

Bioorganic & Medicinal Chemistry Letters 9 (1999) 35-38

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

CHEMICAL AND ENZYMATIC SYNTHESIS OF GLYCOCONJUGATES 5: ONE-POT REGIOSELECTIVE SYNTHESIS OF BIOACTIVE GALACTOBIOSIDES USING A CLONEZYMETM THERMOPHILIC GLYCOSIDASE LIBRARY

Jun Li,^a Dan E. Robertson,^b Jay M. Short,^b and Peng George Wang*.^a

^aDepartment of Chemistry, Wayne State University, Detroit, MI 48202-3489, U. S. A. ^bDiversa Inc., 10665 Sorrento Valley Road, San Diego, CA 92121, U. S. A.

Received 24 September 1998; accepted 10 November 1998

Abstract: Enzymatic synthesis of galactobiosides using a versatile CLONEZYMETM thermostable glycosidase library was studied. One-pot transglycosylation reactions were demonstrated to synthesize $\beta(1\rightarrow 4)$, $\beta(1\rightarrow 6)$, and $\alpha(1\rightarrow 6)$ disaccharide sequences with high regioselectivity and moderate to high yields. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Over the past few years, glycosidases have been used widely for the preparation of a broad spectrum of glycoconjugates and derivatives employing either transglycosylation or reverse hydrolysis reactions.¹ In order to find biocatalysts with unprecedented properties from vast untapped resources of extremophiles, recombinant technologies and robotic screening approaches have been developed by Diversa Inc for the discovery of a novel glycosidase library, namely CLONEZYMETM library, which contains 10 unique thermostable glycosidases.² Recently, we have demonstrated useful transformations for the synthesis of *N*-acetyllactosamine, lactosamine, and β -D-fucopyranosyl- β -D-xylopyranosides with the CLONEZYMETM library.³ It is particularly useful to explore the synthetic repertoire of these robust enzymes catalyzing one-pot oligosaccharide synthesis via either self-transglycosylation or tandem transglycosylation (Figure 1). Using the above strategy, we herein report efficient synthesis of biologically important galactobiosides with this versatile glycosidase library.

Figure 1. One-Pot Oligosaccharide Synthesis Catalyzed by Transglycosylation Reactions.

So far, self-transglycosylation reactions using nitrophenyl glycosides as donors with relatively large scale have been limited to the synthesis of Gal α 1 \rightarrow 3 Gal and Man α 1 \rightarrow 2 Man as major sequences.⁴ Tandem transglycosylation reactions using lactose and raffinose as donors were reported to mainly afford Gal β 1 \rightarrow 3 Gal and Gal α 1 \rightarrow 3 Gal sequences, respectively.⁵ In the course of our screening the commercially available CLONEZYMETM glycosidase library, we found efficient synthesis of three bioactive galactobioside disaccharide sequences with β (1 \rightarrow 4), β (1 \rightarrow 6), and α (1 \rightarrow 6) linkages. Gal β (1 \rightarrow 4)Gal, as a major component of galactooligosaccharide, can promote the growth of Bifidobacteria in human large intestine. Bifidobacteria is a friendly bacteria in human intestine, which has the following therapeutic effects ranging from antitumorigenic activity, reduction of serum cholesterol levels, synthesis of B-complex vitamins, to enhanced absorption of calcium in the prevention of bone loss.⁶ Gal $\beta(1\rightarrow 6$)Gal was found to be responsible for the binding of myeloma immunoglobulins.⁷ Gal $\alpha(1\rightarrow 6)$ Gal is an important disaccharide sequence in glycosylphosphatidylinositol (GPI) anchor of *Trypanosoma brucei* (Figure 2).⁸ Comparing to chemical synthesis of $\beta(1\rightarrow 4)$, $\beta(1\rightarrow 6)$, 10 and $\alpha(1\rightarrow 6)^{11}$ linked galactobiosides elaborated with multistep protecting, glycosylation, and deprotecting sequences, one-pot enzymatic reactions via self-and tandem-transglycosylation are extremely attractive in assembling these bioactive oligosaccharides.



The one-pot reactions via self-transglycosylation were demonstrated using glycosidase Gly-001-06 and Gly-001-10 (Scheme 1). The latter is the only enzyme in the library showing α -galactosidase activity. It is noteworthy that Gly-001-06 is especially efficient for the regioselective synthesis of $\beta 1 \rightarrow 4$ linked galactobioside, while Gly-001-10 is specific for the synthesis of $\alpha 1 \rightarrow 6$ linked galactobioside. Both reactions were in good yields up to 57%. Nevertheless, minor product of $\alpha 1 \rightarrow 3$ linked galactobioside was observed in the use of Gly-001-10. A general procedure is described as follows: 0.36 mmol nitrophenyl D-galactopyranoside was dissolved in 6 mL sodium phosphate buffer (50 mM, pH 6.0). Glycosidase (0.2 mg/mL) from CLONEZYMETM library was added. The reaction was incubated at 70 or 90 °C for 3 h and terminated by freeze-drying. The residue was loaded on silica gel column chromatography and eluted with 'PrOH/EtOAc/H₂O (2/6/1) to afford products 1, 2, and 3. The glycosidic linkages of the products were identified by marked downfield shift of the corresponding C-3, C-4, and C-6 resonances.¹²



Scheme 1. Regioselective synthesis of galactobiosides via self-transglycosylation.

The $\beta 1 \rightarrow 4$ linked galactobioside was found to obtain in high yield in our studies comparing to the results obtained previously from a similar transglycosylation using conventional β -galactosidases.¹³ To the best of our knowledge, Gly001-10 is the only enzyme reported so far to catalyze the high yielding formation of $\alpha 1$ -6 linked galactobioside using self-transglycosylation scheme. The *p*-nitrophenyl group can be selectively removed by a sequence of hydrogenation, *N*-acetylation, and CAN (cerium ammonium nitrate) oxidation reactions.¹⁴ Thus, *p*nitrophenyl- α -D-galactopyranosyl- $(1\rightarrow 6)$ - α -D-galactopyranoside, **2**, can be used as a disaccharide building block for the total synthesis of GPI.

The one-pot reactions via tandem transglycosylation were demonstrated using lactose as a cheap donor and CLONEZYMETM glycosidases as catalysts for double galactosylation of acceptor 2-[2-(2-chloroethoxy)ethoxy]ethanol which can be served as a biocompatible spacer with a good leaving chloride group at one end of the chain. Generally, the yield of double transglycosylation reaction is low. Moreover, the reaction is also plagued by the poor regioselectivity observed at the second transglycosylation step. Here we found two unique enzymes Gly-001-08 and Gly-001-09 catalyzed the double galactosylation reactions with regioselectivity exclusively for $\beta 1 \rightarrow 6$ and $\beta 1 \rightarrow 4$ linkages, respectively (Scheme 2). Although the overall yields are relatively low without optimization, the reactions can be readily scaled up in view of the efficiency of this regioselective approach and the use of cheap donor and acceptor. A general approach for this transglycosylation is shown as follows: To a solution of donor lactose (1 g, 2.9 mmol) and acceptor (212 µL, 1.4 mmol) in 2.9 mL of sodium phosphate buffer (50 mM, pH 6.0) was added CLONEZYME[™] glycosidase (100 µL, 1.8-1.9 mg/mL). The reaction was incubated at 75 °C for 4 h to overnight when the maximum formation of disaccharides was reached. The reaction mixture was concentrated and the residue was fractionated and separated by Biogel P-2 gel. Only $\beta 1 \rightarrow 4$ linked galactobioside 4 (50 mg, 7% yield) and $\beta 1 \rightarrow 6$ linked galactobioside 5 (31 mg, 4% yield) were found for Gly-001-09 and Gly-001-08, respectively.¹² No other types of linkage were observed in the reaction mixtures.



Scheme 2. One-pot regioselective synthesis of galactobiosides via tandem transglycosylation.

In summary, we demonstrated here a versatile CLONEZYMETM thermophilic glycosidase library could be applied for the synthesis of biologically important galactobiosides via highly efficient one-pot regioselective tranglycosylation routes. The yields of the reactions were achieved from relatively low to high with remarkable regioselectivities. All the one-pot reactions shown here provide us with a simple and useful method for the synthesis of complex galactobiosides.

Acknowledgment: This work was generously supported by Hercules Inc, NSF (BES-9728366), and Herman Frash Foundation (449-HF97). JL thanks the Robert Maytag fellowship from the University of Miami.

References and Notes

- (a) Nilsson, K. G. I. Synthesis with Glycosidases In Modern Methods in Carbohydrate Synthesis; Khan, S. H.; O'Neill, R. A. Eds.; Harwood Academic: The Netherlands, 1996, pp 518-547. (b) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. Chem. Rev. 1996, 96, 443. (c) Fernández-Mayoralas, A. Top. Curr. Chem. 1997, 186, 1. (d) Murata, T.; Usui, T. Biosci. Biotech. Biochem. 1997, 61, 1059. (e) Crout, D. H. G.; Vic, G. Curr. Opin. Chem. Bio. 1998, 2, 98.
- (a) Brennan, M. B. Chem. Eng. News 1996, October 14, 31. (b) Adams, M. W. W.; Kelly, R. M. Chem. Eng. News 1995, December 18, 32. (c) Robertson, D. E.; Mathur, E. J.; Swanson, R. V.; Marrs, B. L.; Short, J. M. SIMS News, 1996, 46, 3.
- (a) Li, J.; Wang, P. G. Tetrahedron Lett. 1997, 38, 7967. (b) Fang, J.; Xie, W.; Li, J. Wang, P. G. Tetrahedron Lett. 1998, 39, 919. (c) Li, J. Robertson, D. E.; Short, J. M.; Wang, P. G. Tetrahedron Lett. 1998, in press.
- 4. Nilsson, K. G. I. Carbohydr. Res. 1987, 167, 95.
- 5. Nilsson, K. G. I. Carbohydr. Res. 1988, 180, 53.
- 6. Onishi, N.; Yamashiro A.; Yokozeki, K. Appl. Environ. Microbiol. 1995, 61, 4022.
- 7. Glaudemans, C. P. J. Chem. Rev. 1991, 91, 25.
- 8. Ferguson, M. A. J.; Homans, S. W.; Dwek, R. A.; Rademacher, T. W. Science 1988, 239, 753.
- 9. Kovac, P; Taylor, R. B. Carbohydr. Res. 1987, 167, 153.
- 10. Kovac, P. Carbohydr. Res. 1985, 144, C12.
- 11. Cheung, M. -K.; Douglas, N. L.; Hinzen, B.; Ley, S. V.; Pannecoucke, X. Synlett 1997, 257.
- 12. ¹³C NMR for **1** (100 MHz, $D_2O/5\%$ CD₃OD): δ 171.4, 150.4, 136.0, 126.4, 124.0, 118.5, 105.3 (C-1'), 102.0 (C-1), 77.8 (C-4), 76.1, 75.7, 74.0, 73.9, 72.5, 71.7, 69.6, 61.9 (C-6'), 61.2 (C-6). **2** (100 MHz, D_2O) δ 161.8, 142.7, 126.3, 117.5, 98.1 (C-1'), 96.8 (C-1), 71.2, 71.1, 69.83, 69.80 (2C), 69.5, 68.5, 68.2, 67.0, 61.4 (C-6'). **3** (100 MHz, D_2O) δ 161.2, 142.1, 125.8, 116.6, 96.6 (C-1'), 95.0 (C-1), 73.9 (C-3), 71.9, 70.9, 69.2, 69.1, 68.1, 66.2, 65.2, 61.1 (C-1'), 60.8 (C-1). **4** (100 MHz, $D_2O/20\%$ CD₃OCCD₃) δ 104.7 (C-1'), 103.1 (C-1), 77.5 (C-4), 75.4, 74.5, 73.5, 73.2, 71.8, 71.6, 71.1, 70.1, 69.84, 69.80, 69.0, 68.9, 61.3 (C-6'), 60.7 (C-6), 43.5 (CH₂Cl). **5** (100 MHz, $D_2O/20\%$ CD₃OCCD₃) δ 103.7 (C-1'), 103.2 (C-1), 75.5, 74.1, 73.2, 73.0, 71.1, 71.0, 70.1, 69.9, 69.8, 69.1, 69.0 (2C), 61.3 (C-6'), 43.5 (CH₂Cl).
- 13. Nakano, H; Takenishi, S; Watanabe, Y. Agric. Biol. Chem. 1987, 51, 2267.
- 14. Fukase, K.; Yasukochi, T.; Nakai, Y.; Kusumoto, S. Tetrahedron Lett. 1996, 37, 3343.