Pergamon

Tetrahedron Letters 39 (1998) 8963–8966

TETRAHEDRON LETTERS

Chemical and Enzymatic Synthesis of Glycoconjugates 4. Control of Regioselectivity in High Yielding Synthesis of (β–D-Fucopyranosyl)-*O*-D-xylopyranosyl Disaccharides using a CLONEZYME™ Thermophilic Glycosidase

Jun Li,[†] Dan E. Robertson,[‡] Jay M. Short,[‡] and Peng George Wang*,[†]

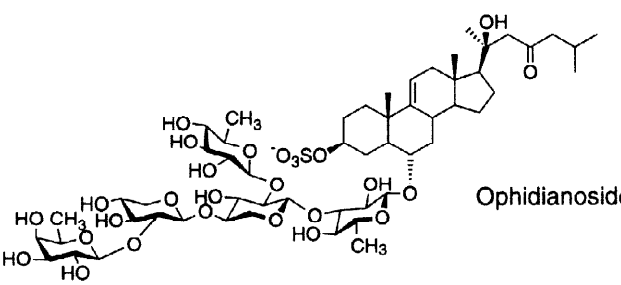
[†]*Department of Chemistry, Wayne State University, Detroit, MI 48202-3489*

[‡]*Diversa Inc., 10665 Sorrento Valley Road, San Diego, CA 92121*

Received 21 August 1998; revised 21 September 1998; accepted 24 September 1998

Abstract: β-D-fucosylation of xylopyranosides catalyzed by glycosidase Gly-001-09 from CLONEZYME™ thermophilic glycosidase library produced β-D-fucopyranosyl-β-D-xylopyranoside disaccharides with 1→2 and 1→3 linkages in high yield up to 88%. Regioselectivity can be controlled by the orientation and size of aglyconic substituents of the acceptor. The enzymatic transglycosylation affords an efficient approach for the preparation of Fucβ(1→2)Xyl disaccharide, an important carbohydrate sequence in asterosaponins.

Asterosaponins as ubiquitous toxic steroidal saponins among starfish are reputed chemical defense agents, precluding infectious aquatic fungi, parasites and predators.[1] They have also shown a variety of pharmacological activities against tumors and viruses.[2] Most of the oligosaccharides found in asterosaponins contain a β-D-fucosylated epitope. The Fucβ(1→2)Xyl is found at the tip of the carbohydrate sequence in ophidianoside F, cosmasteroside A and B, and reticulatoside A and B.[3] Recently, compound ophidianoside F (Figure 1) has been shown to significantly inhibit barnacle attachment, which is the first reported example that bioactive echinoderm compound may play a role in anti-fouling.[4] In our efforts to build up oligosaccharides for the total synthesis of asterosaponins, we found an efficient enzymatic approach to synthesize Fucβ(1→2)Xyl disaccharide.



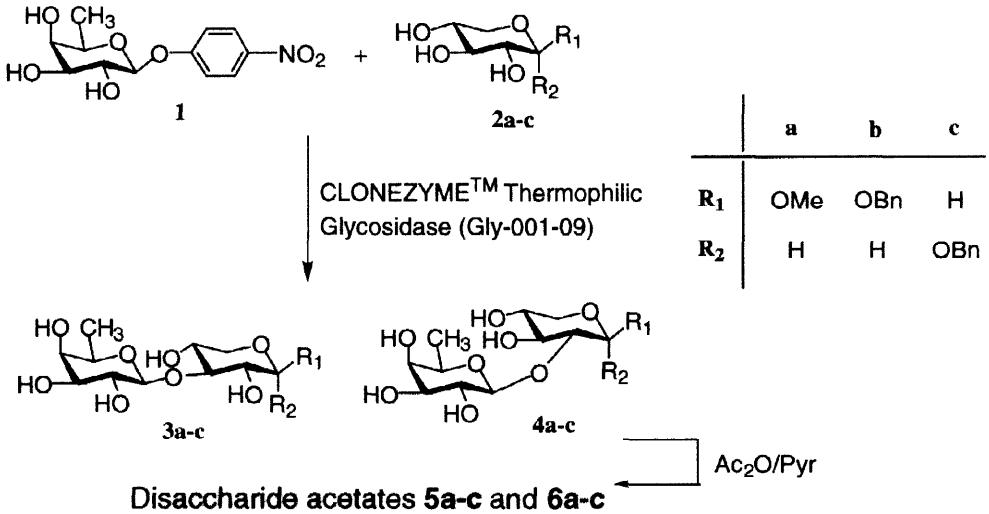
Ophidianoside F

Figure 1.

The use of glycosidases for the synthesis of different glycosides have been a topic of current interest.[5] Recently, we have demonstrated the use of glycosidase CLONEZYME™ library (Diversa Inc.) for some transglycosylation reactions with high regioselectivity.[6] The enzyme sources play an important role on product regioselectivity. After screening 9 glycosidase mutants from CLONEZYME™ library, we found that Gly-001-09 was particularly active for high yielding transfucosylation of xylopyranosides. More importantly, regioselectivity of the glycosidase-catalyzed transglycosylation can be controlled by the orientation and size of aglyconic substituents of the acceptors. Similar features were also observed by Nilsson[7] and Fernández-Mayoralas et al[8] in their previous work.

0040-4039/98/$ - see front matter
PII: S0040-4039(98)02072-3

The reactions of *para*-nitrophenyl-β-D-fucopyranoside (**1**) and xylopyranoside derivatives (**2a-c**) were depicted in Scheme 1. Thermophilic glycosidase Gly-001-09 (400 μL, 1.8 mg/mL) was added to a 12-mL phosphate buffer solution (50 mM, pH 6.0) containing donor **1** (0.6 mmol) and acceptor **2** (3.6 mmol). The reaction was incubated at 70 °C and was monitored by TLC. When the maximum formation of fucosyl-xyloses was reached, the reaction was stopped by lowering the reaction temperature. The mixture was lyophilized, peracetylated, and purified by flash chromatography. It was found that all the reactions produced disaccharides in good yield. With methyl xylopyranoside as an acceptor, the yield is as high as 88% which is much higher than most transglycosylation reactions reported so far. Such high yielding reaction is comparable to glycosyltransferase-catalyzed glycosylation reactions. The 1→3 (**3**) and 1→2 (**4**) products were the only linkages observed in all the reactions. No 1→4 product was found in the reaction mixtures (Scheme 1).



Scheme 1. Enzymatic synthesis of β-D-fucopyranosyl-β-D-xylopyranosides

The linkages of the disaccharides were determined by the studies of ^{1}H-NMR, COSY and 1D NOE difference spectrum of the disaccharide acetates **5** and **6**.[9]

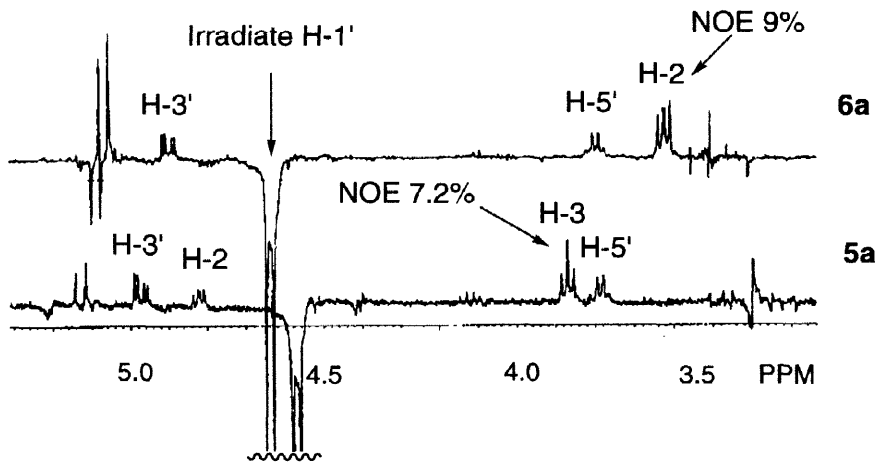


Figure 2. 1D NOE difference spectrum of disaccharide acetates **5a** and **6a**.

Comparing the proton NMR spectrum of peracetylated disaccharides to the corresponding peracetylated xylose derivatives, we clearly assigned the proton on the xylose next to the oxygen which was connected to the fucose. For a specific fucosyl-xylose disaccharide, peracetylation moves all the protons to downfield except that the proton of the glycosidic linkage site remains upfield. The linkage positions were further confirmed by the observation of interglycosidic NOEs via 1D NOE difference spectrum. Upon irradiation of anomeric H-1' of fucose in the disaccharide acetates, more than 5% NOE were observed on H-3 of peracetylated **5** and H-2 of peracetylated **6** (See Figure 2).

The regioselectivity of the fucosylation was studied with the acceptors having different sizes and anomeric orientations of the aglyconic moieties. It is noteworthy that i) with same β anomeric configuration of **2a** and **2b**, product of 1→2 linkage was preferred when small substituent (methyl group) was used. ii) with same size of aglyconic group, benzyl-β-D-xylopyranoside (**2b**) gave more 1→3 linkage disaccharide while benzyl-α-D-xylopyranoside (**2c**) gave rise to 1→2 linkage as a major product (Table 1). The resulting disaccharide **4c** with anomeric position protected by a benzyl group is being used to build oligosaccharides for the synthesis of asterosaponins.

Table 1 Yield and Ratio of Regioselectivity of the Disaccharide Products **3** and **4** from the CLONEZYME™ Thermophilic Glycosidase Catalyzed Reactions

Acceptors	R_1	R_2	Yield (%) **3+4**	Regioselectivity Ratio: **3/4**
2a	OMe	H	88%	1/2.1
2b	OBn	H	76%	2.5/1
2c	H	OBn	67%	1/2.2

In summary, high yielding of fucosylation of xylopyranosides is achieved by thermophilic CLONEZYME™ glycosidase catalyzed reaction. The regioselectivity is tractable by choosing different size and orientation of aglyconic moiety of the acceptors. The transglycosylation affords a simple approach for the preparation of a Fucβ(1→2)Xyl disaccharide building block for the synthesis of asterosaponins of marine origin.

Acknowledgement

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

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* To whom correspondence should be addressed.

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9. **5a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.20 (d, 1H, *J* = 3.6 Hz, H-4'), 5.11 (dd, 1H, *J* = 8, 10.4 Hz, H-2'), 4.97 (dd, 1H, *J* = 3.6, 10.4 Hz, H-3'), 4.89 (td, 1H, *J* = 6.4, 4.4 Hz, H-4), 4.82 (dd, 1H, *J* = 4.8, 6.4 Hz, H-2), 4.57 (d, 1H, *J* = 8 Hz, H-1'), 4.40 (d, 1H, *J* = 4.8 Hz, H-1), 4.09 (dd, 1H, *J* = 4.0, 12.4 Hz, H-5e), 3.87 (t, 1H, *J* = 6.4 Hz, H-3), 3.78 (q, 1H, *J* = 6.4 Hz, H-5'), 3.45 (dd, 1H, *J* = 6.0, 12.0 Hz, H-5a), 3.4 (s, 3H, OMe), 2.17, 2.12, 2.09, 2.05, 1.98 (5s, 15H, acetyl), 1.20 (d, 3H, *J* = 6.4 Hz, H-6'). **6a** : δ 5.21 (d, 1H, *J* = 3.6 Hz, H-4'), 5.14 (dd, 1H, J = 8, 10.4 Hz, H-2'), 5.12 (t, *J* =8.0 Hz, H-3), 4.97 (dd, 1H, *J* = 3.6, 10.4 Hz, H-3'), 4.85 (td, 1H, *J* = 8.0, 4.8 Hz, H-4), 4.69 (d, 1H, *J* = 8 Hz, H-1'), 4.52 (d, 1H, *J* = 6.0 Hz, H-1), 4.04 (dd, 1H, *J* = 5.2, 12.4 Hz, H-5e), 3.81 (q, 1H, *J* = 6.4 Hz, H-5'), 3.61 (dd, 1H, *J* = 5.6, 8.0 Hz, H-2), 3.49 (s, 3H, OMe), 3.41 (dd, 1H, *J* = 7.6, 12 Hz, H-5a), 2.17, 2.08, 2.06, 2.05, 1.97 (5s, 15H, acetyl), 1.22 (d, 3 H, *J* = 6.4 Hz, H-6'). **5b** : δ 7.35-7.27 (m, 5H), 5.19 (dd, 1H, *J* = 3.2, 1.2 Hz, H-4'), 5.11 (dd, 1H, *J* = 8, 10.4 Hz, H-2'), 4.97-4.91 (m, 3H, H-3', H-4 and H-2), 4.80 (d, 1H, *J* = 12.4 Hz, benzylic proton), 4.56 (d, 1H, *J* = 12.8 Hz, benzylic proton), 4.55 (d, 1H, *J* = 7.6 Hz, H-1'), 4.49 (d, 1H, *J* = 6.0 Hz, H-1), 4.13 (dd, 1H, *J* = 4.8, 12.4 Hz, H-5e), 3.85 (t, 1H, *J* = 7.0 Hz, H-3), 3.76 (qd, 1H, *J* = 6.4, 1.2 Hz, H-5'), 3.40 (dd, 1H, *J* = 7.2, 12.4 Hz, H-5a), 2.17, 2.074, 2.07, 1.98, 1.97 (5s, 15H, acetyl), 1.19 (d, 3H, *J* = 6.4 Hz, H-6'). **6b** : δ 7.41-7.29 (m, 5 H), 5.18-5.15 (m, 2H, H-4' & H-2'), 5.13 (t, *J* =8.0 Hz, H-3), 4.93 (dd, 1H, *J* = 3.2, 10.4 Hz, H-3'), 4.91 (d, 1H, *J* = 12.0 Hz, benzylic proton), 4.87 (td, 1H, *J* = 7.6, 4.8 Hz, H-4), 4.73 (d, 1H, *J* = 5.6 Hz, H-1), 4.67 (d, 1H, *J* = 8.0 Hz, H-1'), 4.58 (d, 1H, *J* = 11.6 Hz, benzylic proton), 4.10 (dd, 1H, *J* = 4.8, 12.0 Hz, H-5e), 3.73 (dd, 1H, *J* = 5.6, 8.0 Hz, H-2), 3.63 (q, 1H, *J* = 6.4 Hz, H-5'), 3.44 (dd, 1H, *J* = 7.6, 12 Hz, H-5a), 2.17, 2.08, 2.052, 2.05, 1.97 (5s, 15H, acetyl), 1.12 (d, 3 H, *J* = 6.4 Hz, H-6'). **5c** : δ 7.47-7.44 (m, 5H), 5.19 (dd, 1H, *J* = 3.6, 1.2 Hz, H-4'), 5.08 (dd, 1H, *J* = 7.8, 10.5 Hz, H-2'), 4.99-4.91 (m, 3H, H-3', H-4 and H-1), 4.80 (dd, 1H, *J* = 3.9, 9.9 Hz, H-2), 4.73 (d, 1H, *J* = 11.7 Hz, benzylic proton), 4.58 (d, 1H, *J* = 8.1 Hz, H-1'), 4.48 (d, 1H, *J* = 12.0 Hz, benzylic proton), 4.13 (t, 1H, *J* = 9.6 Hz, H-3), 3.82-3.73 (m, 2H, H-5e, H-5'), 3.63 (t, 1H, *J* = 10.5 Hz, H-5a), 2.16, 2.10, 2.05, 1.97, 1.96 (5s, 15H, acetyl), 1.18 (d, 3H, *J* = 6.3 Hz, H-6'). **6c** : δ 7.41-7.27 (m, 5 H), 5.49 (t, *J* =9.6 Hz, H-3), 5.20 (d, 1H, *J* = 2.7, H-4'), 5.19 (dd, 1H, *J* = 7.8, 10.5 Hz, H-2'), 5.01 (d, 1H, *J* = 3.3 Hz, H-1), 4.95 (dd, 1H, *J* = 3.6, 10.5 Hz, H-3'), 4.91 (td, 1H, *J* = 10.5, 6.6 Hz, H-4), 4.75 (d, 1H, *J* = 12.6 Hz, benzylic proton), 4.62 (d, 1H, *J* = 12.3 Hz, benzylic proton), 4.57 (d, 1H, *J* =7.8 Hz, H-1'), 3.77-3.70 (m, 3H, H-5', H-5e and H-2), 3.66 (t, 1H, *J* = 10.5 Hz, H-5a), 2.18, 2.07, 2.03, 2.00, 1.98 (5s, 15H, acetyl), 1.12 (d, 3 H, *J* = 6.6 Hz, H-6').