"Sweet" Peptides

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Just by the title, you probably will think in peptide sweeteners (i.e., aspartame), but with the provocative "sweet" term I simply would like to call your attention on Glycopeptide conjugates (GPs), which combine in a single molecule a peptide and a sugar moiety (monomeric or oligomeric). This is an increasingly important topic, because both glycans and peptides are crucial players in different biological processes, and each one can modulate the physical and biological properties of the other. In addition to some natural glycopeptides with important biological activities, the covalent attachment of carbohydrates to bioactive peptides can serve, on one hand, to improve their pharmacological properties, affecting binding affinity and/or potency, enhancing metabolic stability, modifying biodistribution (targeted delivery), and increasing penetration across biological membranes (i.e., blood brain barrier, BBB).[1] On the other hand, the sugar moieties of GPs, when imitating glycoprotein epitopes, are recognized as important elements for immunostimulation and vaccines.[2] It is well known that glycoproteins are fundamental biomolecules in key biological and pathological processes, like cell-cell communications, recognition of viruses and bacteria by host cells, and tumor progression (many times linked to bad prognosis).[2]

To illustrate recent advancements in the GP field, this short informative article will cover a selection of some representative examples published in last years, to give you an idea of their current applications and future prospects.

Probably you know that Vancomycin was the first natural glycopeptide antibiotic approved for use in the clinic (1958) to treat Gram-positive infections. Vancomycin, along with Telavancin (2009), Dalvavancin (2014) and Oritavancin (2017), constitute the "last choice" armamentarium to fight against drug resistant bacteria, a worldwide crisis ascribed to the abuse and misuse of antibiotics.[3] For this reason, it is not surprising that the modification of these glycopeptide antibiotics focused the attention of many research groups, with a number of different derivatives prepared and studied. Recent examples include vancapticins,[4] represented here by conjugate **1** (Figure 1), which incorporates into Vancomycin an additional C-terminal, positively charged peptide, to favor electrostatic interactions with the bacterial membrane (negatively charged), and an element that facilitates membrane insertion, enhancing the contact with Lipid II (a precursor in the synthesis of bacterial cell wall). This semisynthetic lipoglycopeptide displayed improved metabolic stability and induced low levels of resistance, compared to Vancomycin and the antibiotic Daptomycin, respectively. More importantly, GP **1** showed enhanced activity against different Gram-positive bacteria (up to 100-fold more active than Vancomycin), including various GP-resistant strains, and displayed potent bactericidal effects *in vivo*. Experimental evidences point to a multifactorial mechanism of action, involving combined effects like inhibition of peptidoglycan formation, a membrane chelation that enhanced the attachment to cell wall precursors, and a direct membrane perturbation. Thus, vancapticins could represent a promising starting point towards new clinical candidates to treat drug-resistant Gram-positive infections. For much more detailed information on structurally-diverse antibiotic glycopeptides, I suggest you read the recent review article by Baskovich et al.[3]

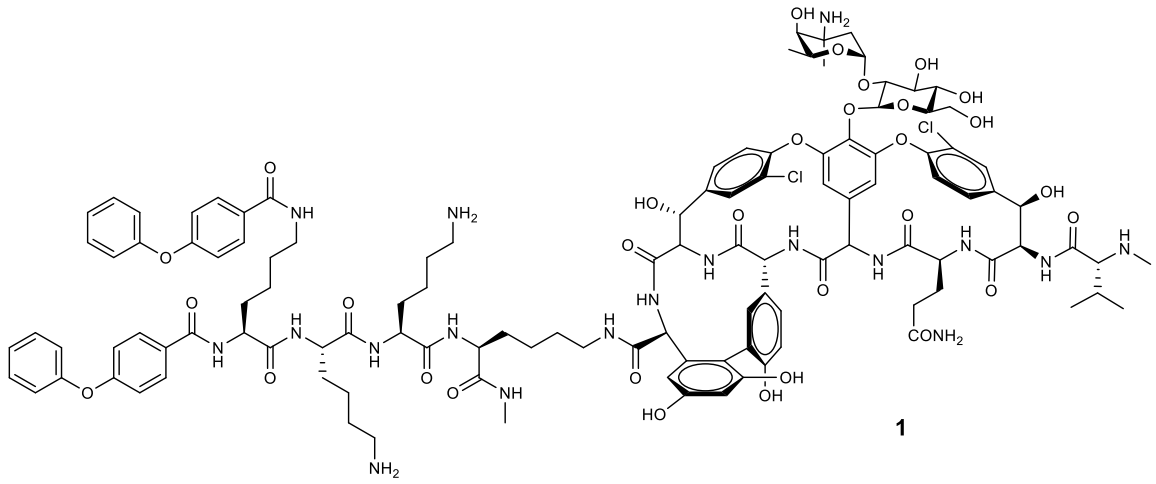

Fig. 1. Glycopeptide **1**, a selected example of vancapticin antibiotics.[4]

The search for inducers of glycan-directed broadly neutralizing antibodies (bNAbs) is a strategy towards HIV-1 vaccines that could surpass virus variability. Structural studies on the interaction of some of these bNAbs and HIV-1 envelope glycoprotein 120 (gp120) revealed that a third variable region (V3) of this glycoprotein, composed of highly glycosylated discontinuous epitopes, is key for antibody contacts. Based on this V3 region, different groups have designed synthetic glycopeptide mimetics able to bind to different bNAbs and to induce immunogenicity. Thus, the high-mannose Man_9-V3 mimetic **2** (with two glycan moieties at N301 and N332, Figure 2) was able to bind to V3-glycan bNAbs, like PTG128 and PTG125, but not to bNAbs that bind more complex glycans.[5] The affinity was higher for mimetic **2** than for the glycan (Man_9) only, indicating the importance of both the polypeptide chain and the oligosaccharide moiety for bNAb recognition. As expected, the corresponding aglycone peptide could not bind these antibodies. In addition, glycopeptide **2** was used to isolate bNABs with neutralizing ability against HIV pseudovirus, and showed modest immunogenic activity in rhesus macaques. Probably, the addition of a T helper epitope and a multimeric presentation would be required for higher immunization efficacy of this GP. Another example of HIV-1 immunogen is the V3-derived glycopeptide **3**, a conjugate of a N334 Man_9-oligopeptide with a peptide T cell epitope and a TLR2 ligand.[6] This three-component GP was able to bind different bNAbs, and to elicit antibody responses in rabbits, with broad recognition of HIV-1 gp120/gp140 envelope proteins (with both N332 and N334 oligosaccharides). Although the N332 to N334 mutation in only present in about 17% of HIV viruses, they normally are highly resistant to bNAB neutralization. Therefore, immunogen **3** could complement other GP mimetics based on N332 V3 epitopes, and could be considered a valuable component for the design of HIV-1 vaccines.

Mucin-1 (MUC1) is a cell surface, tumor-associated glycoprotein overexpressed in a variety of cancers, and the subject of many studies for immunotherapeutic intervention. The extracellular domain of MUC1 contains a number of repeat segments, glycosylated at Ser or Thr residues, but the oligosaccharide composition differs between normal and tumor cells. Therefore, tumor-associated MUC1-derived glycopeptides are considered attractive antigens for antitumor vaccines.

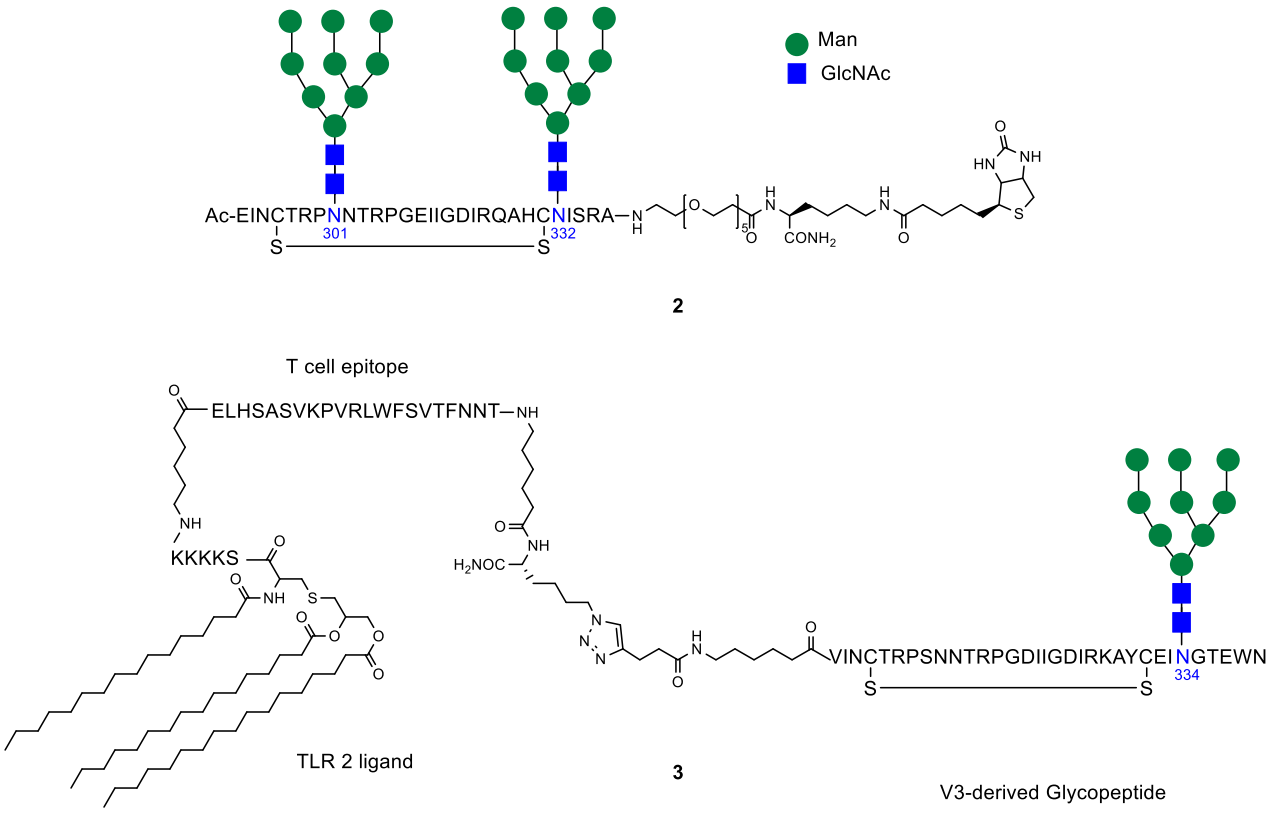

Fig. 2. Representative examples of glycopeptides acting as HIV-1 immunogens.

Glycopeptides active ester **4** (Figure 3), containing a βGal-(1,3)-αGalNAc-disacharide at the Thr residue, was covalently linked to a bacteriophage Qβ carrier and used to immunize MUC1 transgenic mice.[7] This Qβ conjugate induced the generation of antibodies, able to bind to different Mucin glycoforms, and displayed significant activity against metastatic lung cancer *in vivo*. Another approach in this field describes the replacement of O by S/Se at the glycosidic linkage (as in compound **5**, Figure 3).[8] This simple modification not only triggered conformational changes in the glycopeptide, improving its binding affinity to antibodies, but constituted a new strategy toward potent MUC1 antigen mimics. Thus, the N-terminal Cys-containing glycopeptide **5** (only a monosaccharide residue), when conjugated to gold nanoparticles through maleimide appendages, initiated significant immune responses in mice. In fact, the antisera of these mice recognized MUC1 expressing cancer cells, including those from biopsies of breast cancer patients. Therefore, the above described approaches may contribute to the future development of cancer diagnosis systems and vaccines.

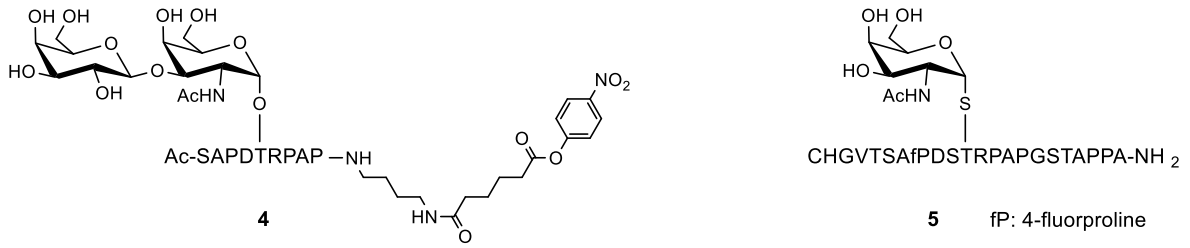

Figure 3. Selected examples of glycopeptide MUC1 antigent mimics.

The preparation and further characterization of N-terminal monosaccharide derivatives of the anticancer cationic peptide R-lycosin have recently been described.[9] In this study, galactose-, arabinose- and glucose-containing conjugates showed improved cytotoxicity compared to the model peptide, while mannose and N-acetylglucosamine displayed reduced activity, thus pointing to the importance of the carbohydrate moiety for antitumor potency. Furthermore, β-D-glucopyranoside derivative **6** (Figure 4) exhibited some selectivity for tumor cells compared to non-tumor normal cells, most probably due to active transport through the GLU1 transporter, overexpressed in numerous cancers (selective targeting). Glucopeptide **6** acted by disrupting cell membranes and mitocondrial-induced apoptosis. It had significant *ex vivo* antitumor activity in tumor spheroids of A549 cells, and inhibited tumor growth after intraperitoneal administration in a Xenograph mice model of A549 human alveolar cell carcinoma.

Protein-protein aggregation of amyloid-b peptide (Ab) results in amyloid plaques associated with Alzheimer's disease (AD). Hence, the search for compound able to reduce transient oligomers and fibril formation could have application for AD treatment. Glycopeptidomimetic **7** was found to interact with monomeric and oligomeric $Ab_{1\text{-}42}$ (NMR and SPR studies), and to inhibit oligomerization and cross-β-sheet elongation (fibrillation) of this amyloid peptide, considering the sugar moiety as the key β-sheet breaker element.[10] Peptide conjugate **7** was highly stable in plasma and, more importantly, increased the survival of SH-SY5Y neuroblastoma cells treated with cytotoxic concentrations of $Ab_{1\text{-}42}$. This is just an example of how glycopeptides could disrupt protein-protein-interaction and, probably, many other examples could follow in the near future.

Sometimes, the conjugation of a peptide with a sugar moiety helps it to cross BBB for targeting the central nervous system. An example in this respect is MMP2200 (**8**, Figure 4), a glycopeptide opioid agonist with good affinities for μ and δ opioid receptors. It displayed 10-fold more potent antinociceptive activity in mice (iv administration) than the corresponding free peptide, an effect that was attributed to improved metabolic stability and BBB penetration. In a recent study, glycopeptide **8** showed antiparkinsonian activity, reducing abnormal involuntary movements (dyskinesia) induced by DA receptor agonists, and reduced the pro-parkinsonian activity of MK-801 (NMDA receptor antagonist), thus suggesting a cross-talk among the three regulatory systems. These results open the opportunity to explore the therapeutic potential of opioid peptide derivatives in combination with DA receptor agonists, currently used for the treatment of Parkinson Disease (PD).

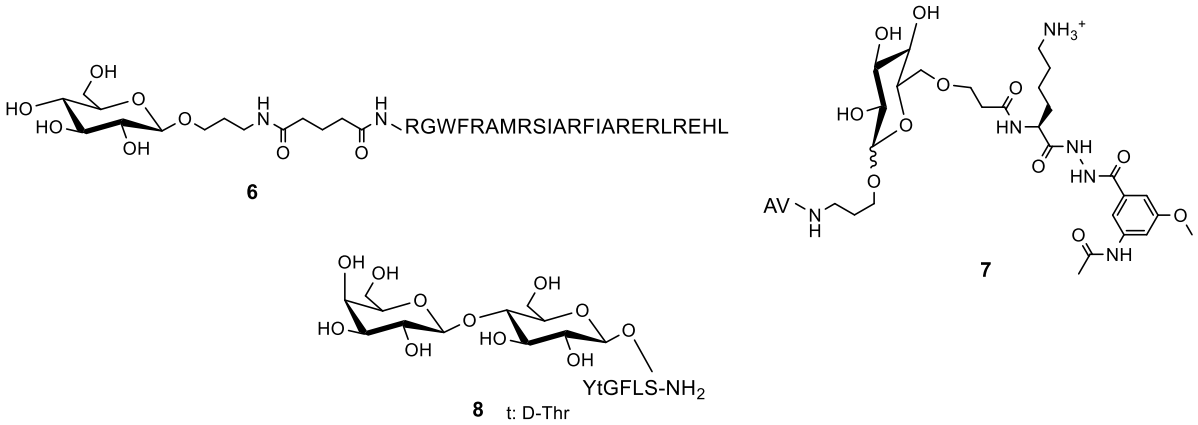

Fig. 4. Other selected examples of glycopeptides of interest in drug discovery.

The advances in efficient strategies for the preparation of GPs, including enzymatic synthesis, click chemistry or native ligation approaches, and the knowledge about biologically relevant glycoproteins, their communication with other biomolecules and their implication in pathological processes greatly improved in recent years. Also, the use of GPs for new materials of interest in drug delivery systems is envisaged already. Hence, all these advancements will undoubtedly predict a bright future for these "sweet" peptides, allowing the preparation and study of increasingly complex conjugates, and expanding the potential fields of their application.

ABBREVIATIONS

Aβ, amyloid-β peptide; AD, Alzheimer's disease; BBB, blood brain barrier; bNAb, broad neutralizing antibody; DA, dopamine; iv, intravenous; MUC1, mucin-1 protein; HIV, human immunodeficiency virus; Gal, galatose; GalNHAc, N-acetyl galatosamine; GlcNHAc, N-acetyl glucosamine; GP, glycopeptide; NMDA, N-methyl-D-aspartic acid; Man, mannose; PD, Parkinson's disease; TLR2, Toll-like receptor type 2.

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