

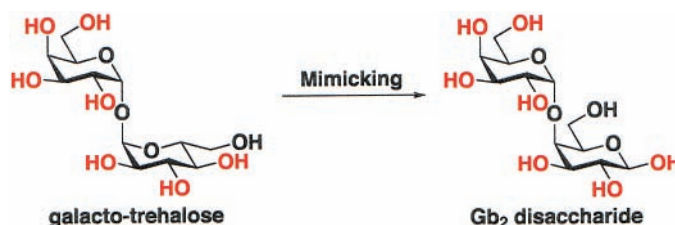
# Molecular Design and Biological Potential of Galacto-Type Trehalose as a Nonnatural Ligand of Shiga Toxins

Hirofumi Dohi,<sup>†</sup> Yoshihiro Nishida,<sup>\*,†</sup> Yuki Furuta,<sup>†</sup> Hirotaka Uzawa,<sup>‡</sup>  
Shin-ichiro Yokoyama,<sup>§</sup> Saori Ito,<sup>§</sup> Hiroshi Mori,<sup>§</sup> and Kazukiyo Kobayashi<sup>\*,†</sup>

Department of Molecular Design and Engineering, Graduate School of Engineering,  
Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan, National Institute of  
Advanced Industrial Science and Technology, 1-1-1 Higashi, Tsukuba,  
305-8565, Japan, and Department of Public Health Pharmacy,  
Gifu Pharmaceutical University, Mitabora-higashi, Gifu 502-8585, Japan  
h996603d@mblox.media.nagoya-u.ac.jp

Received November 27, 2001

## ABSTRACT



Galacto-type trehalose, a “C-4 epimer of trehalose”, possesses a stereochemical structure around the  $\alpha(1-4)$ -linkage analogous to that of the globobiosyl  $\alpha(1-4)$ -linkage in Gb<sub>2</sub> and Gb<sub>3</sub> ceramides, which are known as the ligands of Shiga toxins produced by pathogenic *E. coli*. This paper presents evidence supporting the new idea of using a trehalosyl  $\alpha(1-4)$ -linkage as a substitute for the galactobiosyl  $\alpha(1-4)$ -linkage.

Shiga toxins (Stx-I and Stx-II) produced by *Escherichia coli* O-157:H-7 and other pathogenic *E. coli* species are multi-subunit proteins comprising one toxic A-subunit and five receptor-binding B-subunits.<sup>1</sup> Both Stx-I and Stx-II recognize a galactobiosyl  $\alpha(1-4)$ -linkage in globosyl Gb<sub>2</sub> and Gb<sub>3</sub> ceramides and induce carbohydrate-mediated internalization into host cells.<sup>2</sup> There have been extensive studies on the molecular design of artificial Stx ligands that possess simpler structures and higher activity for blocking the toxin–host cell adhesion than the natural globosyl ceramides. Most of

the synthetic studies have been focused on the replacement of the ceramide group with simpler lipids<sup>3</sup> or on the molecular assembly to construct carbohydrate clusters as glycopolymers, glycodendrimers, and starfish models.<sup>4</sup> However, an effective mimic of the galactobiosyl  $\alpha(1-4)$ -linkage has not yet been found. This is ironic since the synthesis of many mimics has been hampered by the difficulty of constructing the  $\alpha(1-4)$ -linkage.<sup>5</sup> In this communication, we

<sup>†</sup> Nagoya University.

<sup>‡</sup> National Institute of Advanced Industrial Science and Technology.

<sup>§</sup> Gifu Pharmaceutical University.

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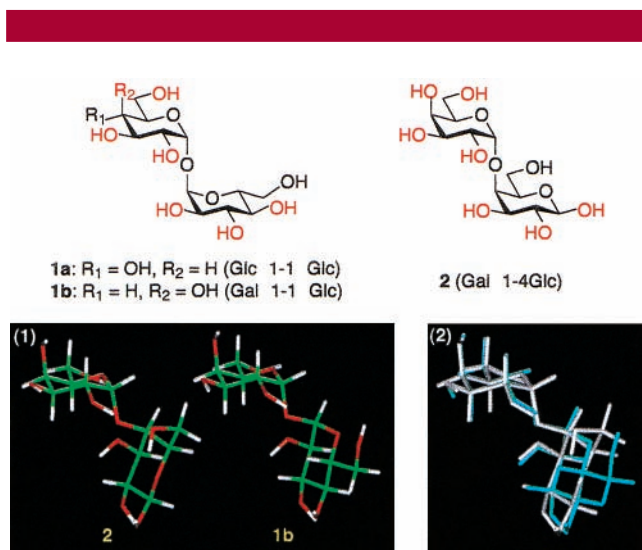
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propose the use of a trehalose  $\alpha(1-1)$ -linkage as the substitute for the galactobiosyl  $\alpha(1-4)$ -linkage and demonstrate the potential of this mimic.

Trehalose **1a** possesses many biological and medicinal potentials applicable to sugar-based therapeutic reagents.<sup>6</sup> This is mainly because the glycoside  $\alpha(1-1)$ -linkage is highly tolerant to both chemical and enzymatic (mammalian  $\alpha$ -glycosidases) degradation compared with glycosyl  $\alpha(1-4)$ -linkages. Here, it can be easily noticed that the trehalose  $\alpha(1-1)$ -linkage has a molecular topology analogous to that of the galactobiosyl  $\alpha(1-4)$ -linkage **2** (Gb<sub>2</sub>) with binding activity to Shiga toxins. A critical difference arises from the configuration at OH-4, which should be axial to be recognized by Shiga toxins. Accordingly, a galacto-trehalose **1b** can be designed as a novel Gb<sub>2</sub> mimic and expected to show a binding affinity to Stxs similar to that of the natural Gb<sub>2</sub> ligand as well as the advantageous property mentioned above. Molecular force field (MM-2) calculation supported the similarity of the stereochemical environment around the glycoside linkage between **2** and **1b** (Figure 1).



**Figure 1.** Preferred conformations of galacto  $\alpha(1-4)$ -bioside **2** and galacto-type trehalose **1b** (1) and superimposed structures (2). The structures were minimized by MM-2 without optimization of all OH bonds in the calculation (Insight II/Discover program and Amber force field); the white and blue lines represent **2** and **1b**, respectively, in panel 2.

In this study, we prepared galacto-type trehalose **6**<sup>7</sup> and its cluster model **7** in order to verify our expectation.<sup>8</sup> The synthesis was carried out via conventional chemical pathways

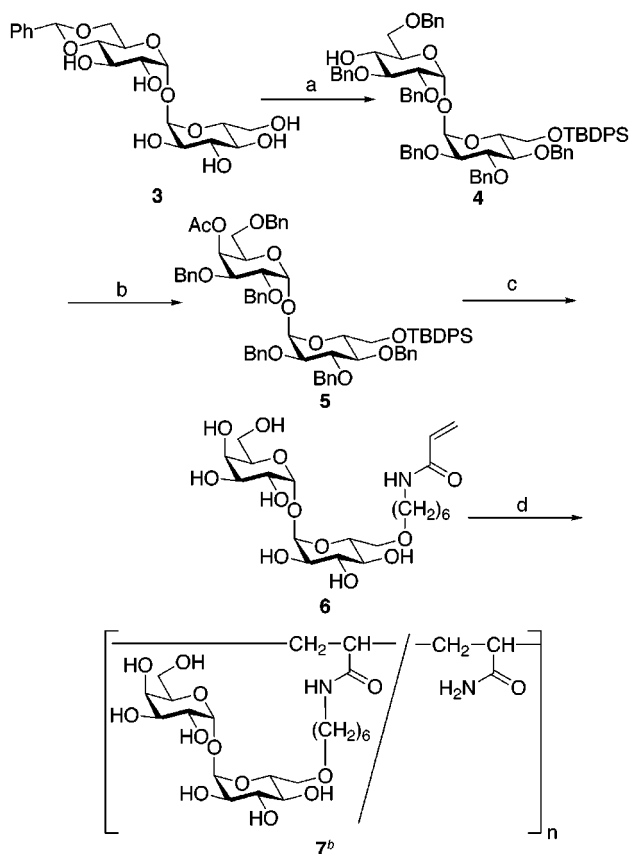
(6) Highly effective use of an  $\alpha$ -D-mannosyl(1-1)- $\beta$ -D-galactosyl linkage as a scaffold constructing a sialyl Lewis X mimic was reported: Hiruma, K.; Kajimoto, T.; Schumidt, G. W.; Ollmann, I.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, 39, 9265–9270.

(7) Compound **6**: <sup>1</sup>H NMR ( $\delta$  ppm, 500 MHz, D<sub>2</sub>O): 5.65–6.24 (dd  $\times$  3, 3H, H-olefin), 5.15 (d, 1H,  $J_{1,2}$  = 3.4 Hz, GalH-1), 5.14 (d,  $J_{1,2}$  = 3.1 Hz, GlcH-1), 4.15 (d,  $J_{3,4}$  = 3.0 Hz, GalH-4), 3.36 (dd, 1H,  $J_{2,3}$  = 6.7 Hz,  $J_{3,4}$  = 9.0 Hz, GlcH-4), 1.40–3.50 (dd  $\times$  2 and m  $\times$  4, 12H, H-methylene).

(8) Various types of glycosylated polyacrylamides have been reported; see: (a) Nishimura, S.-I.; Furuike, T.; Matsuoka, K.; Murayama, K.; Nagata, K.; Kurita, K.; Nishi, N.; Tokura, S. *Macromolecules* **1994**, 27, 4876–4880. (b) Roy, R.; Tropper, F. D. *Glycoconjugate J.* **1988**, 5, 203–206.

starting from 4,6-mono-*O*-benzylidene trehalose **3**<sup>9</sup> as summarized in Scheme 1. As a key reference compound to

**Scheme 1.** Synthesis of Galacto-Trehalose Cluster Model **7**<sup>a</sup>



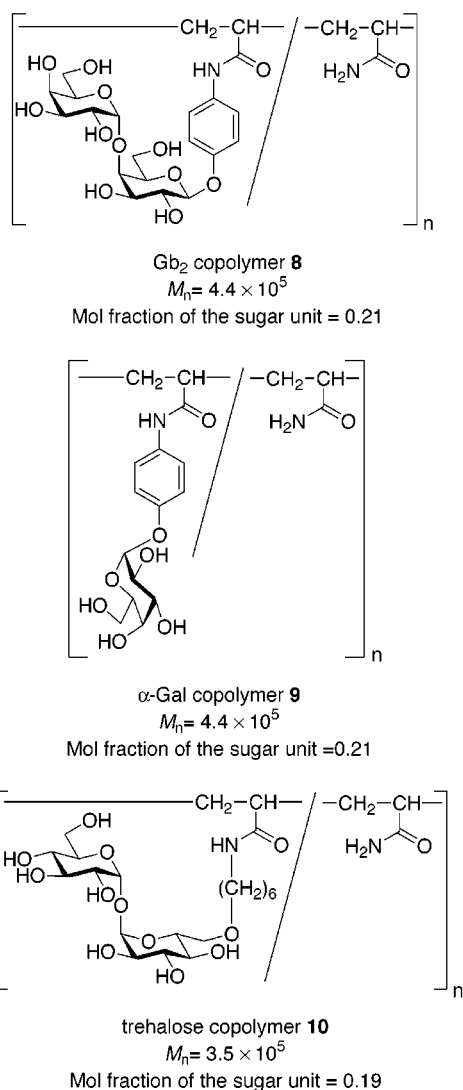
<sup>a</sup> Reaction conditions: (a) (i) TBDPSCl, Et<sub>3</sub>N, DMAP, pyridine, room temperature, 12 h, 91%; (ii) BnBr, NaH, DMF, room temperature, 12 h, 95%; (iii) BH<sub>3</sub>–NHMe<sub>2</sub>, AlCl<sub>3</sub>, THF, 0 °C→room temperature, 2 h, 94%; (b) (i) Tf<sub>2</sub>O, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C→room temperature, 45 min, quant; (ii) CsOAc, 18-crown-6, toluene, ultrasound, 40 °C, 2 h, 97%; (c) (i) NaOMe, MeOH, THF, room temperature, 12 h, quant; (ii) TBAF, THF, room temperature, 24 h, 95%; (iii) 6-azido-1-bromohexane, TBAI, NaH, DMF, 60 °C, 12 h, 92%; (iv) Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, THF, MeOH, room temperature, 1.5 h; (v) acryloyl chloride, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, MeOH, 0 °C→room temperature, 2 h, 91% (2 steps); (d) 2,2-azobis (2-amidinopropane) dihydrochloride, H<sub>2</sub>O, Me<sub>2</sub>SO, 60 °C, 12 h, 72%.

<sup>b</sup> Sloping line in the structure represents the random copolymer comprised of the two constitutional units.

investigate the role of the OH-4 configuration, a cluster model **10** carrying trehalose at the side chain was also prepared in the same way (6-azidohexylation at O-6, reduction of the azido group, *N*-acryloylation, and copolymerization with acrylamide in a redox radical manner) (Figure 2). Acrylamido copolymers **8** and **9** carrying natural Gb<sub>2</sub> and  $\alpha$ -D-galactoside clusters, respectively, were prepared in our previous manner.<sup>5,10</sup>

Hemagglutination inhibition assay<sup>11</sup> was performed for trehalose **1a**, galacto-trehalose **6**, *p*-*N*-acrylamidophenyl Gb<sub>2</sub>,<sup>5</sup>

(9) Richardson, A. C.; Tarelli, E. *J. Chem. Soc. C* **1971**, 22, 3733–3735.



**Figure 2.** Structures and properties of glycoconjugate copolymers **8–10**.

and *p*-nitrophenyl  $\alpha$ -D-galactoside<sup>10</sup> to show that none of these monomeric glycosides possess notable activity to block the Stx binding to erythrocytes (MIC > 10 mM). The result accords well with the report<sup>12</sup> that a monomeric methyl globotriaoside (Gb<sub>3</sub>) has a very low binding affinity to Stx-I ( $K_a < 1.0 \times 10^3 \text{ M}^{-1}$ ). On the other hand, activity was observed clearly for copolymers due to a cluster effect (Table 1).<sup>13</sup> The galacto-trehalose copolymer **7** showed blocking activity for both Stx-I (MIC = ca. 60  $\mu\text{M}$ ) and Stx-II (ca. 240  $\mu\text{M}$ ). Though the activity was slightly lower than that of the natural Gb<sub>2</sub> copolymer **8** (MIC = 40  $\mu\text{M}$  for Stx-I

**Table 1.** Hemagglutination Inhibition Assay and Stx-I Neutralization Activity Assay

copolymer	MIC (M) <sup>a</sup>		neutralization activity against Stx-I <sup>b</sup>
	Stx-I	Stx-II	
<b>7</b>	$6.1 \times 10^{-5}$	$2.4 \times 10^{-4}$	0.88
<b>8</b>	$4.1 \times 10^{-5}$	$1.6 \times 10^{-4}$	0.95
<b>9</b>	$1.1 \times 10^{-3}$	$> 1.1 \times 10^{-3}$	0.65
<b>10</b>	$> 5.7 \times 10^{-2}$	$> 5.7 \times 10^{-2}$	c

<sup>a</sup> Minimum inhibition concentration.<sup>13</sup> <sup>b</sup> Relative activity to that of Gb<sub>3</sub> copolymer. <sup>c</sup> Not determined.

and 160  $\mu\text{M}$  for Stx-II), the activity was apparently higher than that of the  $\alpha$ -galactoside copolymer **9** (MIC = 1 mM for Stx-I and > 1 mM for Stx-II). Moreover, the trehalose copolymer **10** did not show binding activity to Stx-I or Stx-II. These data are suggestive of the structural characteristics of the galacto-type trehalose **1b**. The axial 4-OH in the  $\alpha$ -galactoside moiety is essential for the Stx binding, and the  $\alpha$ -glucoside moiety in **1b**, similarly to the  $\beta$ -galactoside moiety in **2**, plays an important role as a supplementary binding site.

Stx-I neutralization assay using HeLa cells was performed for the binding-active copolymers **7–9**. Under conditions in which the Gb<sub>3</sub>/acrylamide copolymer<sup>10</sup> (10  $\mu\text{M}$ ) can keep all cells alive in the presence of Stx-I, galacto-trehalose copolymer **7** could rescue 88% of the cells. The relative activity matches well with the preceding hemagglutination inhibition activity and also supports our expectation that the galacto-type trehalose **1b** serves as a nonnatural carbohydrate ligand of Shiga toxins.

In conclusion, we have found a potential utility of the galacto-trehalose  $\alpha(1-1)$ -linkage as a promising substitute for the globosyl  $\alpha(1-4)$ -linkage. Though the binding of the galacto-trehalose to Stx-I and Stx-II, particularly to the latter, is still weaker than that of the natural Gb<sub>2</sub> and Gb<sub>3</sub> globosides, galacto-trehalose may integrate the binding affinity significantly by additionally introducing a  $\beta$ -glucoside moiety or other related units. Such efforts are in progress in our group and will be reported elsewhere. Moreover, the idea of applying  $\alpha$ - (or even  $\beta$ -) (1–1)-linked sugars, instead of natural and labile glycosyl linkages, will open a promising pathway leading to sugar-based therapeutic agents.

**Acknowledgment.** Dedicated to Professor Joachim Thiem of the University of Hamburg on the occasion of his 60th birthday. We thank the Japan Society for the Promotion of Science Research for a research fellowship for young scientists (DC) for H. Dohi.

**Supporting Information Available:** Experimental procedures and full characterization for all new compounds (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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