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A chemo-enzymatic approach to the study of carbohydrate-based biological recognitions

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Abstract: A combination of chemical and enzymatic synthetic approaches can be effectively used to study the role of carbohydrates and their conjugates involved in various biological recognition processes. Both natural ligands and designed mimetics can be prepared chemo-enzymatically in a practical manner and used as inhibitors to control the function of glycoconjugates.

Introduction

Carbohydrates are found in all places in biological systems. They can serve as structural components of biomolecules, as energy sources or as recognition elements for signaling and communication processes. Their interaction with receptors is generally weak, although quite specific, and involves multivalency. They are not easily accessible in large quantities for functional study and for clinical evaluation. The recently developed chemo-enzymatic strategy[1] for the synthesis of this class of complex molecules and their conjugates and mimetics, however, has resolved some of the problems in the field and has provided new opportunities for new drug discovery and development. Perhaps one of the most interesting subjects which has emerged recently is the discovery of selectins and their interaction with carbohydrate ligands involved in inflammatory reactions and metastasis.[2-4] Both E- and P-selectins recognize sialyl Lewis x (SLe^x)[5], and L-selectin recognizes sulfated sialyl Lewis x as a ligand.[6] Knowing the ligands of these selectins provides useful information for the development of selectin blockers as potential antiinflammatory and anticancer agents. We have recently developed chemo-enzymatic methods to enable the large scale synthesis of oligosaccharides and glycopeptides, including SLe^x, sialyl Lewis a, Lewis y and hyaluronic acid.[1] We have also developed an enzymatic method for the incorporation of sulfate group into oligosaccharides regioselectively.[1] N-acetyl lactosamine 6-sulfate prepared using the Nod factor sulfotransferase coupled with regeneration of phosphoadenosylphosphosulfate (PAPS),[7] for example, can be converted *in situ* to the L-selectin ligand SLe^x-6-sulfate as both sialyl- and fucosyltransferase are known for the subsequent reactions.[8] The newly developed galactosidase-catalyzed synthesis of N-acetyl lactosamine using 6-oxogalactoside as substrate provides a new practical route to the disaccharide.[9]

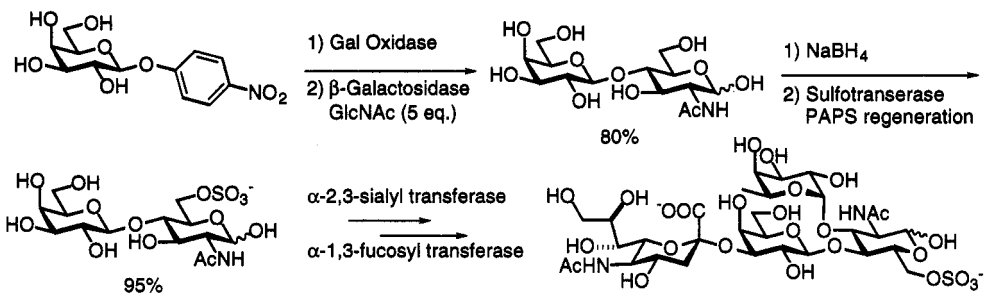

Fig 1. Synthesis of SLe^x-6-sulfate, a ligand for L-selectin.

In our efforts directed toward the development of new methods for the synthesis of homogenous glycoproteins, we have developed an enzymatic method for the semisynthesis of ribonuclease B glycoforms (Fig. 2).[10] Treatment of the heterogeneous glycoprotein ribonuclease B with endo H gave a homogeneous monoglycosylated ribonculease which can be converted to different glycoforms by incorporating additional sugars enzymatically using glycosyltransferases. A SLe^x group linked to Asn-34 via an N-glycosidic linkage has been prepared. When the monoglycosylated protein was treated with subtilisin BPN', about 5 pieces of peptides were generated. Without isolation this mixture of peptides can be religated to the glycoprotein upon addition of 9 volumes of DMSO. This observation suggests that enzymatic synthesis of glycoproteins can be accomplished by ligation of synthetic peptides and glycopeptides with subtilisin BPN' or its engineered variants followed by glycosyltransferase-catalyzed glycosylation. This enzymatic method for the synthesis of a glycoprotein in a single form can be used to study the effects carbohydrates have on glycoprotein structure and function.

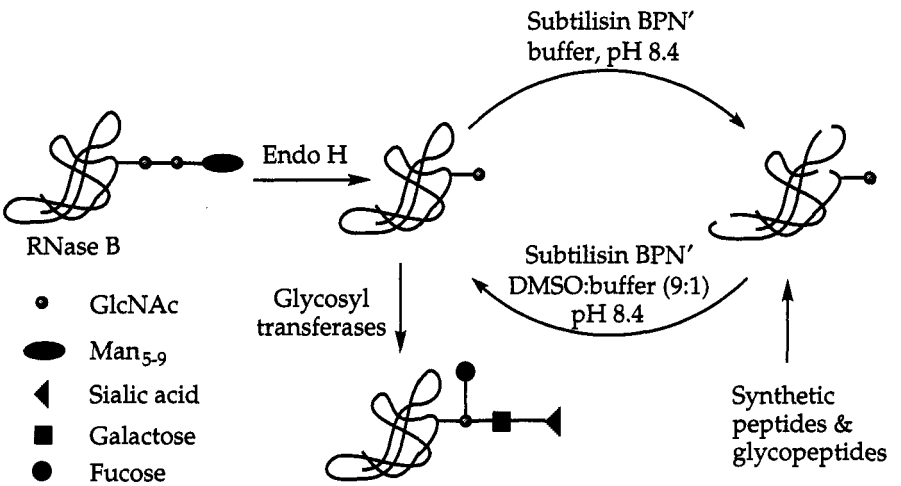

Fig. 2. Enzymatic synthesis of homogenous ribonuclease glycoforms.

As mentioned before, carbohydrate-protein interactions are generally weak[11] and often require a multivalent approach or incorporation of additional groups into sugars or preparation of a rigid structure to mimick the bound conformation of the carbohydrate to enhance binding. Since the solution conformation[12] of SLe^x is different from that bound to E-selectin,[13] less flexible structures which mimick the bound active conformation of SLe^x may exhibit higher affinity for E-selectin. Many groups have been active in pursuing this approach, and those presented in Fig. 3 were prepared in our laboratory.[14-15] Some of the mimetics are better than SLe^x as inhibitors of E-selectin. It is expected that further modification of the good mimetics by incorporating additional hydrophobic groups or by converting to multivalent species such as liposome conjugates will enhance the inhibition potency.[16-17]

Another approach to control the function of carbohydrates is to inhibit the enzymes involved in the biosynthesis or processing of carbohydrates of interest. Glycosidases and glycosyltransferases represent interesting targets in this regard.[1] In our recent work on the development of inhibitors of human -1,3-fucosyltransferase to block the biosynthesis of SLe^x, we have developed a relatively good aza-saccharide inhibitor. In the presence of GDP (50 μM) the designed aza-trisaccharide exhibits micromolar inhibition (IC_{50} ~ 30 μM) against the enzyme.[18] The key aza-sugar used in the inhibitor synthesis was prepared via a chemo-enzymatic route shown in Fig. 4. This synergistic inhibition approach is expected to be generally applicable to any glycosyltransferase, and work is in progress to use this strategy to develop better

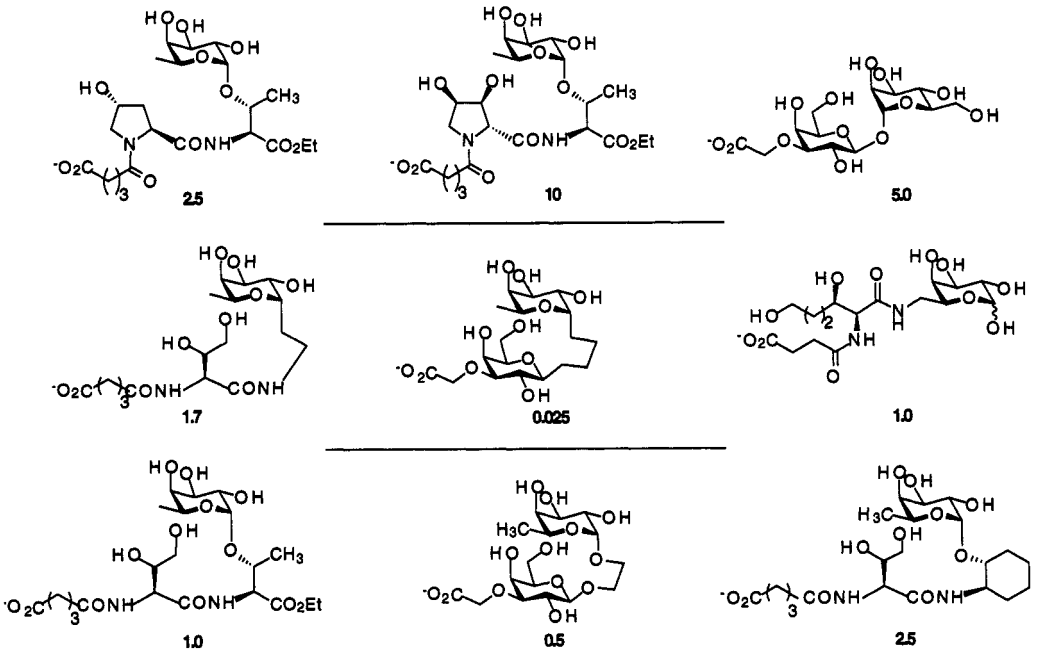

Fig. 3 SLe^x mimetics and their relative activities as compared to SLe^x (**1**).

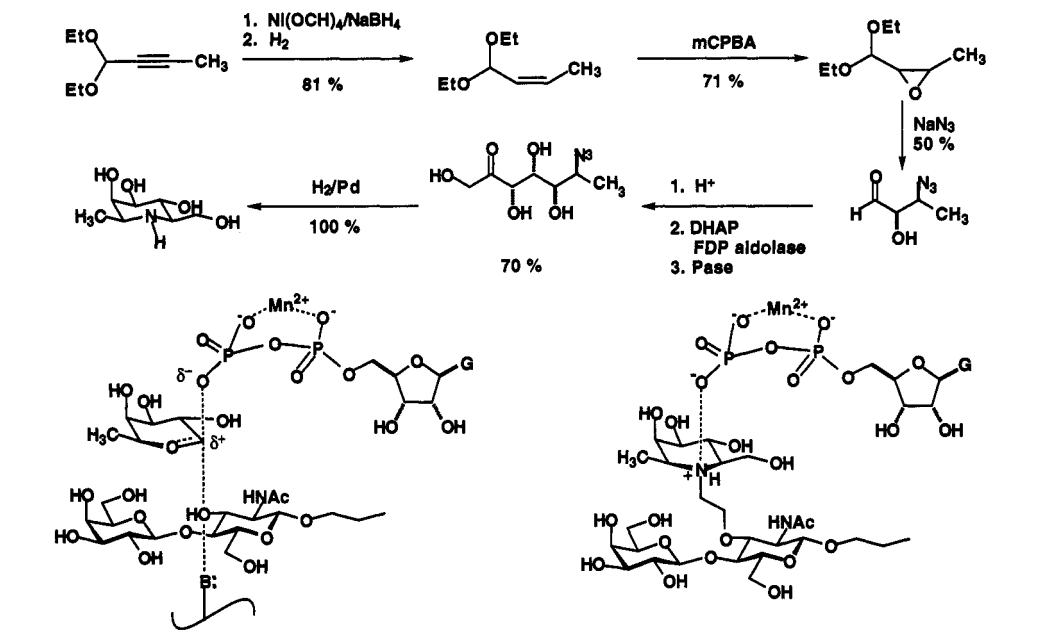

Figure 4. Synthesis of a designed aza-trisaccharide as a synergistic inhibitor of human α-1,3-fucosyltransferase. DHAP, dihydroxyacetone phosphate; Pase, phosphatase.

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References
1. For a review and references cited, H.J.M. Gijsen, L. Qiao, W. Fitz, C.-H. Wong. *Chem. Rev.* **96**, 443 (1996).
2. For a review and references cited, M.P. Bevilacqua, R.M. Nelson. *J. Clin. Invest.* **91**, 379 (1993).
3. A. Varki. *Proc. Natl. Acad. Sci. USA* **91**, 7390 (1994).
4. L.A. Lasky. *Science* **258**, 964 (1992).
5. M.L. Phillips, E. Nudelman, F.C.A. Gaeta, M. Perez, A.K. Singhal, S. Hakamori, J.C. Paulson. *Science* **250**, 1130 (1990).
6. S. Hemmerich, C.R. Bertozzi, H. Leffler, S.D. Rosen. *Biochemistry* **33**, 4820 (1994).
7. C.-H. Lin, G.-J. Shen, C.-H. Wong. *J. Am. Chem. Soc.* **117**, 8031 (1995).
8. E.V. Chandrasekaran, R.K. Jain, R.D. Larsen, K. Wlasichuk, K.L. Matta. *Biochemistry* **34**, 2925 (1995).
9. T. Kimura, S. Takayama, H. Huang, C.-H. Wong. *Angew. Chem. Int. Ed. Engl.* 1996, in press.
10. K. Witte, C.-H. Wong. *J. Am. Chem. Soc.*, submitted.
11. Y.C. Lee, R.T. Lee. *Acc. Chem. Res.* **28**, 321 (1995).
12. Y. Ichikawa, Y.-C. Lin, D.P. Dumas, G.-J. Shen, E. Garcia-Junceda, M.A. Williams, R. Bayer, C. Ketcham. L.E. Walker, J.C. Paulson, C.-H. Wong. *J. Am. Chem. Soc.* **114**, 9282 (1992).
13. K. Scheffler, B. Ernst, A. Katopodis, J.L. Maganani, W.T. Wang, R. Seisemann, T. Peters. *Angew. Chem. Int. Ed. Engl.* **34**, 1841 (1995).
14. T. Uchiyama, V.P. Vassilev, T. Kajimoto, W. Wong, H. Huang, C.-C. Lin, C.-H. Wong. *J. Am. Chem. Soc.* **117**, 5395 (1995).
15. C.-C. Lin, M. Shimazaki, M.-P. Heck, S. Aoki, R. Wang, T. Kimura, H. Ritzen, S. Takayama, S.-H. Wu, G. Weitz-Schmidt, C.-H. Wong. *J. Am. Chem. Soc.*, in press and references cited.
16. T. Murohara, J. Margiotta, L.M. Phillips, J.C. Paulson, S. DeFrees, S. Zalipsky, L.S.S. Guo, A.M. Lefer. *Cardiovascular Res.* **30**, 965 (1995).
17. W. Spevak, C. Foxall, D.H. Charych, F. Dasgupta, J.O. Nagy. *J. Med. Chem.* **39**, 1018 (1996).
18. L. Qiao, B.W. Murray, M. Shimazaki, J. Schultz, C.-H. Wong. *J. Am. Chem. Soc.*, in press.