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Development of Co- and Post-Translational Synthetic Strategies to *C*-Neoglycopeptides

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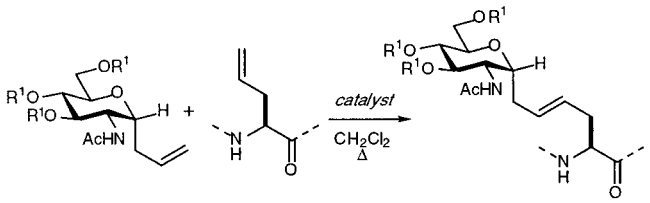
ABSTRACT



The synthesis of stable, *C*-linked analogues of glycopeptides is being investigated with two complementary synthetic strategies, *co-translational* and *post-translational* glycopeptide synthesis. The key feature of the present approach lies in an effective olefin cross-metathesis reaction that allows formation of both glycoamino acids and glycopeptides.

Glycoconjugates are ubiquitous components of cell surfaces that are intimately involved in cellular communication events.[1] Significantly, glycoproteins have shown important correlations with a variety of disease states, often including a primary role in infection.[2] Much of the function of these biomolecules can be attributed to highly selective recognition events involving the oligosaccharide components. Unfortunately, detailed molecular studies of these critical cellular components have been hampered by problems in isolating unique glycoforms of these proteins, as well as the intrinsic lability of the oligosaccharide linkages. It was with these issues in mind that we initiated an investigation toward the synthesis of homogeneous, stable glycopeptide analogues to realize pure glycoconjugates that may prove useful in biological studies and as potential therapeutic agents.

The general strategies by which glycopeptide arrays can be assembled may be reduced to two distinct approaches: (i) a *co-translational* approach wherein the carbohydrate component, in the form of a preformed glycoamino acid (e.g., **1**), is introduced during the course of peptide assembly, and (ii) a *post-translational* approach that features attachment of the carbohydrate component to the intact peptide fragment (Figure 1).[3] The co-translational strategy has dominated glycopeptide synthesis to date, which, in turn, has stimulated much interest in the synthesis of glycoamino acids of the general type **1**.[4] In contrast, post-translational glycopeptide assembly has been only rarely employed, despite the fact that it embodies several features that may recommend its implementation.[5] For example, the application of solid-state peptide synthesis to the co-translational assembly of glyco-

(1) For reviews addressing cell surface carbohydrates, see: (a) *Cell Surface and Extracellular Glycoconjugate*; Roberts, D. D., Mecham, R. P., Eds.; Academic Press: New York, 1993. (b) Feizi, T. *Curr. Opin. Struct. Biol.* **1993**, *3*, 701−710. (c) Hakomori, S.-i. *Biochem. Soc. Trans.* **1993**, *21*, 583−595. (d) Hakomori, S.-i.; Igarashi, Y. *J. Biochem.* **1995**, *118*, 1091−1103. (e) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2755−2794. (f) Hollenbaugh, D.; Bajorath, J.; Aruffo, A. In *Bioorganic Chemistry: Carbohydrates*; Hecht, S., Ed.; Oxford University Press: New York, 1999; Chapter 8, pp 313−334.

(2) For reviews in this area see: (a) *Glycoproteins and Disease*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: New York, 1996. (b) *Glycoproteins and Human Disease*; Brockhausen, I., Kuhns, W., Eds.; Chapman and Hall: New York, 1997. (c) Brockhausen, I.; Schutzbach, J.; Kuhns, W. *Acta Anat.* **1998**, *161*, 36−78.

(3) For reviews on glycopeptide synthesis see: (a) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579−601. (b) Seitz, O. *Chembiochem* **2000**, *1*, 214−246. (c) Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495−4537.

(4) Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1997**, *8*, 2839−2876.

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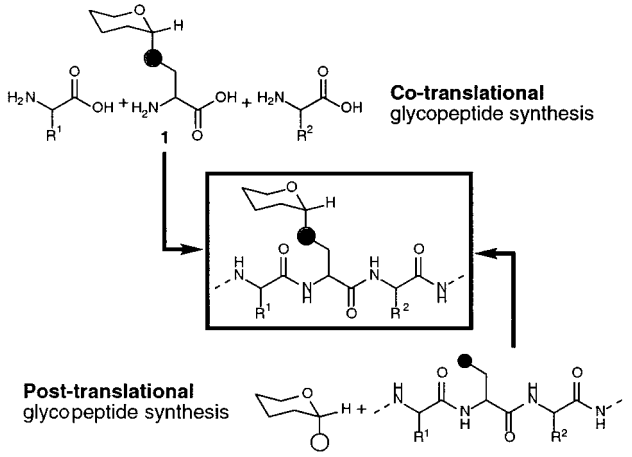


Figure 1. Synthetic approaches to glycopeptides.

peptides that incorporate complex glycoamino acid components can be expected to prove problematic. Late-stage attachment of the carbohydrate segment in a post-translational approach would circumvent this problem and, importantly, would minimize the loss of this valuable component. In addition, biological studies would benefit from the modular nature of this strategy as recognition properties of glycopeptide arrays could be varied though simple attachment of different carbohydrate structures.

In conjunction with studies to prepare well-defined models for mucin cell surface glycoproteins, we examined olefin cross metathesis as a promising means of implementing both of the above synthetic strategies[6] to afford stable *C*-glycoconjugates.[7] We initially focused our attention on the metathetic fusion of *C*-glycosides of 2-deoxy-2-amino hexoses with an appropriate olefinic amino acid to afford *C*-glycoamino acids **1** in forms suitable for subsequent incorporation into (co-translational) glycopeptide synthesis.[8] With this in mind, we sought to prepare carbohydrate and amino acid substrates bearing protecting group schemes compatible with solution and solid-phase peptide synthesis methodology.[3] Using Horton's radical allylation method,[9] stereodefined *C*-allyl glucosamines bearing both acetate **2** and benzyl protection **3** were prepared (Figure 2). A noteworthy advantage conferred by this approach is that the important anomeric stereochemistry is established at an early

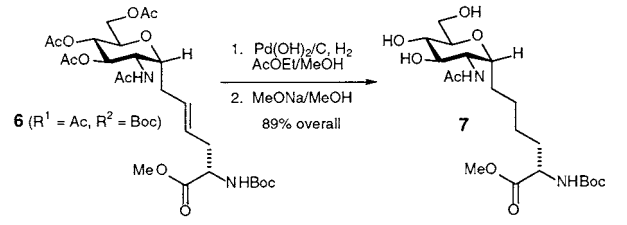
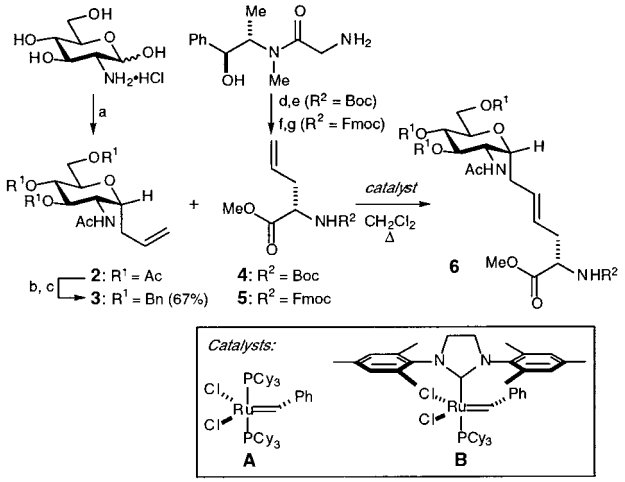


Figure 2. Completion of *C*-tethered glycoamino acid.

stage in the synthetic sequence.[10] Suitable amino acid substrates, in the form of Boc (**4**) and Fmoc (**5**) protected L-allyl glycines, were realized by using the protocols reported by Myers' (Scheme 1).[11]

Scheme 1. Olefin Methathesis to *C*-Glycoamino Acids



[a] Reference 9. [b] NaOMe, MeOH. [c] BnBr, NaH, DMF, rt. [d] Reference 8a. [e] $TMSCH_2N_2$, MeOH (83%). [f] Reference 8b. [g] $SOCl_2$, MeOH (83%).

With these substrates in hand, an examination of the effects of various reaction parameters on cross metathesis leading to *C*-glycoamino acid **6** was examined (Table 1, see Figure 2). Unlike a recent study,[8a] metathesis with Grubb's catalyst **A** was found to be sensitive to the nature of the carbohydrate protecting groups (entries 1 and 2). Whereas the benzyl-protected glucosamine (**3**) led to modest yields of the desired cross-metathesis products **6** (entries 2 and 3), the acetate-protected carbohydrate **2** was virtually unreactive under the same reaction conditions. It was gratifying to find that the improved Grubb's catalyst (**B**) significantly improved the cross-metathesis reaction with **3**, affording products **6** (R^2

(5) (a) Tsuda, T.; Nishimura, S.-I. *Chem. Commun.* **1996**, 2779−2780. (b) Cohen-Anisfeld, S.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 10531−10537. (c) Paulsen, H.; Scheyer, A.; Nathieux, N.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 281−293. (d) Marcaurelle, L. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2001**, *123*, 1587−1595. (e) Grandjean, C.; Gras-Masse, H.; Melnyk, O. *Chem. Eur.* **2001**, *7*, 230−239. (f) Hang, H. C.; Bertozzi, C. R. *Acc. Chem. Res.* **2001**, *34*, 727−736.

(6) For reviews of olefin metathesis applied to carbohydrate chemistry see: (a) Roy, R.; Das, S. K. *Chem. Commun.* **2000**, 519−529. (b) Jorgensen, M.; Hadwiger, P.; Madsen, R.; Stutz, A. E.; Wrodnigg, T. M. *Curr. Org. Chem.* **2000**, *4*, 565−588.

(7) For a review on *C*-neoglycopeptides, see: Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100*, 4395−4421.

(8) For related studies see: (a) Dominique, R.; Liu, B.; Das, S. K.; Roy, R. *Synthesis* **2000**, 862−868. (b) Dondoni, A.; Giovannini, P. P.; Marra, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2380−2388.

(9) Ciu, J.; Horton, D. *Carbohydr. Res.* **1998**, *309*, 319−330.

(10) The advantage of early, unambiguous establishment of anomeric stereochemistry has been noted previously: Chen, X. T.; Sames, D.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 7760−7769.

(11) (a) For the Fmoc protected form see: Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung *J. Am. Chem. Soc.* **1997**, *119*, 6656−673. (b) For the Boc protected form see: Myers, A. G.; Gleason, J. L. *Org. Synth.* **1999**, *76*, 57−76.

Table 1. Cross Metathesis Affording *C*-Glycoamino Acid Derivatives

entry	*C*-glycoside	amino acid	catalyst (mol %)	time (h)	product (R^1, R^2)	yield, %
1	**2**	**5**	**A** (20)	24	**6** (Ac, Fmoc)	
2	**3**	**5**	**A** (20)	22	**6** (Bn, Fmoc)	41
3	**3**	**4**	**A** (20)	12	**6** (Bn, Boc)	50
4	**3**	**5**	**B** (10)	16	**6** (Bn, Fmoc)	74
5	**3**	**4**	**B** (10)	16	**6** (Bn, Boc)	78
6	**3**	**5**[a]	**B** (10)	16	**6** (Bn, Fmoc)	49
7	**2**	**5**	**B** (20)[b]	48	**6** (Ac, Fmoc)	65
8	**2**	**5**[c]	**B** (20)[b]	48	**6** (Ac, Fmoc)	70
9	**2**	**4**	**B** (20)[b]	48	**6** (Ac, Boc)	60
10	**8**	**5**	**A** (20)	48	**10** (Bn, Fmoc)	
11	**8**	**4**	**A** (20)	48	**10** (Bn, Boc)	
12	**8**	**5**	**B** (10)	24	**10** (Bn, Fmoc)	82
13	**8**	**4**	**B** (10)	48	**10** (Bn, Boc)	77
14	**8**	**5**[a]	**B** (10)	24	**10** (Bn, Fmoc)	68
15	**9**	**5**	**B** (20)[b]	48	**10** (Ac, Fmoc)	69
16	**9**	**4**	**B** (20)[b]	48	**10** (Ac, Boc)	77
17	**11**	**5**	**B** (20)[b]	48	**13** (−, Fmoc)	57
18	**11**	**4**	**B** (20)[b]	48	**13** (−, Boc)	73
19	**12**	**5**	**B** (20)[b]	48	**14** (−, Fmoc)	60

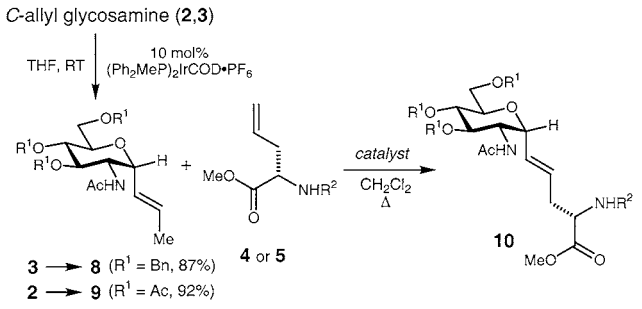
[a] One equivalent of the amino acid component used. [b] The catalyst was introduced in two equal portions at 24-h intervals. [c] Three equivalents of the amino acid component used.

= Boc, Fmoc) in very good yields with only 10 mol % of the catalyst (entries 4 and 5). Important to the objective of realizing fully functional *C*-glycopeptides, it was found that the acetate-protected carbohydrate **2** was an acceptable substrate with the improved catalyst **B**, affording products **6** in good yield (entries 7 and 9). In the course of studies to optimize the yield of this cross-coupled product (**6**, R^1 = Ac, R^2 = Boc, Fmoc), it was noted that consistently good yields could be obtained with 20 mol % of the catalyst introduced in two equal portions (24 h intervals) and that a larger excess of the amino acid (**5**) resulted in only a modest increase in yield (entry 8). It bears noting that metathesis adducts such as **6** (R^1 = Ac, R^2 = Boc) may be efficiently manipulated for subsequent glycopeptide synthesis as indicated by the high yield reduction/deprotection leading to the *C*-tethered glycoconjugate **7** (Figure 2).

It is noteworthy that equimolar quantities of the substrates led to the statistically predicted yield under these conditions, leading to the conclusion that the rate of cross metathesis is on the same order as that for self-condensation (entry 6).[12] It was speculated that cross metathesis could be improved by suppressing competing self-condensation. Toward this goal, it was discovered that the *C*-allyl glycosides **2** and **3** could be efficiently isomerized by using catalytic $(Ph_2MeP)_2$-IrCOD•PF_6 to afford the *C*-vinyl glycosides **8** and **9** (Scheme 2).[13] The diminished reactivity of this disubstituted double bond was demonstrated by the lack of detectable cross-coupled products, using substrate **8** with Grubb's catalyst **A** (entries 10 and 11). However, the improved catalyst **B** led to the desired products in generally excellent yields (entries 12−16). The improved effectiveness of this coupling reaction is clearly demonstrated by the remarkable yield that is obtained when equimolar quantities of **8** and **5** are used, lending credence to the notion that suppression of self-condensation has led to improved cross reactivity (entry 14). Again, 10 and 20 mol % of the catalyst proved optimal for the benzyl-protected and acetate-protected carbohydrate substrates, respectively.

In an effort to probe the scope of this *C*-glycoamino acid synthesis, the readily available *C*-allyl lactose **11**[14] was exposed to the ally glycines **4** and **5** in the presence of catalyst **B** to afford the desired products **13** (R^2 = Fmoc, Boc) in serviceable yields (Scheme 3, see entries 17 and 18). In addition, isomerization of the double bond under the conditions previously described afforded the *C*-vinyl glycoside **12** in excellent yield that, in turn, acted as a viable substrate for cross-metathesis formation of glycoamino acid **14** (R^2 = Fmoc, entry 19).[15] These results offer a preliminary

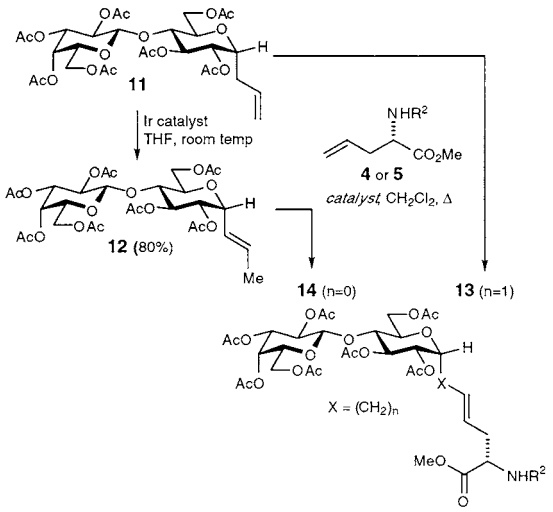
Scheme 2. Cross Metathesis with Isomerized Substrates



(12) All the starting materials were consumed in this reaction to afford a mixture of the three possible metathesis products (1 cross + 2 self-metathesis products). It is important to note that participation of these products in further metathesis reactions is insignificant under these reaction conditions. This will be reported in due course.

(13) Oltvoort, J. J.; van Boeckel, C. A. A.; de Koning, J. H.; van Boom, J. H. *Synthesis* **1981**, 305−308.

Scheme 3. Lactose Metathesis Reactions



suggestion that olefin metathesis may provide a general, convergent means to *C*-glycoamino acids of the type **1** for use in co-translational glycopeptide synthesis.

Our attention was next turned toward that application of olefin metathesis to the post-translational assembly of glycopeptide arrays. Olefin metathesis is particularly well suited to this approach as unique, stable olefin functionality can be easily inserted into a peptide array by using stable, unsaturated amino acid components. To test the metathesis reaction in a more complex setting, model tripeptide **15** was assembled (Figure 3).[16] Exposure of *C*-ally glycoside **3** to 2

(14) Giannis, A.; Sandhoff, K. *Tetrahedron Lett.* **1985**, *26*, 1479−1482.

(15) That only a very modest improvement in yield is observed with **12** relative to **11** is consistent with the noticeably lower reactivity of the lactose substrates.

(16) See Supporting Information for the synthesis of tripeptide **15**.

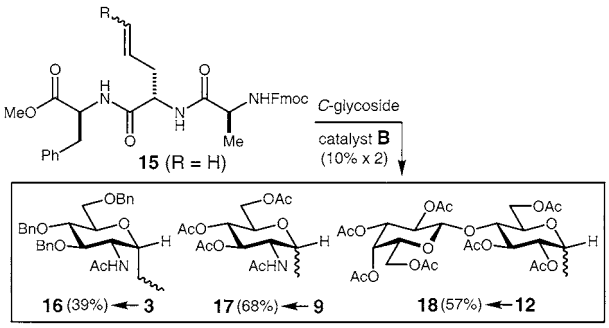


Figure 3. Post-translational metathesis.

equivalents of the tripeptide in the presence of 20 mol % of catalyst **B** led to a modest yield of the desired glycopeptide **16** (39%, Figure 3). However, it was gratifying to find that the same conditions with isomerized *C*-glycosides **9** and **12** led to the corresponding glycopeptides **17** and **18**, respectively, in very good yields (68% and 57%).

The above results demonstrate the potential for applying olefin metathesis to both co-translational and post-translational assembly of *C*-neoglycopeptides. Studies to define conditions to improve the efficiency of this cross-metathesis reaction, as well as its application to specific, biorelevant glycopeptide targets, will be reported in due course.

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Supporting Information Available: Experimental details and physical data are available for the new compounds described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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