New Directions in the Synthesis of Glycopeptide Mimetics

Lisa A. Marcaurelle and Carolyn R. Bertozzi*[a]

Abstract: For more than a decade, glycopeptides have attracted the attention of synthetic chemists as challenging targets for total chemical synthesis. Great strides have been made, but the chemical synthesis of complex glycopeptides still remains an arduous task. In recent years, discoveries at the forefront of biological research that have identified glycoproteins as key modulators of cell–cell communication have created an urgent demand for quantities of homogeneous glycoconjugates for therapeutic and basic research applications. In response, chemists have begun to explore various approaches to the construction of glycopeptide mimetics that have superior properties for therapeutic applications, or lend themselves to a more facile synthesis. In this Concepts article, we summarize contemporary approaches to the design of stable glycopeptide analogues, including *C*- and *S*-glycopeptides and glycopeptoids, as well as creative new strategies for generating glycopeptide mimics with use of the chemoselective ligation technique.

Keywords: chemoselective ligation · *C*-glycosides · glycopeptides · peptoids · *S*-glycosides

Introduction

Glycoproteins have assumed a rather notorious position among biologists and chemists. Owing to their oligosaccharide substituents, glycoproteins are not under direct genetic control but are instead the products of secondary metabolism. Glycoproteins have defied traditional genetic approaches to biological study because of the heterogeneous and template-independent nature of their biosynthesis. This feature has frustrated efforts on the part of biologists to elucidate the biological functions of glycoproteins, particularly in circumstances in which the precise structure of the glycan determines biological activity. It is becoming apparent that oligosaccharides on a polypeptide can modulate protein folding, intra- and intercellular trafficking, and receptor binding and signaling.[1] These discoveries at the forefront of biological research have created a requirement of some urgency for glycoproteins of well-defined structure.

In parallel, chemists have discovered glycoproteins to be alluring yet formidable synthetic targets. Glycoproteins embody all of the complexity of oligosaccharide chemistry, including difficult glycosyl coupling reactions and extensive protecting group manipulations, compounded with the challenges of solid-phase peptide synthesis. Consider the structures of prototypical glycoprotein glycans from the *N*-linked (**1**) and *O*-linked (**2**) families (Figure 1). The synthesis of merely the core fragments, the regions in closest proximity to the polypeptide backbone, can require complex orthogonal protection schemes to form branched oligosaccharide structures. The methods for such transformations that are currently at our disposal are not compatible with the complex functionality of the polypeptide backbone, precluding the chemical elaboration of the oligosaccharide from the polypeptide scaffold. The convergent assembly of separately prepared oligosaccharides and proteins is also marked by several obstacles. Despite these challenges, in recent years several groups have conquered glycopeptide fragments, and some impressive structures have been reported.[2] In particular, advances in enzymatic glycosylation methods promise to lift the burden of protecting-group manipulations in future glycoprotein syntheses.[3]

Still, while chemists continue to progress toward a general and accessible synthesis of native glycoproteins, there are pressing issues in biology that have inspired parallel efforts toward the synthesis of glycoprotein mimetics. For example, a native glycoprotein may lack the stability or bioavailability required for a specific therapeutic or basic science application. Isosteric analogues have been designed to circumvent these problems. The field of glycoprotein mimicry is also driven by the urgency of certain biological problems, which overrides the preference for a native structure. The goal shifts to obtaining as rapidly as possible a synthetic mimic with comparable biological activity. At present, the synthesis of native glycoproteins is a time-consuming and encumbered process, but glycoprotein mimics with subtly altered structures can be made with reasonable facility. In this article, we highlight recent concepts in glycoprotein mimicry that address these issues at the chemistry/biology interface.

[a] Prof. C. R. Bertozzi, L. A. Marcaurelle
Department of Chemistry, University of California
Berkeley, CA 94720 (USA)
Fax: (+1) 510-643-2628
E-mail: bertozzi@cchem.berkeley.edu

0947-6539/99/0505-1384 $ 17.50+.50/0

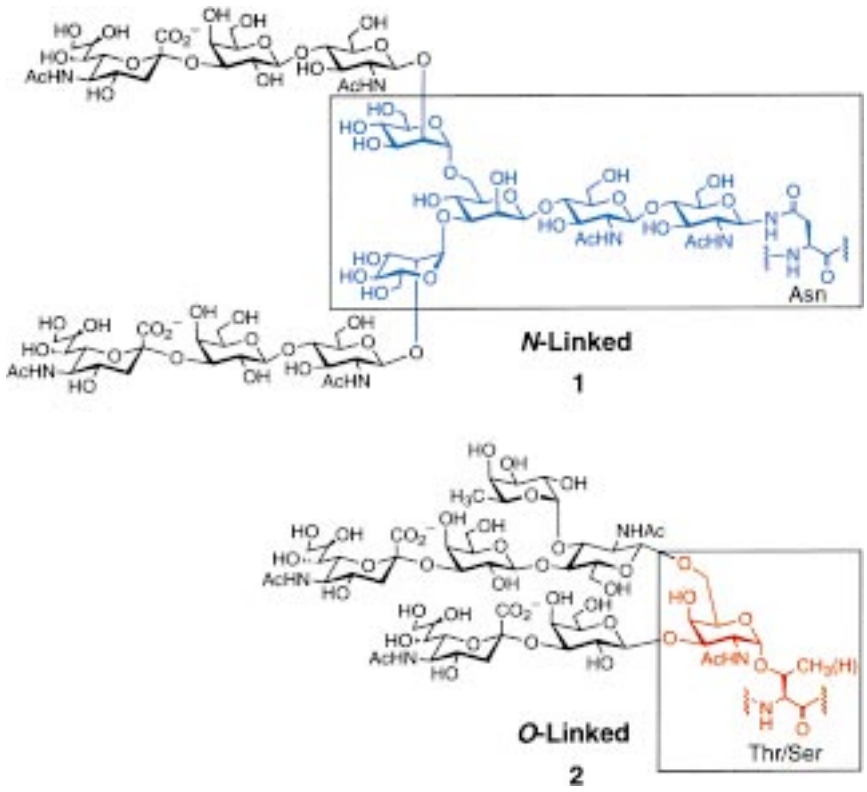


Figure 1. Representative *N*-linked (**1**) and *O*-linked (**2**) glycans. The conserved core structures within each family are indicated in color.

Discussion

Stable glycopeptide isosteres: It is well established that glycosylation of peptide drugs can dramatically alter their pharmacokinetic properties. Indeed, many native glycoproteins have been considered as therapeutic agents and a handful are now in clinical use.[4] But there is room for improvement of these compounds, particularly with respect to the stability of the sugar-peptide linkage. The primary pathway for glycoprotein degradation involves enzymatic cleavage of the glycan from the peptide backbone, an event that, if thwarted, could increase the lifetime of glycopeptide drugs. Chemists have observed that substitution of the glycosidic C–O or C–N bonds found in *O*-linked and *N*-linked glycoproteins, respectively, with a C–S or C–C bond confers resistance to enzymatic hydrolysis.[5] In addition, *C*- and *S*-glycosides demonstrate solution conformations and biological activities similar to their native counterparts.[6] Accordingly, much effort has been devoted to the synthesis of *C*- and *S*-glycopeptide analogues.

The replacement of the oxygen atom of an *O*-linked glycopeptide with a sulfur atom is a conceptually straightforward approach to isosteric mimicry. In practice, this has been accomplished by coupling an activated sugar derivative with a protected cysteine residue. Several *S*-glycosylated cysteine derivatives have been reported[7] (a representative example (**4**) is shown in Figure 2), and some of these analogues have been incorporated into peptides by standard solid-phase methods.[7a, 8] *S*-Glycopeptides have also been prepared using a building block derived from glycosylation of 3-mercaptopropionic acid.[7c]

While structurally similar to *S*-glycopetides, *C*-glycopeptides present a greater synthetic challenge because of the requirement of stereoselective C–C bond formation at the anomeric center. Several groups have met this challenge with the synthesis of *C*-glycosyl serine analogues, which were incorporated into glycopeptides by traditional methods.[9] For example, Beau and co-workers[9c] prepared a *C*-linked analogue of the Tn antigen (GalNAcα-Ser) (**5**, Figure 2), an *O*-linked motif found in several epithelial tumor glycoproteins.[10] Synthetic glycopeptides comprising the *C*-linked Tn analogue may serve as future generations of tumor vaccines.

In contrast to *O*-linked glycopeptides which are degraded through glycosidic bond hydrolysis, *N*-linked glycopeptides are hydrolyzed at the glycosyl amide (**6**, Figure 3) by enzymes from the *N*-glycanase family.[11]

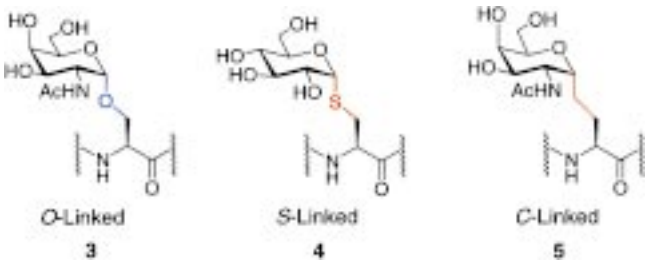


Figure 2. Comparison of a native *O*-linked glycan (**3**) and two stable glycopeptide isosteres, an *S*-glycoside (**4**)[7b] and a *C*-glycoside (**5**).[9c]

Several groups have sought analogues of *N*-linked glycopeptides which resist the action of these enzymes. In one approach, Kessler et al. reversed the orientation of the glycosyl amide affording *C*-glycosyl analogue **7**.[12] Such derivatives may be resistant to *N*-glycanase cleavage just as retro-inverso peptides resist proteolytic cleavage.[13] In an elegant alternative approach, Lee and co-workers[14] combined *C*-glycoside chemistry with enzymatic chemistry to construct a *C*-linked analogue of a high-mannose *N*-linked glycopeptide (**8**, Figure 3). The synthetic strategy consisted of two key steps: the chemical synthesis of a peptide bearing a *C*-linked GlcNAc residue followed by enzymatic transfer of a $Man_9GlcNAc$ moiety onto the core GlcNAc. The *C*-glycopeptide (**8**) was found to be resistant to *N*-glycanase-catalyzed hydrolysis and, furthermore, displayed inhibitory activity toward the enzyme.

  0947-6539/99/0505-1385 $ 17.50+.50/0

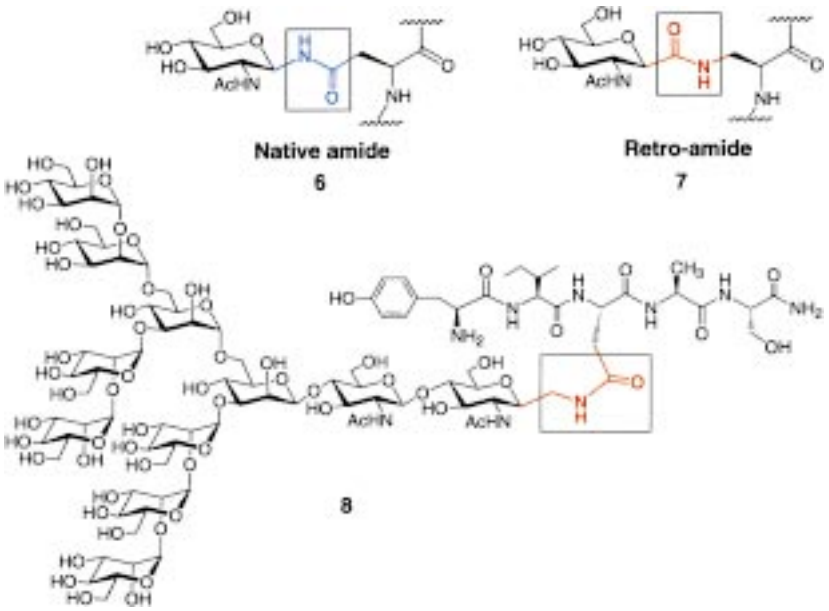


Figure 3. Comparison of a native *N*-linked glycopeptide (**6**), a retro-amide *C*-glycosyl analogue (**7**),[12] and a high-mannose *C*-linked glycopeptide (**8**).[14]

Glycopeptoids: Peptoids, or *N*-substituted oligoglycines (**9**, Figure 4), are achiral peptide mimics in which side chains are sited on the amide nitrogen atom of each glycine monomer rather than the α-carbon atom of each amino acid.[15] Peptoids were designed in order to overcome several shortcomings of synthetic peptides as potential drugs, including susceptibility

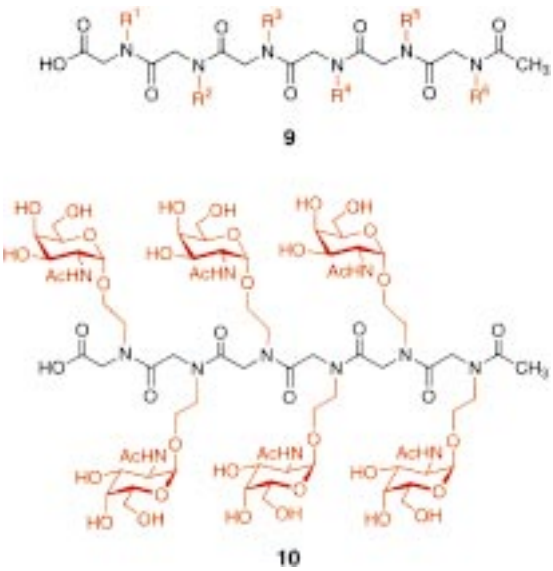


Figure 4. General structure of a peptoid (**9**)[15] and an *O*-linked glycopeptoid (**10**) bearing the Tn antigen.[16c]

to proteolytic degradation and poor bioavailability. While these highly flexible molecules are indeed resistant to proteolysis, the problem of oral bioavailability still exists. To address this problem, Roy and co-workers designed a new class of mimetics which they named glycopeptoids.[16] The *O*-linked glycopeptoid **10** (Figure 4), which bears multiple Tn antigens, was prepared in order to evaluate the role of multivalency in antigen presentation.[16c] A method for the synthesis of a *C*-linked glycopeptoid building block has recently been reported by Kessler and co-workers.[17] When incorporated into glycopeptoids, the *C*-glycoside should provide additional stability towards chemical and enzymatic hydrolysis.

Synthesis of glycopeptide mimics by chemoselective ligation: The concepts that have been described thus far were motivated by the need for metabolically stable glycopeptide mimetics. While glycopeptide isosteres and glycopeptoids are resistant to hydrolysis, the construction of these analogues is still a time-consuming and challenging endeavor. The synthesis of such analogues becomes especially difficult when dealing with larger oligosaccharide structures, such as the naturally occurring *O*- and *N*-linked glycans depicted in Figure 1). Moreover, biologists eager to study the roles of protein-associated glycans in biological systems typically do not have the synthetic expertise to prepare native complex glycoproteins. Their collaborators in synthetic chemistry may require several years to generate small quantities of a relatively simple structure, stalling the progress of research at the biological forefront.

As early as the 1970's, it was apparent that oligosaccharides could be attached via non-native linkages to normally unglycosylated proteins, such as bovine serum albumin (BSA), generating neoglycoproteins that have since found use in a myriad of biological studies.[18] Methods for the synthesis of neoglycoproteins capitalized on the inherent reactivity of certain amino acid side chains, in particular the ε-amino group of lysine and the sulfhydryl group of cysteine, and were of sufficient simplicity that extensive synthetic expertise was not required. The protein component of these reagents was considered largely irrelevant, serving merely as a scaffold for multivalent presentation of the carbohydrates and for the simultaneous attachment of convenient molecular probes. More recently, chemists have shifted their attention to the construction of glycoprotein mimetics in which the sequence and structure of the underlying protein, and the specific sites of glycosylation, are of biological relevance. Some of the methods developed for classical neoglycoprotein synthesis are now being revisited in the context of structurally defined glycopeptide mimetics with unnatural sugar–peptide or glycosidic linkages.

A technique that promises to be extremely powerful for the site-specific attachment of carbohydrates to peptides is based on the concept of chemoselective ligation. Originating in the field of protein chemistry, the technique involves the coupling of two mutually and uniquely reactive functional groups in an

0947-6539/99/0505-1386 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 5

aqueous environment.[19] Chemoselective ligation reactions are of such high selectivity that no protecting groups are needed. Even in the presence of a multitude of potentially reactive functionalities, two chemoselective ligation partners will react only with each other. Reactions that have proven useful for chemoselective ligation include the coupling of aminooxy groups with ketones or aldehydes to afford the corresponding oximes, and reactions of thiolate anions with α-haloacetamides to form the corresponding thioesters or thioethers.

Modifications of endogenous cysteine residues: The chemoselective reaction of cysteine thiols with electrophilic carbohydrates originated with neoglycoproteins and is now widely used for the construction of glycopeptide mimetics of defined structure. For example, α-haloacetamido[20] or bromoethyl glycosides[21] react selectively with cysteine sulfhydryl groups on unprotected peptides to afford modified glycopeptides with non-native sugar–peptide linkages (**11** and **12**, Figure 5).

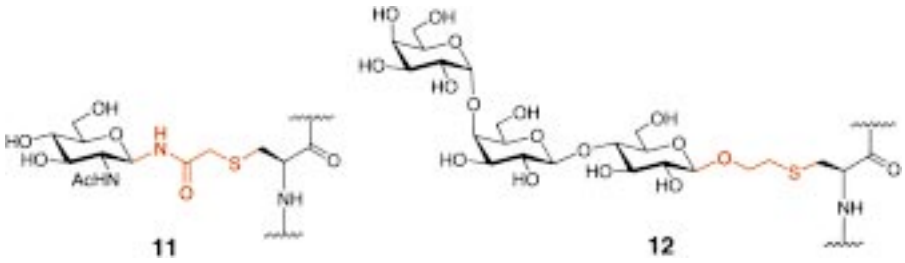
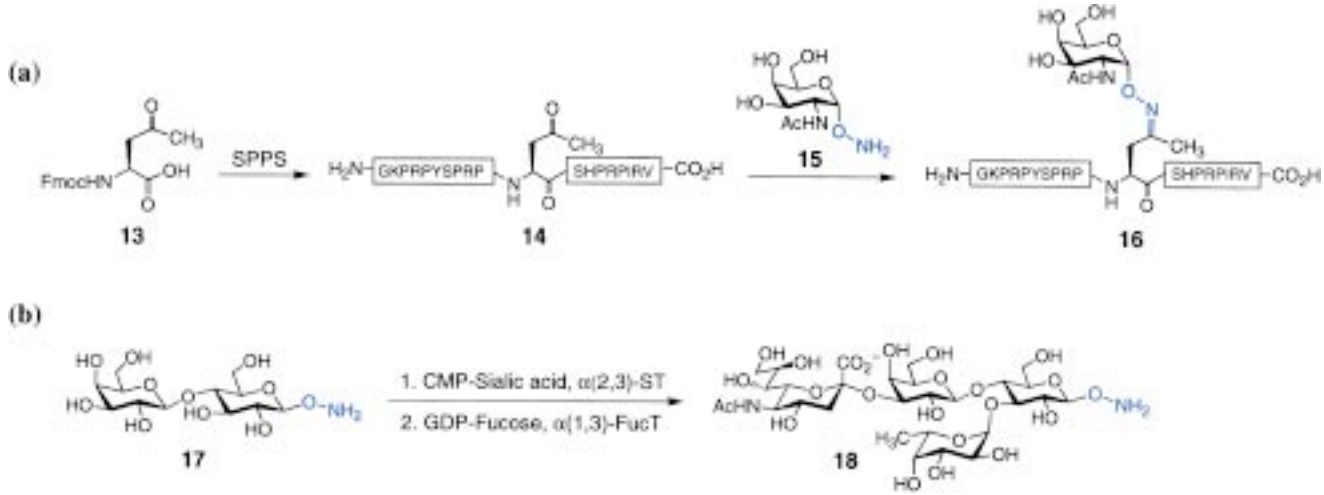


Figure 5. α-haloamides[20] and bromoethyl glycosides[21] have been conjugated to cysteine thiols to generate glycopeptide mimetics such as **11** and **12**.

With respect to synthetic facility, the α-haloacetamido glycosides are superior, as they can be prepared from unprotected free oligosaccharides through glycosylamine intermediates.[20b] In principle, complex oligosaccharides from natural sources[2f] could be attached to peptides in this fashion without any protecting group manipulations. It should be noted, however, that multiple cysteine residues cannot be differentiated, that is, a single cysteine residue cannot be glycosylated site-specifically in the presence of others. In order to maintain an unmodified cysteine residue, one must employ orthogonal cysteine protecting groups during solid-phase peptide synthesis.

Exploiting orthogonal functionality: An alternative strategy for the generation of glycopeptide analogues could proceed by the introduction of a novel functional group into the peptide scaffold, one that is not normally found among amino acid side chains and is chemically orthogonal to native amino acid components. The ketone group fulfills these requirements, and toward this end a method for the synthesis of glycopeptide mimetics has recently been described which exploits the highly selective condensation reaction of ketones with aminooxy groups to afford oximes.[22] Ketone-containing peptides were generated by solid-phase peptide synthesis (SPPS) with amino acid **13**,[23] which bears an unnatural ketone side chain (Scheme 1a). Reaction of ketopeptide **14** with aminooxy sugars such as compound **15** afforded glycopeptide mimetics in which an oxime substitutes for the native sugar–peptide bond. In this fashion, an oxime-linked analogue (**16**) of the antimicrobial glycopeptide drosocin was prepared.[22]

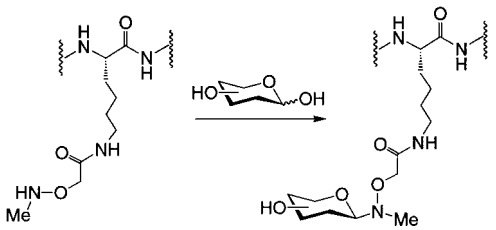
This chemoselective ligation strategy is not limited to the synthesis of peptides bearing simple monosaccharides. More complex oligosaccharides can be generated from simple aminooxy sugars using established enzymatic methods. For example, elaboration of aminooxy lactose (**17**, Scheme 1b) by enzymatic sialylation and fucosylation afforded sialyl Lewis *x* analogue **18**, which was then allowed to react with ketopeptide **14** to generate an oxime-linked glycopeptide bearing a structural motif similar to that found in *O*-linked mucin-like glycoproteins.[24] Alternatively, unprotected glycosylamines can be converted to aminooxy,



Scheme 1. a) Synthesis of an oxime-linked glycopeptide (**16**) by means of an unnatural ketoamino acid (**13**).[22] b) Enzymatic conversion of aminooxy lactose to sialyl Lewis *x* analogue **18**.[24] α(2,3)-ST = α(2,3)-sialyltransferase and α(1,3)-FucT = α(1,3)-fucosyltransferase.

hydrazide, or thiosemicarbazide derivatives in a straightforward manner, and each of these can be chemoselectively ligated to ketopeptides.[24] Like α-haloacetamides, these latter nucleophilic carbohydrate derivatives can be generated from small quantities of unprotected free oligosaccharides through glycosylamine intermediates. Thus, precious oligosaccharides isolated from natural sources could potentially be converted to coupling partners suitable for conjugation to ketone-containing peptides.

An alternate strategy for the chemoselective synthesis of glycopeptide analogues with non-native sugar–peptide linkages has recently been described by Mutter and co-workers.[25] Free reducing sugars were reacted with peptides bearing internal *N*,*O*-disubstituted aminooxy groups (**19**, Scheme 2) to



19 **20**

Scheme 2. Reaction of free reducing sugars with peptides bearing *N*,*O*-disubstituted aminooxy groups (**19**) to generate β-*N*-linked glycopeptide analogues (**20**).[25]

produce β-*N*-linked glycopetide analogues (**20**). The reducing terminal pyranose unit was preserved and the peptide was glycosylated with high anomeric stereoselection without the need for activation at the anomeric center or protection of functional groups. Free reducing oligosaccharides have also been appended to the N-terminus of synthetic peptides adorned with an N-terminal aminooxy group.[26]

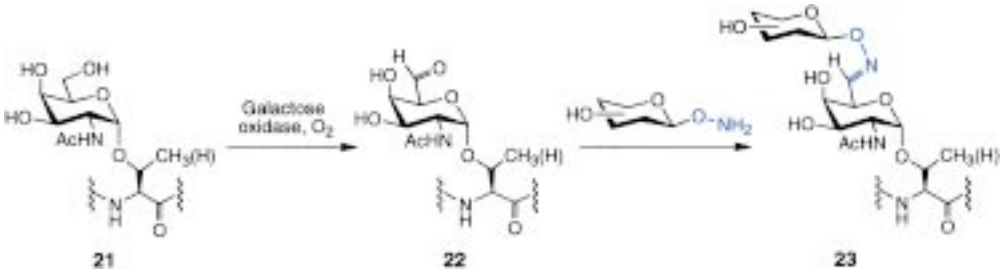
As the synthesis of glycopeptide mimetics is motivated by a need for biologically active structures, a crucial question is: how does the substitution of the native sugar–peptide bond with a non-native linkage affect bioactivity? Despite a multitude of strategies for their construction, very few glycopeptide mimetics have actually been evaluated for activity relative to their native counterparts. One study has shown that replacement of the sugar–peptide bond with an oxime linkage has no detrimental effect on function, even in a molecule whose activity is normally dependent on glycosylation. The oxime-linked analogue of drosocin (**16**, Scheme 1a), a 19-amino-acid glycopeptide with a single site of *O*-linked glycosylation,[27] was found to be comparable in bacteriostatic activity to the native molecule.[22]

It may be that in some cases the loss of the native sugar–peptide linkage results in reduced bioactivity of the glycoprotein. Indeed, it has been demonstrated in model glycopeptides that the monosaccharide units most proximal to the peptide backbone can profoundly influence local peptide structure.[28] These observations have directed attention to new sites of unnatural linkage substitution such as glycosidic bonds within the pendant glycan. As an example of this alternative approach, chemoselective ligation chemistry was combined with enzymatic chemistry to generate modified *O*-linked glycopeptides with native sugar–peptide linkages.[29] First, a glycosylated amino acid building block was incorporated into a peptide using standard solid-phase methods to produce a glycopeptide bearing a proximal GalNAc (**21**, Scheme 3). Next, a chemically unique functional group for chemoselective ligation was introduced by means of the enzyme galactose oxidase, which selectively converts galactose or GalNAc residues to the corresponding C-6 aldehydes.[30] Finally, the aldehyde (**22**) was coupled to a synthetic aminooxy sugar to afford an oxime-linked analogue (**23**) of the $\beta 1 \rightarrow 6$ glycosidic linkage that is commonly found in native *O*-linked glycoproteins. Flexibility in the elaboration of outlying glycoforms could be achieved while the native peptide-proximal GalNAc-α-Thr linkage was maintained.

These ketone- or aldehyde-based chemoselective ligation strategies have the advantage over traditional methods for neoglycoprotein synthesis in that they are site-selective. Ketone, aldehyde, and aminooxy groups do not normally reside within polypeptides, so once installed at a specific position, they can be glycosylated in a controlled manner. In addition, these ligation reactions are orthogonal to thiol alkylations, allowing one to conjugate two different oligosaccharides to ketone and thiol groups within a peptide in one synthetic step (see Scheme 4). The one-step dual modification of synthetic peptides using these mutually compatible reactions has recently been achieved with biophysical probes.[23]

Future Directions

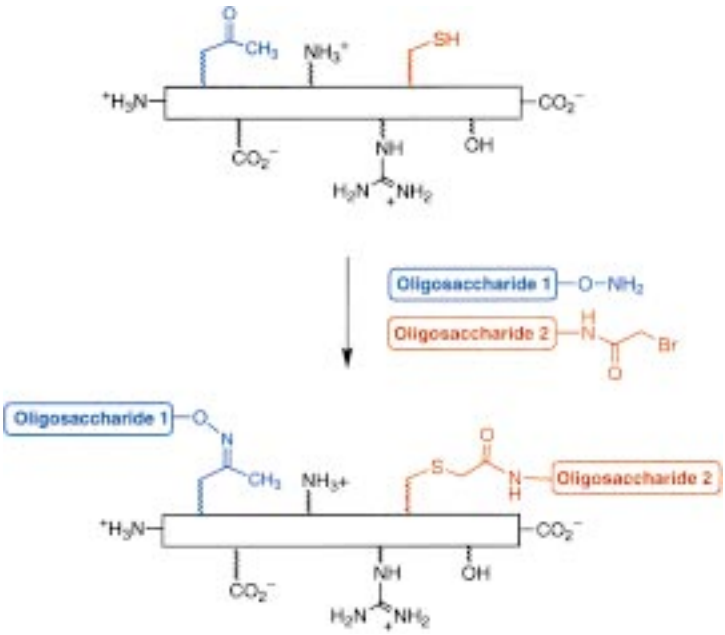
These novel concepts in glycopeptide mimicry have enabled the synthesis of glycopeptide analogues with improved pharmacokinetic properties or impressive glycan structures that are difficult to achieve in their native form. Still, most of



21 **22** **23**

Scheme 3. General strategy for the synthesis of glycopeptide mimics with C-6 oximes.[29]

0947-6539/99/0505-1388 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 5



Scheme 4. General strategy for the one-step modification of peptides with two different oligosaccharides.

the synthetic targets described were rather small with respect to peptide length, representing fragments of glycoproteins several orders of magnitude larger in size. Glycopeptide synthesis must by definition be restricted by the limitations of solid-phase peptide synthesis, currently executed in a routine fashion for peptides of around 60 amino acids or less. Protein chemists are now addressing this limitation with the development of new strategies for convergent segment condensation. Two recent technologies, native chemical ligation[19a, 31] and the related expressed protein ligation,[32] have taken the forefront in this regard. The strategies described above for synthesizing glycopeptide mimetics could be merged with these protein synthesis techniques to afford full-length glycoprotein mimetics of unlimited size.

Finally, in nature many glycoprotein molecules reside on the surfaces of cells, anchored to the plasma membrane. The glycans of cell-surface glycoproteins contribute to many extracellular recognition events that govern the life of the cell. Perhaps it is now time to ask whether we can construct glycoproteins or their mimetics in the context of a cell surface. While this is a somewhat radical notion, the ability to generate glycoproteins of defined structure on cell surfaces would provide avenues for biological investigation for which there is no current alternative. The exogenous addition of structures onto cells is not a new concept, having been demonstrated with recombinant GPI-anchored proteins which passively insert into the plasma membrane when added to cells in culture.[33] However, the attachment of chemically synthesized glycoproteins or mimetics has not been reported and would represent a landmark experiment. Chemically synthesized glycoproteins could be attached to cell surfaces through a suitable cell surface anchor. Several groups have taken steps in this direction with the conjugation of synthetic oligosaccharides to endogenous glycoprotein glycans using enzymatic and chemical methods.[34] In this fashion, novel glycoprotein landscapes were created on living cells. The extension of these approaches to include synthetic glycoproteins and their mimetics is a major frontier in cell surface engineering.

[1] a) R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683; b) A. Varki, *Glycobiology* **1993**, *3*, 97.

[2] a) N. Mathieux, H. Paulsen, M. Meldal, K. Bock, *J. Chem. Soc. Perkin Trans. 1* **1997**, *6*, 2359; b) D. Sames, X.-T. Chen, S. Danishefsky, *Nature* **1997**, *389*, 587; c) S. J. Danishefsky, S. Hu, P. F. Cirillo, M. Eckhardt, P. Seeberger, *Chem. Eur. J.* **1997**, *3*, 1617; d) Z.-G. Wang, X.-F. Zhang, Y. Ito, Y. Nakahara, T. Ogawa, *Carbohydr. Res.* **1996**, *295*, 25; e) J. Seifert, C. Unverzagt, *Tetrahedron Lett.* **1996**, *37*, 6527; f) E. Meinjohanns, M. Meldal, H. Paulsen, R. A. Dwek, K. Bock, *J. Chem. Soc. Perkin Trans. 1* **1998**, *549*.

[3] a) C. Unverzagt, *Carbohydr. Res.* **1998**, *305*, 423; b) O. Seitz, C.-H. Wong, *J. Am. Chem. Soc.* **1997**, *119*, 8766.

[4] D. A. Cumming, *Glycobiol.* **1991**, *1*, 115.

[5] a) T. Bar, R. R. Schmidt, *Liebigs Ann. Chem.* **1991**, 185; b) D. Horton, J. D. Wander, *Carbohydrates: Chemistry and Biochemistry*, *Vol. 4B* (Eds. : W. W. Pigman, D. Horton), 2nd ed., Academic Press, New York, **1990**, pp. 799–842; c) M. A. Sparks, K. W. Williams, G. M. Whitesides, *J. Med. Chem.* **1993**, *36*, 78.

[6] a) D. E. Levy, *The Chemistry of C-Glycosides,* Pergamon, Oxford, **1995**; b) Y. Kishi, *Pure Appl. Chem.* **1993**, *65*, 771.

[7] a) M. Gerz, H. Matter, H. Kessler, *Angew. Chem.* **1993**, *105*, 311; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 269; b) L. Käsbeck, H. Kessler, *Liebigs Ann.* **1997**, *1*, 165; c) M. Elofsson, B. Walse, J. Kihlberg, *Tetrahedron Lett.* **1991**, *32*, 7613.

[8] K. Michael, V. Wittman, W. König, J. Sandow, H. Kessler, *Int. J. Peptide Protein Res.* **1996**, *48*, 59.

[9] a) C. R. Bertozzi, P. D. Hoeprich, Jr., M. D. Bednarski, *J. Org. Chem.* **1992**, *57*, 6092; b) S. D. Debenham, J. S. Debenham, M. J. Burk, E. J. Toone, *J. Am. Chem. Soc.* **1997**, *119*, 9897; c) D. Urban, T. Skrydstrup, J.-M. Beau, *Chem. Commun.* **1998**, *9*, 955; d) U. Tedebark, M. Meldal, L. Panza, K. Bock, *Tetrahedron Lett.* **1998**, *39*, 1815; e) P. Arya, R. N. Ben, H. Qin, *Tetrahedron Lett.* **1998**, *39*, 6131; f) A. Dondoni, A. Marra, A. Massi, *Chem. Commun.* **1998**, 1741.

[10] G. F. Springer, *Science* **1984**, *224*, 1198.

[11] T. Suzuki, K. Kitajima, S. Inoue, Y. Inoue, *Glycobiology* **1994**, *4*, 777.

[12] a) O. Frey, M. Hoffman, H. Kessler, *Angew. Chem.* **1995**, *107*, 2194; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2026; b) M. Hoffman, F. Burkhart, G. Hessler, H. Kessler, *Helv. Chim. Acta* **1996**, *79*, 1519.

[13] M. Chorev, M. Goodman, *Acc. Chem. Res.* **1993**, *26*, 266.

[14] L.-X. Wang, M. Tang, S. Tadashi, K. Kitajima, Y. Inoue, S. Inoue, J.-Q. Fan, Y. C. Lee, *J. Am. Chem. Soc.* **1997**, *119*, 11137.

[15] R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, D. C. Spellmeyer, R. Tan, A. D. Frankle, D. V. Santi, F. E. Cohen, P. A. Bartlett, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9367.

[16] a) U. Saha, R. Roy, *Tetrahedron Lett.* **1995**, *36*, 3635; b) U. K. Saha, R. Roy, *Tetrahedron Lett.* **1997**, *38*, 7697; c) J. M. Kim, R. Roy, *Tetrahedron Lett.* **1997**, *38*, 3487; d) J. M. Kim, R. Roy, *Carbohydr. Res.* **1997**, *298*, 173.

[17] M. A. Dechantsreiter, F. Burkhart, H. Kessler, *Tetrahedron Lett.* **1998**, *39*, 253.

[18] a) C. P. Stowell, Y. C. Lee, *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225; b) S. Y. C. Wong, *Curr. Opin. Struct. Biol.* **1995**, *5*, 599; c) R. T. Lee, Y. C. Lee in *Glycoproteins II* (Eds.: J. Montreuil, J. F. G. Vliegenhart, H. Schachter), Elvesier, New York, **1997**, pp. 601–620.

[19] a) T. W. Muir, P. E. Dawson, S. B. H. Kent, *Methods Enzymol.* **1997**, *289*, 266; b) T. W. Muir, *Structure* **1995**, *3*, 649; c) G. A. Lemieux, C. R. Bertozzi, *Trends Biotechnol.* **1998**, *16*, 506; d) J. Shao, J. P. Tam, *J. Am. Chem. Soc.* **1994**, *116*, 30.

[20] a) N. J. Davis, S. L. Flitsch, *Tetrahedron Lett.* **1991**, *32*, 6793; b) S. Y. C. Wong, G. Guile, R. Dwek, G. Arsequell, *Biochem. J.* **1994**, *300*, 843.

[21] M. Bengtsson, J. Broddefalk, J. Dahmén, K. Henriksson, J. Kihlberg, H. Lohn, B. R. Srinivasa, K. Stenvall, *Glycoconj. J.* **1998**, *15*, 223.

[22] L. A. Marcaurelle, E. C. Rodriguez, C. R. Bertozzi, *Tetrahedron Lett.* **1998**, *39*, 8417.

[23] a) L. A. Marcaurelle, C. R. Bertozzi, *Tetrahedron Lett.* **1998**, *39*, 7279; b) R. M. Werner, O. Shokek, J. T. Davis, *J. Org. Chem.* **1997**, *62*, 8243.
[24] E. C. Rodriguez, L. A. Marcaurelle, C. R. Bertozzi, *J. Org. Chem.* **1998**, *63*, 7134.
[25] F. Peri, P. Dumy, M. Mutter, *Tetrahedron* **1998**, *54*, 12269.
[26] a) Y. Zhao, S. B. H. Kent, B. T. Chait, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 1629; b) S. E. Cervigni, P. Dumy, M. Mutter, *Angew. Chem.* **1996**, *108*, 1325; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1230.
[27] a) P. Bulet, J.-L. Dimarcq, C. Hetrû, M. Languex, M. Charlet, G. Hegy, A. V. Dorsselaer, J. A. Hoffman, *J. Biol. Chem.* **1993**, *68*, 14893; b) P. Bulet, L. Urge, S. Ohresser, C. Hetrû, L. Otvos, *Eur. J. Biochem.* **1996**, *238*, 64.
[28] a) A. H. Andreotti, D. Kahne, *J. Am. Chem. Soc.* **1993**, *115*, 3352; b) R. Liang, A. H. Andreotti, D. Kahne, *J. Am. Chem. Soc.* **1995**, *117*, 10395; c) N. J. Maeji, Y. Inoue, R. Chujo, *Biopolymers* **1987**, *26*, 1753; d) S. E. O'Connor, B. Imperiali, *Chem. Biol.* **1998**, *5*, 427.
[29] E. C. Rodriguez, K. A. Winans, D. S. King, C. R. Bertozzi, *J. Am. Chem. Soc.* **1997**, *119*, 9905.
[30] a) G. Avigad, D. Amaral, C. Asenio, B. L. Horecker, *J. Biol. Chem.* **1962**, *237*, 2736; b) C. G. Gahmberg, *J. Biol. Chem.* **1976**, *251*, 510.
[31] a) P. E. Dawson, T. W. Muir, I. Clarke-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776.
[32] T. W. Muir, D. Sondhi, P. A. Cole, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6705.
[33] L. K. Mahal, C. R. Bertozzi, *Chem. Biol.* **1997**, *4*, 415.
[34] a) G. Srivastava, K. J. Kaur, O. Hindsgaul, M. M. Palcic, *J. Biol. Chem.* **1992**, *267*, 22356; b) K. J. Yarema, L. K. Mahal, R. E. Bruehl, E. C. Rodriguez, C. R. Bertozzi, *J. Biol. Chem.* **1998**, *273*, 31168.

Received: October 29, 1998 [C1420]

  0947-6539/99/0505-1390 $ 17.50+.50/0