁸ but not as strongly as the following vaccine assembly. Similarly, Boons *et al.* also reported the efficacy of a fully synthetic vaccine harboring the MUC1-Tn antigen built on a TLR-2 **Pam**₃**CSK**₄ lipopeptide.⁴⁹ Unfortunately, the formulation occurred as liposomes and, consequently, will likely encounter the problems associated with structural analysis previously met with protein carriers. Danishefsky and his group have also greatly contributed to our understanding of the chemistry and immunology of TACAs.^{5,50} A fully synthetic cancer vaccine incorporating a trimeric Lewis^y oligosaccharide built on the TLR-2 ligand (**Pam**₃**CS**) but missing the required Th-cell epitope only provoked IgM stimulation. They also prepared a multi-antigenic construct which, when anchored to KLH produced both IgM as well as IgG against each of the TACAs present. These results further pointed toward the need for a Th-cell epitope in the vaccine formulation.

Bay *et al.* fully demonstrated the feasibility and efficacy of eliciting complete immune responses with memory effects using carbohydrate cancer antigens built on multiple antigen glycopeptide (MAG) scaffolds (*e.g.* trimeric T_N (**19**), T_N (**20**)) (Fig. 5).⁵¹ Thus the immunogenicity of synthetic multiple antigenic glycopeptides displaying four clustered T_N-epitopes anchored to an oligomeric branched lysine core was examined. Synthesis of MUC1 multi-antigenic glycopeptide dendrimer vaccines by employing multiple-epitope presentation such as the one using the non-immunogenic hyperbranched poly-L-lysine core⁵¹ a carrier-induced immune suppression that can occur during immunization with carrier protein vaccines could be avoided. Vaccines based on multiple antigen presentation⁵² could be favourable for the uptake and presentation of the antigen by antigen presenting cells (Fig. 6). Syntheses of sialyl-T_N (**21**) (Fig. 6) glycopeptide dendrimer vaccines have recently been reported.⁵³ Using a resin-linked lysine core, the tetrameric 16 amino acid tandem repeat of mucin MUC4, -Thr-Ser-Ser-Ala-Ser-Thr-Gly-His-Ala-Thr-Pro-Leu-Pro-Val-Thr-Asp-, was

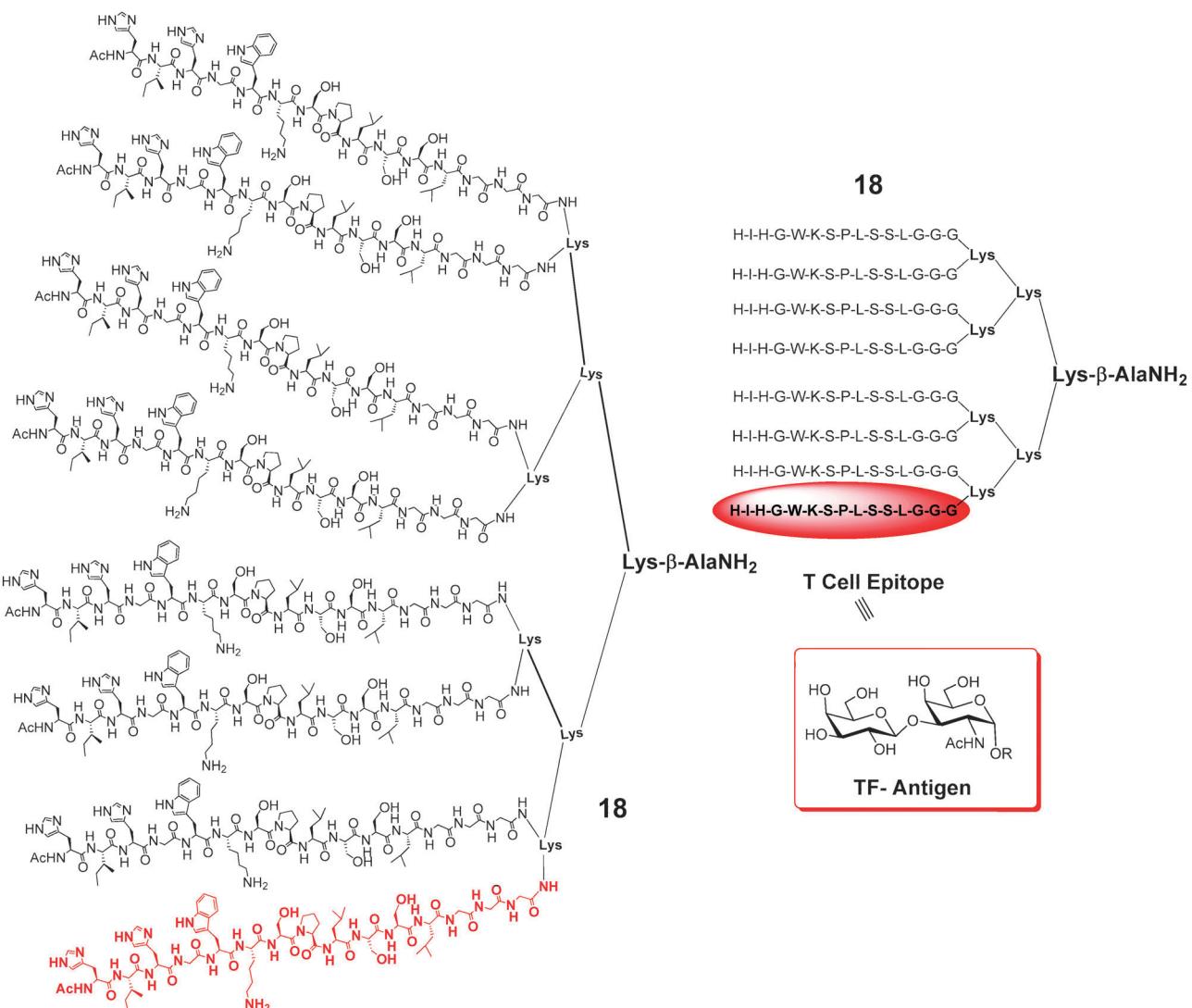


Fig. 4 Antigenic carbohydrate epitopes can be functionally replaced by peptide sequences. When multimerized into a dendritic molecular architecture, the resulting neoglycoconjugates can not only be recognized by anti-carbohydrate antibodies but can also act to trigger a successful immune response.⁴⁰

assembled by automated solid-phase synthesis. Such vaccines were prepared in both forms, with and without additional extra immunostimulating poliovirus peptide (KLFAVWKITYKDT)⁵¹ or tetanus toxoid Th-cell peptide epitopes. In order to separate the B- and T-cell epitope and the di-lysyl lysine core from each other, triethylene glycol spacer amino acid was incorporated in between them.

According to the principle of multiple antigen presentation (MAP), a di-lysyl lysine core was used for the synthesis of tetramers of a glycododecapeptide antigen from the tandem-repeat sequence of the tumor-associated mucin, MUC1, which carries a sialyl-T_N-antigen (**21**) saccharide side chain (Fig. 6).⁵³ The methodology was extended to the analogous construction of a tetrameric vaccine consisting of a T cell epitope from tetanus toxoid and the sialyl T_N glycododecapeptide of MUC1. A linear construct based on trivalent globoside built on the MUC5 mucin scaffold and linked to KLH has also been prepared.⁵⁴

The construction of octameric (**22**)⁵⁵ (Fig. 7) glycopeptide antigens from the tandem repeat region of tumor-associated

glycoproteins MUC1 and MUC4 was accomplished according to the MAP principle introduced by Tam *et al.*⁵⁶ First layer and second layer lysyl-lysine cores were applied in solid-phase syntheses in order to obtain tetrameric and octameric T_N antigen glycopeptide antigens. As a rule, the NMR spectra of these dendrimeric compounds in water show only one set of signals indicating a very similar flexibility of the glycopeptide portions of these dendrimers. This result is considered promising for the use of these dendritic glycopeptide antigens in immunological treatment of epithelial tumors. In particular, not only a B cell, but also a T cell immune response should be inducible with these multimeric antigens. The NMR spectra of these dendritic compounds in water showed only one set of signals indicating structural equivalency as well as very similar flexibility of the glycopeptide portions of these dendrimers. This result was considered promising for the use of such constructs in immunological treatment of epithelial tumors. In particular, not only a B cell, but also a T cell immune response should be inducible with these multimeric antigens.

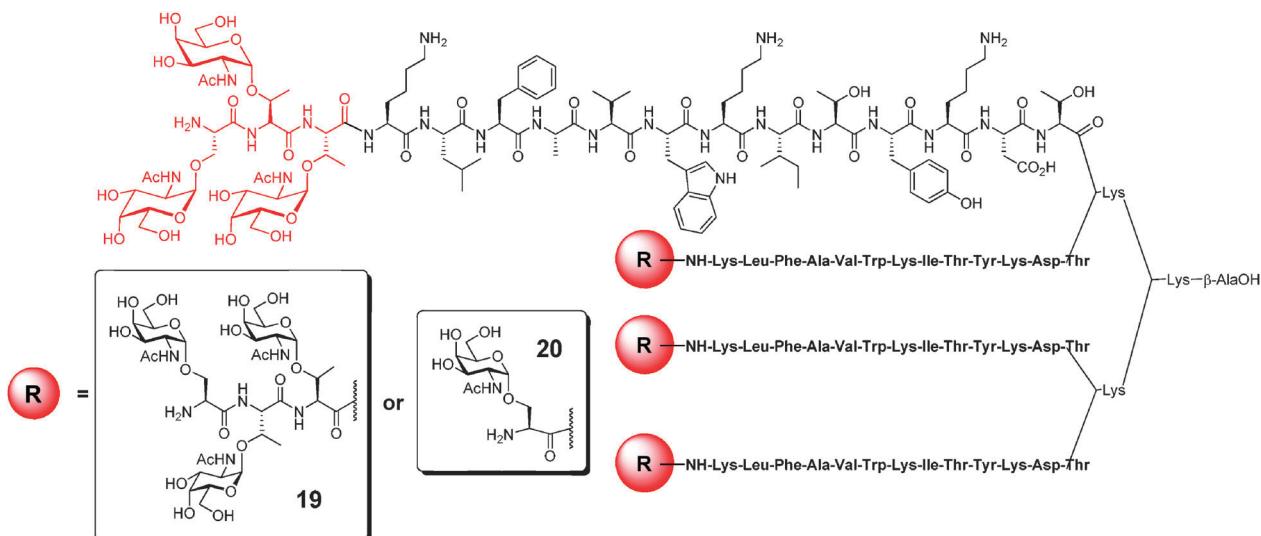


Fig. 5 Tetravalent glycodendrons with monomeric and trimeric T_N-antigens possessing four copies of the poliovirus CD4⁺ Th-cell epitopes which conferred high murine immunogenicity.⁵¹

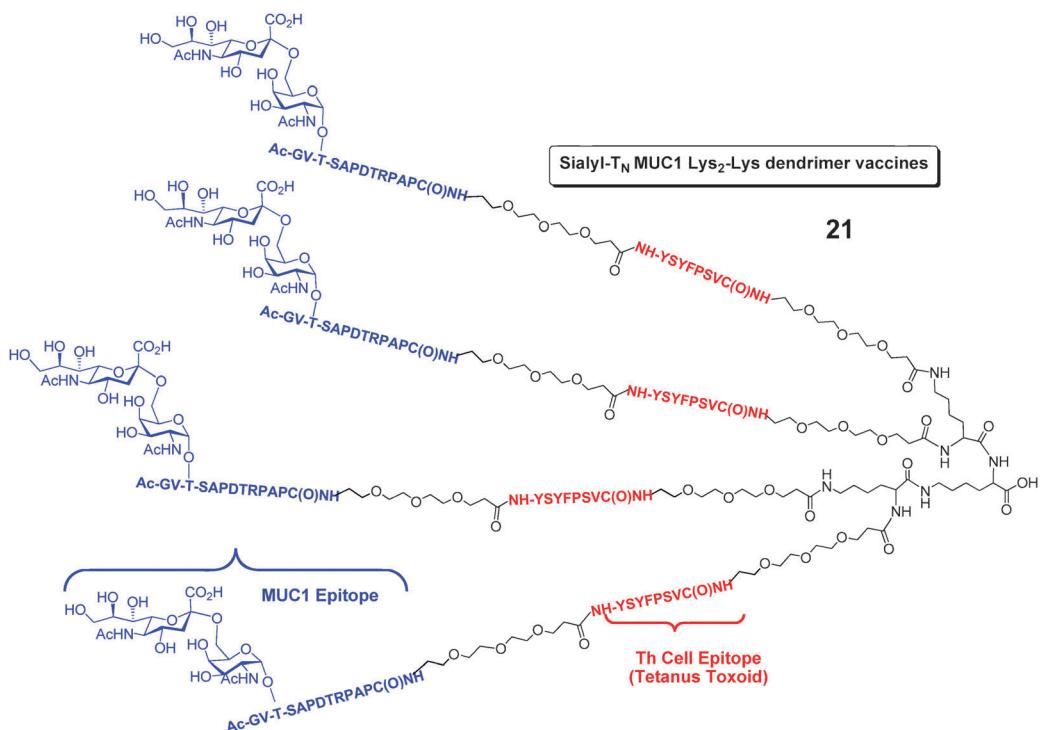


Fig. 6 Tetravalent two component glycodendron vaccines incorporating the sialyl-T_N antigen on its natural MUC1 peptide (B-cell epitope) together with a universal T. toxoid CD4⁺ Th-cell epitope.⁵³

In their most recent report, Kunz *et al.* reported the solid-phase synthesis (SPS) of the natural and non-natural 6,6'-fluorinated analog of the TF-antigens.⁴⁸ The fluorinated analog was introduced to determine whether more enzymatically stable glycomimetic would influence the immunological efficiency of the vaccine. The SPS was done on a Tentagel R resin using standard coupling reagents. After sequential build up of the TF-MUC1 glycopeptides serving as B-cell epitopes, triethylene glycol linker introduction as spacer and deprotection, the intermediate was coupled to diethyl squarate used as a linker.

Ligation of the remaining ester of the squarate moiety to either BSA or T. toxoid afforded two vaccine formulations. After mice immunization, IgG antibody titers against the natural TF-antigen were very high and shown to be 25 times stronger than in the case of the strongest responding mice immunized with the fully synthetic MUC1-ovalbumin vaccine⁴⁷ and more than 100 times stronger than that of the **Pam**₃**CSK**₄ lipopeptide-MUC1 vaccine.⁵⁷ The antibody titers of the mice immunized with the F₂-TF-antigen assembly were approximately half those from the natural B-cell epitope. Additionally, the MUC1 glycopeptide

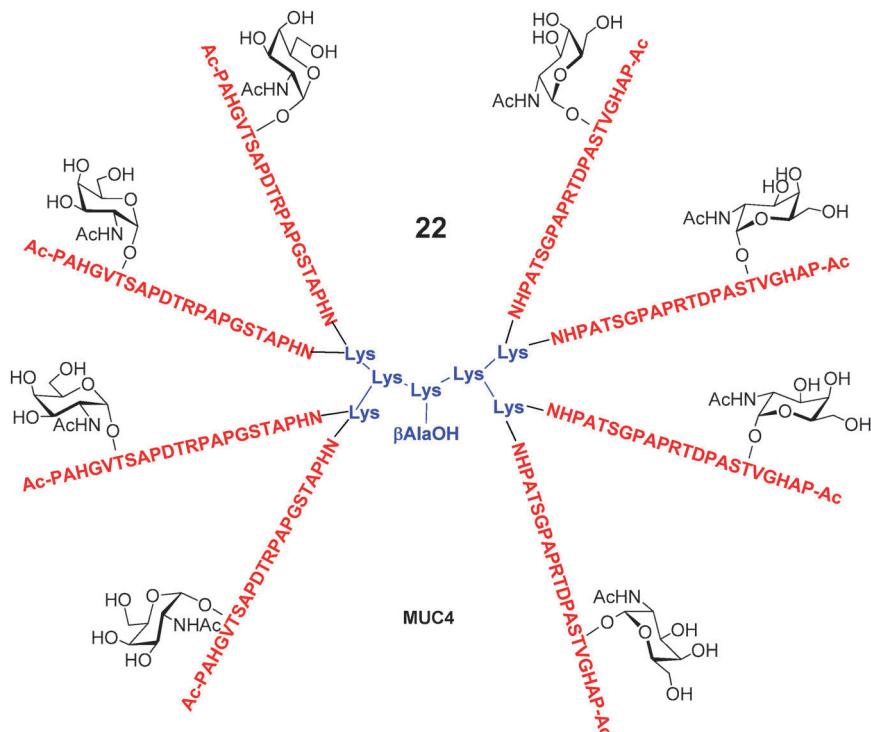


Fig. 7 Multiple Antigen Peptide (MAP) dendritic scaffold harbouring octameric T_N -MUC1 TACA residues.⁵⁵

truncated to 12 amino acids, rather than 20, was only weakly recognized. The resulting antibodies from both vaccines were shown to bind to the epithelial tumor cell line MCF7 (breast cancer) and the MUC1 glycopeptides could inhibit the binding. Hence, the fluorinated analog did not break the immunogenicity of the vaccine and furthermore, both vaccines could override the natural tolerance of the immune system against traces of the antigenic determinant intrinsically occurring on mammalian cells.

Using a similar approach, short synthetic glycopeptides bearing multiple T_N -antigens and various CD4⁺ T-cell epitopes were shown to induce T_N -specific antibody responses.⁵¹ The dendrimeric target immunoconjugate **23** (Fig. 8) contains clusters

of three consecutive T_N -epitopes linked to three different T-cell epitopes. Again, the superior efficacy of the MAG strategy over the traditional KLH glycoconjugates to elicit an anti-carbohydrate IgG response was clearly established. The effect of varied aglyconic carrier elements for their recognition by the immune system was also discussed. The introduction of three different promiscuous HLA-restricted T-helper epitopes from the poliovirus (PV-103–115) (KLFAVWKITYKDT), tetanus toxoid (QYIKANSKFIG ITEL) or PADRE[®] (AKXVAATL-KAAA; X = cyclohexylalanine) was evaluated and immunological evaluation in primates was investigated. To our knowledge, this was the first example wherein a dendritic TACA was used in higher

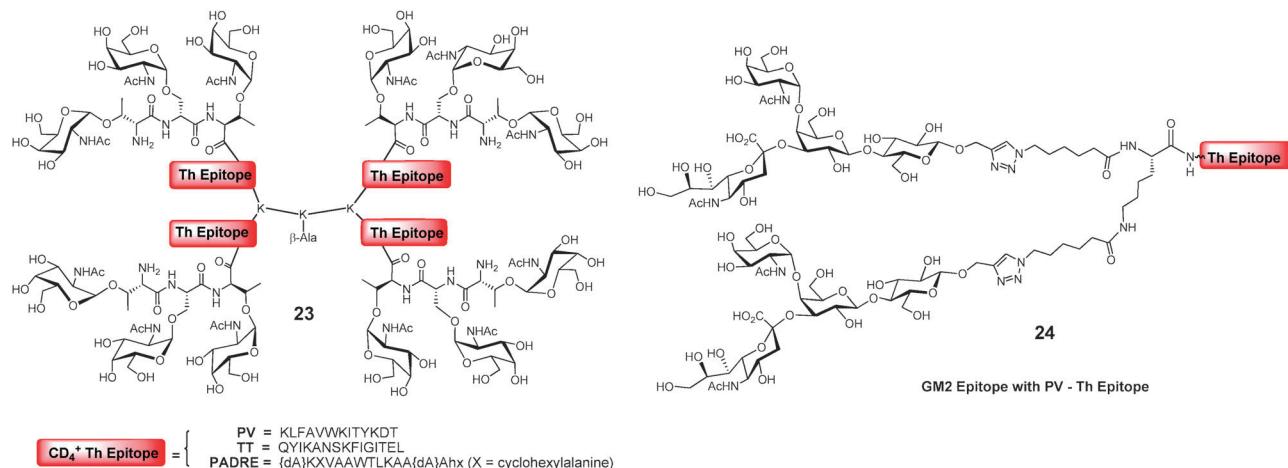


Fig. 8 (left) Tetraovalent dendritic vaccine bearing trimeric T_N -antigen (B epitope) built on a poly-L-lysine scaffold and four copies of the T-cell peptide epitopes taken from either poliovirus, tetanus toxoid, or PADRE[®], a synthetic universal MHC II restricted epitope. (right) Dimeric GM2 B-cell epitope (TACA) at the tip of a poliovirus T-cell peptide.^{51,58}

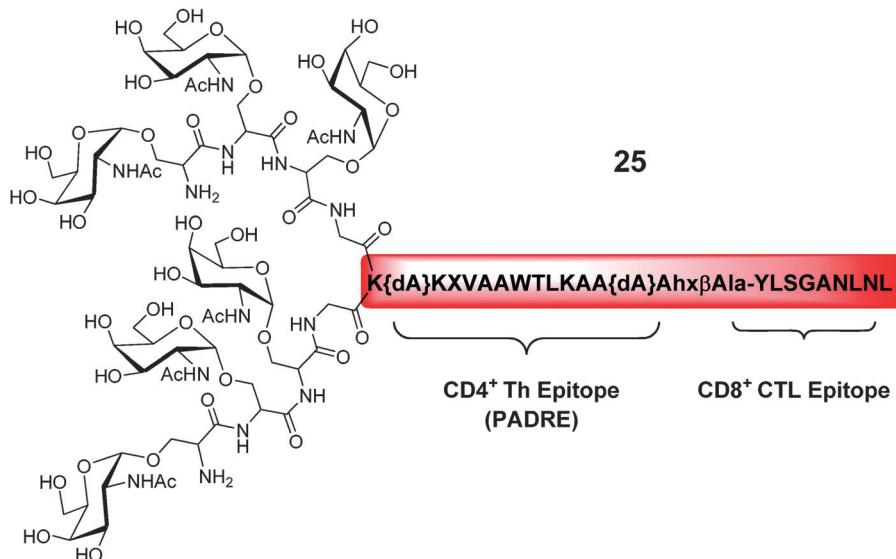


Fig. 9 A three component vaccine TACCA with trimeric T_N -antigen together with both $CD4^+$ and $CD8^+$ T cell peptide epitopes.⁵⁹

animals. The MAG- T_N vaccines induced in all of the animals strong tumor-specific anti- T_N antibodies that could mediate antibody-dependent cell cytotoxicity (ADCC) against human tumor. The two different $CD4^+$ T-cell peptides, PADRE and TT, introduced into the MAG were capable of providing help for anti- T_N antibody production in all of the immunized primates. Again, these antibodies could be induced with a mild adjuvant setting (alum), although the addition of CpG oligonucleotides strongly improved the immunogenicity of the MAG by eliciting a quantitatively higher and more rapid response. Importantly, in all of the experimental settings, no adverse reaction was observed in any animal such as local inflammation at the sites of injection or weight loss assessing the safety of the MAG. Therefore, the preclinical evaluation of the MAG- T_N vaccine demonstrated that it represented a safe and highly promising immunotherapeutic agent with molecularly defined architecture for targeting breast, colon, and prostate cancers that express the carbohydrate T_N antigen.

The GM2 ganglioside (Fig. 4) is another member of the TACA family which also represents an important target for specific anticancer immunotherapy. A synthetic neoglycopeptide immunogen displaying one or two copies (**24**) of the GM2 tetrasaccharide was recently described (Fig. 8).⁵⁸ The glycopeptides were prepared using the “click chemistry” (Huisgen cycloaddition), by coupling propargylated GM2 with an N-terminal azido $CD4^+$ T-cell peptide epitope from poliovirus. The fully synthetic glycopeptide induced human tumor cell-specific antibodies after immunization in mice. Notably, the monovalent, but not the divalent GM2 vaccine construct provoked anti-melanoma antibodies. Therefore, such a carbohydrate-peptide conjugate represents a promising cancer vaccine strategy for active immunotherapy targeting gangliosides. It is not clear however, why in this case, a multivalent presentation failed to provide the adequate immune response.

Another glycoform (**25**)⁵⁹ was similarly designed that contained a synthetic glycopeptide vaccine containing a cluster of the T_N -antigen as a B-cell epitope covalently linked to a $CD4^+$ T-cell peptide corresponding to the Pan DR ‘universal’

T -helper epitope (PADRE[®]) and to a cytotoxic T lymphocyte (CTL) epitope from the carcinoembryonic antigen (CEA) (Fig. 9). The immunogenicity of the construct was evaluated in outbred mice as well as in HLA transgenic mice (HLA-DR1 and HLA-DR4). A strong T-cell dependent antibody response specific for the T_N antigen was elicited in both outbred and HLA transgenic mice. The antibodies induced by the glycopeptide construct efficiently recognized a human tumor cell line underlying the biological relevance of the response.

In summary, glycoconjugate vaccines harbouring “universal” $CD4^+$ Th-epitopes can address the limitations of the genetic restriction in humans by providing effective T-cell helps. The resulting synthetic immunogens are predominantly attractive for both their ease of preparation in a pure modular fashion, purification steps and, perhaps more importantly, by their straightforward characterization in accurate chemical compositions which are essential features for safe clinical vaccines. Previous results showed that, although simple, monovalent glycopeptides are efficient immunogens, the dendritic MAG system can induce an even stronger immune response. Moreover, using a combination of immunogenic peptides, multivalent constructs bearing ‘universal’ T-cell epitopes have already demonstrated their superior immunogenic properties.

6. Toll-like receptors: glycolipid conjugates

As mentioned in the Introduction, lipid-bound antigens can be directly recognized by TLRs from which they are internalized within the APCs, processed, and finally exposed on the surface for $CD8^+$ natural killer cell activation (Fig. 1). The Lipid A portion of Gram-negative bacteria has been co-crystallized with TLR4,¹⁵ while TLR2 was co-crystallized with Pam₂CSK₄ (Fig. 10, inset).¹⁶ The overall consequences of this and similar observations were that glycochemists initiated intensive research programs aimed at grafting carbohydrate antigens onto lipopeptides. A powerful example of this situation has been described for the TACAs’ antigens described above,^{49,57} and bacterial CPS.⁶⁰

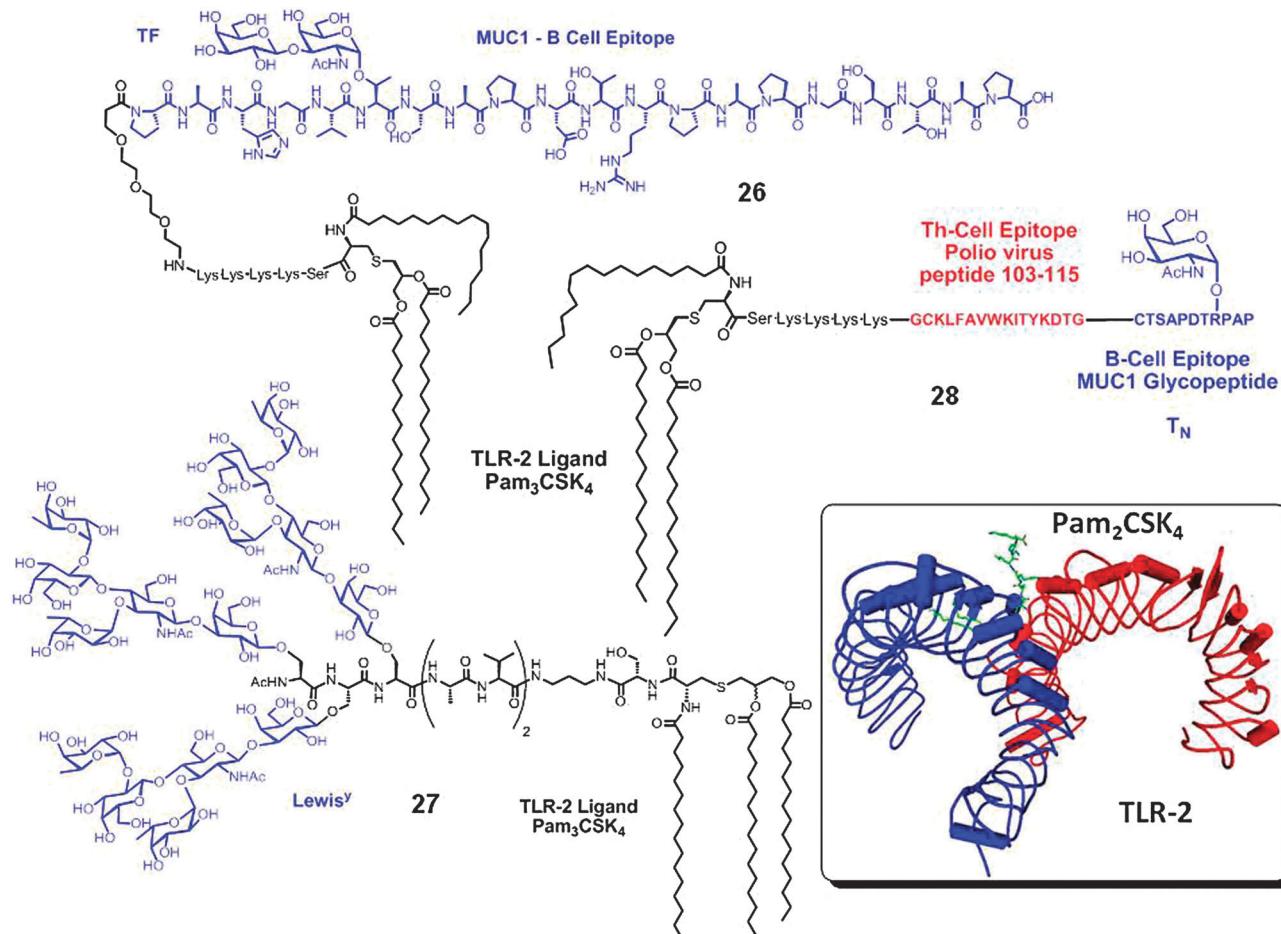


Fig. 10 Inset: TLR2 cocrystallized with the lipopeptide Pam₂CSK₄ (PDB No. 3A79); chemical structures of fully synthetic carbohydrate-based two (**26**)⁵⁷ and three (**28**) component vaccines bearing the TF-antigen (**26**);⁵⁷ dendritic Lewis^y (**27**)⁶² and the T_N construct (**28**).⁴⁹

The initial observation of Toyokuni *et al.*^{37,61} who provided the first example demonstrating that a synthetic carbohydrate vaccine could generate a robust immune response without the use of a protein carrier or external adjuvant paved the way for the next generation of multiepitopic vaccines. Toyokuni's vaccine was simply composed of a di-T_N-Pam₃Cys containing a dimeric T_N epitope and an immunologically active lipopeptide, tripalmitoyl-S-glyceryl-cysteinylserine (**Pam₃CS**), derived from the N-terminal sequence of an *E. coli* lipoprotein.

Several molecular combinations using the TLR ligand (**Pam₃CS**) have followed to provide TACAs-based vaccines (Fig. 10). For instance, Kunz *et al.*^{57a} used either the T_N, TF, or STF carbohydrate antigens linked to the MUC1 B cell epitope (Fig. 10, TF-Ag shown, **26**). To minimize the influence of the lipopeptide and its basic side chains on the conformation of the MUC1 glycopeptide antigen, an oligoethylene glycol spacer was again introduced between the TLR2 ligand and the B-cell epitope. In the more recent version,^{57b} the researchers constructed a tetravalent MUC1 dendrimer (L-lysine scaffold, click chemistry) having the Pam₃CSKKK as the focal point. Alternatively, Danishefsky *et al.* combined the principle of multivalency and the TLR agonist to synthesize a two component vaccine having three copies of the Lewis^y antigens (**27**) (Fig. 10).⁶² The vaccine elicited only IgM antibodies as a result of T-cell epitope lacking. A small antibody class switch to produce IgG was observed in the

presence of the QS21 adjuvant. A fully synthetic three-component vaccine (**28**) synthesized from a TLR2 Pam₃CSK₄ lipopeptide ligand, a promiscuous T-helper epitope identified from a well-documented mouse major histocompatibility complex (MHCII) from the polio virus (PV₁₀₃₋₁₁₅) together with the tumor-associated glycopeptide T_N-MUC1 has been shown to elicit remarkably high antibody titers of IgG isotype in mice (Fig. 10).⁴⁹ The antibodies were shown to recognize cancer cells expressing the tumor-associated carbohydrate antigen. The superior properties of the vaccine candidate were attributed to the local production of cytokines, upregulation of costimulatory proteins, enhanced uptake by macrophages and dendritic cells and avoidance of epitope suppression. The vaccine was however prepared as a poorly defined liposome composition, hence multivalency was artificially created. Here again, the IgG titer was greatly augmented in the presence of the QS21 adjuvant.

Danishefsky *et al.* cleverly conceptualized an additional and interesting strategy based on multiepitopic (B-cell) carbohydrate antigens that they coined "cassette-vaccines" (**29**) (Fig. 11).⁶² Again, when the approach was used on the highly immunogenic KLH protein carriers, high IgG antibody titers were obtained against each of the antigen present. Unfortunately, when applied on a single TLR2 receptor (**29**) (Fig. 11), the construct failed to elicit a useful antibody class switch.⁶³

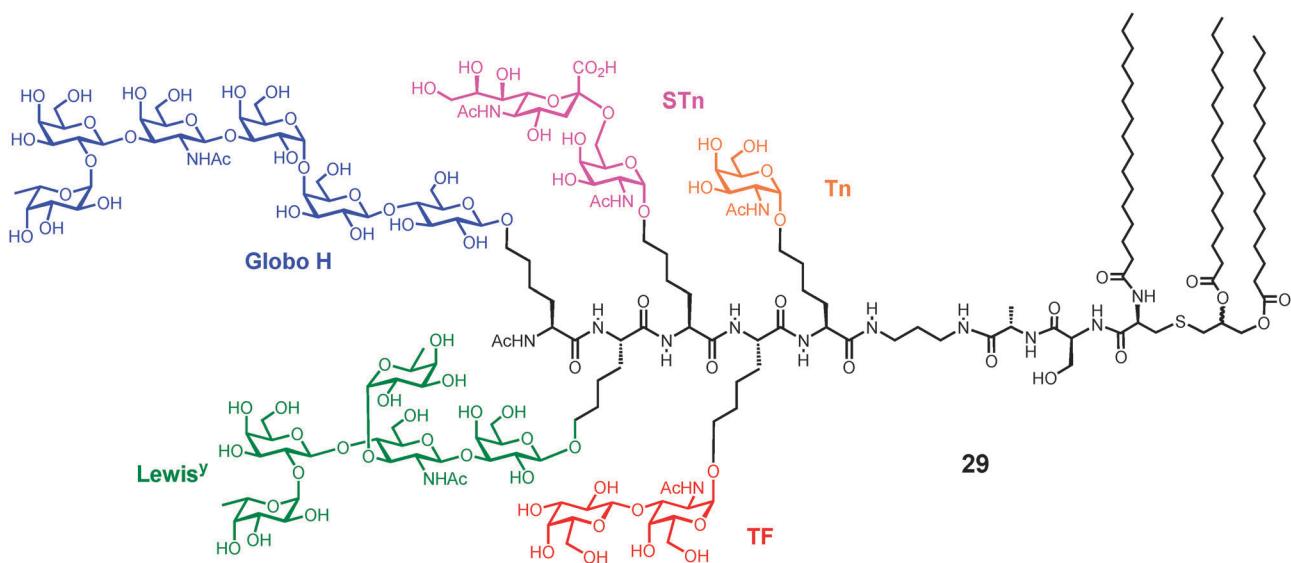


Fig. 11 A pentaepitopic vaccine composition built on the lipophilic TLR ligand was first conceptualized by Danishefsky *et al.*⁶³

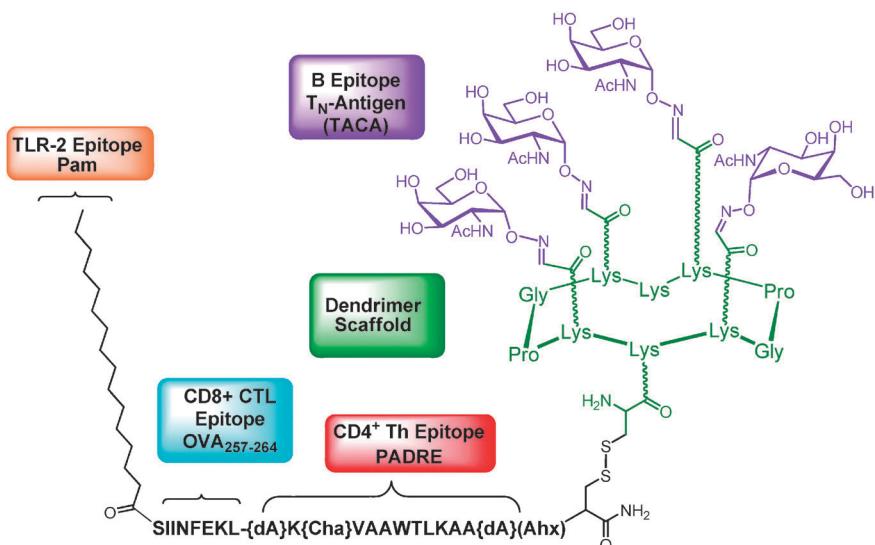
As it has been observed so far, optimum conditions were identified to construct successful glycoconjugate vaccines. Thus, the components of an ideal vaccines should contain: (1) a CD4⁺ Th cell epitope as well as (2) CD8⁺ CTL epitopic peptides to trigger both humoral and cytotoxic immunity, respectively; (3) an adjuvant that can be added externally to the construct or in the best scenario covalently bound to the multicomponent vaccine ensuring Toll-like receptor (TLR-(2)) uptake; (4) obviously the key TACA serving as a B-cell epitope against which antibodies should be raised. Additionally, a fifth component seems to arise from the above observations and that is (5) multivalency. This particular aspect may naturally emerge if the Pam₃Cys TLR is present as it is likely to form liposomes, particularly if QS21 adjuvant is coinjected. Alternatively, multivalency could be obtained through dendrimer scaffolding. The latter two constituents may afford a complex system of sufficiently high molecular weight (or size) that could lead itself to endocytosis of the vaccines as a productive uptake mechanism. Moreover, glycodendrimers of certain sizes (generation) may well be, on their own, capable of cross-linking immunoglobulin receptors on B-cells and thus trigger signalization leading ultimately to B-cell proliferations.

Capitalizing on these cumulated observations and toward achieving a fully synthetic vaccine construct (**30**), Dumeny and his group have successfully synthesized such complex architectures (Fig. 12).⁶⁴ They investigated the use of regioselectively addressable functionalized templates (RAFTs) as scaffolding dendrimers.⁶⁵ They reported the synthesis and the immunological evaluation of a few well defined multiepitopic RAFT scaffolds. The most favourable conjugates exhibited clustered T_N analogues as tumor-associated carbohydrate antigens (TACA, B-cell epitope), the CD4⁺ helper T-cell peptide from the type 1 poliovirus (see above) together with a CD8⁺ CTL from ovalbumin (OVA_{257–264}; SIINFEKL) to which was covalently added the palmitic acid adjuvant (TLR-2 agonist). The saccharidic and peptidic epitopes were separately synthesized and combined regioselectively to the RAFT core using their established⁶⁵ sequential oxime ligation. B- and T-antigenicity as well as

immunogenicity of the vaccine candidates were clearly demonstrated *in vitro* and *in vivo*.

This study established that the saccharidic part of the conjugates was recognized by T_N-specific monoclonal antibodies. Moreover, the antibodies elicited by immunization of mice with the new vaccine candidates recognized the native form of the T_N epitope expressed on human breast cancer cell line MCF-7. This observation, together with the one discussed above in the PAMAM section, points toward the lacking necessity of the full MUC1 glycopeptide linked together. In their latest constructs,^{64b} a CD8⁺ CTL peptide taken from the sequence of human epidermal growth factor 2 (HER-2_{420–429}), a transmembrane receptor-like glycoprotein with tyrosine kinase activity, they positioned the TLR-2 palmitic acid agonist in different positions of the peptide sequences and they concluded that the attachment at the peptide N-terminal afforded most advantageous results. After challenge inoculation with cancerous MO5 cells, vaccinated mice showed 100% survival after 90 days. These results suggest that the RAFT scaffold provides a promising and suitable tool for engineering other potent synthetic anticancer vaccines.⁶⁴

Most successful carbohydrate-based antitumor vaccine candidates have been synthesized in softly clustered modes. An additional promising “twist” to the overall concepts discussed above using multiple copies of tumor-associated carbohydrate antigens T_N and ST_N has been recently described.⁶⁶ The two TACAs were readily assembled on a single cyclic peptide scaffold (RAFT) in a highly convergent manner (**31**) (Fig. 13). Ring-closing metathesis (RCM)-mediated cross-linking of internal anchors provided access to epitopes with limited conformational freedom (**32**), thus offering an additional possibility for precise epitopic presentation toward the aforementioned carbohydrate receptors on immune cells. Of interest, these rigidified internally cross-linked constructs may enhance cluster-recognition antibody responses by retaining an appropriate distance between the glycans linked to the RAFT scaffold. It is anticipated that these highly clustered anticancer constructs will help in gaining further insight into the way in which carbohydrate clusters are recognized by the immune system.



30

Fig. 12 Recent self-adjuvanted multicomponent TACA vaccine incorporating all of the elements necessary to raise both humoral and cytotoxic immunity.⁶⁴

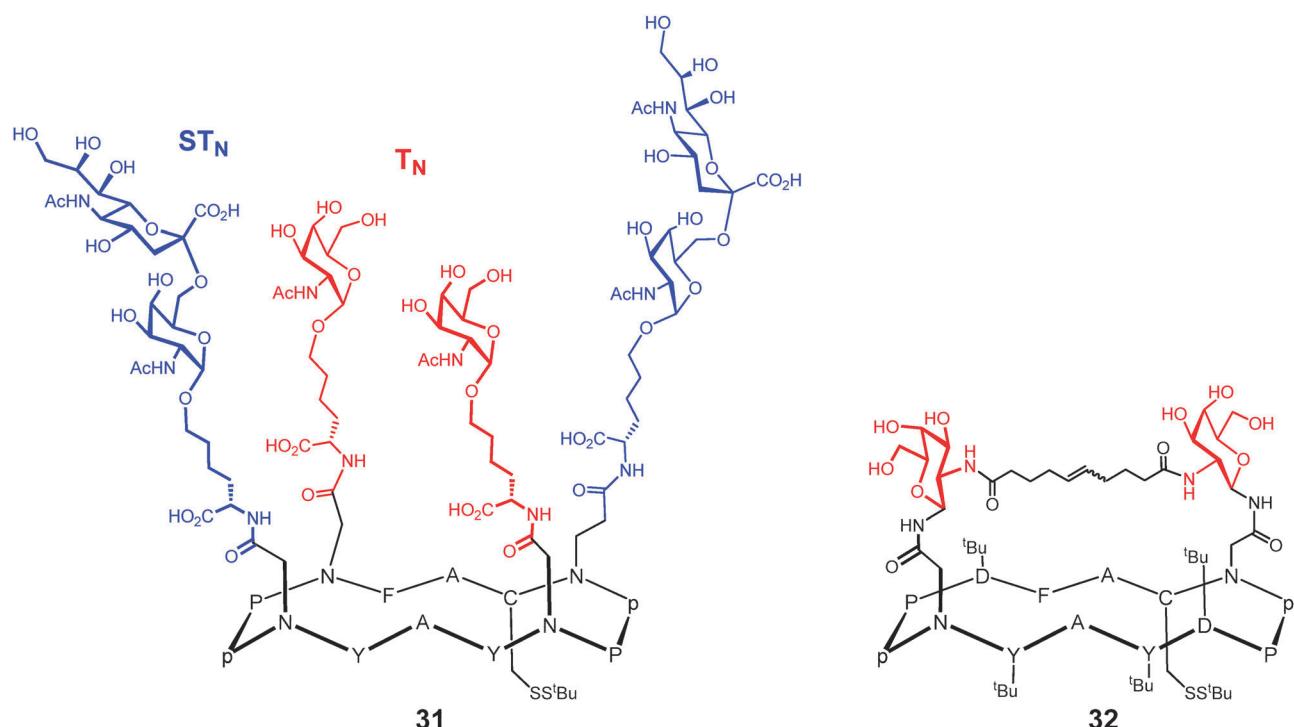


Fig. 13 Multiepitopic RAFT-based vaccine precursors, including (right) a novel concept in which cross-linking of the TACA epitopes may provide further controllable presentation elements (p denotes D-Pro).⁶⁶

7. Other vaccines and conclusion

As clearly evidenced from the cumulated information gained on the synthetic design and immunological investigations disclosed throughout, the time seems to be rapidly approaching wherein several potential vaccine candidates should become commercial reality. In general, synthetic TACAs-based vaccines are somewhat behind schedule when compared to the numerous other

successful and already commercial carbohydrate-based antibacterial vaccines.^{1-3,67,68} Obviously, several other human pathologies are being investigated through prophylactic vaccine treatments and glycobiologists have accordingly focused their efforts in that direction. Given our deeper understanding of antigen uptake mechanisms, the creativity of organic chemists has greatly contributed to the great versatility of vaccine design. Additionally, the sophistication of modern analytic tools to

better identify naturally occurring carbohydrate antigens including crystallography, our improved capacity to screen ligands and receptors through microarrays,⁶⁹ and the many novel strategies to synthesize complex oligosaccharides and create glycomimetics thereof,⁷⁰ have greatly improved our capacity to undertake glycoimmunochemistry. Due to space limitation, this brief review could not cover several other beautiful synthetic applications in the design of multivalent, glycopolymers-based vaccines and a forthcoming review should cover these topics together with other emerging applications of glycopolymers. For several other disease applications, the readers are strongly encouraged to consult the previous reviews by the author.^{1–3}

In conclusion, through the many permutations in the chemical design of carbohydrate-based vaccine candidates, we have learned about the fundamental requirements toward the elaboration of winning cases (Fig. 14). On the one hand, in situations in which multivalency was involved, it appeared that its application⁷¹ contributed positively to the success. Additionally, the presence of both CD4⁺ and CD8⁺ peptide epitopes also constituted critical elements, a situation apparently not necessary in the cases of non-TACAS-based carbohydrate vaccines against pathogenic infections. The absolute requirement for Toll-like receptor (TLR) ligands such as the universally accepted Pam₃CS lipid or its simpler palmitic acid did not seem major since several reports provided valuable immunological responses in their absence. In fact, it could well be that this particular vaccine constituent is also indirectly

contributing to multivalency. This assessment is strongly supported by the need for another adjuvant such as the naturally occurring glycolipid QS21 and by the formation of liposomes.

The recent four component vaccine construct discussed herein⁶⁴ could be further improved by incorporating structural components elegantly introduced by the Danishefsky group, *i.e.* multiepitopic antigens together with conformationally-constrained carbohydrates.^{63,66} If, in addition to these improvements, one adds the fact that carbohydrates themselves could be advantageously replaced by peptide mimotopes,⁴⁰ the day could be close when the anti-carbohydrate vaccines would not contain carbohydrate at all.

Acknowledgements

The generous financial support by a Canadian Research Chair (CRC) in Therapeutic Chemistry from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

References

- R. Roy and T. C. Shiao, *Chimia*, 2011, **65**, 24–29.
- Carbohydrate-based Vaccines*, ed. R. Roy, ACS Symp. Ser., 2008, vol. 989.
- R. Roy, *Drug Discovery Today: Technol.*, 2004, **1**, 327–336.
- Carbohydrate-Based Vaccines and Immunotherapies*, ed. Z. Guo and G. J. Boons, John Wiley & Sons, Hoboken, New Jersey, 2009.
- (a) R. M. Wilson, J. D. Warren, O. Ouerfelli and S. J. Danishefsky, in *Carbohydrate-based Vaccines*, ed. R. Roy, ACS Symp. Ser., 2008, vol. 989, pp. 258–292; (b) S. J. Danishefsky and J. R. Allen, *Angew. Chem., Int. Ed.*, 2000, **39**, 836–863.
- Z. Guo and Q. Wang, *Curr. Opin. Chem. Biol.*, 2009, **13**, 608–617.
- (a) A. Liakatos and H. Kunz, *Curr. Opin. Mol. Ther.*, 2007, **9**, 35–44; (b) T. Becker, S. Dziadek, S. Wittrock and H. Kunz, *Cancer Drug Targets*, 2006, **6**, 491–517; (c) U. Westerlind and H. Kunz, *Chimia*, 2011, **65**, 30–34.
- (a) L. Morelli, L. Poletti and L. Lay, *Eur. J. Org. Chem.*, 2011, 5723–5777; (b) M. C. Galan, D. Benito-Alfonso and G. M. Watt, *Org. Biomol. Chem.*, 2011, **9**, 3598–3610.
- B. A. Cobb and D. L. Kasper, *Eur. J. Immunol.*, 2005, **35**, 352–356.
- L. P. Icart, V. Fernandez-Santana, R. C. Veloso, T. Carmenate, S. Sirois, R. Roy and V. Verrez Bencomo, in *Carbohydrate-based Vaccines*, ed. R. Roy, ACS Symp. Ser., 2008, vol. 989, pp. 1–19.
- (a) Y. M. Chabre and R. Roy, *Adv. Carbohydr. Chem. Biochem.*, 2010, **63**, 165–393; (b) Y. M. Chabre and R. Roy, in *The Sugar Code. Fundamentals of glycosciences*, ed. H.-J. Gabius, Wiley-VCH, Weinheim, 2009, pp. 53–70.
- C. G. Figgdr, Y. van Kooyk and G. J. Adema, *Nat. Rev. Immunol.*, 2002, **2**, 77–84.
- Y. M. Chabre and R. Roy, *Dendrimer-coated carbohydrates as drug delivery Trojan horses in glycosciences*, in *Dendrimer-based Drug delivery Systems: From Theory to Practice*, ed. Y. Y. Cheng, Wiley-VCH, Weinheim, in press, 2011.
- R. Spohn, U. Buwitt-Beckmann, R. Brock, G. Jung, A. J. Ulmer and K.-H. Wiesmüller, *Vaccine*, 2004, **22**, 2494–2499.
- B. S. Park, D. H. Song, H. M. Kim, B.-S. Choi, H. Lee and J.-O. Lee, *Nature*, 2009, **458**, 1191–1195.
- J. Y. Kang, X. Nan, M. S. Jin, S.-J. Youn, Y. H. Ryu, S. Mah, S. H. Han, H. Lee, S.-G. Paik and J.-O. Lee, *Immunity*, 2009, **31**, 873–884.
- F. Y. Avci and D. L. Kasper, *Annu. Rev. Immunol.*, 2010, **28**, 107–130.
- R. A. De Silva, Q. Wang, T. Chidley, D. K. Appulage and P. R. Andreana, *J. Am. Chem. Soc.*, 2009, **131**, 9622–9623.
- (a) S.-I. Hakomori, *Adv. Exp. Med. Biol.*, 2001, **491**, 369–402; (b) G. Ragupathi, *Cancer Immunol. Immunother.*, 1996, **43**, 152–157.
- G. F. Springer, *Science*, 1984, **224**, 1198–1206.

Fig. 14 Proposed structure of an idealized vaccine illustrating the key components described herein.

- 21 (a) P. O. Livingston, *Immunol. Rev.*, 1995, **145**, 147–166; (b) T. Freire, S. Bay, S. Vichier-Guerre, R. Lo-Man and C. Leclerc, *Mini-Rev. Med. Chem.*, 2006, **6**, 1357–1373; (c) A. Franco, *Anti-Cancer Agents Med. Chem.*, 2008, **8**, 86–91.
- 22 C. L. Hattrup and S. J. Gendler, *Annu. Rev. Physiol.*, 2008, **70**, 431–457.
- 23 R. C. Bast Jr., D. Badgwell, Z. Lu, R. Marquez, D. Rosen, J. Liu, K. A. Baggerly, E. N. Atkinson, S. Skates, Z. Zhang, A. Lokshin, U. Menon, I. Jacobs and K. Lu, *Int. J. Gynecol. Cancer*, 2005, **15**(s3), 274–281.
- 24 (a) O. J. Finn, *Nat. Rev. Immunol.*, 2003, **3**, 630–641; (b) P. O. Livingston and G. Ragupathi, *Cancer Immunol. Immunother.*, 1997, **45**, 10–19.
- 25 M.-G. Baek and R. Roy, *Rev. Mol. Biotechnol.*, 2002, **90**, 291–309.
- 26 R. R. Koganty, D. Yalamati and Z.-H. Jiang, in *Carbohydrate-based Vaccines*, ed. R. Roy, ACS Symp. Ser., 2008, vol. 989, pp. 311–334.
- 27 (a) R. Roy, D. Zanini, S. Meunier and A. Romanowska, *J. Chem. Soc., Chem. Commun.*, 1993, 1869–1872; (b) R. Roy, D. Zanini, S. Meunier and A. Romanowska, *ACS Symp. Ser.*, 1994, **560**, 104–119.
- 28 T. Buskas, P. Thompson and G.-J. Boons, *Chem. Commun.*, 2009, 5335–5349.
- 29 E. I. Buzás, B. György, M. Pásztói, I. Jelinek, A. Faluz and H.-J. Gabius, *Autoimmunity*, 2006, **39**, 691–704.
- 30 M.-G. Baek and R. Roy, *Bioorg. Med. Chem.*, 2002, **10**, 11–17.
- 31 K. Rittenhouse-Diakun, X. Xia, D. Pickhardt, M.-G. Baek and R. Roy, *Hybridoma*, 1998, **17**, 165–173.
- 32 (a) M.-G. Baek and R. Roy, *Biomacromolecules*, 2000, **1**, 768–770; (b) M.-G. Baek and R. Roy, *Macromol. Biosci.*, 2001, **1**, 305–311.
- 33 R. S. Donavan, A. Datti, M.-G. Baek, Q. Wu, I. J. Sas, B. Korczak, E. G. Berger, R. Roy and J. W. Dennis, *Glycoconjugate J.*, 1999, **16**, 607–615.
- 34 M.-G. Baek, K. Rittenhouse-Olson and R. Roy, *Chem. Commun.*, 2001, 257–258.
- 35 M.-G. Baek and R. Roy, *Bioorg. Med. Chem.*, 2001, **9**, 3005–3011.
- 36 R. Roy, M.-G. Baek and K. Rittenhouse-Olson, *J. Am. Chem. Soc.*, 2001, **123**, 1809–1816.
- 37 T. Toyokuni and A. K. Singhal, *Chem. Soc. Rev.*, 1995, **24**, 231–242.
- 38 (a) W. G. Bessler, M. Cox, A. Lex, B. Suhr, K. H. Wiesmuller and G. Jung, *J. Immunol.*, 1985, **135**, 1900–1905; (b) P. Hoffmann, K. H. Wiesmuller, J. Metzger, G. Jung and W. G. Bessler, *Biol. Chem. Hoppe-Seyler*, 1989, **370**, 575–582; (c) J. Metzger, G. Jung, W. G. Bessler, P. Hoffmann, M. Strecker, A. Lieberknecht and U. Schmidt, *J. Med. Chem.*, 1991, **34**, 1969–1974.
- 39 J. Heimborg, J. Yan, S. Morey, O. V. Glinskii, V. H. Huxley, L. Wild, R. Klick, R. Roy, V. V. Glinsky and K. Rittenhouse-Olson, *Neoplasia*, 2006, **8**, 939–948.
- 40 J. Heimborg-Molinaro, A. Almogren, S. Morey, O. V. Glinskii, R. Roy, G. E. Wilding, R. P. Cheng, V. V. Glinsky and K. Rittenhouse-Olson, *Neoplasia*, 2009, **11**, 780–792.
- 41 H. Kunz, S. Dziadek, S. Wittrock and T. Becker, in *Carbohydrate-based Vaccines*, ed. R. Roy, ACS Symp. Ser., 2008, vol. 989, pp. 293–310.
- 42 U. Westerlind and H. Kunz, *Carbohydr. Chem.*, 2010, **36**, 1–37.
- 43 S. Dziadek and H. Kunz, *Chem. Rec.*, 2004, **3**, 308–321.
- 44 (a) S. Dziadek, A. Hobel, E. Schmitt and H. Kunz, *Angew. Chem., Int. Ed.*, 2005, **44**, 7630–7635; (b) U. Westerlind, A. Hobel, N. Gaidzik, E. Schmitt and H. Kunz, *Angew. Chem., Int. Ed.*, 2008, **47**, 7551–7556.
- 45 G.-A. Cremer, N. Bureauaud, V. Piller, H. Kunz, F. Piller and A. F. Delmas, *ChemMedChem*, 2006, **1**, 965–968.
- 46 H. Kunz and S. Birnbach, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 360–362.
- 47 A. Kaiser, N. Gaidzik, U. Westerlind, D. Kowalczyk, A. Hobel, E. Schmitt and H. Kunz, *Angew. Chem., Int. Ed.*, 2009, **48**, 7551–7555.
- 48 A. Hoffmann-Röder, A. Kaiser, S. Wagner, N. Gaidzik, D. Kowalczyk, U. Westerlind, B. Gerlitzki, E. Schmitt and H. Kunz, *Angew. Chem., Int. Ed.*, 2010, **49**, 8498–8503.
- 49 T. Buskas, S. Ingale and G.-J. Boons, *Angew. Chem., Int. Ed.*, 2005, **44**, 5985–5988.
- 50 (a) 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; (b) V. Y. Dudkin, M. Orlova, X. Geng, M. Mandal, W. C. Olson and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2004, **126**, 9560–9562; (c) X. Geng, V. Y. Dudkin, M. Mandal and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2004, **43**, 2562–2565.
- 51 (a) S. Bay, R. Lo-Man, E. Osinaga, H. Nakada, C. Leclerc and D. Cantacuzene, *J. Pept. Res.*, 1997, **49**, 620–625; (b) R. Lo-Man, S. Bay, S. Vichier-Guerre, E. Deriaud, D. Cantacuzene and C. Leclerc, *Cancer Res.*, 1999, **59**, 1520–1524; (c) R. Lo-Man, S. Vichier-Guerre, S. Bay, E. Deriaud, D. Cantacuzene and C. Leclerc, *J. Immunol.*, 2001, **166**, 2849–2854; (d) S. Vichier-Guerre, R. Lo-Man, S. Bay, E. Deriaud, H. Nakada, C. Leclerc and D. Cantacuzene, *J. Pept. Res.*, 2000, **55**, 173–180.
- 52 C. Brocke and H. Kunz, *Bioorg. Med. Chem.*, 2002, **10**, 3085–3112.
- 53 S. Keil, A. Kaiser, F. Syed and H. Kunz, *Synthesis*, 2009, 1355–1369.
- 54 J. Zhu, Q. Wan, G. Ragupathi, C. M. George, P. O. Livingston and S. Danishefsky, *J. Am. Chem. Soc.*, 2009, **131**, 4151–4158.
- 55 T. Becker, A. Kaiser and H. Kunz, *Synthesis*, 2009, 1113–1122.
- 56 (a) J. P. Tam, *Proc. Natl. Acad. Sci. U. S. A.*, 1988, **85**, 5409–5413; (b) L. Crespo, G. Sanclimens, M. Pons, E. Giralt, M. Royo and F. Albericio, *Chem. Rev.*, 2005, **105**, 1663–1681.
- 57 (a) A. Kaiser, N. Gaidzik, T. Becker, C. Menge, K. Groh, H. Cai, Y.-M. Li, B. Gerlitzki, E. Schmitt and H. Kunz, *Angew. Chem., Int. Ed.*, 2010, **49**, 3688–3692; (b) H. Cai, Z.-H. Huang, L. Shi, Y.-F. Zhao, H. Kunz and Y.-M. Li, *Chem.-Eur. J.*, 2011, **17**, 6396–6406.
- 58 S. Bay, S. Fort, L. Birikaki, C. Ganneau, E. Samain, Y.-M. Coïc, F. Bonhomme, E. Dériau, C. Leclerc and R. Lo-Man, *ChemMedChem*, 2009, **4**, 582–587.
- 59 S. Vichier-Guerre, R. Lo-Man, L. BenMohamed, E. Dériau, S. Kovats, C. Leclerc and S. Bay, *J. Pept. Res.*, 2003, **62**, 117–124.
- 60 (a) F. Bélot, C. Guerreiro, F. Baleux and L. A. Mulard, *Chem.-Eur. J.*, 2005, **11**, 1625–1635; (b) L. A. Mulard and A. Phalipon, in *Carbohydrate-based Vaccines*, ed. R. Roy, ACS Symp. Ser., 2008, vol. 989, pp. 105–136; (c) K. Wright, C. Guerreiro, I. Laurent, F. Baleux and L. A. Mulard, *Org. Biomol. Chem.*, 2004, **2**, 1518–1527.
- 61 T. Toyokuni, B. Dean, S. Cai, D. Boivin, S.-I. Hakomori and A. K. Singhal, *J. Am. Chem. Soc.*, 1994, **116**, 395–396.
- 62 V. Kudryashov, P. W. Glunz, L. J. Williams, S. Hintermann, S. J. Danishefsky and K. O. Lloyd, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 3264–3269.
- 63 (a) S. J. Keding and S. J. Danishefsky, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 11937–11942; (b) G. Ragupathi, D. M. Colart, L. J. Williams, F. Koide, E. Kagan, J. R. Allen, C. R. Harris, P. W. Glunz, P. O. Livingston and S. J. Danishefsky, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 13699–13704; (c) Y. S. Cho, Q. Wan and S. J. Danishefsky, *Bioorg. Med. Chem.*, 2005, **13**, 5259–5266.
- 64 (a) O. Renaudet, L. BenMohamed, G. Dasgupta, I. Bettahi and P. Dumy, *ChemMedChem*, 2008, **3**, 737–741; (b) O. Renaudet, G. Dasgupta, I. Bettahi, A. Shi, A. B. Nesburn, P. Dumy and L. BenMohamed, *PLoS One*, 2010, **5**, 1–16; (c) I. Bettahi, G. Dasgupta, O. Renaudet, A. A. Chentoufi, X. Zhang, D. Carpenter, S. Yoon, P. Dumy and L. BenMohamed, *Cancer Immunol. Immunother.*, 2009, **58**, 187–200.
- 65 (a) S. Grigalevicius, S. Chierici, O. Renaudet, R. Lo-Man, E. Dériau, C. Leclerc and P. Dumy, *Bioconjugate Chem.*, 2005, **16**, 1149–1159; (b) O. Renaudet and P. Dumy, *Open Glycosci.*, 2008, **1**, 1–7.
- 66 I. Jeon, D. Lee, I. J. Krauss and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2009, **131**, 14337–14344.
- 67 V. Verez-Bencomo, V. Fernández-Santana, E. Hardy, M. E. Toledo, M. C. Rodriguez, L. Heynngnez, A. Rodriguez, A. Baly, L. Herrara, M. Izquierdo, A. Villar, Y. Valdés, K. Cosme, M. L. Deler, M. Montane, E. Garcia, A. Ramos, A. Aguilar, E. Medina, G. Toraño, 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.
- 68 V. Verez Bencomo, R. Roy, M. C. Rodriguez, A. Villar, V. Fernandez-Santana, E. Garcia, Y. Valdes, L. Heynngnez, I. Sosa and E. Medina, in *Carbohydrate-based Vaccines*, ed. R. Roy, ACS Symp. Ser., 2008, vol. 989, pp. 71–84.
- 69 C.-H. Liang, S.-K. Wang, C.-W. Lin, C.-C. Wang, C.-H. Wong and C.-Y. Wu, *Angew. Chem., Int. Ed.*, 2011, **50**, 1608–1612.
- 70 (a) A. Imbert, Y. M. Chabre and R. Roy, *Chem.-Eur. J.*, 2008, **14**, 7490–7499; (b) Y. M. Chabre and R. Roy, *Curr. Top. Med. Chem.*, 2008, **14**, 1237–1285.
- 71 R. Roy, *Curr. Opin. Struct. Biol.*, 1996, **6**, 692–702.