



Cite this: *Biomater. Sci.*, 2017, 5, 953

Received 25th January 2017,

Accepted 3rd March 2017

DOI: 10.1039/c7bm00072c

rsc.li/biomaterials-science

Cyclopeptide scaffolds in carbohydrate-based synthetic vaccines

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Cyclopeptides have been recently used successfully as carriers for the multivalent presentation of carbohydrate and peptide antigens in immunotherapy. Beside their synthetic versatility, these scaffolds are indeed interesting due to their stability against enzyme degradation and low immunogenicity. This mini-review highlights the recent advances in the utilization of cyclopeptides to prepare fully synthetic vaccines prototypes against cancers and pathogens.

Introduction

Carbohydrates represent the most abundant and complex class of biomolecules in nature. For decades, our knowledge in this field has been limited to their metabolic or structural function, but their scientific interest has been reassessed more recently in light of their primary relevance in cell–cell communication, host–pathogen interaction, cancer metastasis or fertilization. Carbohydrates are usually expressed on the cellular surface conjugated to membrane protein or lipid, composing the glycocalyx (Fig. 1) which provides a characteristic “glyco-signature” for both mammalian cells and pathogens¹ and participates to diverse biological events. For example, they are involved in the initial infection step of pathogens by adhering to proteins expressed at the surface of host cells. Moreover infected organisms detect the presence of pathogens through recognition of carbohydrates and pattern recognition receptors (PRRs) which stimulates defense responses by the host.² Carbohydrate–protein interactions play therefore key roles both for pathogen to initiate infection and for the host to trigger immune response. By contrast, the majority of human cancers are characterized by aberrant glycosylation, *i.e.* tumor cells may over-express truncated versions of oligosaccharides, unusual terminal oligosaccharide sequences and show an increased sialylation of cell-surface glycolipids and O- and N-linked glycoproteins. The expression of these altered glycans, also called tumor-associated carbohydrate antigens (TACAs) participates in various stages of the tumour progression and is associated with malignancy. For these reasons, these oligosaccharides represent relevant structures for the design of synthetic vaccines. It should be mentioned that the chemical synthesis of carbohydrate antigens is a very large

topic and will not be covered in this manuscript. We suggest the readers to refer to excellent reviews in this field.^{3–6}

Problems associated with carbohydrate-based vaccines

The major hurdle associated with the development of carbohydrate-based vaccines is however due to the poor quality of antibody responses to carbohydrates.⁷ The principal cause of this problem is the incapacity of carbohydrate antigens to stimulate T cell dependent response.⁸ Indeed they are able to activate B cells upon binding to membrane receptors (BCR) such as immunoglobulins, which induces their clustering and leads to the B cell activation (Fig. 2a). Except in rare cases,⁹ this primary response results in the production of low affinity and short-living IgM whereas no memory effect is induced due to the absence of T cells stimulation.

To achieve antibody class switching, B cells need the participation of helper T cells (T_h cells). This requires the processing and presentation of peptidic antigens (CD4⁺ epitopes) by MHC-II molecules by antigen presenting cells (APCs) such as B cells, followed by its recognition by the surface protein CD4⁺ expressed by T_h cells.

Design of synthetic vaccines

Semi-synthetic approach

As mentioned above, T cells-dependent responses are typically generated by protein and peptide epitopes. For this reason, carbohydrate-based vaccine are classically prepared by coupling glycans to immunogenic carrier proteins,^{5,10–14} such as keyhole limpet hemocyanin (KLH), ovalbumin (OVA), bovine serum albumin (BSA) or tetanus toxoid (TT). These proteins indeed provide CD4⁺ epitopes upon processing by APCs whose combination with carbohydrate epitope is now known to trigger a long-lasting and IgG-mediated antibody response that could not be achieved by the separated administration of these

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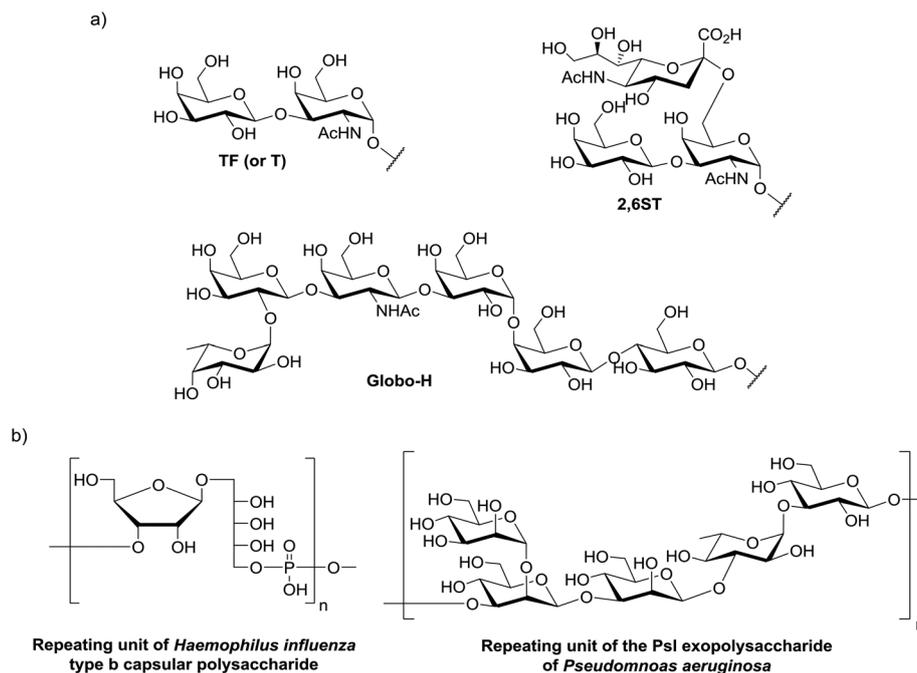


Fig. 1 Examples for (a) bacterial antigen repeating units and (b) tumor associated carbohydrate antigens.

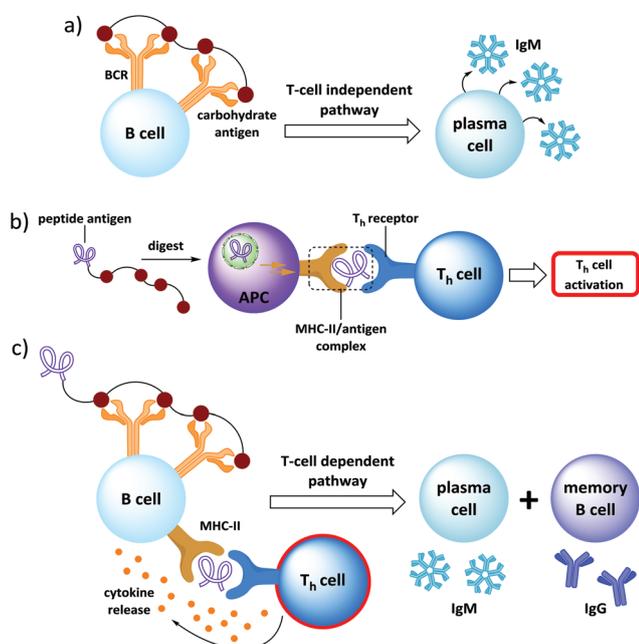


Fig. 2 (a) Carbohydrate binding to B-cell receptors, initiating a weak T-cell independent immunological response; (b) activation of T_h cells through antigen presenting cells (APCs: dendritic cells, macrophages and B-cells); (c) T-cell dependent immune response.

single elements.^{2,15–17} The efficiency of the immune response induced by these carbohydrate–protein conjugates depends on several factors which all contribute to the structure of the vaccine: the size and the total number (density) of conjugated carbohydrate epitopes, the nature of the carrier protein, the

type and the length of the linker between the carbohydrate and the carrier.

Many semi-synthetic vaccines have been developed on these structural bases. For example, Kunz's and Li's groups have synthesized vaccine candidates composed of a MUC1 glycopeptide displaying both the Tn and STn antigens,^{18–21} which was further conjugated to the bovine serum albumin (BSA) carrier. Immunization of Balb/c mice was found to promote a remarkable Ab response with predominant IgG1 isotype antibodies and a strong binding to breast MCF-7 tumor cell line.²² However they show some major disadvantages. First, the difficulty to control the conjugation chemistry may lead to ambiguities in composition and structure and batch to batch variations of the vaccine, and as a consequence, the reproducibility of the immune response. The second major drawback is related to the use of carrier proteins that are often highly immunogenic by themselves and will necessarily elicit strong immune responses, thus leading to carrier-induced epitope suppression. The result is an increase in the antibody response to the carrier and a decrease in the response to conjugated peptides or polysaccharides.^{23–29} In other words, the grafted epitope represents only a small part of the total conjugate, and it is randomly distributed on the protein carrier surface, which contains several T_h cell epitopes.³⁰ Therefore, immune responses to the carrier molecule may lead to a low level of the desired antibodies as compared to the total amount of antibodies produced.

Fully synthetic approach

With the aim to circumvent these drawbacks, Leclerc and co-workers has designed fully synthetic multiple antigen glyco-



peptides³¹ (MAGs) composed of clustered Tn antigens and CD4⁺ peptide epitope from the type I-poliovirus protein (PV) within a well-defined lysine-based dendritic core, paving the way to the fully synthetic approach in vaccine design. This new system offers several advantages: (i) the carbohydrate content is much higher than in traditional protein conjugates; (ii) the core dendron, representing a minor fraction of the total construct, is non immunogenic thus avoiding unwanted immune responses, and (iii) the resulting construct has a precise and well-characterized chemical structure. After these key findings in 1997, more attention has been focused on subunit of vaccines, which are devoid of any unnecessary immunogenic components, combining only elements indispensable for evoking an antigen-specific immune response.

A key element in this fully synthetic approach is the central scaffold which should ensure the correct presentation of the different units, without interfering with their activity and possibly improving recognition properties by means of multivalent presentation. In this mini-review we focused our attention on the various peculiarities that make cyclopeptide interesting scaffolds for carbohydrate-based vaccines candidates.

Fully synthetic vaccine based on cyclopeptide scaffolds

The group of Dumy and Renaudet have selected cyclopeptide carriers to construct synthetic vaccines.³² In particular, they focused their attention to the RAFT cyclodecapeptide platform (Regioselectively Addressable Functionalised Template)^{33–35} which is composed of two adjacent proline-glycine as β -turn inducers that constrain the backbone conformation into an antiparallel β sheet (Fig. 3).

The platform can thus be easily prepared by solid phase peptide synthesis of the linear sequence, followed by cyclization in solution, with high level of efficiency and reproducibility. Up to six lysine residues can be introduced in the decapeptide sequence, in positions 3-4-5-8-9 and 10. The resulting scaffold thus present two separate spatial domains where the Lys 3-5-8-10 are oriented in the upper plane, while the Lys 4-9 residues delineate a second domain in the opposite plane. Lysine side-chain can be orthogonally functionalized by using orthogonal protecting groups (*e.g.* Boc, Alloc, Dde, *etc.*).³⁶

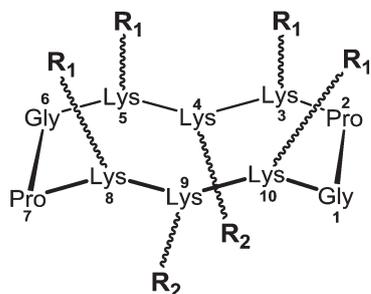


Fig. 3 RAFT platform.

Moreover, modified Lys can be introduced as building blocks during SPPS, giving readily available orthogonal functionalities for a further extension of the platform.

Together with these structural features, the cyclic nature of such scaffolds offers an improved stability towards enzymatic degradation compared to linear analogues, which makes cyclopeptides attractive scaffolds for biological application. For example, it was recently proven suitable in diverse research fields, in particular for the synthesis of multivalent glycosylated structures with nanomolar affinity towards lectins.^{37–41}

For all these reasons, cyclopeptides represent interesting carriers to conjugate carbohydrate and peptide epitopes for the preparation of fully synthetic carbohydrate vaccines.

Anti-cancer vaccine design

Renaudet and Dumy in collaboration with the group of Leclerc have developed a first generation of anticancer vaccine prototypes by grafting four Tn antigen analogues as B-cell epitope, and the CD4⁺ peptide from the type 1 poliovirus as T-cell epitope. Tn antigen is one of the most overexpressed carbohydrate antigen found in the membrane-bound glycoprotein MUC-1 (Fig. 4).⁴²

The native Tn antigen is depicted as a α GalNAc unit linked to a Ser/Thr residue through a glycosidic bond. For synthetic considerations, Renaudet and Dumy have decided to introduce an oxyamine function on the anomeric carbon of the α GalNAc unit to conjugate four copies of this Tn analogue on the upper side of the RAFT scaffold by oxime ligation.⁴³ The lower face of the construct was functionalized with the PV_{103–115} peptide sequence from type I-poliovirus protein (Fig. 5; compound 1), since it was previously proved efficient in triggering T_h cells for the induction of antibody responses in BALB/c mice models.⁴⁴ As for the conjugation of the Tn analogue, the peptide antigen was attached to the scaffold by oxime ligation. This strategy has proved efficient for the convergent assembly of diverse bioconjugates and was found compatible with a wide range of chemical functions without requiring any need of coupling agents.^{45–57} Moreover, the oxime bond has been shown to be stable under physiological conditions.^{58–60}

The B cell antigenicity was assessed by ELISA using two monoclonal antibodies (mAbs) specific for the Tn antigen: an IgG3 (6E11) and an IgM (83D4). They were both able to interact with compound 1, but not with the control molecules lacking the sugar moiety or presenting another saccharide. This clearly demonstrates that the recognition is due to the unnatural Tn analogue. Its multivalent presentation at the cyclopeptide surface indeed seems able to mimic the carbohydrate

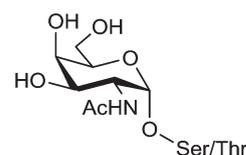


Fig. 4 Tn antigen.



(SIINFEKL)⁸³ was used as a target CD8⁺ T-cell epitope, and synthesized in line with the Pan-DR universal CD4⁺ Th peptide (PADRE, {dA}K{Cha}VAAWTLKAA{dA}{Ahx}).⁸⁴ The resulting construct was extended at the N-terminal end with a palmitic acid moiety to confer the self-adjuvanting property of this four-component glyco-lipo-peptide vaccine.

The immunological properties of the glycolipopeptide 2 were investigated in the MO5/BALB/c tumor mouse model. A strong production of antibodies that recognize human tumor cell lines (MCF7) as well as a stimulation of T-cells without administration of external adjuvant were clearly shown. More interestingly, an improved survival rate and a significant tumor regression were also observed. Among twenty mice challenged subcutaneously with MO5 cells, none of them developed a tumor after 90 days. In addition, all the mice survived after this delay, whereas the survival was less than 30% in non-immunized control group.

Compounds 3 and 4 have been further synthesized by the same group on the model 2, except for the peptide that contains a HER₄₂₀₋₄₂₉ sequence (PDSLRLDSVFK) from the HER-2/neu glycoprotein that is over-expressed by breast carcinomas. This peptide act as a CD8⁺ T cell epitope, replacing the OVA protein. Moreover, the palmitoyl moiety was attached at the N-terminal end (linear glyco-lipo-peptide 3), or in between the CD4⁺ and CD8⁺ T cell epitope sequences (branched glyco-lipo-peptide 4). The uptake, processing and cross-presentation pathway of 3 and 4 were investigated to assess whether the position of the lipid moiety would affect the B- and T-cell immunogenicity and protective efficacy. While more potent HER₄₂₀₋₄₂₉-specific IFN- γ CD8⁺ T cell responses was induced by the linear construct 3, the branched compound 4 generated stronger tumor-specific IgG responses. Furthermore, although both constructs enter dendritic cells *via* TLR2 and induce their maturation, each construct appear to be processed and presented to T cells differently. Thus, the position of the lipid moiety within synthetic glyco-lipo-peptide constructs profoundly affects the phenotypic and functional maturation process of DCs, the processing of the glyco-peptide molecules and their cross-presentation in DCs, as well as the magnitude of IgG and CD8⁺ T-cell responses. Although cellular and molecular mechanisms underlying the immunogenicity of 3 and 4 remain to be fully elucidated, data suggests that the position of the lipid moiety strongly impact the immunogenicity of these constructs and is superior compared to non-lipidated analogues. It indeed not only affected the uptake and cross-presentation pathways in DCs, but modulated the magnitude of antitumor antibody and CD8⁺ T-cell protective immunity. It can be expected that the presence of the lipid tail may promote the self-organisation of the vaccine candidate in solution, which may impact the resulting immunogenicity. However, this aspect has not been discussed by the authors.

A third generation of vaccine construct was elaborated using the same scaffold. This vaccine prototype includes four clustered Tn-antigen mimetics as B-cell epitopes and the PADRE sequence in line with the OVA₂₅₇₋₂₆₄ peptide, respectively as CD4⁺ Th and CD8⁺ CTL epitopes conjugated on the

cyclopeptide carrier (Fig. 7; compound 5).⁸⁵ It has been hypothesized that TACA-based vaccines displaying these mimetics instead of native Tn antigens could be more stable towards enzymatic degradation by endogenous glycosidases.

The bioactive Tn-antigen mimetic, developed by Nativi and co-workers, is a 2-deoxy-2-thio- α -D-galactoside that retains the ⁴C₁ chair conformation of the native antigen (Fig. 8).⁸⁶

It was conjugated through amidic linkage to the Lys residues on the scaffold, and the immunostimulant peptide was linked on the lower domain of the scaffold *via* disulfide bond ligation.

The safety and immunogenicity were evaluated in B10.D1 mice inoculated with 5 in presence of CpG₁₈₂₆ adjuvant. High levels of mucin-specific IgG/IgM antibodies were induced (IgG/IgM titers >8000), and approximately half of the titer was still present in the serum 240 days after the last immunization. Moreover, antibodies were found to bind to human (MCF7)

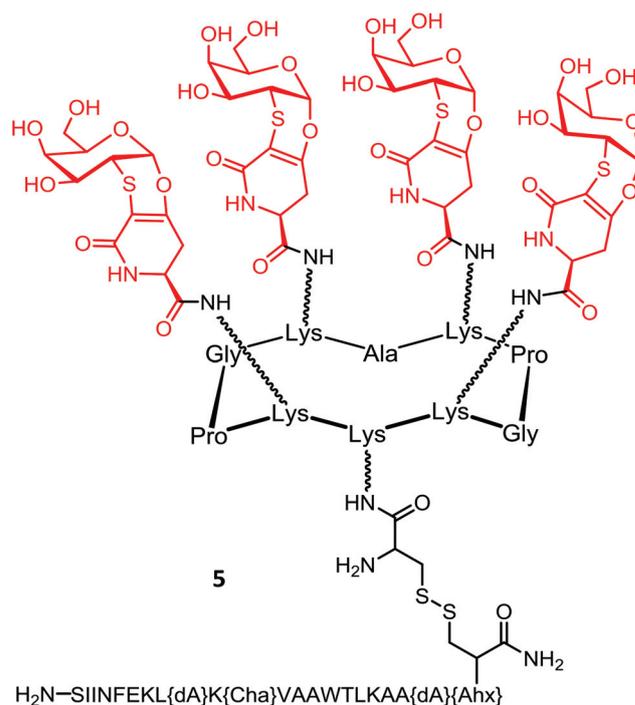


Fig. 7 Structure of the clustered α -Tn antigen mimetic based vaccine prototype.

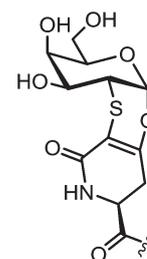


Fig. 8 Structure of the α -Tn antigen mimetic residue.



cancer cell lines expressing the native carbohydrate antigens. Immunotherapeutic efficacy studies of **5** determined by assessing tumor growth and survival rate in female B10.D1 mice implanted with NT2 cells showed that out of ten mice vaccinated, seven were still alive eight weeks after tumor inoculation, whereas only one or none survived in negative control groups. Finally, *in vivo* depletion of B cells, CD4⁺ cells, or CD8⁺ T cells in immunized mice by using specific mAbs showed that only depletion of B cells abrogated the protection induced by **5** against tumor progression and death, suggesting that the protection is mainly due to B cells.

In the frame of the design of anticancer vaccine, the group of Danishefsky proposed a prototype displaying multiple copies of two TACAs, the Tn and STn antigens, assembled on a cyclic peptidic scaffold using a convergent synthesis.¹¹ These two carbohydrate epitopes are over-expressed on the surfaces of a variety of epithelial cancers and are presented in broadly conserved clusters of 2–4 carbohydrate units. The cyclopeptidic scaffold contains two pairs of D-Pro-L-Pro as β -turn inducers and located opposite one another in the sequence,⁸⁷ four

aspartic acid residues as glycan points of attachment and one unique cysteine residue for carrier protein conjugation. The resulting cyclic 12-mer peptide conformation directs the side chain groups of the aspartic acid residues above the plane determined by the scaffold and the cysteine side chain residue projecting from the bottom (Fig. 9).

The Tn and STn functionalized building blocks, respectively **7** and **8**,⁸⁸ were used as handles for coupling to the peptide scaffold (Fig. 10). Tetra- and hexadecaivalent homo-antigenic vaccines prototypes were first constructed, compounds **9** and **10** displaying respectively four and six Tn residues and compound **11** being decorated by STn units (Fig. 10).

Cyclic peptides containing six or four aspartate residues, for accessing to compounds **9–11**, were prepared through automated solid-phase synthesis; after *tert*-butyl ester deprotection building blocks **7** or **8** were coupled under conditions known to minimize aspartimide formation.^{89–93} Next, Danishefsky and coworkers turned to the synthesis of the unimolecular multi-hetero-antigenic construct, **12**, wherein both Tn and STn antigens are displayed on the cyclopeptidic scaffold. This type of clusters is intended to reflect the degree of carbohydrate heterogeneity associated with most cancers.^{94,95} It is postulated that such hetero-glyco-clusters could activate different populations of B cells to produce Abs with multiple selectivity and that these Abs could target tumors at different stage of the disease progression. This unimolecular multi-epitope construct was obtained following a sequential attachment of building blocks **7** and **8** onto a scaffold bearing orthogonal protected aspartate residues and subsequent global deacetylation. Another original feature of this work concerns the utilization of ring-closing metathesis (RCM) to incorporate an internal cross-linking element between carbohydrates, in order

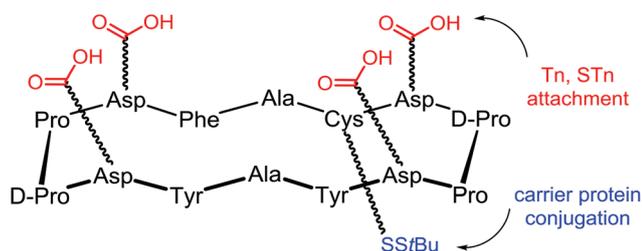


Fig. 9 Scaffold used by Danishefsky to attach Tn and STn antigens.

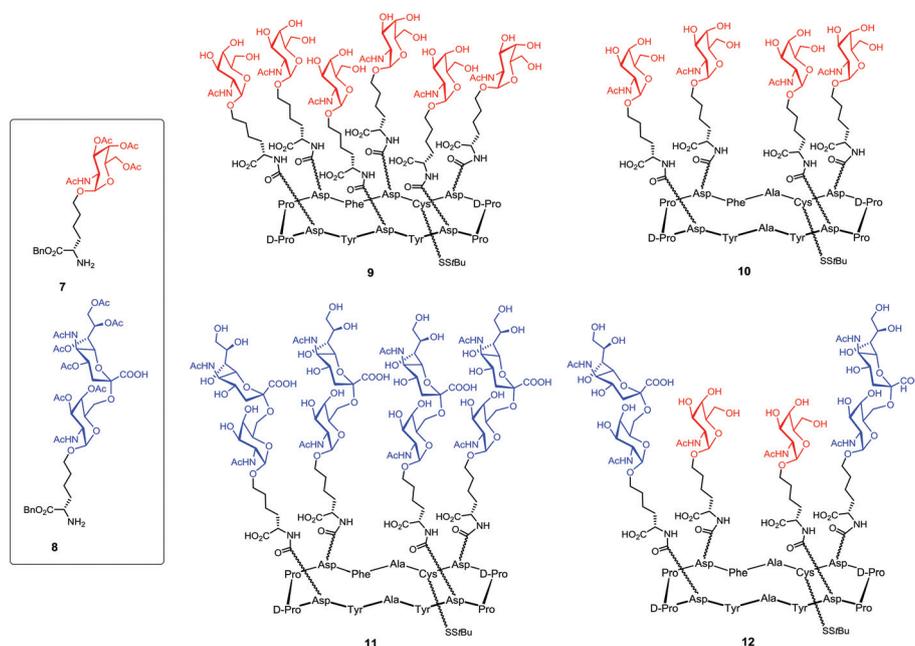


Fig. 10 Homo- and hetero-displayed Tn/STn-containing clusters synthesized by Danishefsky's group.



to rigidify the construct and minimize the spreading of glycan units. This type of constrained architecture is expected to better mimic tightly clustered B cell carbohydrate epitope and thus increase the antibody response. Construct **13** has been obtained from intermediate **14** performing a RCM reaction followed by reduction of the resulting olefin⁹⁶ was found to give good yield and avoid terminal olefin isomerisation as well (Scheme 1).

However, immunological investigations of these promising constructs have not been reported yet.

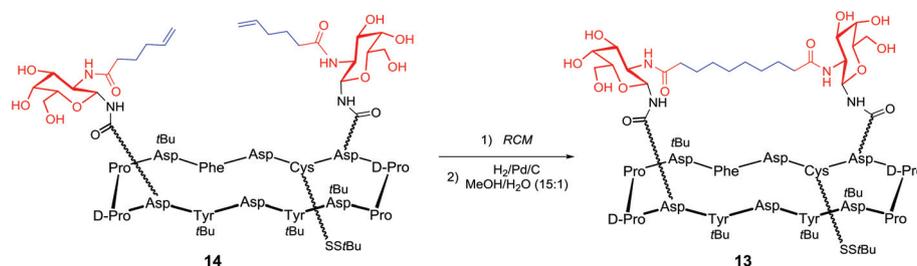
Anti-viral vaccine design

A few studies describing the utilization of cyclopeptide scaffolds as carrier in the design of vaccines against viruses have been reported. For example, Danishefsky and co-workers have synthesis of carbohydrate-based anti-HIV vaccine prototypes.⁹⁷ Only few antibodies have been identified to bind the heavily glycosylated exposed faces of the HIV envelope spike protein gp120. Among them, the human 2G12 antibody was found to neutralize a broad range of HIV strains by interacting with the Man₉GlcNAc₂ epitope expressed in the silent region of gp120 glycoprotein. Several experiments^{98,99} including a co-crystal structure^{100,101} showed that clusters of the high-

mannose glycan moiety **15** can bind to 2G12 efficiently (Fig. 11). Although synthetic structures containing several copies of the glycan **15** have been already synthesized along with the proximal portion of a gp120 amino acid sequence, no affinity for 2G12 has been observed. Danishefsky and co-workers suggested that the peptide component should present the glycans in a suitable orientation to ensure recognition.

To confirm this hypothesis, they chose a 14-residue cyclic peptide scaffold to achieve much greater design flexibility compared to the natural sequence (Fig. 12). This platform allowed for variation in the number of glycan attachments and distances between glycans units. Moreover, the presence in this scaffold of a chemical handle, spatially oriented in the opposite side of glycan units, provided a suitable anchorage point for conjugation to a carrier protein for further immunological assays. After conversion of fully deprotected glycan moiety **15** to glycosyl amine **16** by Kotchetkov amination,¹⁰² peptidic scaffolds bearing two to three aspartate residues were reacted to give the double and triple aspartylation products, respectively **17** and **18**.

The binding properties of these construct were evaluated by surface plasmon resonance on a freshly prepared 2G12 surface with the non- and mono-glycosylated peptides as controls.



Scheme 1 Constrained Tn-bearing cluster synthesized by Danishefsky and co-workers.

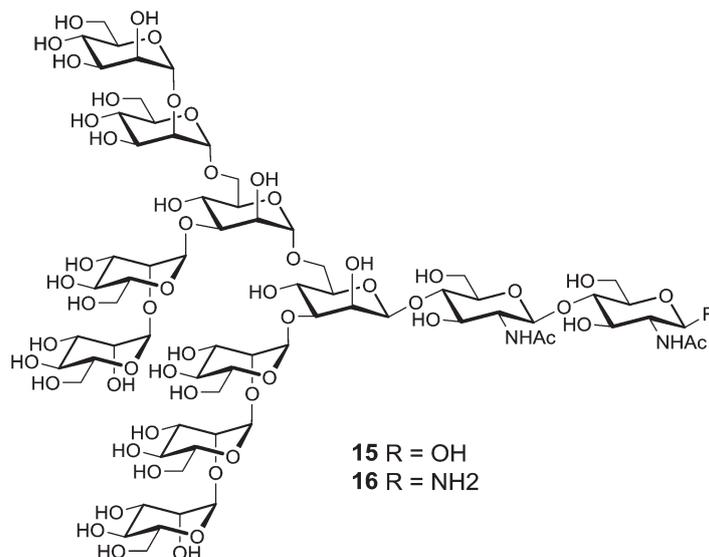


Fig. 11 Structure of high-mannose oligosaccharide epitope Man₉GlcNAc₂.



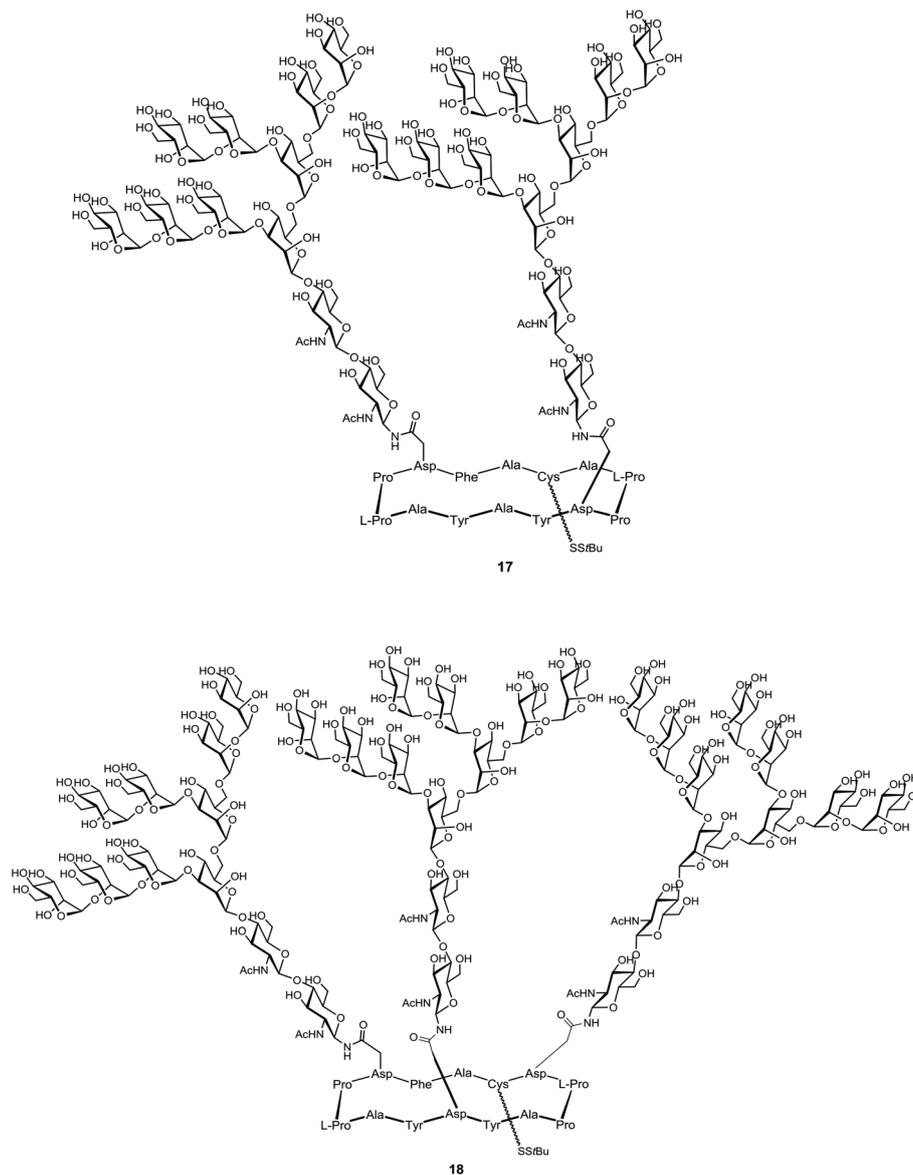
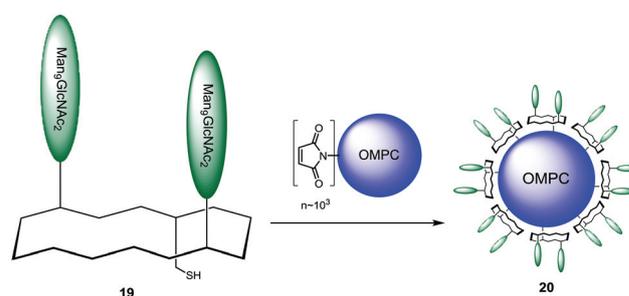


Fig. 12 2G12-epitope mimics by Danishefsky and co-workers.

Only divalent and trivalent glycopeptides **17** and **18** showed strong binding to the 2G12 surface, highlighting the importance of multivalent interactions and suggesting homology to the natural epitope on gp120. Having proved the ability of their synthetic glycopeptides to mimic 2G12 epitope, Danishefsky and co-workers next proceeded by coupling the selected divalent construct **17** to the outer membrane protein complex (OMPC) derived from *Neisseria meningitidis*, a macromolecular lipoprotein complex used as the immunogenic carrier.^{103–105,112} For this purpose, surface-accessible lysine residues of OMPC were maleimidated and the activated carrier was reacted with unmasked thiol group of **19** to give the conjugate **20** (Scheme 2).

The thiol–maleimide coupling was controlled by quantification of dicarboxyethylcysteine produced upon acid hydro-



Scheme 2 Conjugation of epitope mimic to immunogenic carrier.

lysis. The peptide loading was found to be ranged from ~2000 to 3000 mol peptide per mol carrier complex, indicating that ~50% of available maleimide groups were derivatized.¹⁰⁶ The



ability of the glycoconjugate **20** to bind human 2G12 antibody was qualitatively investigated by an ELISA sandwich assay. For this, plates coated with gp160, compound **20** or OMPC alone were incubated with 2G12. HRP antihuman IgG was used as secondary antibody. The results showed that even though compound **20** gave a clear response compared to the negative control, it was still less efficient than gp160 positive control. The immunogen capacity of the glycoconjugate **20** to induce a 2G12-like neutralizing antibody response was then evaluated. Immunization of guinea pigs and rhesus macaques was shown to promote carbohydrate-specific Ab responses in both species; unfortunately these antibodies were not able to neutralize HIV strains and although compound **20** was able to bind 2G12, it was poor competitor for binding of the mAb to native gp120.

Similarly, Wang and co-workers designed a new class of template-assembled oligomannose cluster as epitope mimics for HIV-neutralizing antibody 2G12.¹⁰⁷ A previous work, in which an oligomannose cluster Tetra-Man9 was conjugated to the keyhole limpet hemocyanin (KLH), has shown that the majority of antibody responses were directed to the linkers and the template, thus diluting drastically the immune response to the oligomannose cluster. For this reason, they decided to design new conjugate changing the carrier nature.¹⁰⁸ Based on immunization studies that have demonstrated the non-immunogenicity of RAFT scaffold,¹⁰⁹ they designed a novel cyclopeptide displaying the oligomannose cluster, in which four α -linked Man4 oligosaccharides corresponding to the tetramannose D1 arm of Man₉GlcNAc₂ epitope were introduced at one face of the scaffold, and two copies of universal T-helper epitopes corresponding to the sequence (TT₈₃₀₋₈₄₄) of tetanus toxoid¹¹⁰ were introduced at the other face (Fig. 13).

The D1 arm tetrasaccharide derivative containing an azido functionality at the aglycon portion was synthesized in two variants regarding the linker length (**21** and **22**), along with a fluorinated derivative of **21** (**23**) and the mannose arm **24** (Fig. 14).

These arms were conjugated to a tetrapropargylated scaffold using copper(I)-catalyzed azido-alkyne cycloaddition

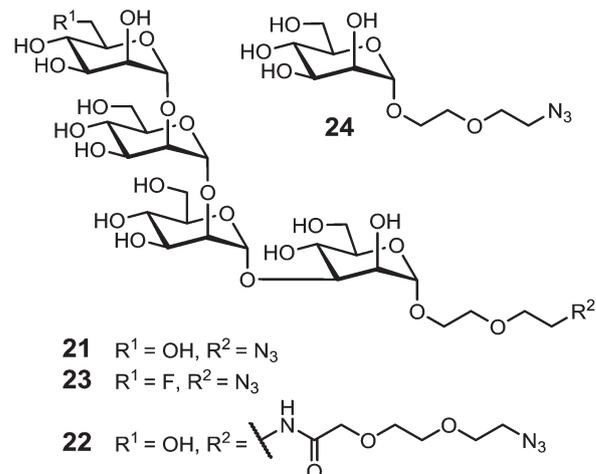


Fig. 14 Azido-bearing D1 arm tetrasaccharide derivatives and mono-mannose analogue.

(CuAAC) to give the corresponding glycoclusters (**25–28**) with excellent yields (Scheme 3).

Finally, for the two T-helper peptides introduction, the fluorinated oligosaccharide cluster **27** was derived with two azide linkers in the lower face of the platform, to give compound **29**, which underwent CuAAC reaction with the propargyl moiety previously introduced in the peptide moiety-terminus, to give the fully synthetic vaccine prototype **30** with good yields (Scheme 4).

The binding abilities of the obtained clusters to human 2G12 antibody were evaluated by SPR experiments.¹¹¹ In agreement with the previous observation of Danishefsky and co-workers,¹¹² it was found that at 10 μM , all the synthetic monomers **21–23**, as well as the high-mannose type *N*-glycan Man₉GlcNAc₂Asn, did not show apparent binding to antibody 2G12. The template assembled mannosyl-based glycocluster **25** neither showed detectable binding, while the synthetic oligosaccharide clusters carrying four units of the D1 arm tetrasaccharide (**26–28**) have demonstrated apparent affinity to the antibody 2G12 in terms of response units (RU). Also the

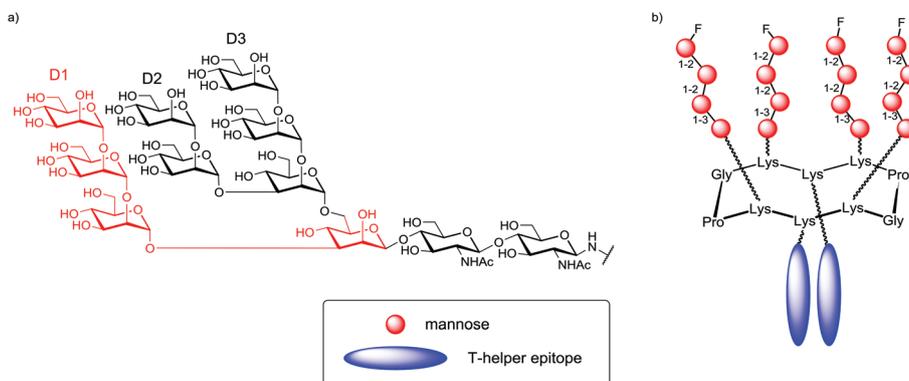
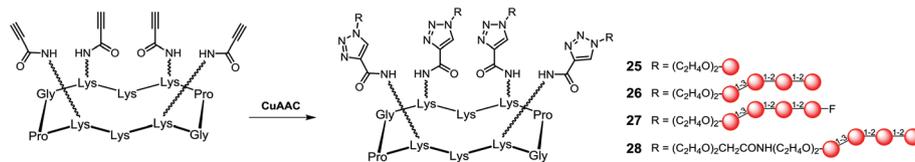
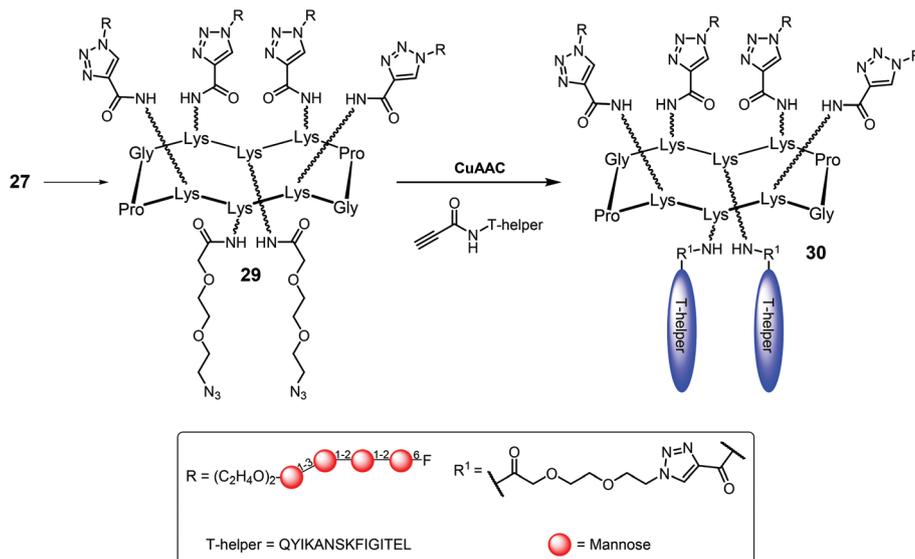


Fig. 13 (a) Structures of Man₉GlcNAc₂, and (b) the designed template-assembled glycopeptide prototype vaccine.





Scheme 3 Mannose-functionalized clusters as mimics of the 2G12 epitope.



Scheme 4 Conjugation of the T_h epitope.

fluorinated compound **27**, despite a reduced efficiency in comparison with compounds **26** and **28**, exhibits significant affinity for 2G12 (43 RU). This data suggests that an appropriate distance between the scaffold and the sugar moiety allows an improvement for 2G12 antibody binding.

Finally, the 2G12 binding of the fully synthetic vaccine prototype **30**, containing two T-helper peptides was examined. This compound demonstrated almost identical binding profiles and kinetic outcome to those of the corresponding fluorinated glycocluster **27**, which suggests that the introduction of the T-helper moiety onto the cyclic decapeptide template did not affect the structural integrity of the oligosaccharide cluster formed at the other face of the template.

Conclusion and prospect

This article presents an overview of the utilization of cyclopeptide as scaffold for multivalent and heterogeneous presentation of carbohydrate B-cell and peptidic T-cell epitopes for the design of synthetic vaccines. We highlighted the numerous advantages offered by cyclopeptide templates for vaccine constructs: (i) its non-immunogenicity; (ii) its two independent functional faces that can be regioselectively functionalized

with several carbohydrate units as B cell antigens, and the other for the conjugation of T cell peptide; (iii) the possibility to easily combine diverse epitopes; (iv) the control in the glycan composition to ensure a reproducible immune response. To date, the most sophisticated structures display four different immunogenic components that were shown to elicit efficient responses in mice models against cancer. In addition, carbohydrates based-vaccine candidates were also reported for antiviral applications and have demonstrated promising biological activities. While fully synthetic carbohydrate-based vaccine candidates have not been yet evaluated in clinical trial, recent promising results undoubtedly open new perspectives for immunotherapy to promote either for therapeutic or prophylactic effects. Due to their synthetic modularity, cyclopeptides seem ideal to combine several carbohydrate antigens on the same vaccine prototype in order to promote a multifaceted immune response against different types of cancers, and to respond to a wider population of tumor cells and at different stage of the disease progression. Other structural modifications such as the presentation of different display and/or higher density of carbohydrate epitopes, of combinations of different antigenic peptides and of glycopeptide mucin fragments represent interesting directions for future development towards immunological optimization.



Acknowledgements

Part of these studies was supported by CNRS, Université Grenoble Alpes, the “Communauté d'agglomération Grenoble-Alpes Métropole” (Nanobio program) and Labex ARCANE (ANR-11-LABX-0003-01). O. R. acknowledge, the French Agence Nationale de la Recherche (ANR-12-JS07-0001-01 “VacSyn”) and the European Research Council Consolidator Grant “LEGO” (647938).

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