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Review

Glycopeptide dendrimers, Part III – a review: Use of glycopeptide dendrimers in immunotherapy and diagnosis of cancer and viral diseases

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Abstract: Glycopeptide dendrimers containing different types of tumor associated-carbohydrate antigens (T_N, TF, sialyl-T_N, sialyl-TF, sialyl-Le^x, sialyl-Le^a etc.) were used in diagnosis and therapy of different sorts of cancer. These dendrimeric structures with incorporated T-cell epitopes and adjuvants can be used as antitumor vaccines. Best results were obtained with multiantigenic vaccines, containing, e.g. five or six different TAAs. The topic of TAAs and their dendrimeric forms at molecular level are reviewed, including structure, syntheses, and biological activities. Use of glycopeptide dendrimers as antiviral vaccines against HIV and influenza is also described. Their syntheses, physico-chemical properties, and biological activities are given with many examples.

Keywords: anticancer vaccines; antiviral vaccines; dendrimers; glycobiology; glycocluster; glycoconjugates; glycodendrimers; glycopeptides; glycotope; ligation chemistry; multiple antigen glycopeptides (MAGs); review; synthetic vaccine; tumor associated antigen

INTRODUCTION

This review is a continuation of our earlier reviews [1–3], where the reader will find general information about glycopeptide dendrimers including explanation of many terms. Therefore, we do not explain all terms, syntheses, properties, etc. and the reader is referred to the aforementioned reviews. We included literature since 2000, in special cases earlier. Many topics from the area of oligosaccharides, glycopeptides, and glycopeptide dendrimers have been reviewed: biological and immunological properties of glycopeptides and glycopeptide dendrimers [4–30], synthesis both in solution and on the solid phase including different coupling reagents, resins, protecting groups, and discussion about side reactions [4,6–8,11,12,14,18–20,22,23,27,30–52]; the use of glycopeptides in the immunotherapy of cancer [7,11,14–16,18,20–22,25,27–29,32,53–56] and dendrimeric MRI contrast agents for imaging tumor angiogenesis [57]. Examples of the use of derivatized dendrimers to inhibit viral infection (influenza, HSV1, HSV 2, HIV, Ebola, foot and mouth disease, and sendai) were reviewed [58–61]. Recent progress in bioinspired applications of dendrimers as protein mimics, anticancer or antiviral agents, vaccines, nanomaterials, drug and gene delivery vehicles has been discussed [16,30,62–66].

Scope and Limitations

In comparison with Parts I and II of this review [1–3] we use the term 'dendrimer' in a more free sense, i.e. as a more or less branched, chemically defined and characterized structure with biological and chemical properties, which are not only the sum of the given substructures but create a new quality. In other words, the 'cluster effect' [18,48,67] or 'multivalency' [12,21,68] were the guidelines, together with the branched structure. To choose what, why, and how to write was difficult. In contrast to Part I and Part II, this Part III is ordered first in accordance with the given antigen and activity and then in accordance with the chemical structure (branches, core) of the dendrimer. We also included compounds with interesting structure and activity, independently on the fact that they are glycodendrimers and not glycopeptide dendrimers. The unifying idea is cluster effect. In the Table 3, only entries with clear immunobiological impact and exact data are given.

Abbreviations: Standard abbreviations have been followed throughout this paper (*J. Pept. Sci.*, 2006; **12**: 1–12). Other abbreviations are in the following Table 1. When not stated otherwise, amino acids are of L-configuration and carbohydrates are of D-configuration.

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BIOGRAPHY

Dr. Petr Niederhafner was born in 1966 in Mlada Boleslav, Czechoslovakia. In 1988 he graduated from the Institute of Chemical Technology, Prague. He taught at the secondary school (1990–1996). He carried out research in industry from1996 to 2000, and from 2000 has been at the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, where he is a PhD student (supervisor, Jan Hlavacek, PhD.). His research areas include peptide synthesis on PEG carriers and synthetic vaccines. His hobbies include sports and reading books.

Jan Ježek, PhD., was born in 1951 in Strážnice, Czechoslovakia. In 1974 he graduated from Charles University, Prague. He joined the Institute of Organic Chemistry and Biochemistry, Czechoslovak (from 1993 Czech) Academy of Sciences, Prague in 1978 for the doctoral degree, and was awarded the PhD degree in 1981(for both synthesis and structure–activity studies in the newly established area of muramyl glycopeptides; tutor Dr M. Zaoral, DSc.). The following studies have been carried out abroad: (i) at the Shemyakin Institute of Bioorganic Chemistry, Moscow, with Prof. V.T. Ivanov and T.M. Andronova, PhD. in 1988 on the synthesis of oligosaccharide muramyl peptides and lipoglycopeptides; (ii) at the Rockefeller University, New York, with Prof. R.B. Merrifield on glucagone analogs and Torrey Pines Institute for Molecular Studies, San Diego, with R.A. Houghten, PhD. on the simultaneous multiple peptide synthesis, T-bag method (1989–1990); (iii) at the Institute for Biochemistry and Biophysics, Friedrich Schiller University, Jena, Germany, with Prof. S. Reissmann on bradykinine analogs with backbone-to-backbone cyclization, synthesis and structure–activity studies (1992–1993). His research areas include SPPS, MAPs, MAGs, peptide and glycopeptide dendrimers, coupling reagents, synthetic peptide, and glycopeptide vaccines. His hobbies include postage stamp collection, body building, and minerals.

BIOGRAPHY

Jaroslav Šebestík PhD. was born in 1977 in Ostrava, Czechoslovakia. In 2000, he obtained the MSc. Degree (Organic and Medicinal Chemistry) from the Institute of Chemical Technology, Prague. In 2005, he was awarded the BSc. degree (Teaching of Chemistry) by the Institute of Chemical Technology, Prague. At the end of 2006, he obtained the PhD. degree (Organic Chemistry), working with Dr Jan Hlaváček and Prof. Ivan Stibor from the Institute of Chemical Technology, Prague. Since 2007, he has been a postdoc with Dr Petr Bour, Department of Molecular Spectroscopy, Institute of Organic Chemistry and Biochemistry, AS CR, Prague.

Milan Reiniš, PhD. Born in 1961 in Prague, Czechoslovakia. In 1985 graduated from Charles University, Prague. In 1993 PhD degree at the Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague. Postdoctoral positions: 1995–1997, Laboratory of Immunogenetics headed by Prof. Fabio Malavasi, University Torino, Italy; 2000–2002, HIV Research Laboratory headed by Prof. Barbara Weiser and Prof. Harold Burger; 1997–2000, the National Reference Laboratory on AIDS, Institute of Public Health, Prague; from 2002 onward the Institute of Molecular Genetics, Prague, Laboratory of Tumor Immunology. Current research area: tumor immunoediting, MHC class I–deficient tumors, immunoepigenetics, experimental antitumor vaccines for a therapy of minimal residual tumor disease using animal models for HPV-16–associated tumors.

GLYCOPEPTIDE DENDRIMERS WITH TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS

Tumor-Associated Carbohydrate Antigens (TACAs): Occurrence, Structure and Biological Significance

Many glycoproteins of the outer cell membrane are decisive ligands and receptors involved in biological recognition processes including immunodifferentiation, cell adhesion, cell differentiation, and regulation of cell growth. Glycosylation stabilizes the conformations of glycoproteins, modifies their physicochemical properties, and protects them from proteolysis. The conformation of a glycoprotein has a deep impact on its immunogenicity. Aberrant glycosylation can be associated with autoimmune diseases as well as with cancer. Glycosylation plays a crucial role in protein antigenic properties and conformation [7,9,69–75]. The effects of glycosylation are diverse: glycosylation can 'inactivate' the peptidic epitope or can be required for its reactivity with Ab, depending on the structure of the antigenic site and Ab fine specificity. Cell surface carbohydrates mediate interactions between themselves and other cells and also between cells and antibodies, viruses, bacteria, peptide hormones, toxins

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Table 1 Abbreviations used

ADCC	antibody-dependent cell-mediated cytotoxicity
BHA	benzhydrylamine
CCR5 and CXCR4	the main coreceptors for cellular entry of HIV-1
CD	cyclodextrin
CDC	cell-dependent cytotoxicity
Con A	plant lectin concanavalin A
CTL	cytotoxic T lymphocytes
GSL	glycosphingolipid
HA	hemagglutinin
HAI	hemagglutination inhibition assay
HLA	human leucocyte-associated antigen
HSV	herpes simplex virus
KLH	keyhole limpet hemocyanine
mAb	monoclonal antibody
MHC	major histocompatibility complex
MRI	magnetic resonance imaging
NKR-P1A	natural killer receptor protein 1; activating
NKR-P1B	natural killer receptor protein 1; inhibitory
PADRE	Pan-HLA-DR-binding epitope is a synthetic non-natural T helper epitope of the sequence AKchxAVAAWTLKAAA (chxA = cyclohexylalanine)
Pal	palmitoyl
Pal_3Cys or P_3C	*N*-α-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-L-cysteine
PAMAM dendrimers	poly(amidoamine) dendrimers
PPI dendrimers	poly(propyleneimine) dendrimers
PS Gal 64mer	polysulfated galactose functionalized dendrimer, generation 5
QS-21	*Quillaja saponaria* Molina-based immunological adjuvant; an investigational immune adjuvant
RAFT	regioselectively addressable functional template
SARS	severe acute respiratory syndrome
Sialyl-T_N antigen	NeuNAcα(2 $\rightarrow$ 6)GalNAcα(1 $\rightarrow$ O)Ser/Thr
α-2,3-sialyl-TF antigen	α-D-Neu5Ac(2 $\rightarrow$ 3)β-D-Gal(1 $\rightarrow$ 3)α-D-GalNAc
α-2,6-sialyl-TF antigen	β-D-Gal(1 $\rightarrow$ 3)[α-D-Neu5Ac(2 $\rightarrow$ 6)]α-D-GalNAc
SLe^a	neolactoseries antigens sialyl-Lewis a
SLe^x	neolactoseries antigens sialyl-Lewis x
SPGS	solid phase glycopeptide synthesis
SPR	surface plasmon resonance
TAAs	tumor-associated antigens
TACAs	tumor-associated carbohydrate antigens
TF antigen	Galβ(1 $\rightarrow$ 3)GalNAcα(1 $\rightarrow$ O)Ser/Thr
T_N antigen	GalNAcα(1 $\rightarrow$ O)Ser/Thr
TT	tetanus toxin

etc. [9]. Glycoconjugates expressed predominantly on cancer cells which represent TAAs are called tumor-associated glycopeptide antigens (TAGAs) or tumor-associated carbohydrate antigens (TACAs) (Figure 1(a) and (b)) [11,14,15,19–22,25,28,29,51,56,74,76–78]. We will use the term TACAs. They are of two main classes: *O*-glycopeptides containing carbohydrate antigens [T_N, TF, sialyl-T_N, sialyl-TF, α(2 $\rightarrow$ 6)sialyl-TF, α(2 $\rightarrow$ 3)sialyl-TF, KH-1, Globo-H (= MBr 1) [51,78–80], GM1, fucosyl-GM1 [80,81], GM2, GM3, GD2, GD3 [11,15,20–22,25,74,75,82,83], Le^y [15,80] (Figure 1(a))] and *N*-glycopeptides containing carbohydrate antigens (sialyl-Le^x, sialyl-Le^a) (Figure 1(b)). In *O*-glycopeptides, the sugars are bound to OH group in the side chain of Ser or Thr. In *N*-glycopeptides containing carbohydrate antigen, the sialyl-Le^x and sialyl-Le^a are bound to the amide group of Asn.

Carbohydrates are clinically relevant antigens of those tested and subsequently developed for vaccines against infectious diseases [51,53,54]. Unfortunately, many of the defined TACAs are really altered 'self' antigens and the body does not react to them immunologically. The development of anticancer vaccines is therefore more difficult compared with those for infectious diseases owing to the difficulty connected with breaking the body's immunological tolerance to the antigen. In comparison with bacterial antigens, the TACAs lack the inherent immunogenicity.

TACAs are not only tumor markers but constitute essential machinery to induce tumor metastasis and

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invasiveness [25,75,77,84]. Tumors expressing a high level of certain types of TACAs exhibit greater metastasis and progression than those expressing low level of TACAs, as reflected in decreased patient survival rate. Well documented examples of such TACAs are: Globo-H/Le^y/Le^a in primary non–small cell lung carcinoma; SLe^x and SLe^a in various types of cancer; T_N and sialyl-T_N in colorectal, lung, breast, and many other cancers; GM2, GD2, and GD3 gangliosides in neuroectodermal tumors; Globo-H in breast, ovarian,

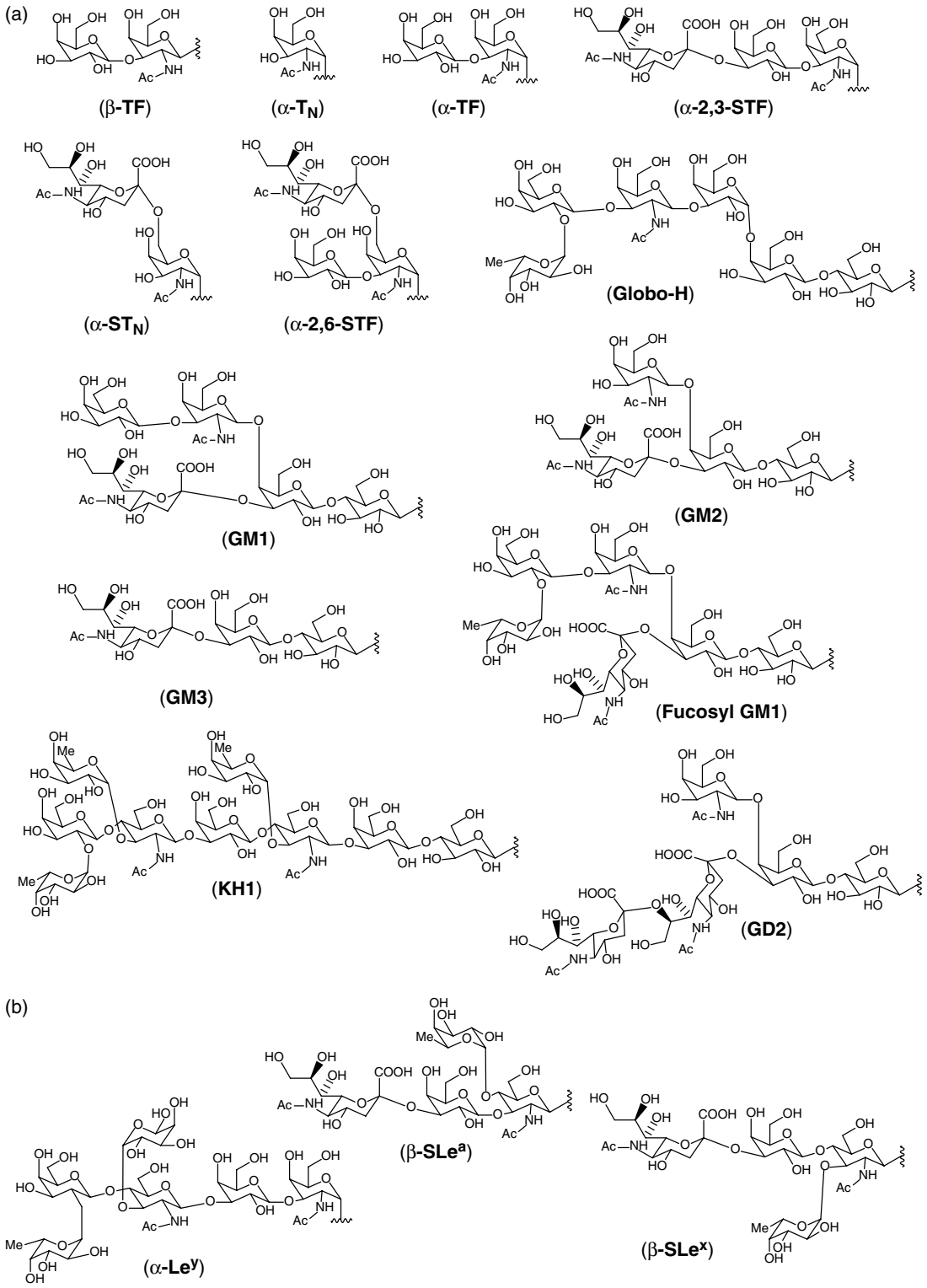

Figure 1 Examples of tumor-associated carbohydrate antigens [11,15,20–22,25,51,74,75,78–83] (a) *O*-glycopeptides (b) *N*-glycopeptides.

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and prostate cancer, etc. For details see Table 2. Not all TACAs affect tumor malignancy, and anticancer vaccines should target the TACAs that do affect malignancy. Some glycosylation and TACAs suppress invasiveness and metastatic potential. Well documented examples are: blood group A antigen in primary lung carcinoma, bisecting $\beta(1 \rightarrow 4)$GlcNAc of *N*-linked structure in melanoma and other cancers, and galactosylgloboside (GalGb4) in seminoma. Seminoma is a rare cancer in adults, but it is a major type of urogenital cancer in infants and children. Anticancer vaccines have to fulfill the following criteria for success [21,84,85]: (i) the antigen is expressed highly on tumor cells; (ii) high Ab production depends on clustering of the antigen used in the vaccine and choice of appropriate carrier and adjuvant; (iii) high T-cell response is specific against tumor antigens; (iv) expression of the same antigen in normal epithelial tissues must not pose a major obstacle, i.e. these tissues must not be damaged during anticancer vaccine-induced immune response. Unfortunately, there are some daunting obstacles [21]. Effective antitumor immune responses can be hampered not only because of the fact that tumor antigens are in general auto- or self-antigens but also due to the local or systemic immunosuppression. Further, antigens that could serve as efficient immunogens are typically masked by the many auto- and self-antigens surrounding them on the cell surface. Another complication stems from the heterogeneity of the TACAs, even within a particular cancer type. 'Any approach to the treatment and prevention of cancer must face the daunting reality that each cancer may be as individual as the patient in whom it has evolved' [86].

Both humoral and cellular arms of the immune system can play a role in specific antitumor immune responses elicited by TACAs. Tumor cells can be eradicated by mechanisms based on induction of Ab responses, mainly of IgG and IgM production, by complement-dependent cytotoxicity or ADCC [29]. T_h cells are important for both Ab production and for CTL-mediated cytotoxicity. There is a mounting evidence that not only peptides but also carbohydrate moieties of TACAs are able to serve as CTL-epitopes and CTLs specific for carbohydrate structures are able to lyze the tumor cells [87,88]. However, the mechanisms of carbohydrate antigen processing and presentation in the context of MHC class I molecules is not yet fully understood.

Another way for development of T- and B-cell dependent type responses to carbohydrate antigens is the use of peptide or polypeptide mimetics of carbohydrate antigens [76]. It has been demonstrated that conversion of carbohydrate epitopes to peptides could enable effective antigen presentation and development of peptide-specific T-cells capable of also binding carbohydrate antigens [89]. Immunizations with these peptide mimotopes, which can be formulated in a variety of ways [multiple antigen peptides (MAPs), DNA vaccines], induce carbohydrate crossreactive humoral and cellular responses. These improvements in the area of carbohydrate-based vaccines bring new dimensions in anticancer vaccine development.

Table 2 Carbohydrate cancer cell-surface targets for immune attack [28,29,53,54]

	Carbohydrate cancer cell-surface targets for immune attack
Tumor	Antigens[b]
Neuroectodermal cancers	
Melanoma	GM2, GD2, GD3L, GD3
Neuroblastoma	GM2, GD2, GD3L, GD3, polysialic acid
Sarcoma	GM2, GD2, GD3L, GD3
Small-cell lung cancer	GM2, fucosyl GM1, polysialic acid, globo-H, SLe^a
Epithelial cancers	
Breast	GM2, globo-H, Le^y, TF, T_N, ST_N, SLe^a
Prostate	GM2, T_N, ST_N, TF, Le^y
Ovary	GM2, globo-H, ST_N, TF, Le^y
Colon	GM2, ST_N, TF, Le^y, SLe^a, T_N
Stomach	GM2, Le^y, SLe^a, Le^a
Lung	GM2, globo-H, Le^y

[b] Antigens present on at least 50% of cancer cells in at least 60% of biopsy specimens

TACAs were discovered first in mucins [11,22,27,75, 90,91]. Chemically, mucins are excessively *O*-glycosylated proteins found on the surface of various types of epithelial cells. Mucins contain domains of tandem repeat sequences rich in serine, threonine, and proline. Mucin core structures, namely, core-1 to core-8 are given in Figure 2 [27]. The protein backbone in normal tissue carries complex oligosaccharides derived from glycan core structures. Characteristic feature is GalNAc unit α-*O*-linked to serine or threonine. The expression of mucins in tumor cells is usually increased, and the carbohydrate side-chains are altered due to incomplete glycosylation and premature sialylation. The incompletely formed glycans change and influence the conformation of the peptide chain (and vice versa) and thus form new tumor-specific glycopeptide epitopes. Both the peptide and the carbohydrate portion of the glycoprotein contribute to changed immunological behavior in comparison with a healthy tissue. Therefore, TACAs play an important role in cancer diagnosis [75] and preparation of semisynthetic or synthetic anticancer vaccines [1,2,4,11,14,21,22,25,27–29,32,51,53–55,77,92–99].

TACAs, which are overexpressed in clusters at the malignant cell surface, represent targets for epithelial cancer immunotherapy but their weak immunogenicity

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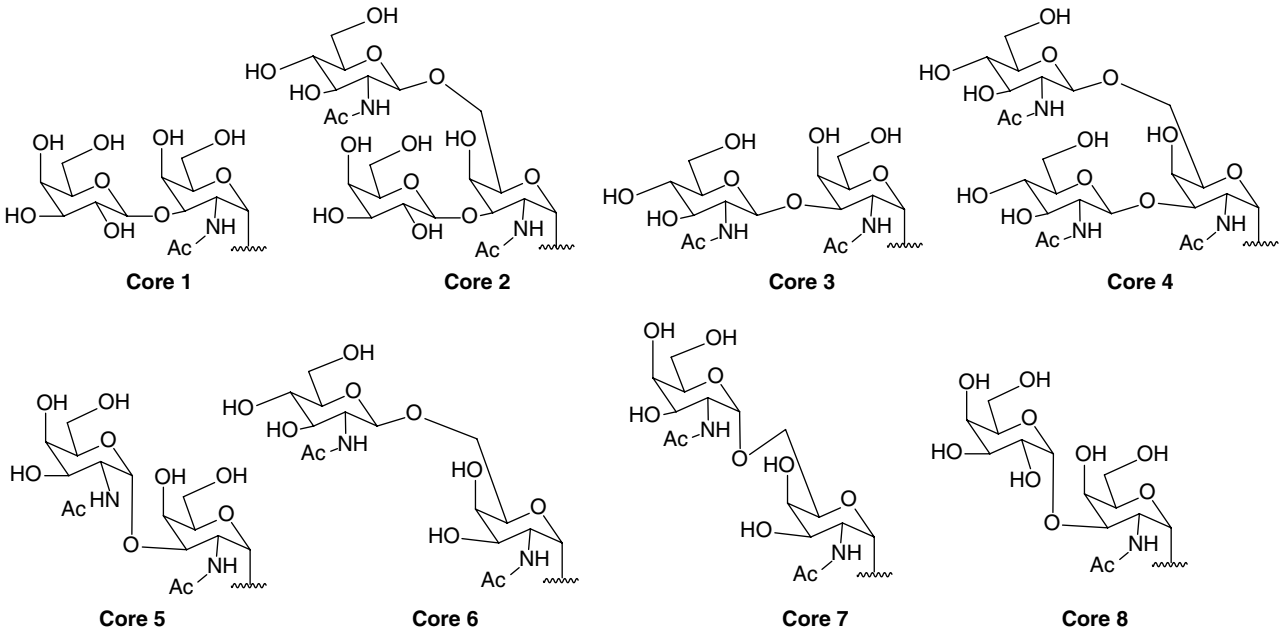

Figure 2 Core structures of mucin-type *O*-linked glycans [27].

is a limiting factor for the design of efficient synthetic anticancer vaccines. In the first generation of vaccines, TACAs were conjugated to an immunogenic carrier protein such as KLH, BSA, or ovine serum albumin (OSA). These conjugates have significant limits: low level of the desired antibodies, irrelevant response against the carrier, undefined composition of the molecule, and low molecular ratio of the antigens over the carrier protein.

In spite of the striking development of gene techniques, genetic production of glycopeptides or glycoproteins is still limited. Consequently, chemical synthesis is the best way of supplying these compounds in large amounts and in defined structures. Chemical syntheses of tumor antigens are therefore of utmost importance for the study and diagnostics of tumor processes and for the preparation of antitumor vaccines with the aim of suppressing tumor growth.

Next generation of fully synthetic vaccines based on dendrimeric lysine scaffolds, namely MAPs or multiple antigen glycopeptides (MAGs), have far better qualities. MAPs and MAGs have been used as nonimmunogenic carriers for B-cell antigens and T-cell helper peptides. The advantages of MAPs and MAGs are defined chemical composition, reproducible purity, and high density of clustered antigens at the surface of a small peptidic scaffold. These compounds elicit a strong, reproducable, and specific Ab response.

The role of lipids and carbohydrate-based adjuvants and carriers for the preparation of synthetic carbohydrate (glycopeptide) containing vaccines have been reviewed elsewhere [51,85,100].

The topic of TACAs in general, their occurrence, structure, synthesis, physicochemical and immunobiological properties, and development of synthetic antitumor carbohydrate vaccines and their activities have been reviewed [1,4,11,14,15,21,22,25,27–29, 31–33, 42,44,51,53–55,75,76,78,83,84,96–99,101, 102].

Tumor-Associated Antigens of the T_N Type

The biological activities of glycopeptide dendrimers are summarized in Table 3. Different antigen constructs and carrier molecules for augmenting the immunogenicity of T_N type TACAs have been compared. Nakada *et al.* [103] have shown that the essential epitopic structure of T_N antigen recognized by a T_N-specific IgG3 mAb, MLS 128 is a cluster composed of three or four consecutive GalNAc-serines/threonines. Cancer mucins may have repeating sequences of two serines or threonines as in the case of MUC1 tandem repeats, or four or five serines or threonines as in the case of MUC2 tandem repeats. These clusters can be mimicked by serine or threonine trimers with GalNAc1-α-*O*-linked to each amino acid [104]. Vaccination with (sialyl-T_N)$_c$-KLH plus a QS-21 adjuvant is the most effective approach for inducing antibodies against sialyl-T_N-positive cancer cells in breast cancer patients [105].

Linear glycopeptides containing T_N antigen (mono-, Ser* Thr* Thr*-, or hexa-T_N antigen; $* = T_N$ antigen) and $CD4^+$ T-cell epitope [polio virus (PV) or tetanus toxin (TT] were synthesized [110]. The $(T_N)_3$ and $(T_N)_6$ glycopeptides were recognized by MLS 128. The antigenicity is not altered by the position of the tri-T_N motif in the peptide sequence. The glycopeptides induced high titers of anti-T_N antibodies in mice, in the absence of a carrier molecule. The antibodies generated in mice recognized the native T_N antigen on human

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Table 3 Biological activities of glycopeptide dendrimers

Tumor-associated carbohydrate antigens	[11,28,29,53,54,74, 78,96–99,106]
T_N antigen	[11,32]
Tetravalent MAGs containing dimeric T_N antigen [Ac-$(T_N)_2$-γ-Abu]$_4$-(Lys-X)$_2$-Lys-β-Ala (1: X = 0; 2: X = γ-Abu) and [Ac-$(T_N)_2$-γ-Abu]$_8$-(Lys-X)$_4$-(Lys-X)$_2$-Lys-β-Ala (3: X = 0; 4: X = γ-Abu), immobilized on biocompatible TentaGel S NH_2 support were synthesized. Rosetting tests of **1**, **2**, **3**, and **4** showed positive reactions with anti-T_N (DAKO) and T_N+ erythrocytes, with anti-T_N/A (BRIC 66) and T_N+ and A erythrocytes, other combinations were negative. In all the animals immunized with **4**, a remarkable increase in the level of anti-T_N (titer 2000–64 000, score 105–167) and no change of anti-A levels (titer 8, score 13–17) was found. Immunized mice did not exhibit any sign of adverse reaction to the administered conjugates.	[107]
(S*KLFAVWKITYKDT)$_4$-MAG was able to induce T_N-specific Abs in a T-cell-dependent manner. A therapeutic immunization performed with this MAG was shown to increase the survival of tumor-bearing mice.	[108,109]
Glycopeptides containing T_N antigen (tri-, or hexa-T_N antigen) and $CD4^+$ T-cell epitope [poliovirus (PV) or tetanus toxin (TT)] were recognized by MLS 128, a T_N-specific mAb. The antigenicity is not altered by the position of the tri-T_N motif in the peptide sequence. The glycopeptides induced high titers of anti-T_N Abs in mice, in the absence of a carrier molecule. The generated antibodies recognized the native T_N antigen on cancer cells. The results show that short synthetic glycopeptides are able to induce anticancer Ab response.	[110]
In both prophylactic and therapeutic vaccinations, (Ser*Thr*Thr*-KLFAVWKITYKDT)$_4$-Lys$_2$-Lys-β-Ala-OH (Figure 3) afforded good protection against the development of T_N-expressing tumor cells. All nonvaccinated mice died within 30 days after tumor challenge. 80% of mice vaccinated three times with [$(T_N)_3$-PV]$_4$-MAG with alum survived the tumor challenge, whereas 10% of mice survived in control groups receiving $(PV)_4$-MAP mixed with alum.	[108,111,112]
(H-S*S*S*G)$_2$KAKchxAVAAWTLKAAAβ-AlaYLSGANLNL (T_N6–PAD) was prepared as a model of fully synthetic antitumor vaccine. The T_N6-PAD induced carbohydrate-specific Abs (IgG and IgM) in both outbred and HLA-DR transgenic mice and these Abs reacted *in vitro* with the native carbohydrate antigens expressed on the surface of human tumor cells. In both types of mice, the PADRE provides efficient T-cell help for carbohydrate-specific Ab response. OF_1 mice immunized with T_N6-PAD developed T_N-specific IgG Abs. The binding of T_N6-PAD-induced Abs from mouse serum to human Jurkat T-lymphoma cells that express the T_N antigen, was studied. Positive binding of Abs from mice vaccinated with T_N6-PAD was detected.	[82]
The immunogenicity of (H-hSer*hSer*hSer*-KLFAVWKITYKDT)$_4$-K$_2$-K-β-Ala-OH was analyzed in BALB/c mice. Immunization with the MAG induced specific IgG Abs, which recognized the $(hSer*)_3$ cluster. When injected in mice, the MAG induces a strong Ab response, which recognizes native TACAs at the surface of human tumor cells. The incorporation of non-natural T_N analogs will prolong their biological half-life and protect them from *in vivo* degradation. These results open new perspectives for synthetic anticancer vaccine development.	[113]
The influence of glycosylation of the immunodominant DTR motif of MUC1 on its antigenicity was studied using synthetic glycopeptides A(HGVTSAPDT*RPAPGSTAPPA)$_n$ where $n = 1–5$. Binding patterns of DTR-specific Abs reveal a glycosylation-conditioned tumor-specific epitope of the epithelial mucin MUC1. Biological functions of epithelial mucins are deeply influenced by glycosylation.	[73]
RAFT scaffolds presenting four T_N (B-cell epitope) and one or two $CD4^+$ helper T-cell peptides from the type 1 PV were investigated *in vitro* by ELISA and T-cell stimulation assays. The Abs elicited by immunization of mice with RAFT(4GalNAc,1PV) and RAFT(4GalNAc,2PV) constructs recognize the native form of T_N epitope expressed on human tumor cells.	[114]
Enzymatic large-scale synthesis of MUC6-T_N glycoconjugates was elaborated using UDP-*N*-acetylgalactosamine: polypeptide *N*-acetylgalactosaminyltransferases (ppGalNAc-Ts). The MUC6-T_N glycoconjugates were recognized by two anti-T_N mAbs that are specific to human cancer cells. Besides, the MUC6-T_N glycoconjugate glycosylated using MCF7 extracts as the ppGalNAc-T source induced IgG Abs that recognized a human tumor cell line.	[115]
TF-antigen	[11,22,32,101]
Water-soluble oligoacrylamide-bound TF-antigen glycoconjugates with a high degree of lipophilicity were studied by solid-phase microtiter plate assay with mouse mAb FAA-J11 (IgG3). Increased	[116]

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Table 3 (*Continued*)

lipophilicity of ligands (from ammonia to propylamine) yielded higher degree of adsorption to the surface of the microtiter plates. The relative lipophilicity was also determined by measuring the radioactive count following treatment of the coated TF-antigen polymers with tritium-labeled UDP-GlcNAc substrate and core 2-$\beta(1 \rightarrow 6)$GlcNAc transferase. As the lipophilicity increased, the amount of radioactivity steadily increased. These results support the above data using ELISA, although they were more sensitive.	
Le^y	
Synthetic Le^y-bearing glycopeptides mimicking cell surface Le^y mucin glycoprotein architecture (Figure 7) have been tested as anticancer vaccine candidates. ELISA was used to determine the reactivities of tris(hexasaccharide) construct **5** with anti-Le^y Ab 3S193. The α-O-linked hexasaccharide had reactivity comparable to that of the Le^y-ceramide control. Mice vaccinated with **5** (as an emulsion in Intralipid), without additional adjuvant, responded by a strong IgM immune response. These Abs strongly bind to natural mucin-related Le^y and Le^y-ceramide. The biological results 'demonstrate that a realistic immunogenic mimic of a tumor-associated cell surface-displayed Le^y mucin has been achieved by total synthesis'.	[93,117]
Re-examination of the clustered Le^y-serine Pal$_3$-Cys conjugate with the QS-21 adjuvant resulted in the identification of both IgG and IgM Abs reacting with tumor cells. This demonstrates the feasibility of an entirely synthetic carbohydrate-based anticancer vaccine in an animal model.	[118]
Globo-H	
A phase I trial study of Globo-H-KLH conjugate with adjuvant QS-21 was done with 27 metastatic breast cancer patients. Vaccination of breast cancer patients was able to stimulate moderate IgM Ab titers in the majority of participants. IgG reactivity was stimulated to a lesser extent. Significant binding of the patients IgM antibodies to MCF-7 tumor cells was observed in 16 of the 27 patients by FACS analysis. IgG Ab reactivity was found in only 3 of 27 patients. The encouraging result is that nine patients demonstrated increased CDC because potential antitumor mechanisms of antibodies involve binding to the tumor cell surface and induction of complement-mediated lysis. Evidence of ADCC was observed in 8 of 27 patients.	[119]
Multiantigenic carbohydrate-based anticancer vaccines	[28,29,53,54,78,80, 94–99,120]
Synthetic glycopeptides containing TF, Le^y, and T$_N$ (**8**) and Globo-H, Le^y, and T$_N$ (**9**), respectively (Figure 9), were conjugated to KLH giving conjugates **10** and **11**, respectively. Mice were immunized with these constructs as free glycopeptides (**8** or **9**) or as KLH conjugates (**10** or **11**). QS-21 or the related GPI-0100 were used as adjuvants. Both KLH conjugates induced IgG and IgM Abs against each carbohydrate antigen used. The unnatural Globo-H-Le^y-T$_N$-conjugate (**11**) was more immunogenic than the mucin-based TF-Le^y-T$_N$-conjugate (**10**). The GPI-0100 adjuvants induced higher Ab titers than QS-21.	[94]
Penta- and hexavalent vaccines (Figure 8(b)): a defined molecule containing a glycopeptide, carrying several different antigens is selectively bound to a suitable carrier. This approach reflects the heterogeneity of TACAs present on the surface of transformed cells. The created antibodies react more strongly and have higher therapeutic value than a mixture of monovalent vaccines.	[54,78,96–98]
Viruses	[16,60,61,106]
HIV	[16,59,61,121,122]
Polysulfated galactose- functionalized PPI dendrimer (PS Gal 64mer) (Figure 13) inhibited HIV-1 infection of cultured indicator cells as effectively as a known inhibitor dextran sulfate. Neither the PS Gal 64mer nor dextran sulfate is cytotoxic at the highest concentration tested (3 mg/ml).	[123,124]
Synthetic bis-(triantennary undecasaccharide) **16** bound to gp-120 fragment (Figure 12) competes with gp120 for binding to 2G12, supporting the idea that the dimeric glycopeptide binds to 2G12 mimicking the clustered gp120 epitope.	[125]
Fully synthetic carbohydrate HIV antigens designed on the logic of the 2G12 Ab were prepared. Man$_9$GlcNAc$_2$-NH$_2$ was bound to a RAFT containing suitably placed Asp residues. These glycopeptides were bound to the purified outer membrane protein complex from *Neisseria meningitidis*. The ability of some of the conjugates to bind 2G12 was assessed by ELISA sandwich assay. They showed a clear response, although it was not as robust as that of the positive control, gp160. These multivalent glycopeptide constructs do not have any relation to a native gp120 sequence, yet still mimic gp120's binding to 2G12.	[126]

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Table 3 (*Continued*)

Glycopeptides GlcNAc-C34, LacNAc-C34, and Man_9-$GlcNAc_2$-C34 (bound to C34 via Asn^{637}) (Figure 10) showed potent inhibitory activities against HIV-1 infection at nanomolar concentrations. The presence of di- or undecasaccharide in the molecule of C34 leads to a much better solubility and also higher resistance to protease digestion. The conserved glycan at Asn^{637} profoundly affects the six α-helix-bundle formation essential for viral membrane fusion.	[69]
$(Man_9$-$GlcNAc_2$-$Asn)_3$-cholic acid cluster demonstrated a 46-fold enhancement in 2G12-binding over the triantennary Man_9-$GlcNAc_2$-Asn. The IC_{50} values for the $(Man_9$-$GlcNAc_2$-$Asn)_3$-cholic acid cluster and Man_9-$GlcNAc_2$-Asn were 21 and 960 μM, respectively.	[16,127]
$(Man\alpha$-O-$(CH_2)_3$-$CO)_8$-Lys_4-Lys_2-Lys-gp41(541–555) conjugate elicited polyclonal antibodies (titer 1 : 4000) in rabbit. Immune response of the corresponding KLH conjugate and MAP was much weaker.	[128]
The targeting potential and anti-HIV activity of lamivudine (3TC) loaded mannosylated G5 poly(propyleneimine) (MPPI) dendrimers were investigated. The 3TC loaded MPPI was found to possess superior anti-HIV activity compared to that of free drug and G5 PPI based formulation.	[129]
The influence of G2-G5 PAMAM dendrimers on the inhibition of Tat peptide/trans-acting responsive element (TAR) RNA binding in HIV-1 transcription was studied.	[130,131]
Influenza	[132]
A series of cyclic peptides, cyclo(SerGlyGlyGlnSerHisAsp)$_3$ (**17**) cyclo(GlySerSerGlnSerSerGly)$_3$ (**18**) and cyclo(GlySerGlnSerSerGly)$_3$ (**19**), mono- (**17m, 18m, 19m**), bi- (**17b, 18b, 19b**), and tri-substituted (**17t, 18t, 19t**) with sialotrisaccharide units, Neu5Acα(2 → 3)Galβ(1 → 4)Glc (Figure 14) were tested as inhibitors of virus-induced hemagglutination. Only **17t** and **17b** showed potent inhibitory effects. The binding assay between the HA protein and cyclic glycopeptides was studied by SPR. Only **17t** (K_d 0.63 mM) and **17b** (K_d 1.6 mM) showed significant affinity. Compound **17t** had multidentate effect.	[16,133]
Sialic acid (SA) in multiple form, conjugated to linear polyacrylamide backbone is more effective than monomeric SA at inhibiting influenza-induced agglutination of red blood cells. Spheroidal, linear, linear-dendron, comb-branched and dendrigraft polymeric inhibitors were evaluated for the ability to inhibit virus hemagglutination and to block infection of mammalian cells *in vitro* with various strains of influenza A and sendai. Linear dendron copolymers were 1025–8200-fold more effective against H2N2 influenza, X-31 influenza, and sendai viruses. Comb-branched and dendrigraft inhibitors were the most effective with 50 000-fold increased activity. These dendrimers were not cytotoxic at therapeutic levels.	[134]
Dendronized chitosan-SA hybrids were prepared as model compounds for the study of inhibition of viral pathogens including the influenza virus.	[135–139]
PAMAM dendrimer G4, conjugated with SA (G4-SA) was tested as a means to prevent adhesion of three influenza A subtypes (H1N1, H2N2, and H3N2). Hemagglutination-inhibition assays have shown G4-SA to inhibit all H3N2 and three of five H1N1 influenza subtype strains. *In vivo*, G4-SA completely prevented infection by a H3N2 subtype in a murine influenza pneumonitis model.	[140]
PAMAM dendrimers containing 4, 8, 16, 32, and 64 *N*-acetylneuraminic acid residues were synthesized and their ability to inhibit the HA-mediated adhesion of influenza virus was studied. Biological activity of PAMAM dendrimers was lower than that of multivalent glycoconjugates based on other types of synthetic polymeric carriers. Conformational analysis (molecular dynamics) revealed the tendency of the PAMAM chains toward compaction and formation of dense globules. This can affect the ability to effectively bind the polyvalent carbohydrate-recognizing proteins.	[141]
Carbosilane dendrimers uniformly functionalized with α-thioglycoside of SA were synthesized and tested as a new class of influenza neuraminidase inhibitors.	[142]
Cancer	
Cancer diagnosis and therapy with dendrimeric drugs.	[57,106,143–150]
Adjuvants used to augment immune response of synthetic and semisynthetic vaccines	[78,85,100,150–156]
QS-21 is a purified fraction from the bark extract of the *Quillaja saponaria* tree which has immunoadjuvant activity. QS-21 is currently under investigation as an adjuvant for use in humans.	[29,51,53,54,80,94, 97,105,118–120, 150,157–162]
GPI-0100 is a semisynthetic *Quillaja* saponin analog with modifications designed to augment stability and diminish toxicity.	[29,53,94,150,160]

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Table 3 (*Continued*)

ISCOMATRIX: ISCOMATRIX adjuvant stimulates both cellular and humoral immune responses to endogenously processed target antigens and hence is the preferred adjuvant when CTL responses are desirable. ISCOMATRIX adjuvant is comprised of a highly purified saponin fraction from the bark extract of the *Quillaja saponaria* tree (ISCOPREP), combined with phospholipid and cholesterol.	[163]
Pal_3Cys-Ser or Pal_3Cys [164,165] (Figure 4) is often used as adjuvant in antitumor vaccines.	[93,97,118,157,166, 167]
Muramyl glycopeptides and glycolipopeptides are immunomodulators originating from bacterial cell walls. About 2000 derivatives were synthesized and tested for immunoadjuvant activity.	[168,169]

Jurkat cancer cell line. The results show that short synthetic glycopeptides are able to induce anticancer Ab response.

Oligosaccharidic TACAs (GM2, GD2, Globo-H, Le^y, sialyl-T_N) can display large glycotopic structures available for Ab binding sites. In contrast, T_N, which is a monosaccharidic antigen (α-D-GalNAc-Ser/Thr), is recognized by different mAbs as a T_N cluster. It should be advantageous to mimic naturally occurring T_N structures on the surface of cancer cells. Therefore the same group [111] introduced a cluster of three T_N into the MAG. They presented results of both prophylactic and therapeutic vaccinations using a $[(T_N)_3\text{-PV}]_4$-MAG carrying four copies of the PV peptide further extended with tri-T_N glycotope (Figure 3) [108]. In both prophylactic and therapeutic vaccinations, (Ser*-Thr*-Thr*-KLFAVWKITYKDT)$_4$-Lys_2-Lys-β-Ala-OH (*is T_N antigen) afforded good protection against the development of T_N-expressing tumor cells. The authors verified the capacity of $[(T_N)_3\text{-PV}]_4$-MAG induced Abs to target and reject the highly tumorigenic TA3/Ha adenocarcinoma *in vivo*. Mice were left untreated or were vaccinated three times with 10 µg of $(PV)_4$-MAP or $[(T_N)_3\text{-PV}]_4$-MAG in alum. Then 10 days after the last boost, mice were challenged with 1000 TA3/Ha cells, and survival of mice to the TA3/Ha graft was followed for 100 days. All nonvaccinated mice died within 30 days of tumor challenge. Of the mice vaccinated three times with $[(T_N)_3\text{-PV}]_4$-MAG and alum, 80% survived the tumor challenge, whereas 10% of mice survived in control groups receiving $(PV)_4$-MAP mixed with alum. Using direct ELISA, the MLS 128 Ab can very efficiently bind to the $[(T_N)_3\text{-PV}]_4$-MAG compared with the $[(T_N)\text{-PV}]_4$-MAG carrying a monomeric T_N. To ensure that T_N-specific Abs induced by the $[(T_N)_3\text{-PV}]_4$-MAG can recognize native T_N structures, the authors analyzed by flow cytometry the binding of sera from $[(T_N)_3\text{-PV}]_4$-MAG-immunized mice to tumor cells. The human Jurkat cells were well recognized by Abs induced after immunization with $[(T_N)_3\text{-PV}]_4$-MAG, but not with [(STT)-PV]$_4$-MAG (without sugar). The above data show that a MAG containing a TACA, together with an appropriate $CD4^+$ T-cell epitope is highly immunogenic and can efficiently allow rejection of implanted tumor cells when used in therapeutic treatment. The introduction of glycotope clusters into MAG immunogen is important for the induction of a strong, long-lasting antitumor response.

Livingston *et al.* [170] have compared various T_N constructs and carriers for augmenting the immunogenicity of the ephithelial cancer antigen T_N. Antigens were covalently attached to KLH or TentaGel resin beads using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester, which couples the free cysteine sulfhydryl groups of the antigen to the amino groups of the carrier. The authors demonstrated that among the tested T_N conjugates, trimers $(T_N)_3$ are consistently a better form of T_N for induction of antibodies against two sources of naturally expressed T_N (desialylated ovine submaxillary mucin and tumor cells, respectively). Results have shown that conjugation to KLH is more effective than conjugation to BSA or to TentaGel beads for ELISA test. MUC1 glycosylated at three sites per tandem repeat and conjugated via Cys to KLH (APRT*DPAS*T*VGHAPPAT*S*GPAPRT*DPAS*T*VGH-Cys-linker-KLH) produced significantly higher Ab titers than the T_N(c)-KLH conjugate, and also produced higher Ab titers against MUC1 than unglycosylated MUC1-KLH. The other result is not surprising: mix of $(T_N)_3$ and KLH is significantly less effective than T_N(c)-KLH conjugate. On the basis of the aforementioned data, most immunization protocols for antitumor vaccine development [25] were done with KLH as a carrier.

MAGs containing dimeric T_N antigen $[\text{Ac-}(T_N)_2\text{-}\gamma\text{-Abu}]_4$-(Lys-X)$_2$-Lys-$\beta$-Ala (**1**: X = 0; **2**: X = γ-Abu) and $[\text{Ac-}(T_N)_2\text{-}\gamma\text{-Abu}]_8$-(Lys-X)$_4$-(Lys-X)$_2$-Lys-$\beta$-Ala (**3**: X =

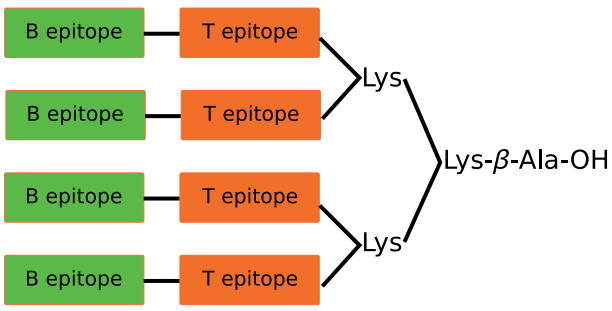

Figure 3 Schematic representation of T_N-PV-MAG molecule containing B- and T-cell epitopes [108]. This figure is available in colour online at www.interscience.wiley.com/journal-/jpepsci.

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0; **4**: X = γ-Abu), immobilized on biocompatible Tenta Gel S NH_2 support were prepared by SPPS [107]. Rosetting tests of **1**, **2**, **3,** and **4** showed positive reactions with anti-T_N (DAKO) and T_N+ erythrocytes, with anti-T_N/A (BRIC 66) and T_N+, and A erythrocytes; other combinations were negative. In all the animals immunized with **4**, we found a remarkable increase in the level of anti-T_N (titer 2000–64 000, score 105–167) and no change of anti-A levels (titer 8, score 13–17). Immunized mice did not exhibit any sign of adverse reaction to the administered conjugates. The biological activities of these synthetic T_N antigen conjugates (good availability for the immunological interactions, highly specific immunogenicity, good biological tolerance) together with their precise chemical characterization seem to be a promising approach to preparation of antitumor vaccine and affinity purification of anti-T_N antibodies.

Lo-Man [108,109,112] and his colleagues have shown that a MAG containing a monosaccharidic T_N motif was able to induce T_N-specific Abs in a T-cell-dependent manner. They used tetravalent lysine MAG. To each arm, a $CD4^+$ T-cell epitope (PV peptide: KLFAVWKITYKDT sequence from the PV type 1) with a single serine T_N residue at the NH_2 terminus was linked. A therapeutic immunization performed with this MAG was shown to increase the survival of tumor bearing mice and induced tumor specific antibodies in nonhuman primates that could mediate ADCC against human tumor cells [112].

The amino acid carrying the T_N antigen (Ser or Thr residues) is important to the Ab recognition [113]. Different mAbs specific for the Ser*Ser*Ser* or Ser*Thr*Thr* sequence (* is T_N antigen) have been obtained. To lay more emphasis on the importance of the amino acid carrying the sugar antigen, (H-hSer*hSer*hSer*-KLFAVWKITYKDT)$_4$-K_2-K-β-Ala-OH has been prepared by SPGS. The T_N building block has been used with free OH groups, as Fmoc-hSer*-OPfp. After detachment from the Wang resin, the tetravalent MAG was purified by RPHPLC and characterized by amino acid analysis and electrospray MS. The immunogenicity was analyzed in BALB/c mice. Sera from immunized mice were tested by ELISA against a biotinylated synthetic hSer* cluster coated on streptavidin plates. Nonglycosylated analog was used as a control for background reactivity. Immunization with the MAG induced specific IgG antibodies, which recognized the (hSer*)$_3$ cluster [113]. When injected in mice, the MAG induces a strong Ab response, which recognizes native TACAs at the surface of human tumor cells. The incorporation of non-natural T_N analogs will prolong their biological half-life and protect them from *in vivo* degradation. For other T_N antigen derivatives with hSer, Orn, Lys, and hCys, see Ref. [11] and the references cited therein. These results open new perspectives for synthetic anticancer vaccine development.

(H-S*S*S*G)$_2$KAKchxAVAAWTLKAAAβ-AlaYLSGANLNL (T_N6-PAD), (H-SSSG)$_2$KAKchxAVAAWTLKAAAβ-AlaYLSGANLNL (PAD), (H-S*S*S*G)$_2$KGGGGGGK(Biot)G (T_N6-PEP) and S*S*S*GGGGGGK(Biot)G (T_N3-PEP) (* is T_N antigen) have been prepared [82] by SPGS using free Fmoc-Ser *-OPfp. For other examples of incorporation of unprotected saccharide, see Refs 111,109,113. The T_N6-PAD molecule contains six T_N antigens (i.e. two tri-T_N glycotopes), the PADRE T-helper epitope (which binds to many murine and human MHC class II molecules), and a CTL epitope (YLSGANLNL) from carcinoembryonic antigen (CEA), which binds to the HLA-A_{0201} molecule. The β-Ala was used as a spacer between the PADRE and CEA peptide. Glycopeptides with an irrelevant peptide (T_N3-PEP and T_N6-PEP) were used to demonstrate the T_N specificity of the immune response. The totally synthetic MAG induced carbohydrate-specific antibodies (IgG and IgM) in both outbred and HLA-DR transgenic mice and these antibodies reacted *in vitro* with the native carbohydrate antigens expressed on the surface of human tumor cells. In both types of mice, the PADRE provides efficient T-cell help for carbohydrate-specific Ab response. PADRE is selected for its capacity to bind with a wide range of HLA class II molecules. OF_1 mice immunized with T_N6-PAD, but not with PAD, developed T_N-specific IgG antibodies. The Abs induced by T_N6-PAD are specific for the T_N antigen because in ELISA, the T_N glycopeptides with an irrelevant peptide (T_N3-PEP and T_N6-PEP) were recognized. On the other site, no binding to the synthetic peptide PAD lacking the T_N antigen occurred. The binding of T_N6-PAD-induced Abs from mouse serum to human Jurkat T-lymphoma cells that express the T_N antigen was studied. Positive binding of Abs from mice vaccinated with T_N6-PAD was detected, whereas no binding was observed when sera from mice immunized with the PAD control peptide (without GalNAc) were used. To our knowledge [82], the prepared T_N6-PAD is the first synthetic glycopeptide containing all three essential components necessary for an anticancer vaccine: T-helper epitope, a CTL epitope, and a highly clustered carbohydrate TACA B-cell epitope. The use of PADRE-based glycoconjugates can address the limitations of the genetic restriction in humans by providing an effective T-cell help for carbohydrate-specific immune response based on a universal T-helper cell epitope.

Biological functions of epithelial mucins (and TACAs in general) both in health and disease are deeply influenced by glycosylation [73,75,90]. The influence of glycosylation of the immunodominant DTR motif of MUC1 on its antigenicity was studied. Glycopeptides containing T_N antigen A(HGVTSAPDT*RPAPGSTAPPA)$_n$ where n = 1,2,3,4, and 5 were synthesized and tested to examine the sole and combined effects of the number of repeats and *O*-glycosylation with GalNAc at the DTR motif on the binding patterns of 22 mAbs

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recognizing this motif. Almost all Abs bound better to unglycosylated peptides in the form of multiple MUC1 tandem repeats. Glycosylation at the DTR caused enhanced binding in 11 cases, whereas ten Abs were not influenced in binding, and one was inhibited. Improved binding to the glycosylated DTR motif was exclusively found with Abs generated against tumor-derived MUC1. The obtained results show that a tumor-specific MUC1 epitope is defined comprising the ...PDTRP... sequence in a particular conformation essentially determined by *O*-glycosylation at the Thr residue with either the T_N antigen or related short glycan. These results can be applied in cancer immunotherapy and development of synthetic anticancer vaccines.

The interactions of a carbohydrate (e.g. TACAs) and the peptide in a molecule of glycopeptide (e.g. MAG, antitumor glycopeptide vaccine) are mutual and influence one another in all possible properties: chemical (stability to acids, bases, pK), physical (solubility, aggregation), and biological (biodegradability, immunogenicity, toxicity, penetration through membranes). The carbohydrate influences the conformation of the peptide and *vice versa*. Therefore, in general, the properties of glycopeptide ...SS*S... are different from ...ST*S... or ...S*SS....

Because active immunotherapy requires both the stimulation of humoral and cellular antitumor response, short synthetic glycopeptides bearing multiple T_N-antigens as B-cell epitopes and PV-derived tridecapeptide $CD4^+$ T-cell epitope were incorporated into the MAG [112]. Tetravalent MAGs of the type $(antigen)_4$-Lys_2-Lys-β-Ala with different antigens S*T*T*, S*T*T*-PV, S*S*S*-PV, hS*hS*hS*-PV, S*T*T*-TT, and S*S*S*-PADRE, respectively, where PV = Lys-Leu-Phe-Ala-Val-Trp-Lys-Ile-Thr-Tyr-Lys-Asp-Thr, TT = Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Leu, were prepared by SP method. These MAGs were compared with analogous linear structures and $(T_N)_{951}$KLH in induction of anticarbohydrate IgG Ab responses for therapeutic vaccination against cancer. The two different CD4 T-cell peptides, PADRE and TT, incorporated into T_N-MAGs were capable of providing the necessary help for anti-T_N Ab production in all immunized primates. The MAGs showed superior efficacy over the traditional KLH glycoconjugate in eliciting anticarbohydrate IgG response against the T_N antigen. The preclinical evaluation of the T_N-MAG vaccines demonstrated safe and promising immunotherapeutic properties suitable for targeting colon, breast, and prostate cancers.

Structure of a synthetic hexaglycosylated decapeptide HT*S*T*T*S*S*S*VTK (* = T_N antigen) representing a part of the extracellular domain of human glycophorin A in comparison with nonglycosylated peptide was studied by NMR, CD, and MD simulations [171]. Glycosylation introduced a structural motif in the peptide that is characterized by alternating positions of T_N residues along the peptide backbone. This is accompanied with a 'wave-type' conformation of the molecule. The GalNAc residues are directed toward the *N*-terminus.

Oxime ligation was used [172] to prepare mucin mimics containing the tumor-related T_N and sialyl-T_N antigens. Multiple ketone residues in a peptide were ligated with the corresponding aminooxy sugars. Glycopeptide dendrimers containing six TACAs were obtained. The oxime-based strategy benefits from convergent assembly of peptides and aminooxy sugars, both of which are straightforward to prepare.

A *C*-glycosidic analog of the T_N epitope [173] has been ligated by aminooxyacetic acid to the T-cell epitope peptide $^{328-340}$OVA. The main innovation is the use of α-*C*-glycosidic analog of the T_N antigen. The *C*-glycosidic bond is stable toward enzymatic hydrolysis, acids, and bases. The product affords two HPLC peaks corresponding to *syn* and *anti* oxime isomers. The glycopeptide was recognized by transgenic T-cells. it can bind to extracellular MHC molecules without the need of internalization and the *C*-glycoside part does not interfere with T-cell receptor recognition. The glycopeptide is aimed as a vaccine against TA3-Ha mouse mammary carcinoma.

Analogous to MAPs and MAGs, RAFTs also provide a tool for multimeric presentation of B- and T-epitopes and were used as a new scaffold for the design of anticancer vaccines. Dumy *et al.* [114] synthesized well-defined orthogonally protected RAFT scaffolds as synthetic vaccine candidates presenting four T_N analogs as TACA (B-cell epitope) and one or two $CD4^+$ helper T-cell peptides from the type 1 PV. The saccharidic and peptidic epitopes were both synthesized separately and combined regioselectively to the RAFT core using a sequential oxime bond formation strategy. The antibodies elicited by immunization of mice with RAFT(4GalNAc,1PV) and RAFT(4GalNAc,2PV) constructs recognize the native form of T_N epitope expressed on human tumor cells. RAFT scaffold is a promising and suitable tool for engineering synthetic anticancer vaccine.

Pal_3Cys-Ser or Pal_3Cys [164,165] (Figure 4) is often used as adjuvant in antitumor vaccines [166,167]. The Ab responses of two conjugates, Ac-S*S*S*-Pal_3Cys and Ac-S*S*S*-KLH, were compared in phase I clinical trial [157] in patients with biochemically relapsed prostate cancer. The saponin immunologic adjuvant, QS-21 was used. Ac-S*S*S*-KLH with QS-21 stimulated the production of high-titer IgM and IgG Abs. An antitumor effect in the form of a decline in post-treatment *versus* pretreatment prostate-specific antigen slopes was also observed. Inferior Ab responses were seen with Ac-S*S*S*-Pal_3Cys. A safe synthetic conjugate vaccine with Ac-S*S*S*-KLH was developed that can break immunologic tolerance by inducing specific humoral responses [157]. The use of QS-21 adjuvant was reviewed [51].

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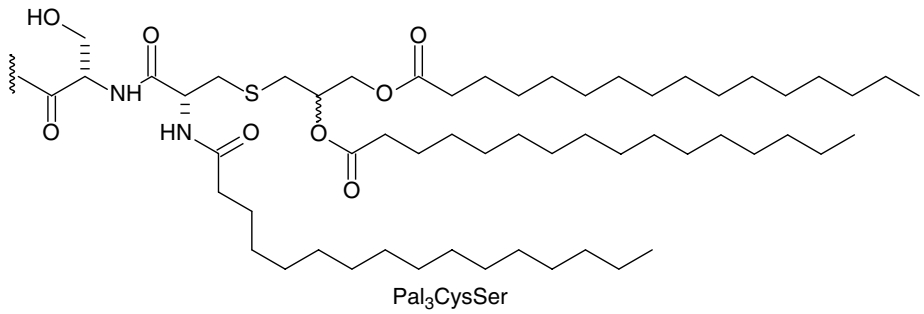

Figure 4 Structure of *N*-α-palmitoyl-*S*-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-Cys-Ser-OH ($Pal_3Cys\text{-}Ser = P_3CS$) [164–167].

In order to generate metabolically stable glycopeptide analogs, the synthesis of T_N-antigen glycopeptidomimetic clusters was elaborated [174] where *N*-substituted oligoglycines (peptoids) [175,176] were used as backbone (Figure 5). Compounds with 2, 3, 4, 6, and 8 α-D-GalNAc units (Figure 6) [174] were prepared using a reiterative blockwise approach and *N*-substituted oligoglycine peptoid backbones. These glycopeptidomimetics showed MS-fragmentation patterns similar to peptides, thus enabling easy structural determination. It is worth mentioning that owing to their secondary amide contents, these peptoids gave mixtures of rotameric isomers, which could be detected by the NMR time scale.

Oligomeric *N*-substituted glycines were developed as a motif for the generation of chemically diverse libraries. The authors [175] refer to these oligomers as 'peptoids', in recognition of their conceptual lineage. They are structurally similar to α-amino peptides, but differ in that the side chain is appended to the amide nitrogen instead of the α-carbon [177]. Peptoids offer several advantages, including stability to proteolysis, biocompatibility, and facile synthesis by the submonomer approach [176].

Danishefsky *et al.* [178] applied a 'click-like' cycloaddition method to join the peptide and carbohydrate part of the target glycopeptide. The method involves introduction of azide group to the saccharide part and

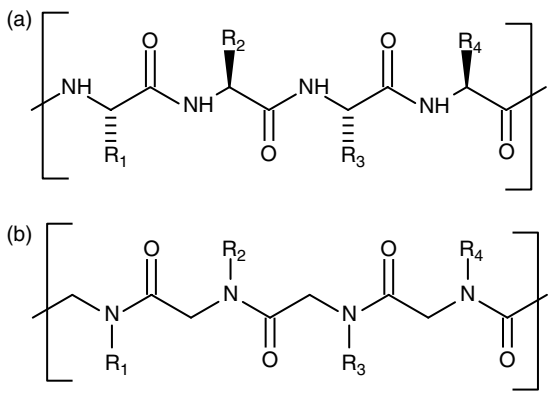

Figure 5 Comparison of the structure of a peptide (a) and peptoid (b) [175,176].

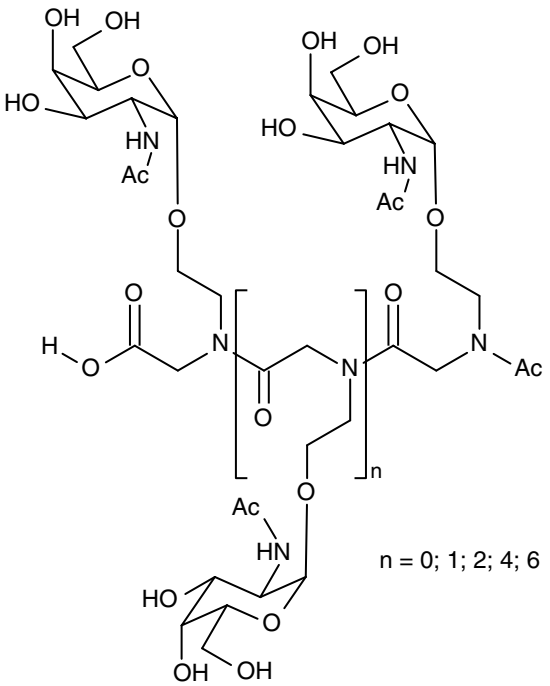

Figure 6 Oligomeric glycopeptide mimetics bearing the T_N antigen [174].

pendant alkynyl functionality to the peptide part. These two sectors are connected by 1,3-dipolar cycloaddition reaction forming a 1,2,3-triazole. The pathway is exemplified by the preparation of a tri-T_N-cluster. This method could be useful in the synthesis of fully synthetic vaccines. The use of aqueous buffer is an advantage.

To develop anticancer vaccines based on the T_N antigen, Bay *et al.* [115] elaborated enzymatic large-scale synthesis of MUC6-T_N glycoconjugates. The GalNAc enzymatic transfer to two recombinant MUC6 proteins expressed in *Escherichia coli* was performed using UDP-*N*-acetylgalactosamine: polypeptide *N*-acetylgalactosaminyltransferases (ppGalNAc-Ts), which catalyze *in vivo* the T_N antigen synthesis. Either a mixture of ppGalNAc-Ts from MCF7 breast cancer cell extracts or a recombinant ppGalNAc-T1 was used. The synthesis of MUC6-T_N glycoconjugates at a semipreparative scale (mg amounts) was achieved in both cases

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in yields of 25–69% (after purification). The average GalNAc number was 20–54 per molecule. The MUC6-T_N glycoconjugates were recognized by two anti-T_N mAbs that are specific to human cancer cells. Besides, the MUC6-T_N glycoconjugate glycosylated using MCF7 extracts as the ppGalNAc-T source induced IgG antibodies that recognized a human tumor cell line. The enzymatic large-scale synthesis of MUC6-T_N glycoconjugates is promising for developing effective anticancer vaccines.

For T_N MAGs with γ-Abu insert [179], see Section on 'Conformation, Molecular Dynamic and Molecular Modeling of Dendrimers'.

Tumor-Associated Antigens of the TF Type

Thomsen-Friedenreich antigen (TF-antigen) is expressed in many carcinomas, including those of the breast, colon, bladder, and prostate. The TF-antigen is also important in adhesion, metastasis, and as a potential immunotherapy target.

Water-soluble TF-antigen containing glycopolymers with a high degree of lipophilicity were synthesized [116] from poly[*N*-(acryloxy)succinimide] by amidation with an amine-ending TF-antigen. Various amines with increasing alkyl chain length were used. Solid-phase microtiter plate assay (ELISA) with mouse mAb FAA-J11 (IgG3) has shown that increased lipophilicity of ligands (from ammonia to propylamine) yielded high degree of adsorption to the surface of the microtiter plates. The more lipophilic glycodendrimer binds more tightly to the surface. The relative lipophilicity was also determined by measuring the radioactive count following treatment of the coated TF-antigen polymers with tritium-labeled UDP-GlcNAc substrate and core 2-$\beta(1 \rightarrow 6)$GlcNAc transferase, which catalyzes the transfer of the GlcNAc residue to the GalNAc 6-OH position. As the lipophilicity increased, the amount of radioactivity steadily increased. These results support the above data using ELISA, although they were more sensitive. The more lipophilic glycodendrimers provided the highest sensitivity and accuracy.

TF-antigen PAMAM dendrimers [101,180,181] with valency 4, 8, 16, and 32 were prepared. The relative efficiency of the various glycodendrimers to inhibit the binding of mouse monoclonal IgG Ab to coating copolyacrylamide TF-α-O-$(CH_2)_2$-S-$(CH_2)_2$-NH_2 was determined using goat antimouse mAb IgG. The degree of inhibition for TF-antigen PAMAM was proportional to the valency. Maximum inhibition was obtained for dendrimer with 32 valencies. Turbidimetric analysis and enzyme-linked lectin assay are consistent with these results. These results represent 460-, 960-, 1700-, and 3800-fold enhancement of inhibitory potentials in relation to TF-α-O-allyl, respectively. In the series of TF-antigen on *N*,*N*′-bis(acrylamido)acetic acid dendrimer, the dimer showed the lowest inhibitory value (120); tetramers were approximately equipotent (120, 128), while hexamer showed a dramatic decrease (48). The activity of TF-antigen PAMAM dendrimers [101,180,181] was compared also with analogous poly(propylene imine) dendrimers. These dendrimers differ only by a three-carbon shorter distance between the anomeric oxygen and the branching amine residues and by one less amide bond in the poly(propylene imine) dendrimers, which should confer less polar behavior. The PAMAM dendrimers were more active.

MAGs with TF-antigen on L-Lys core with valencies of 2, 4, and 8 were prepared. *N*-chloroacetyl-Gly-Gly was used as spacer and capping residue. First the chloroacetyl dendrimer was prepared on SP, split off from the resin, purified, and reacted with 3-thiopropyl glycoside of α-TF antigen [101,182].

Heterobifunctional TF-antigen conjugates with biotine or fluorescein were used for receptor screening [101].

Glycopeptide dendrimers with TF-antigen have been reviewed [11,18,22,32,101].

Tumor-Associated Antigens of the Sialyl-T_N Type

More than 80% of prostate, breast, and ovarian cancers express sialyl-T_N (ST_N). In contrast, the expression levels of ST_N in normal tissue are much reduced and restricted to only a few epithelial tissues [15,183]. Therefore, ST_N is an ideal candidate for boosting the patient's immune system specifically against a unique tumor-associated antigen.

The results of vaccination with Theratope (ST_N-KLH, containing synthetic ST_N; designed by Biomira, Inc., Edmonton, Canada) as treatment for breast cancer have been reviewed [183,184]. The linkage method consists of ozonization of the ST_N crotyl monomer. The created aldehyde is bound to the ε-amino groups of Lys residues on the KLH by reductive amination. Clinical trials have predominantly been carried out in breast cancer patients. Over 1000 women with metastatic breast cancer were enrolled into the program. Overall, Theratope vaccine has been well tolerated with minimal toxicity. In some cases, induration and erythema have been observed at the injection site. The vaccine decreased the risk for relapse and prolonged overall survival.

For preparation of synthetic vaccines with sialyl-T_N antigen (not dendrimeric) and their biological activities see also Refs 11,22,33,44,53,54.

Tumor Associated Antigens of the Sialyl-TF Type

The sialyl-TF antigens are of two types: α-2,3-sialyl-TF and α-2,6-sialyl-TF antigen (Figure 1). In the first, the

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3-position of the Gal residue is sialylated. In the second one, the 6-position of GalNAc is sialylated.

SPGS of an *N*-terminal fragment of human glycophorin AM, with the sequence H-Ser-Ser(R)-Thr(R)-Thr(R)-Gly-OH where R = α-2,3-sialyl-TF antigen, has been described [185]. Fmoc strategy and 2-chlorotrityl or 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyric acid-benzhydrylamine (HMPB-BHA) resins were used. The synthesized deprotected glycopeptide represents the epitope of human blood group type M. For other syntheses of glycopeptides with sialyl-TF antigens see Refs 11,22,31,32.

Synthetic vaccines with α-2,6-sialyl-TF antigen and α-2,3-sialyl-TF antigen have been described [22,33].

Tumor-Associated Antigens of the (2,3-2,6-Bissialyl)-TF Type

A synthetic vaccine with (2,3-2,6-bissialyl)-TF antigen has been reviewed [32,33].

Tumor-Associated Antigens of the Le^y Type

Le^y is a blood group determinant that has been identified as an important epitope for eliciting antibodies against colon and liver cancers. It has also been implicated in prostate, breast, and ovarian tumors.

Design and synthesis of Le^y-bearing glycopeptides mimicking cell surface Le^y mucin glycoprotein architecture have been described [15,93,117,118] (Figure 7). The designed Le^y-mucin mimic [93] has the following features: (i) displays the full Le^y tetrasaccharide, (ii) contains an intervening carbohydrate spacer group so that the structure and immunological properties of the determinants are not affected by direct contact with the peptide, and (iii) immunostimulating Pal_3Cys moiety is incorporated to carboxy terminus. Therefore, the conjugation to a carrier protein such as KLH was not necessary. ELISA was used to determine the reactivities of tris(hexasaccharide) construct **5** with anti-Le^y Ab 3S193. The α-*O*-linked hexasaccharide had reactivity comparable to that of the Le^y-ceramide control. Mice vaccinated with **5** (as an emulsion in Intralipid), without additional adjuvant, responded by a strong IgM immune response. These Abs strongly bind to natural mucin-related Le^y and Le^y-ceramide. The biological results 'demonstrate that a realistic immunogenic mimic of a tumor-associated cell surface-displayed Le^y mucin has been achieved by total synthesis' [93].

Tumor-Associated Antigens of the Globo-H Type

Danishefsky *et al.* [119] studied the immunomodulatory potential of hexasaccharide, Globo-H. This antigen is commonly found in breast cancer cells and was identified in the human breast cancer line MCF-7 as well as in a variety of epithelial cell tumors of ovarian, gastric, pancreatic etc. origin. The hexasaccharide structure of Globo-H (= MBr1 antigen) (Figure 1(a)) was assigned by Hakomori *et al.* [186] and subsequently synthesized by Danishefsky *et al.* [158,159]. The synthetic Globo-H antigen was conjugated to KLH. Twenty-seven metastatic breast cancer patients were treated with Globo-H-KLH conjugate plus the immunologic adjuvant QS-21. Each vaccine contained 10 μg of Globo-H in Globo-H-KLH conjugate and 100 μg of QS-21. This dose was administered subcutaneously during weeks 1, 2, 3, 7, and 19 for a total of five doses for each patient. Vaccination was able to stimulate moderate IgM Ab titers in a majority of participating breast cancer patients. IgG reactivity was stimulated to a lesser extent with lower peak titers. Fluorescence-activated cell sorting (FACS) analyses were performed on pretreatment and post-treatment sera to determine whether IgM or IgG Abs were able to bind to MCF-7 tumor cells that express Globo-H. Significant binding of the patients'

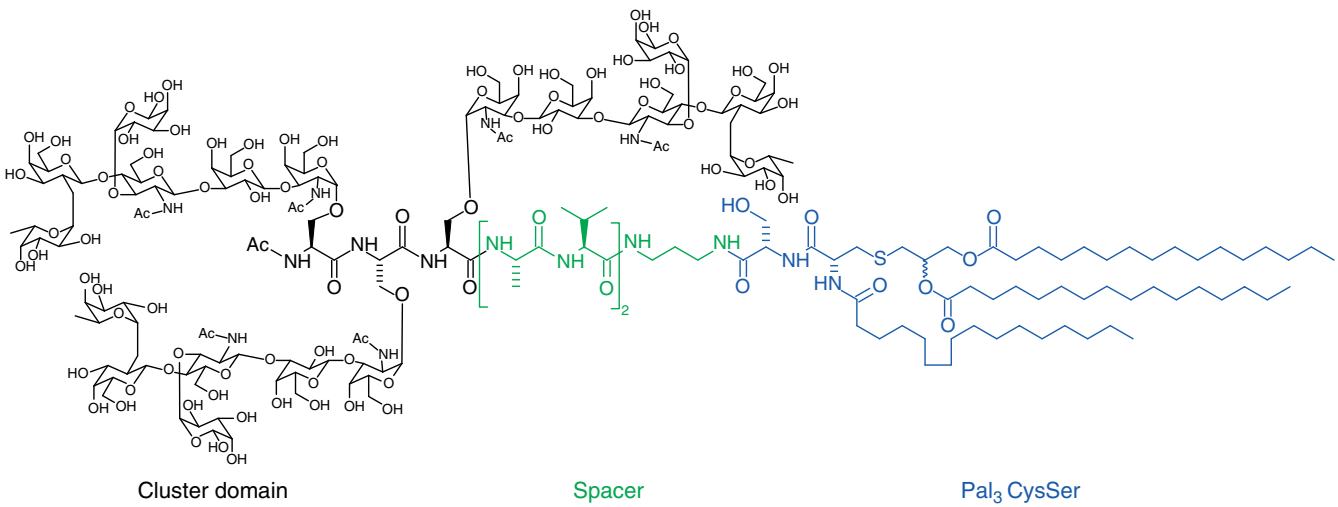

Figure 7 Le^y mucin-based synthetic vaccine **5** [117].

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IgM Abs to MCF-7 tumor cells was observed in 16 of the 27 patients. IgG Ab reactivity was found by FACS analysis in only three patients. The encouraging result is that nine patients demonstrated increased CDC because potential antitumor mechanisms of Abs involve binding to the tumor cell surface and induction of complement-mediated lysis. It is important to note that not all breast tumor cells may display the Globo-H antigen and therefore, a vaccine having only Globo-H alone has limited clinical impact. The combination of Globo-H with several other antigens (Le^y, T, sialyl-T_N etc.) may result in much better clinical impact; see also Section on 'Multiantigenic carbohydrate-based anticancer vaccines' [54].

The topic of Globo-H antigen synthesis was reviewed, including corresponding vaccines [51,15].

Multiantigenic Carbohydrate-Based Anticancer Vaccines

Transformed cancer cells exhibit abnormal cell-surface glycosylation patterns; these carbohydrates are potentially a class of antigens. Indeed, patients with natural or vaccine-induced antibodies against GM2, sialyl-T_N, TF, and a number of other cell-surface carbohydrate-based antigens have been reported to survive longer than patients lacking these antibodies [187].

Monovalent vaccines (i.e. with one sugar epitope) were promising in early clinical trials. This approach, however, does not respect the degree of heterogeneity of carbohydrate epitopes present on the surface of transformed cells. To overcome this challenge, three approaches of the polyvalent antigen strategy are possible [97]. In the first one, a mixture of different monovalent KLH constructs (each containing only one epitope) is used (Figure 8(a)). In the second one, different cancer-related antigens (2, 3, 4, 5, 6) are displayed on a single polypeptide, and this multivalent molecule (**6** unimolecular pentavalent; **7** unimolecular hexavalent) is bound to a suitable carrier, e.g. KLH (unimolecular multivalent vaccine, Figure 8(b)). A third approach is the conjugation of more different sugar antigens to the same carrier molecule (Figure 8(c)). Most sophisticated is the second ('dendrimeric') approach, where a defined molecule containing a glycopeptide, carrying several different antigens is selectively bound to a suitable carrier.

A heptavalent-KLH conjugate vaccine with seven different glycotopes separately bound on one KLH molecule (analogy to Figure 8(c)) containing the seven epithelial cancer antigens GM2, Globo-H, Le^y, TF(c), T_N(c), sialyl-T_N(c), and glycosylated MUC1 (for the given antigen structures, see Figure 1) was synthesized by Danishefsky *et al.* [160]. The antigens were conjugated to KLH by reductive amination (GM2), or by the use of heterobifunctional linkers 4-(4-maleimidomethyl)cyclohexane-1-carboxyl hydrazide (Globo-H and Le^y) or *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (TF(c), T_N(c), sialyl-T_N(c), and glycosylated MUC1). The immunological protocols varied. The authors found that immunization with the heptavalent-KLH conjugate plus GPI-0100 adjuvant induces Abs against the seven antigens of comparable titer in comparison with the individual-KLH conjugate vaccines. High titers of Abs against T_N, sialyl-T_N, TF, MUC1, and Globo-H, while lower titers of Abs against Le^y, and only occasional Abs against GM2 were induced. The Abs obtained reacted with naturally expressed antigens on the cancer cell surface by FACS and with the purified synthetic antigens by ELISA. An optimal dose of QS-21 (50 μg) or GPI-0100 (1000 μg) is necessary for optimal Ab titer. It is to be noted that the same authors [97] use the term 'unimolecular pentavalent' vaccine for a construct **6**, where five antigens are connected on one common backbone and then bound to KLH.

Multifunctional gold glyconanoparticles containing sialyl-T_N and Le^y antigens, T-cell helper peptides (TT), and glucose (analogy to Figure 8(c)) in well-defined average proportions and different density have been synthesized [188] in one step and characterized by NMR and transmission electron microscopy. The nature and size of the linker were crucial to control kinetics of S–Au bond formation and the ratio of ligands on the gold clusters. The mean gold core diameters ranged from 1.45 to 2.25 nm. This corresponds to an average of 116–309 gold atoms, respectively. The type, ratio, and density of TACAs and T-cell helper peptides on the multivalent construct can be varied. The obtained gold glyconanoparticles were water soluble and stable for months in solution. They have been tested *in vivo* (female Balb/mice). The generated reactive antisera detect sialyl-T_N/Le^y epitopes on the surface of the gold nanoparticles. This technology can be used for synthesis of polyvalent anticancer vaccines.

Unimolecular multivalent vaccines (analogy to Figure 8(b)) represent the most sophisticated class of antitumor vaccines. Fully synthetic multivalent glycopeptides as models of carbohydrate-based antitumor vaccines were prepared by Danishefsky *et al.* [53,80,94]. The synthetic trivalent multiantigenic unimolecular glycopeptides containing TF, Le^y, and T_N (**8**) and Globo-H, Le^y, and T_N (**9**), respectively, were conjugated to KLH giving conjugates **10** and **11** (Figure 9). Mice were immunized with these constructs as free glycopeptides (**8** or **9**) or as KLH conjugates (**10** or **11**). QS-21 or the related GPI-0100 were used as adjuvants. Both KLH conjugates induced IgG and IgM Abs against each carbohydrate antigen used; however, the unnatural Globo-H-Le^y-T_N-conjugate **11** was more immunogenic than the mucin-based TF-Le^y-T_N-conjugate **10**. The GPI-0100 adjuvants induced higher Ab titers than QS-21. The multiantigenicity of these compounds did not suppress the response against any of the incorporated antigens. Abs stimulated in response to **11**

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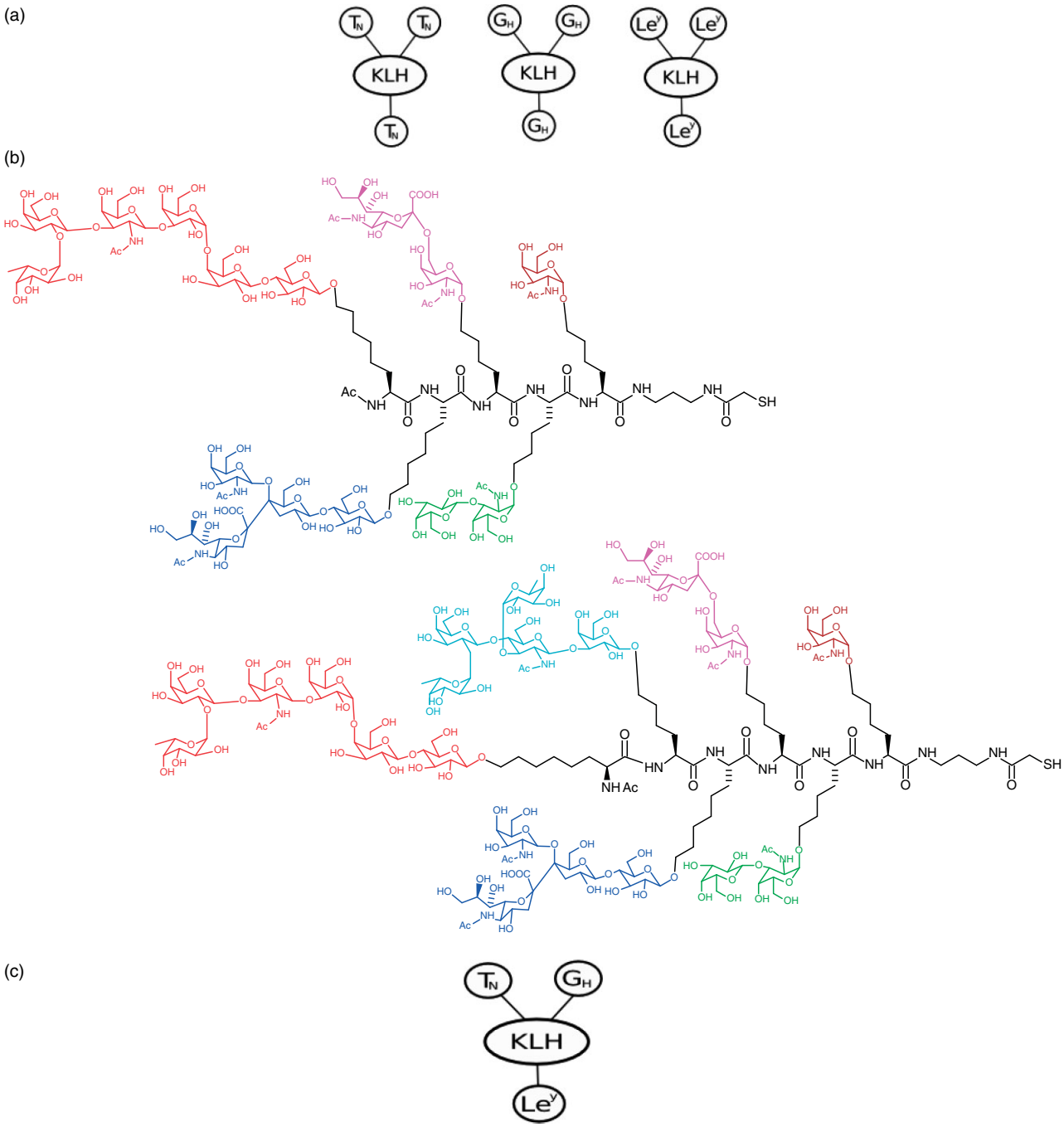

Figure 8 Different approaches to vaccine preparation – (a) A mixture of different monovalent KLH constructs (each containing only one epitope) is used; (b) Structure of unimolecular penta- and hexavalent vaccine (**6** and **7**, respectively) [95–98]; (c) Different sugar antigens bound to the same carrier molecule [188].

reacted with tumor cells known to selectively express the individual antigens. This study provides 'an important proof of principle for the concept that single vaccine constructs, bearing several different carbohydrate antigens, have the potential to stimulate a multifaceted immune response necessary for optimal targeting of the heterogenous population of cells associated with a particular cancer type'.

The methodology of unimolecular multiantigenic carbohydrate-based anticancer vaccine synthesis was improved, and it enabled the synthesis of penta- and hexavalent antitumor vaccines [54,95–98] targeted against prostate and breast cancer. The pentavalent antigenic construct **6** (Figure 8(b)) contained (from amino to carboxy terminus) Globo-H, GM2, sialyl-T_N, TF, and T_N antigens [97], and Globo-H, Le^y, sialyl-T_N, TF, and T_N antigens **12** [95–98], respectively. The hexavalent one contained Globo-H, Le^y, GM2, sialyl-T_N, TF, and T_N antigens **7** [97,98]. The unimolecular pentavalent glycopeptides **6** and **12** were conjugated to

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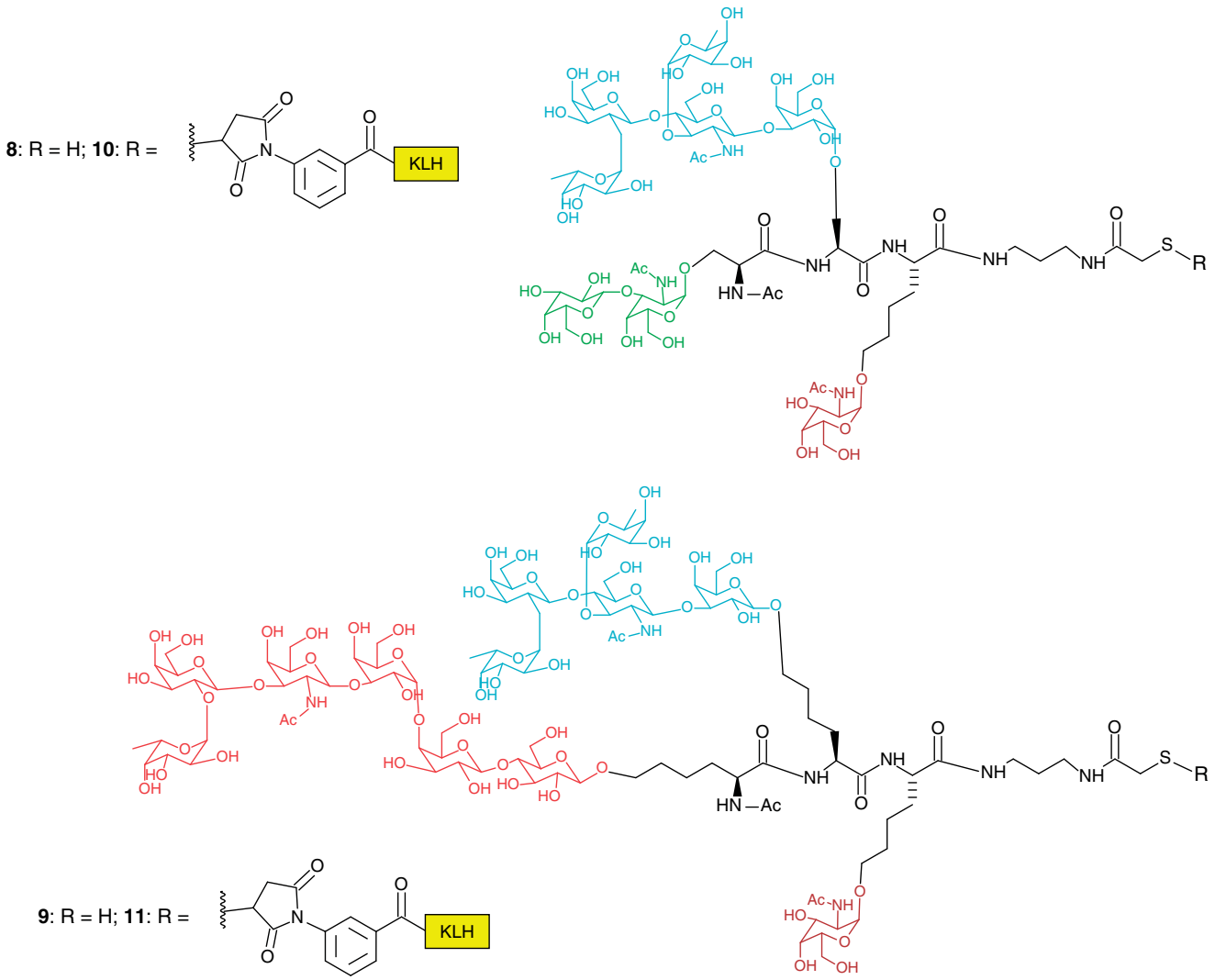

Figure 9 Synthetic trivalent multiantigenic unimolecular glycopeptides containing TF, Le^y, and T_N (**8**) and Globo-H, Le^y, and T_N (**9**) antigens, respectively, and their conjugates to KLH (**10** and **11**, respectively) [53,80,94].

two different immunogenic carriers, KLH and P_3C, respectively [95–98]. In the case of KLH conjugate, the ratio of glycopeptide-to-protein was 228 : 1. These vaccine constructs have the potential to stimulate a multifaceted immune response against prostate cancer [95]. The authors say that 'this approach could be applied to create tailor-made patient-driven cancer vaccines provided that rapid, automatable determinations of antigenic cell surface populations could be achieved'. The immunogenicity of unimolecular pentavalent vaccine **12** was compared with corresponding pooled monovalent vaccines [97]. Groups of five mice were immunized three times in 1-week intervals with: (i) 10 μg KLH-conjugated pentavalent vaccine **13**, (ii) 30 μg Pal_3Cys conjugated pentavalent vaccine **14**, (iii) 10 μg unconjugated pentavalent vaccine **12**, (iv) 10 μg unconjugated pentavalent vaccine and KLH, and (v) the pooled monovalent vaccines corresponding to each of the antigens displayed on the unimolecular construct, each conjugated to KLH (analogy with Figure 8(a)) (3 μg of each antigen conjugated to KLH). All vaccines were administered subcutaneously with QS-21. Performed ELISA has revealed that unconjugated pentavalent vaccine **12** either alone or mixed with KLH, induced lower titers than did the KLH-conjugated construct **13**. Pal_3Cys conjugate **14** was more immunogenic than unconjugated pentavalent vaccine **12,** but the titers of IgG and IgM were lower than with KLH-conjugated pentavalent vaccine **13**. Evaluation of the pooled monovalent vaccines has shown a superior IgM response against T_N, but response against the Globo-H ceramide, Le^y, sialyl-T_N, and TF were much lower in comparison with unimolecular KLH-conjugated pentavalent vaccine **13**.

From the above-mentioned data, it follows that the best results are obtained with unimolecular multivalent vaccines, where the epitopes are a part of one glycopeptide molecule, which is coupled to a suitable carrier, like KLH. The role of a suitable adjuvant is also of utmost importance [54,78,85,100,151–156,163]. Any

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successful vaccine must reflect the immunodominant TACAs, which are characteristic for a given type of cancer. These antigens are also responsible for the course of disease, and last but not the least, they will be the target of multifaceted immune response, which should include both humoral and cellular immunity. Also the unique immune status of any patient must be taken into consideration.

For synthesis of other multiantigenic vaccines via cystein-based and cysteine-free native chemical ligation, see Refs 189,190.

MUCIN ARCHITECTURE

To study the effect of glycosylation on peptide conformation, Ac-Orn-Ile-Thr-Pro-Asn(R)-Gly-Thr-Trp-Ala-amide (peptide **15** R = H; glycopeptide **15-**α R = GlcNAc-$\beta(1 \rightarrow 4)$GlcNAc-α; glycopeptide **15-**β R = GlcNAc-$\beta(1 \rightarrow 4)$GlcNAc-β) were synthesized and studied by NMR (TOCSY, ROESY) and MD simulations [72]. The core β-D-$(GlcNAc)_2$ remains conserved in all *N*-linked glycoproteins. NMR data indicate that the conformation of glycopeptide **15-**α is more similar to that of peptide **15** than to that of the glycopeptide **15-**β, which has a type I β-turn in the peptide sequence around the carbohydrate moiety. Results from MD simulations show that glycopeptide **15-**α and peptide **15** differ from the behavior of the glycopeptide **15-**β, which has relatively stable conformations for the peptide-backbone ϕ torsion angles. It was shown that 'the molecular composition of the core disaccharide has a critical and unique conformational effect on the peptide backbone'. The conformations of the peptide and α- and β-linked glycopeptides are uniquely influenced by the attached saccharide.

A series of mucin glycopeptide motifs derived from the *N*-terminal fragment STTAV of the cell surface glycoprotein CD43 has been prepared by total synthesis and studied by NMR, CD, and MD calculations [92]. They included the peptide itself, three glycopeptides having clustered T_N, TF, and sialyl-TF carbohydrate antigens, respectively, and one with β-O-TF antigen. A glycopeptide S*S*S*AVAV, in which each of the three side-chain hydroxyls bears the full α-Le^y blood group determinant, was synthesized too. NMR and MD calculations revealed that across the spectrum of the α-*O*-linked glycopeptides (including the tris-α-Le^y glycopeptide) a distinct NOE fingerprint was evident, clearly indicating a high level of molecular organization in the examined mucin-like structures. The mode of attachment of the first sugar and the peptide is very important in establishing the organization of the core (see also Ref. 72). Remarkably, while there is a profound organizational effect on the peptide backbone with the α-linked glycans, the attachment by β-linkage has little apparent consequence.

CONFORMATION, MOLECULAR DYNAMICS, AND MOLECULAR MODELING OF DENDRIMERS

The role of dynamically correlated conformational equilibria in the folding of dendrimers has been reviewed [191]. The data suggest that the mechanism by which the residual motions contribute to the thermodynamic stability is related to the chiral amplification phenomenon observed in helical polymers and supramolecular assemblies.

Molecular dynamics study of the effect of the γ-Abu insert on the conformational behavior of the T_N MAGs based on the oligolysine scaffold in *N*,*N*′-dimethylformamide was studied [179]. MD techniques were used to examine structural differences taking place during synthesis of two classes of tetravalent MAGs that differ only by the γ-Abu insert in the structure of the oligolysine core (for structures, see above [107]). The synthetic intermediates were modeled in DMF and geometrically characterized (distances of free or extended termini from the anchor, interatomic distances between free or substituted termini, radius of gyration, and spatial distribution of molecular density). Conformational analysis of 16 MAGs shows the distinct behavior of the inserted *versus* noninserted constructs already during the initial steps of the synthesis. Character as well as the length of the insert has a major impact on the spatial characteristics and behavior of the MAGs. The γ-Abu insert can increase a tendency of dendrimers to establish a high-density core. This is similar to the effect of a higher generation. Therefore, a careful consideration is necessary before inserting some spacer into a molecule. Sometimes, disastrous effects on the synthesis can take place instead of synthetic improvement.

For molecular modeling, see also Refs. 133,192,193, 141 in the text. Molecular dynamics simulations of glycoclusters and glycodendrimers have been reviewed [194].

VIRUSES

Dendrimer-Based Anti-HIV Vaccines and Therapeutics

Effective HIV vaccines have to induce both humoral and cellular immunity. The most difficult problem in HIV vaccine design is to identify epitopes for inducing broadly neutralizing Abs. If the broadly neutralizing mAbs (e.g. 2F5, 2G12, and 4E10) were induced in sufficient concentrations, passive immunization was successful in some animal models. Major HIV defense mechanisms are (i) frequent mutation of neutralizing epitopes, (ii) conformational masking of receptor binding sites, (iii) extensive glycosylation (e.g. formation of an evolving glycan shield) to evade immune recognition of the underlying protein domains, and (iv)

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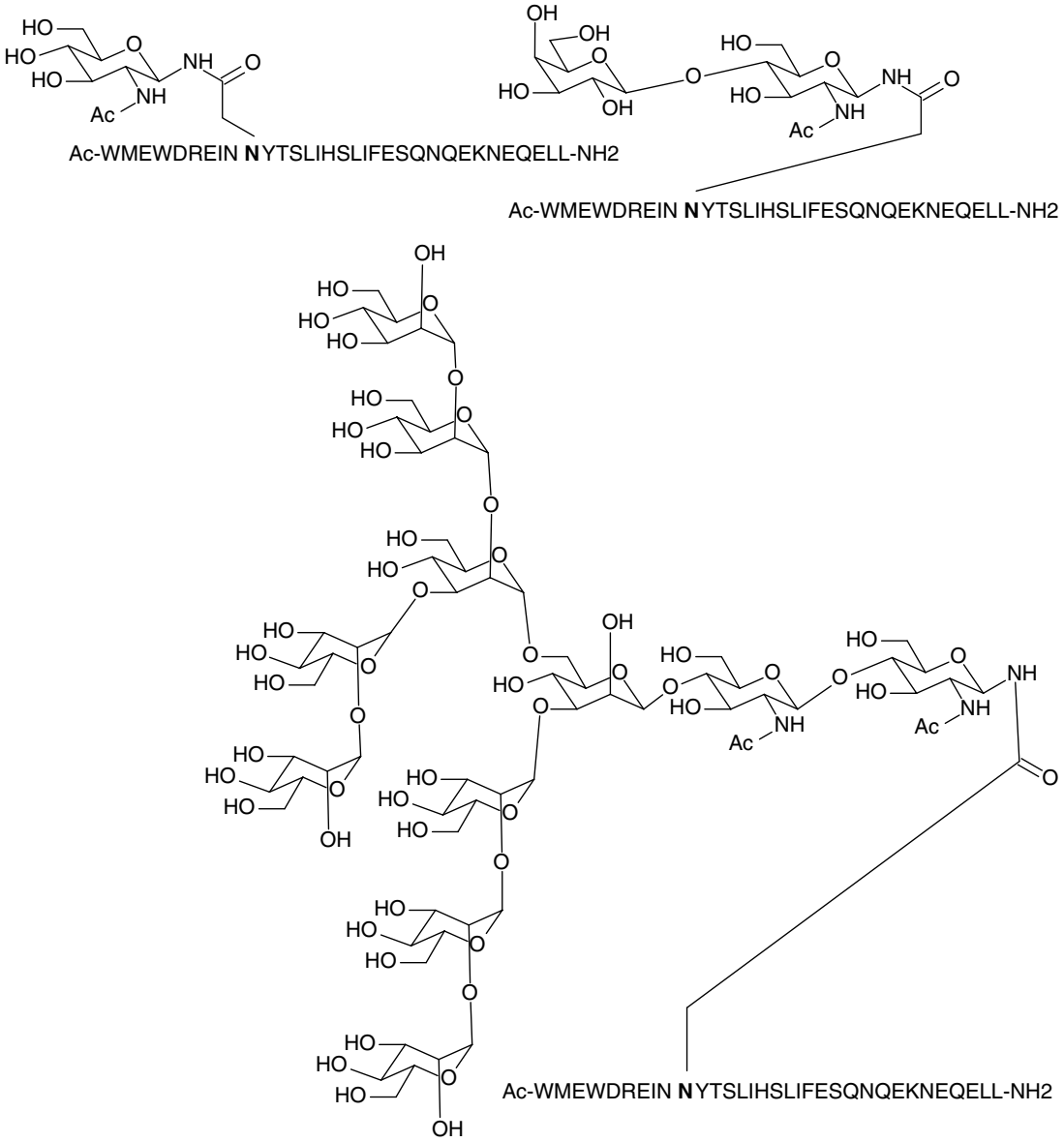

Figure 10 Synthetic glycopeptides derived from C34 peptide of HIV-1 gp41 [59,69].

formation of envelope glycoprotein complexes to occlude conserved epitopes [16,59,121,122,126].

The HIV-1 is heavily glycosylated. The transmembrane envelope glycoprotein, gp41, carries four conserved *N*-glycans. The outer envelope glycoprotein, gp120, has typically 24 *N*-glycans. The carbohydrates are important in viral immune evasion, and serve as ligands for dendritic-cell specific lectin DC-SIGN during HIV-1 transmission [59,69]. Chemoenzymatic synthesis of the HIV-1, gp41 glycopeptides which correspond to peptide C34 was described [69]. Peptide C34 is a 34-peptide derived from the *C*-terminal ectodomain region of gp41 (AAs 628–661). This sequence tends to form six-helix bundles with the *N*-terminal peptide (N36) of gp41. Synthetic peptide C34 is a potent inhibitor of HIV-1 infection. This sequence in the native gp41 carries an *N*-glycan at Asn^{637}. The authors evaluate the effect of glycosylation on anti-HIV activity and the helix-bundle forming ability of peptide C34. Three glycopeptides were prepared: GlcNAc-C34, LacNAc-C34, and triantennary undecasaccharide M9-C34 containing 9 Man units (Man_9-$GlcNAc_2$-C34) (Figure 10) [59,69]. The saccharide moieties are bound to C34 via Asn^{637}. The GlcNAc-C34 was constructed on automated SP synthesizer, using Fmoc chemistry and Fmoc-Asn(Ac_3GlcNAc)-OH as a building block. A glycopeptide with a disaccharide moiety, LacNAc-C34 was synthesized by enzymatic transfer of a galactose residue to the GlcNAc-C34 from UDP-Gal under the catalysis of $\beta(1 \rightarrow 4)$-galactosyltransferase. Glycopeptide M9-C34 that carries a native high-mannose type *N*-glycan, was prepared by elegant chemoenzymatic way using the *Arthrobacter endo-β-N-acetylglucosaminidase* (Endo-A). Endo-A

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is able to transfer an intact oligosaccharide to a suitable GlcNAc-containing peptide to form a new $\beta(1 \rightarrow 4)$-glycosidic linkage. Their anti-HIV-1 activity was determined by a cell-fusion assay. All the three glycoforms of C34 showed potent inhibitory activities against HIV-1 infection at nanomolar concentrations. Attachment of monosaccharide (GlcNAc) or disaccharide moiety (LacNAc) to C34 ($IC_{50} = 1.1$ nM) had no significant effect on its anti-HIV activity; M9-C34 ($IC_{50} = 7.7$ nM) demonstrated a moderate decrease in anti-HIV activity. The presence of di- or undecasaccharide in the molecule of C34 leads to much better

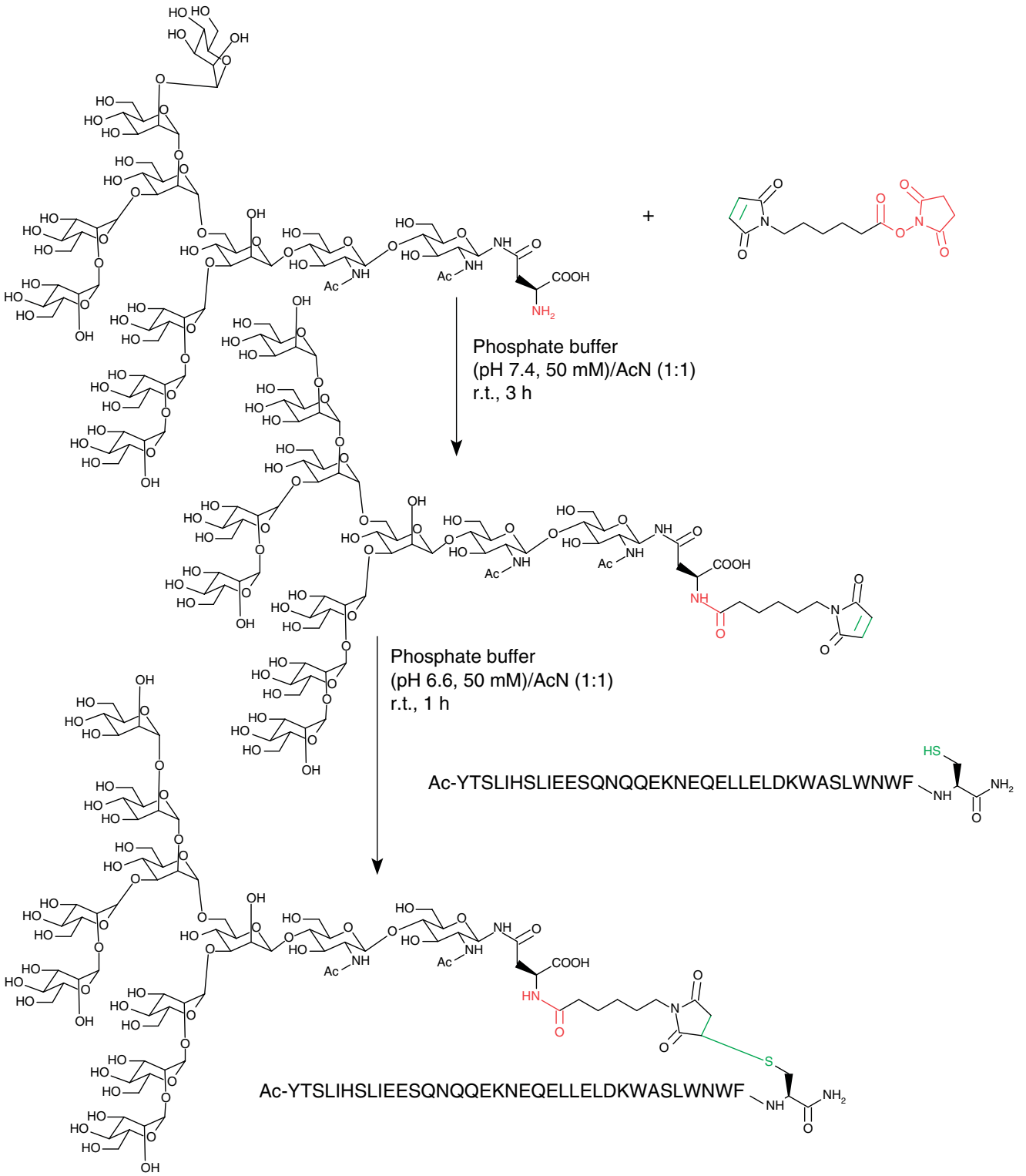

Figure 11 Ligation of $Man_9GlcNAc_2Asn$ to a 36mer HIV-1 gp41 peptide [195].

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solubility and also higher resistance to protease digestion.

The synthesis and Ab-binding affinity of a novel template-assembled oligomannose cluster as an epitope mimic for human anti-HIV Ab 2G12 were described [127]. Cholic acid was modified as a rigid scaffold and three high-mannose oligosaccharide (Man_9-$GlcNAc_2$-Asn) moieties were selectively attached at the 3α, 7α, and 12α positions of the scaffold through a series of regioselective transformations. This glycosylated cholic acid was designed to mimic the oligosaccharide cluster formed by the *N*-glycans at the Asn^{332}, Asn^{392}, and Asn^{339} sites on gp120. The affinity of the synthetic glycosylated cholic acid cluster was evaluated by competitive inhibition of 2G12 binding to the immobilized HIV-1 gp120 in an ELISA assay. The synthetic oligosaccharide cluster based on cholic acid is 46-fold more effective than the subunit Man_9-$GlcNAc_2$-Asn in inhibiting 2G12-binding to immobilized gp120. The IC_{50} values for the triantennary oligosaccharide cholic acid cluster and Man_9-$GlcNAc_2$-Asn were 21 and 960 μM, respectively.

Mannose-binding proteins on the surface of dendritic cells play an important role in recognition and capture of a variety of pathogens displaying D-Man, L-Fuc, or D-GlcNAc on their surface. To highlight the role of LLSGIV sequence (gp41 544–549), which is located on the *N*-terminal portion of the HIV-1, gp41 ectodomain, in the docking of the *C*-terminal portion during the viral entry into the host cell, octamannosylated MAG-peptide conjugates were prepared. The authors described a direct, linear approach based on machine-assisted Fmoc/Bu^t SPGS toward octamannosylated MAG-peptide conjugates [128]. Rink amide Tentagel resin was used as carrier and

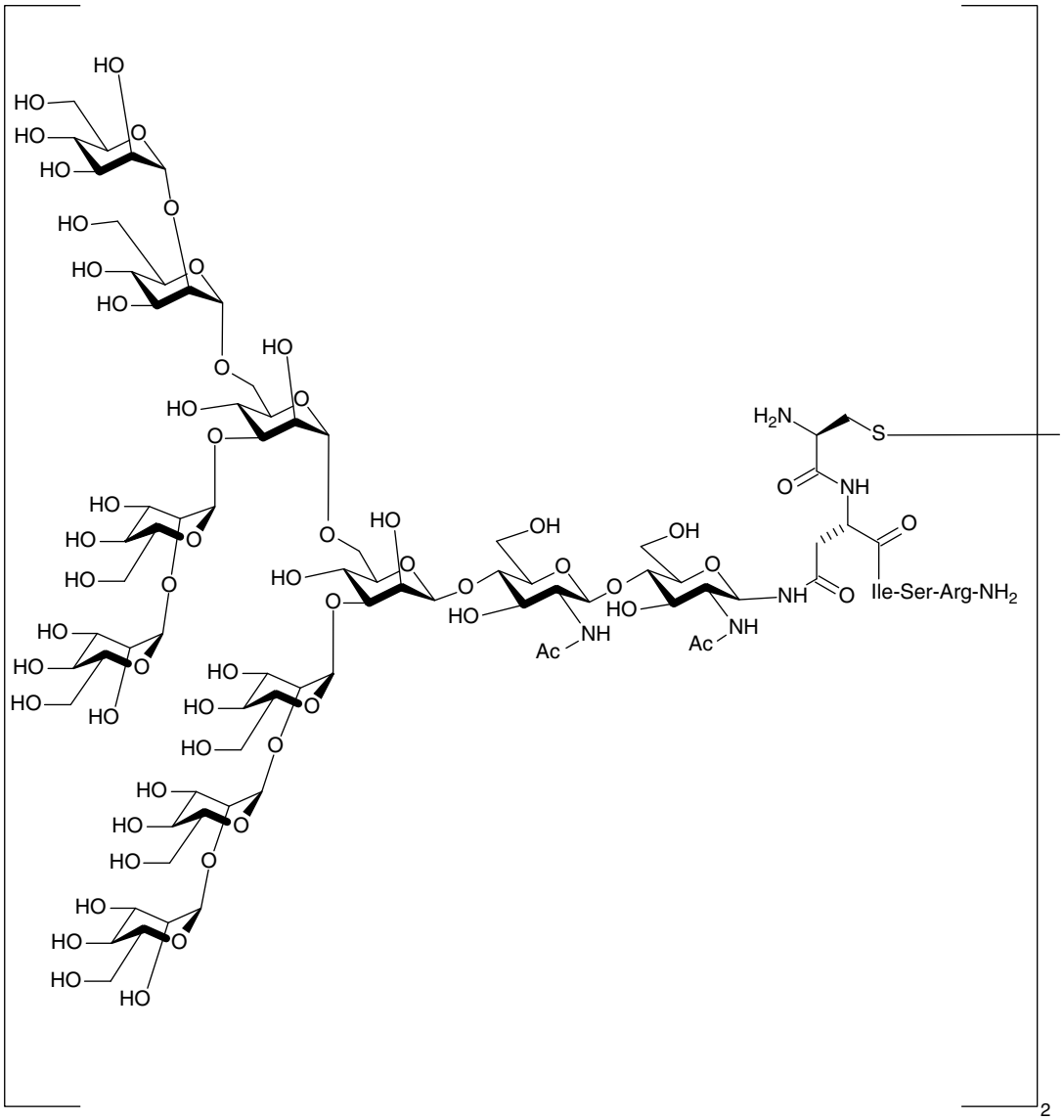

Figure 12 High-mannose dimer **16** [125].

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all steps included peptide bond formation. Mannose was condensed as 4-[(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)oxy]-butanoic acid. The structure of synthesized MAG-peptide conjugates was (Manα-O-$(CH_2)_3$-CO)$_8$-Lys$_4$-Lys$_2$-Lys-peptide-amide. Peptides incorporated were HIV-1 gp41(541–555), HIV-1 gp41 (553–567), SARS S2 (1081–1105), SARS S2 (1144–1187), and influenza HA2 (1–25), respectively. All free MAG-peptide conjugates showed good solubility in aquous medium. The MAG-HIV-1, gp41(541–555) conjugate elicited polyclonal antibodies (titer 1 : 4000) in rabbit. The corresponding KLH conjugate and MAP induced much weaker immune response. The authors suppose that the multiple mannose groups stabilize the immunogenicity of the peptide gp41(541–555) by protecting it from proteolysis and by enhancing the interaction with the receptors on the immune cells. Further immunological studies are ongoing.

Triantennary undecasaccharide $Man_9GlcNAc_2Asn$ was converted into its maleimide-activated form by taking advantage of the existing amino group in the Asn [195] (Figure 11). This maleimide-activated undecasaccharide was ligated with the Cys SH group of a 36mer HIV-1 gp41 peptide, T20, which is a potent inhibitor of HIV infection. For sequence, see Ref. 196. The chemoselective ligation is efficient, fast, and nearly quantitative. The oligomannose part of the conjugate should be useful for targeting to macrophage and dendritic cells, the primary targets for HIV-1 infection.

Ab 2G12, isolated from a long-term survivor of HIV infection, is able to efficiently neutralize a wide spectrum of different HIV isolates *in vitro* and to protect macaques from simian–human immunodeficiency virus challenge. The Ab 2G12 recognizes either high-mannose or hybrid-type glycans on Asn^{332}, Asn^{339}, and Asn^{392} residues of gp120. The synthesized constructs mimicking the 2G12 epitope could be potential antigen candidates in HIV vaccines. Therefore, Danishefsky *et al.* [125] prepared several triantennary undecasaccharides bound to fully synthetic $gp120^{331-335}$ peptide, H-Cys-Asn-Ile-Ser-Arg-NH_2. The oxidized form (Figure 12) carrying high-mannose oligosaccharide and different hybrid-type fragments were tested for binding with 2G12 using SPR technology. The dimeric form of high-mannose glycopeptide **16** showed the strongest binding to 2G12. On the other hand, hybrid glycans are not recognized by 2G12. The results of competition-binding experiments indicate that gp120 and high-mannose glycopeptide **16** compete for binding to 2G12. The clustered synthetic dimer **16** binds 2G12 significantly stronger than the monomeric dimer.

The same authors [126] prepared fully synthetic carbohydrate HIV antigens designed on the logic of the 2G12 Ab. Triantennary undecasaccharide $Man_9GlcNAc_2$-NH_2 was bound to cyclic tetradecapeptide (RAFT) containing suitably placed Asp residues. These glycopeptides were bound to the purified outer membrane protein complex from *Neisseria meningitidis*, in order to increase the immunogenicity. The ability of some of the conjugates to bind 2G12 was qualitatively assessed by an ELISA sandwich assay. They showed a clear response, although it was not as robust as that of the positive control, gp160. These multivalent glycopeptide constructs do not contain any relation to a native gp120 sequence, yet still mimic gp120s binding to 2G12.

Some selected glycosylation patterns of the HIV viral protein, gp120 can probably themselves serve as epitopes for potent, broadly neutralizing Abs (e.g. Ab 2G12). The 2G12 Ab recognizes a cluster of $\alpha 1 \rightarrow 2$ linked mannose residues on the HIV surface. Synthetic construct that is able to elicit a strong immune response to a cluster of gp120 high-mannose glycans could be potentially a valuable candidate for a HIV vaccine. Danishefsky *et al.* [197,198] have reported the first chemical synthesis of gp120 glycopeptide high mannose-type fragments gp120 (316–335) and gp120 (331–335). The triantennary undecasaccharide was assembled by two efficient methods and then conjugated with gp120 peptide segments through direct aspartylation.

Cell surface GSLs in lipid rafts are used by a variety of pathogens for cellular entry. GSLs consist of a ceramide (*N*-acetylated sphingosine) backbone and a carbohydrate moiety β-linked to the terminal hydroxy group of sphingosine. GSLs, galactosyl ceramide (GalCer), and its 3′-sulfated derivative, sulfatide (SGalCer) are receptors for HIV-1, gp120. It is known that gp120 contains high affinity binding sites for GalCer. To study the interaction of HIV-1, gp120 with GalCer and its 3′-sulfated derivative, SGalCer and to develop carbohydrate-based inhibitors of this interaction, the authors [123] synthesized four different series of PPI glycodendrimers (without peptide) derivatized with galactose residues, either sulfated or nonsulfated, generation 1–5. One series of glycodendrimers was prepared from purified natural GalCer, and another was from chemically synthesized 3-(β-D-galactopyranosylthio)propionic acid. The fourth series was made by random sulfation of the 3-(β-D-galactopyranosylthio)propionic acid-functionalized dendrimers. SPR studies found that recombinant gp120 IIIB bound to the derivatized dendrimers with nanomolar affinity, and to dextran sulfate (DxS) with picomolar affinity. DxS was reported to bind gp120 at the V3 loop, and is known as a potent binding antagonist of HIV infection *in vitro*. *In vitro* tests of synthesized glycodendrimers at inhibiting infection of U373-MAGI-CCR5 cells by HIV-1, Ba-L indicated that the sulfated glycodendrimers were better inhibitors than the nonsulfated glycodendrimers, but not as effective as DxS. The authors hypothesized that the lack of inhibition by the nonsulfated glycodendrimers reflects their inability to block the interaction of the positively charged V3 loop of gp120 with negatively charged,

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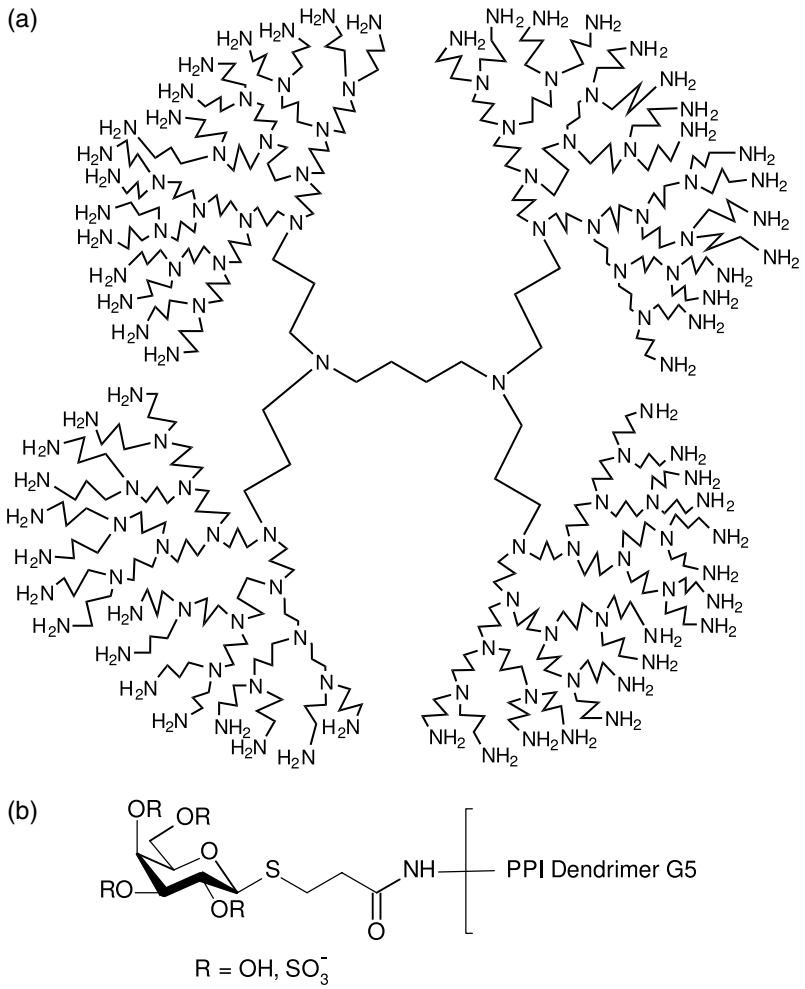

Figure 13 (a) PPI dendrimer, generation 5; (b) randomly polysulfated galactose functionalized PPI dendrimer, generation 5 [124].

sulfated-tyrosine residues of the *N*-terminus of the chemokine coreceptors. Despite GalCer binding to the V3 loop of gp120, GalCer does not inhibit the interaction of V3 loop with sulfated-amino terminus of the chemokine coreceptors (CXCR4 and CCR5). Therefore GalCer does not prevent the formation of fusion pore complex for HIV. The degree of sulfation of DxS (50 kD) is approximately 2.3 sulfate groups per repeating glycosyl residue; the degree of sulfation of the most potent sulfated glycodendrimer (SGal 64mer) is on average less than one sulfate group per galactose residue. This comparison allows the hypothesis that higher sulfation of dendrimers described above might result in a potent class of HIV-1-binding antagonists. This hypothesis was confirmed (see later [124]).

In non-CD4-expressing cells, GSLs have been implicated as alternative receptors for the docking of HIV-1 virions and migration to the chemokine coreceptors. There were several studies that led to the hypothesis that lipid rafts represent binding and entry sites for HIV-1. Membrane fusion is a cooperative process requiring multiple copies of several different receptors. Concentrating these receptors in lipid rafts increases the probability of forming a fusion pore. GSLs can act as glue that holds together the components needed for HIV-1 entry. Therefore, GSL rafts are potential sites for therapeutic intervention. The authors [124] hypothesized that binding antagonists of HIV-1 based on multivalent GSL raft-mimicking glycoconjugates could inhibit viral transmission and potentially reduce viral load. The PS Gal 64mer that contained an average of ~2 sulfate groups per galactose residue has been synthesized (Figure 13) and its ability to inhibit infection of cultured indicator cells by HIV-1 was compared to that of DxS (50 kD). DxS is a known potent inhibitor of HIV-1 infectivity. The results of the viral inhibition assays indicate that for the three HIV-1 isolates tested [HIV-1 IIIB (X4), NL4-3 (X4), and 89.6 (X4/X5)], the novel PS Gal 64mer glycodendrimer was an inhibitor of HIV-1 infectivity fully comparable with DxS (50 kD) with EC_{50} values in the nanomolar range. The cytotoxicity studies revealed that neither the glycodendrimer PS Gal 64mer nor DxS are toxic to cells at the highest tested concentration (3 mg/ml). Despite the fact that the DxS is nearly twice as large as the PS Gal 64mer and contains ~3.7-fold more sulfate groups, the two compounds are

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comparable in their ability to inhibit HIV-1 infection of both X4 and R5 indicator cells.

Carbohydrate core with branches containing maleimide was used as a new type of template for multivalent peptide assembly [196]. Synthesis of two MAGs with two different branches was given. The peptide inhibitor T20 is a 36mer peptide from the *C*-terminal ectodomain of HIV-1, gp41 with sequence Ac-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFC-NH_2. The two MAGs have been obtained by ligation chemistry, i.e. addition of the SH group from Cys of the peptide to the double bond of maleimide on the branches. The immunogenicity of the given glycopeptide dendrimers is currently being evaluated.

Dendrimer-based drugs were used as antivirals and microbicides [121]. The best antiviral agent, SPL7013 was synthesized by reaction of Lys_{16}-Lys_8-Lys_4-Lys_2-Lys-BHA with sodium 1-(carboxymethoxy)naphthalene-3,6-disulfonate mediated by PyBOP. STARPHARMA submitted an investigational new drug application for SPL7013 gel (VivaGel) to the US FDA in June 2003. This was the first submission for a dendrimer-based drug. In 2004, the first clinical trial was completed. Dendrimers of this type can be used in prevention of HIV and sexually transmitted infections.

The targeting potential and anti-HIV activity of lamivudine (3TC) loaded mannosylated G5 poly (propyleneimine) (MPPI) dendrimers were investigated [129]. Lamivudine (3TC) is an important antiretroviral drug belonging to reverse transcriptase inhibitors. The entrapment efficiency of 3TC loaded MPPI and G5 PPI dendrimers were 43 and 36%, respectively. The *in vitro* release profile shows that while PPI releases the drug by 24 h, the MPPI shows prolonged release up to 144 h. The ligand agglutination assay *in vitro* indicated that even after conjugation with PPI, mannose displayed binding specificity toward Con A. Cellular uptake of 3TC significantly increased when MPPI was used, which was 21 and 8.3 times higher than that of free drug and PPI at 48 h, respectively. 3TC-loaded PPI and MPPI were found to possess higher anti-HIV activity at a concentration as low as 0.019 nM/ml, in comparison to that of free drug ($p < 0.001$). The proposed carrier has the potential to increase the efficacy and reduce the toxicity of antiretroviral therapy.

The special binding of Tat protein to trans-acting responsive element (TAR) RNA leads to the transcription of HIV-1 virus. In the absence of Tat, most of the viral transcripts terminate prematurely. The influence of G2-G5 PAMAM dendrimers on the inhibition of Tat peptide/TAR RNA binding in HIV-1 transcription was studied [130,131]. The combining activity of G3 is the strongest and this compound should be the best candidate for a future drug. Two criteria were used: the combination coefficient using microgravimetric quartz crystal microbalance and charge/M_r ratio obtained by capillary electrophoresis analysis. These two criteria

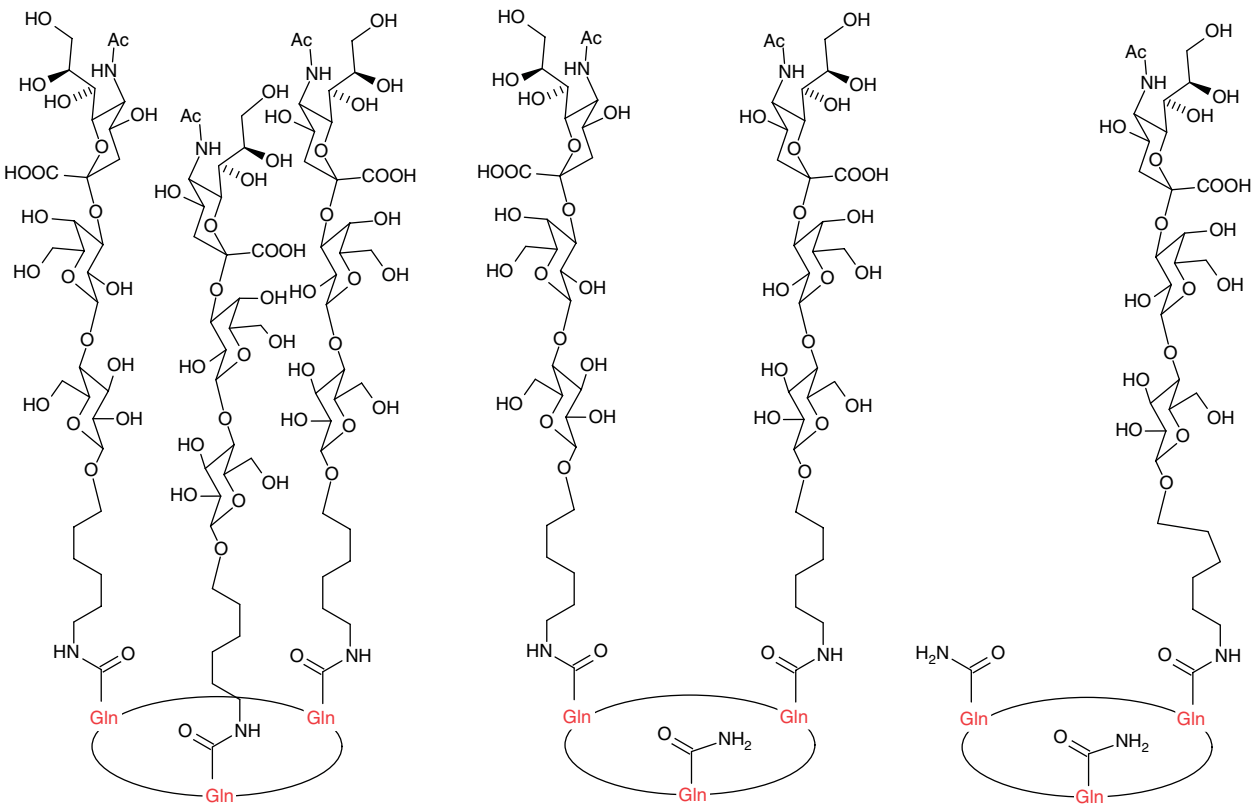

Figure 14 Cyclic glycopeptides containing 1–3 sialotrisaccharide units [133].

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can be applied for screening of numerous candidates of HIV drugs *in vitro* simply and rapidly.

Oligosaccharide, peptide, and glycopeptide-based HIV vaccines have been reviewed [61,122].

Influenza

The binding of HA, a viral carbohydrate-binding membrane protein to SA-containing oligosaccharides, such as GM3 trisaccharide [Neu5Acα(2 → 3)Galβ(1 → 4)Glc], on the host cell surfaces plays an important role in infectivity. Therefore, compounds with high affinity to HA would be possible candidates for blocking the influenza virus. On the basis of known HA structure, Nishimura *et al.* [133] developed a chemoenzymatic synthesis of a series of cyclic peptides (analogy with RAFTs) containing three sialotrisaccharide units Neu5Acα(2 → 3)Galβ(1 → 4)Glc (Figure 14). The cyclic peptides cyclo(SerGlyGlyGlnSerHisAsp)$_3$ (**17**), cyclo(GlySerSerGlnSerSerGly)$_3$ (**18**), and cyclo(GlySerGlnSerSerGly)$_3$ (**19**) were prepared on chlorotrityl resin. These cyclic peptides were coupled with 6-aminohexyl β-lactoside (large excess) using transglutaminase from guinea pig liver and afforded a mixture of mono-, di-, and trisubstituted intermediates. SA was attached by recombinant rat α-2,3-(*N*)-sialyltransferase in the presence of CMP-Neu5Ac, yielding a mixture of mono- (**17m, 18m, 19m**), bi- (**17b, 18b, 19b**), and tri-substituted (**17t, 18t, 19t**) derivatives, respectively. Purification by lectin-based affinity chromatography, DEAE-Sepharose, Sephadex G-25, and RP-HPLC afforded the trisubstituted trisaccharide derivatives in yield 8–9% (based on the peptide). The inhibitory effect on virus-induced hemagglutination was studied by using A/PR/8/34(H1N1) influenza virus. From the compounds tested, only **17t** and **17b** showed potent inhibitory effects. The binding assay between the HA protein and cyclic glycopeptides was studied by SPR. Only **17t** (K_d 0.63 mM) and **17b** (K_d 1.6 mM) showed significant affinity to HA on the sensor surface. All other compounds had $K_d > 20$. This is in agreement with the results of the HAI titer experiment. These data show that the amino acid sequence of the cyclic peptide has decisive influence on the direction and flexibility of the Gln side chains, even though their circular sizes were identical. The solution structures of cyclic peptides **17** and **19** determined by molecular modeling using restraints derived from 2D NOESY NMR showed that for **17**, all three Gln side chains are directed outward from the peptide ring (open formation), as expected. However, in compound **19**, the side chains are directed inward (close formation). This explains that the three attached trisaccharides in **17t** could reach the binding sites of HA simultaneously and a multidentate effect was obtained (i.e. the three oligosaccharides bound to cyclic peptides occupy the three binding sites of HA simultaneously). These results offer new insight into the design of antiviral agents and carbohydrate-based drugs. For other multidentate ligands, see Refs 199–203.

Influenza A viral infection starts by the binding of HA glycoproteins on the viral envelope to cell membrane SA. Free SA *in vivo* is toxic. PAMAM dendrimer G4, conjugated with SA (G4-SA) was tested [140] as a means to prevent adhesion of three influenza A subtypes (H1N1, H2N2, and H3N2). Hemagglutination-inhibition assays have shown G4-SA to inhibit all H3N2 and three of five H1N1 influenza subtype strains at concentrations 32–170 times lower than those of SA monomers. G4-SA had no ability to inhibit hemagglutination with H2N2 subtypes or two of five H1N1 subtype strains. *In vivo*, G4-SA completely prevented infection by a H3N2 subtype in a murine influenza pneumonitis model. Dendrimers containing SA are perspective antiviral therapeutics. The strain specificity needs further examination.

PAMAM dendrimers containing 4, 8, 16, 32, and 64 *N*-acetylneuraminic acid (Neu5Ac) residues were synthesized [141] and their ability to inhibit the HA-mediated adhesion of influenza virus was studied. Biological activity of PAMAM dendrimers was higher than that of free carbohydrate ligands, but lower than that of multivalent glycoconjugates based on other types of synthetic polymeric carriers. Conformational analysis (molecular dynamics method using the vacuum approximation) of PAMAM matrices and their glycoconjugates revealed the tendency of the PAMAM chains toward compaction and formation of dense globules. This can affect the ability of PAMAM-glycoconjugates to effectively bind the polyvalent carbohydrate-recognizing proteins. Moreover, the conformation and biological activity are deeply influenced by the charge of the carbohydrate ligand.

Carbosilane dendrimers (without peptide) uniformly functionalized with α-thioglycoside of SA were synthesized and tested as a new class of influenza neuraminidase inhibitors [142]. Dendrimers with valency 3, 4, and 6 showed inhibitory potencies not only for H3N2 sialidase but also for H1N1 type sialidase. Monomeric and dimeric compounds including other type of 3, 4, and 6 valent dendrimers did not show any activity. The topological orientation of each sialoside and distances between the sugar moieties are also important for the activity.

BIOMEDICAL APPLICATIONS OF DENDRIMERS

Data presented in this review have emphasized the potential therapeutic capacity of glycopeptide dendrimers as antitumor and antiviral prophylactic or therapeutic vaccines, as well as antiviral agents (Table 3). Indeed, a number of preparations have been tested in clinical trials, e.g. as vaccines against breast [105,119,161], prostate [157], and small cell lung

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cancers [81] with encouraging results. In these phase I studies, immunologic responses in patients were recorded. Antitumor effect was noticed in the form of a decline in post-treatment *versus* pretreatment prostate-specific antigen slopes in a phase I trial for treatment of prostate cancer with α-*N*-acetylgalactosamine-*O*-serine/threonine conjugate vaccine [157].

A phase II study was performed on 30 patients with biochemically relapsed prostate cancer [120]. The hexavalent (not unimolecular) vaccine, which comprised GM2, Globo-H, Le^y, glycosylated MUC-1-32mer, T_N, and TF in a clustered formation and conjugated to KLH, was used with QS-21 adjuvant. All patients responded serologically and increased levels of Ab titers at least against two antigens were recorded. However, the responses were unexpectedly lower than those against monovalent vaccines and, further, no clinical responses were noticed.

Taken together, the results from both preclinical and clinical studies clearly indicate that dendrimers have a potential therapeutic outcome. Their usage as prophylactic and therapeutic vaccines, as well as antiviral drugs represents a modality for possible dendrimer biological applications, besides their usage as carriers in drug delivery, in gene therapy etc [10,16]. For other biomedical applications, see also Part II [2].

CONCLUSIONS

From the aforementioned data we can conclude that life is dendrimeric or less strictly, life is branched.

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