JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by American Chemical Society

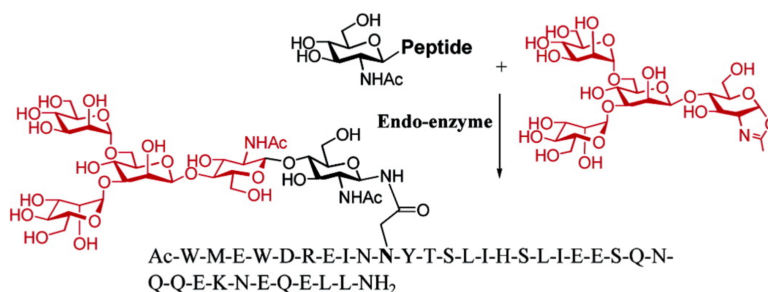
Communication

Highly Efficient Endoglycosidase-Catalyzed Synthesis of Glycopeptides Using Oligosaccharide Oxazolines as Donor Substrates

Bing Li, Ying Zeng, Steven Hauser, Haijing Song, and Lai-Xi Wang

J. Am. Chem. Soc., **2005**, 127 (27), 9692-9693• DOI: 10.1021/ja051715a • Publication Date (Web): 15 June 2005

Downloaded from http://pubs.acs.org on March 25, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 12 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 06/15/2005

Highly Efficient Endoglycosidase-Catalyzed Synthesis of Glycopeptides Using Oligosaccharide Oxazolines as Donor Substrates

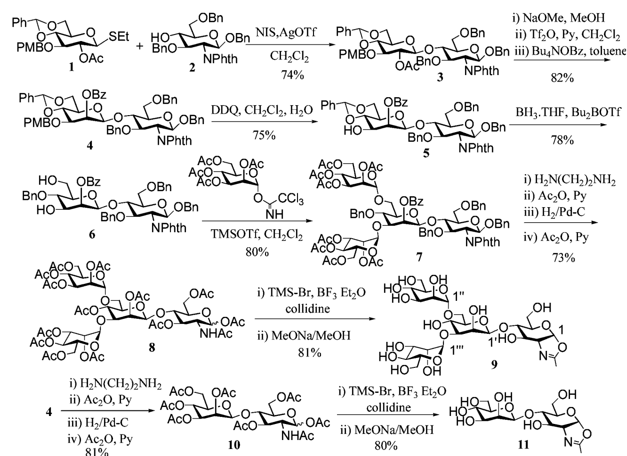
Bing Li, Ying Zeng, Steven Hauser, Haijing Song, and Lai-Xi Wang*

Institute of Human Virology, University of Maryland Biotechnology Institute, University of Maryland, 725 West Lombard Street, Baltimore, Maryland 21201

Received March 17, 2005; E-mail: wangx@umbi.umd.edu

Glycosylation is a common post-translational modification of proteins in eukaryotes.[1] The oligosaccharide components of glycoproteins affect a wide range of protein functions and are involved in many important cellular recognition processes.[2] However, natural glycoproteins typically exist as a mixture of glycoforms differing in oligosaccharide structures, and pure individual glycoforms are difficult to isolate for detailed structural and functional studies.[3] Thus, a variety of synthetic methods have been explored to generate homogeneous glycopeptides and glycoproteins.[4] Among others, the chemoenzymatic approach using the endo-β-*N*-acetylglucosaminidase (ENGase)-catalyzed transglycosylation seems particularly promising.[5] ENGases are a class of endoglycosidases that hydrolyze the β-1,4-glycosidic bond in the core *N*,*N*′-diacetylchitobiose moiety of *N*-glycoproteins to release the *N*-glycans. However, some ENGases, such as Endo-A from *Arthrobacter protophormiae*[6] and Endo-M from *Mucor hiemalis*,[7] possess transglycosylation activity and are able to transfer the releasing *N*-glycan to a GlcNAc-peptide acceptor to form a new glycopeptide.[5] In contrast to common glycosyltransferases and exoglycosidases that transfer only monosaccharides, Endo-A and Endo-M can transfer a large intact oligosaccharide to a GlcNAc-peptide acceptor in a single step to form a new glycopeptide, thus allowing a highly convergent glycopeptide synthesis without the need of protecting groups. A number of large *N*-glycopeptides were synthesized by the chemoenzymatic method for structural and functional studies.[8−11] Nevertheless, the chemoenzymatic method suffers with a low transglycosylation yield (generally 5−20%), the product hydrolysis, and the limitations of using only natural *N*-glycans as the donor substrates. To solve these problems, we report in this paper the use of synthetic oligosaccharide oxazolines, the mimics of the presumed oxazolinium ion intermediate formed in a retaining mechanism, as donor substrates for glycopeptide synthesis, which not only broadened the substrate availability but also led to a high-yield synthesis of large *N*-glycopeptides.

The method was based on the assumption that the ENGase-catalyzed reaction proceeds via a mechanism of the substrate-assisted catalysis involving an oxazolinium ion intermediate, as demonstrated for some chitinases[12] and *N*-acetyl-β-hexosaminidases.[13] Although a detailed mechanism of ENGase-catalyzed transglycosylation is yet to be characterized, Fujita and co-workers recently reported that a disaccharide oxazoline of Manβ1,4GlcNAc could serve as a substrate for ENGase-catalyzed transglycosylation.[14] This observation suggested that the Endo-A- and Endo-M-catalyzed transglycosylation might indeed proceed via an oxazolinium ion intermediate. To test whether oligosaccharide oxazolines would be kinetically more favorable substrates for an efficient *N*-glycopeptide synthesis than natural *N*-glycans, we synthesized the di- and tetrasaccharide oxazolines corresponding to the core of *N*-glycans (Scheme 1). The synthesis of the Manβ1,4GlcN disaccharide core was achieved through stereocontrolled β-glycosylation of intermediates **1** and **2**, followed by selective inversion of the *Glc* C-2 configuration to give **4**. Compound **4** was changed to **6** via protecting group manipulations and was glycosidated with the mannosyl imidate to give the tetrasaccharide **7**, which was then converted to the fully acetylated derivative **8**. Finally, treatment of **8** with TMS-Br/BF_3 Et_2O for oxazoline formation,[15] followed by de-*O*-acetylation gave the desired tetrasaccharide oxazoline **9**. The disaccharide oxazoline **11** was synthesized from **4** by similar protecting group manipulations and oxazoline formation (Scheme 1).

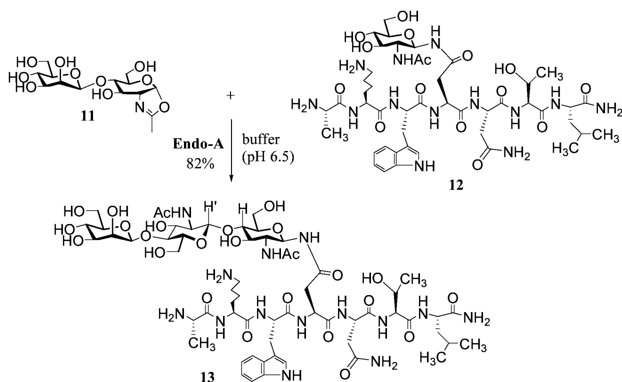


Scheme 1. Chemical Synthesis of the Di- and Tetrasaccharide Oxazolines

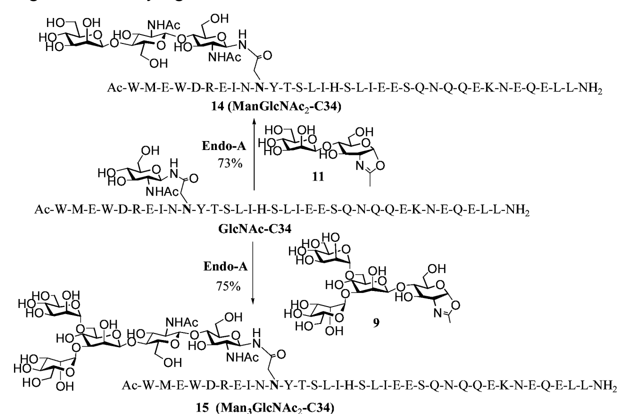
To examine the synthetic oligosaccharide oxazolines as donor substrates for constructing *N*-glycopeptides, we synthesized two typical GlcNAc-peptides, a GlcNAc-heptapeptide **12** derived from HIV-1 gp120 and a 34-mer peptide GlcNAc-C34 derived from HIV-1 gp41 to serve as the glycosyl acceptors.[9,11] It was found that the Endo-A-catalyzed reaction between the oxazoline **11** and the GlcNAc-heptapeptide **12** (3:1) in phosphate buffer (pH 6.5) proceeded smoothly to form the glycopeptide **13**, which was isolated in 82% yield (Scheme 2). Endo-M was also effective in catalyzing the reaction to give the glycopeptide **13** in 78% yield. The newly formed glycosidic bond was unambiguously determined to be the expected GlcNAcβ1,4GlcNAc−Asn linkage by detailed NMR (TOCSY and NOESY) analysis of **13** (see Supporting Information). A doublet at δ 4.65 with a large coupling constant (J = 8.5 Hz) for H-1′ indicated a β-glycosidic bond, and an apparent NOE correlation between H-1′ and the H-4 of the Asn-linked GlcNAc indicated that the newly formed glycosidic linkage was a β-1,4-type. The results confirm that the ENGase-catalyzed transglycosylation using oligosaccharide oxazoline as the donor substrate proceeds in both a stereo- and regiospecific manner to afford the desired glycopeptide, as previously observed when natural *N*-glycans were used as the donor substrates.[9−11,16]

10.1021/ja051715a CCC: $30.25 © 2005 American Chemical Society

Scheme 2. ENGase-Catalyzed Synthesis of a HIV-1 gp120 Fragment Carrying the Core Trisaccharide



Scheme 3. ENGase-Catalyzed Synthesis of HIV-1 gp41 Fragments Carrying the Core Tri- and Pentasaccharides



We next tested the Endo-A-catalyzed transglycosylation of the di- and tetrasaccharide oxazolines with the large acceptor, GlcNAc-C34.[11] It was found that the oligosaccharides could also be effectively transferred to the large GlcNAc-C34 by Endo-A to form the glycopeptides **14** (73%) and **15** (75%), respectively (Scheme 3). The glycopeptides were again characterized by ESI-MS and NMR analysis. Further structural characterization of glycopeptide **15** was performed by Pronase digestion that yielded a single Asn-linked oligosaccharide, which was identical to the authentic Asn-linked core pentasaccharide $Man_3GlcNAc_2Asn$ by ^1H NMR, ESI-MS, and Dionex HPAEC analysis. It was also observed that while the Manβ1,4GlcNAc−oxazoline and $Man_3GlcNAc$−oxazoline acted as an efficient substrate for transglycosylation, the resulting glycopeptide $ManGlcNAc_2$-C34 (**14**) was resistant to Endo-A hydrolysis, and the glycopeptide $Man_3GlcNAc_2$-C34 (**15**) was hydrolyzed only slowly by Endo-A (data not shown). This suggests that the oligosaccharide oxazolines are much more active substrates than the ground state *N*-glycopeptides, thus being kinetically favorable for product accumulation.

In conclusion, a highly efficient chemoenzymatic synthesis of *N*-glycopeptides was achieved. The use of synthetic oligosaccharide oxazolines as the donor substrates for the ENGase-catalyzed transglycosylation not only expanded the substrate availability but also resulted a substantial enhancement of the synthetic efficiency, allowing a high-yield synthesis of large *N*-glycopeptides. When combined with the novel in vivo suppressor tRNA technology that enables efficient overproduction of the acceptor GlcNAc-protein in *E. coli*,[17] the high-yield transglycosylation may be also very useful for total glycoprotein synthesis and remodeling.

Acknowledgment. We thank Prof. K. Takegawa for kindly providing the pGEX-2T/Endo-A plasmid, and Prof. K. Yamamoto for kindly providing Endo-M. The work was supported by the National Institutes of Health (AI054354 and AI051235).

Supporting Information Available: Experimental procedures for the synthesis of oligosaccharide oxazolines; ESI-MS spectra of glycopeptides **13**, **14**, and **15**; and detailed NMR analysis (TOCSY and NOESY) of glycopeptide **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Apweiler, R.; Hermjakob, H.; Sharon, N. *Biochim. Biophys. Acta* **1999**, *1473*, 4−8.
(2) (a) Varki, A. *Glycobiology* **1993**, *3*, 97−130. (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683−720. (c) Rudd, P. M.; Elliott, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. *Science* **2001**, *291*, 2370−2376.
(3) Fukuda, M.; Hindsgaul, O. *Molecular and Cellular Glycobiology*; Oxford University Press: New York, 2000.
(4) (a) Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1999**, *10*, 3045−3094. (b) Sears, P.; Wong, C. H. *Science* **2001**, *291*, 2344−2350. (c) Grogan, M. J.; Pratt, M. R.; Marcaurelle, L. A.; Bertozzi, C. R. *Annu. Rev. Biochem.* **2002**, *71*, 593−634. (d) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579−601. (e) Geng, X.; Dudkin, V. Y.; Mandal, M.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2004**, *43*, 2562−2565. (f) Warren, J. D.; Miller, J. S.; Keding, S. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 6576−6578.
(5) (a) Yamamoto, K. *J. Biosci. Bioeng.* **2001**, *92*, 493−501. (b) Wang, L. X.; Singh, S.; Ni, J. In *Synthesis of Carbohydrates through Biotechnology*; Wang, P. G., Ichikawa, Y., Eds.; ACS Symposium Series 873; American Chemical Society: Washington, DC, 2004; pp 73−92.
(6) Takegawa, K.; Yamaguchi, S.; Kondo, A.; Iwamoto, H.; Nakoshi, M.; Kato, I.; Iwahara, S. *Biochem. Int.* **1991**, *24*, 849−855.
(7) Yamamoto, K.; Kadowaki, S.; Watanabe, J.; Kumagai, H. *Biochem. Biophys. Res. Commun.* **1994**, *203*, 244−252.
(8) (a) Takegawa, K.; Tabuchi, M.; Yamaguchi, S.; Kondo, A.; Kato, I.; Iwahara, S. *J. Biol. Chem.* **1995**, *270*, 3094−3099. (b) Haneda, K.; Inazu, T.; Yamamoto, K.; Kumagai, H.; Nakahara, Y.; Kobata, A. *Carbohydr. Res.* **1996**, *292*, 61−70. (c) Wang, L. X.; Tang, M.; Suzuki, T.; Kitajima, K.; Inoue, Y.; Inoue, S.; Fan, J. Q.; Lee, Y. C. *J. Am. Chem. Soc.* **1997**, *119*, 11137−11146. (d) Mizuno, M.; Haneda, K.; Iguchi, R.; Muramoto, I.; Kawakami, T.; Aimoto, S.; Yamamoto, K.; Inazu, T. *J. Am. Chem. Soc.* **1999**, *121*, 284−290. (e) O'Connor, S. E.; Pohlmann, J.; Imperiali, B.; Saskiawan, I.; Yamamoto, K. *J. Am. Chem. Soc.* **2001**, *123*, 6187−6188.
(9) Singh, S.; Ni, J.; Wang, L. X. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 327−330.
(10) Li, H.; Singh, S.; Zeng, Y.; Song, H.; Wang, L. X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 895−898.
(11) Wang, L. X.; Song, H.; Liu, S.; Lu, H.; Jiang, S.; Ni, J.; Li, H. *ChemBioChem* **2005**, *6*, 1068−1074.
(12) (a) Brameld, K. A.; Shrader, W. D.; Imperiali, B.; Goddard, W. A., III. *J. Mol. Biol.* **1998**, *280*, 913−923. (b) Terwisscha van Scheltinga, A. C.; Armand, S.; Kalk, K. H.; Isogai, A.; Henrissat, B.; Dijkstra, B. W. *Biochemistry* **1995**, *34*, 15619−15623. (c) Tews, I.; Terwisscha van Scheltinga, A. C.; Perrakis, A.; Wilson, K. S.; Dijkstra, B. W. *J. Am. Chem. Soc.* **1997**, *119*, 7954−7959.
(13) (a) Mark, B. L.; Vocadlo, D. J.; Knapp, S.; Triggs-Raine, B. L.; Withers, S. G.; James, M. N. *J. Biol. Chem.* **2001**, *276*, 10330−10337. (b) Williams, S. J.; Mark, B. L.; Vocadlo, D. J.; James, M. N.; Withers, S. G. *J. Biol. Chem.* **2002**, *277*, 40055−40065.
(14) Fujita, M.; Shoda, S.; Haneda, K.; Inazu, T.; Takegawa, K.; Yamamoto, K. *Biochim. Biophys. Acta* **2001**, *1528*, 9−14.
(15) Colon, M.; Staveski, M. M.; Davis, J. T. *Tetrahedron Lett.* **1991**, *32*, 4447−4450.
(16) Fan, J. Q.; Takegawa, K.; Iwahara, S.; Kondo, A.; Kato, I.; Abeygunawardana, C.; Lee, Y. C. *J. Biol. Chem.* **1995**, *270*, 17723−17729.
(17) (a) Zhang, Z.; Gildersleeve, J.; Yang, Y. Y.; Xu, R.; Loo, J. A.; Uryu, S.; Wong, C. H.; Schultz, P. G. *Science* **2004**, *303*, 371−373. (b) Xu, R.; Hanson, S. R.; Zhang, Z.; Yang, Y. Y.; Schultz, P. G.; Wong, C. H. *J. Am. Chem. Soc.* **2004**, *126*, 15654−15655.

JA051715A