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

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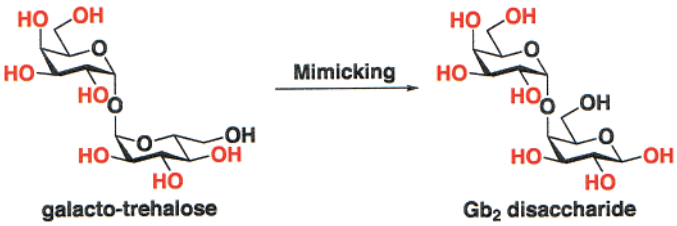
ABSTRACT



Galacto-type trehalose, a "C-4 epimer of trehalose", possesses a stereochemical structure around the α(1–1)-linkage analogous to that of the globobiosyl α(1–4)-linkage in Gb_2 and Gb_3 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 α(1–1)-linkage as a substitute for the galactobiosyl α(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 multisubunit proteins comprising one toxic A-subunit and five receptor-binding B-subunits.[1] Both Stx-I and Stx-II recognize a galactobiosyl α(1−4)-linkage in globosyl Gb_2 and Gb_3 ceramides and induce carbohydrate-mediated internalization into host cells.[2] 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[3] or on the molecular assembly to construct carbohydrate clusters as glycopolymers, glycodendrimers, and starfish models.[4] However, an effective mimic of the galactobiosyl α(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 α(1−4)-linkage.[5] In this communication, we

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(1) (a) Kozlov, Y. V.; Chernaia, M. M.; Fraser, M. E.; James, M. N. *J. Mol. Biol.* **1993**, *232*, 704−706. (b) Picking, W. D.; McCann, J. A.; Nutikka, A.; Lingwood, C. A. *Biochemistry* **1999**, *38*, 7177−7184.

(2) (a) Lingwood, C. A.; Law, H.; Richardson, S.; Petric, M.; Brunton, J. L.; Grandis, S. D.; Karamali, M. *J. Biol. Chem.* **1987**, *262*, 8834−8839. (b) Lindberg, A. A.; Brown, J. E.; Strömberg, N.; Westling-Ryd, M.; Schultz, J. E.; Karlsson, K. A. *J. Biol. Chem.* **1987**, *262*, 1779−1785. (c) Arab, S.; Lingwood, C. A. *Glycoconjugate J.* **1996**, *13*, 159−166.

(3) (a) Debenham, S. D.; Cossrow, J.; Toone, E. J. *J. Org. Chem.*, **1999**, *64*, 9153−9163. (b) Mylvaganam, M.; Lingwood, C. A. *Biochem. Biophys. Res. Commun.* **1999**, *257*, 391−394. (c) Ling, H.; Boodhoo, A.; Hazes, B.; Cummings, M. D.; Armstrong, G. D.; Brunton, J. L.; Read, R. J. *Biochemistry* **1998**, *37*, 1777−1788.

(4) (a) Nishida, Y.; Dohi, H.; Uzawa, H.; Kobayashi, K. *Tetrahedron Lett.* **1998**, *39*, 8681−8684. (b) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839−7842. (c) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669−672. (d) Lundquist, J. J.; Debenham, S. D.; Toone, E. J. *J. Org. Chem.* **2000**, *65*, 8245−8250.

(5) Dohi, H.; Nishida, Y.; Tanaka, H.; Kobayashi, K. *Synlett* **2001**, *9*, 1446−1448.

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

Trehalose **1a** possesses many biological and medicinal potentials applicable to sugar-based therapeutic reagents.[6] This is mainly because the glycoside α(1−1)-linkage is highly tolerant to both chemical and enzymatic (mammalian α-glycosidases) degradation compared with glycosyl α(1−4)-linkages. Here, it can be easily noticed that the trehalose α(1−1)-linkage has a molecular topology analogous to that of the galactobiosyl α(1−4)-linkage **2** (Gb_2) 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_2 mimic and expected to show a binding affinity to Stxs similar to that of the natural Gb_2 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).

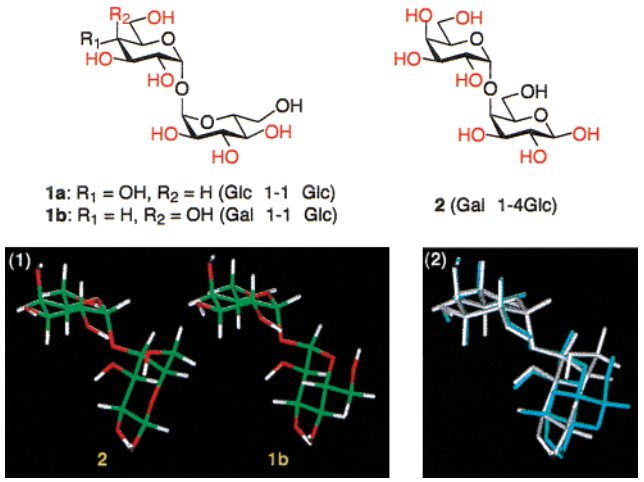
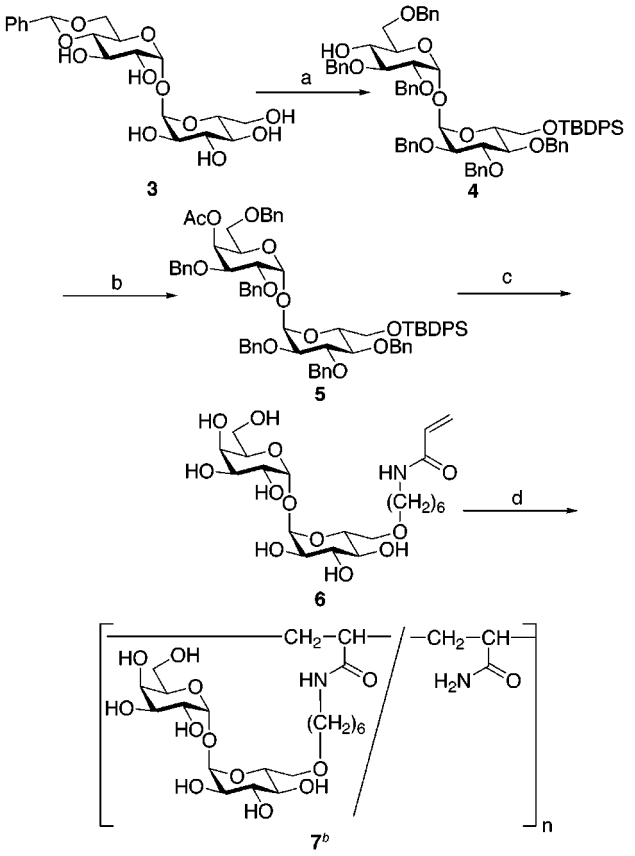


Figure 1. Preferred conformations of galacto α(1−4)-bioside **2** and galacto-type trehalose **1b** (1) and superimposed structures (2). The structures were minimized by MM-2 without optimization of all OH bonds in the calculation (Insight II/Discover program and Amber force field); the white and blue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

In this study, we prepared galacto-type trehalose **6**[7] and its cluster model **7** in order to verify our expectation.[8] The synthesis was carried out via conventional chemical pathways starting from 4,6-mono-*O*-benzylidene trehalose **3**[9] as summarized in Scheme 1. As a key reference compound to 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_2 and α-D-galactoside clusters, respectively, were prepared in our previous manner.[5,10]

Scheme 1. Synthesis of Galacto-Trehalose Cluster Model **7**[a]



[a] Reaction conditions: (a) (i) TBDPSCl, Et_3N, DMAP, pyridine, room temperature, 12 h, 91%; (ii) BnBr, NaH, DMF, room temperature, 12 h, 95%; (iii) BH_3-NHMe_2, $AlCl_3$, THF, 0 °C→room temperature, 2 h, 94%; (b) (i) Tf_2O, pyridine, CH_2Cl_2, 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) $Pd(OH)_2/C$, H_2, THF, MeOH, room temperature, 1.5 h; (v) acryloyl chloride, Et_3N, CH_2Cl_2, MeOH, 0 °C→room temperature, 2 h, 91% (2 steps); (d) 2,2-azobis (2-amidinopropane) dihydrochloride, H_2O, Me_2SO, 60 °C, 12 h, 72%. [b] Sloping line in the structure represents the random copolymer comprised of the two constitutional units.

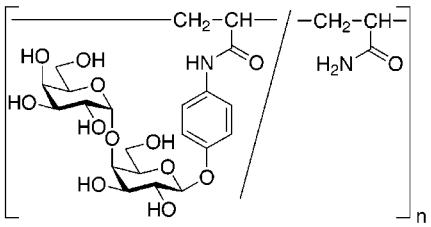
Hemagglutination inhibition assay[11] was performed for trehalose **1a**, galacto-trehalose **6**, *p*-*N*-acrylamidophenyl Gb_2,[5]

(6) Highly effective use of an α-D-mannosyl(1−1)-β-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.

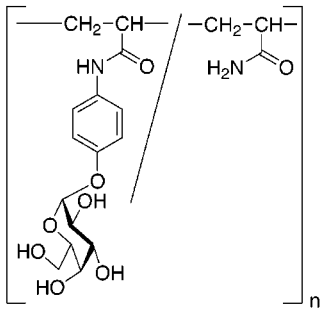
(7) Compound **6**: ^1H NMR (δ ppm, 500 MHz, D_2O): 5.65−6.24 (dd × 3, 3H, H-olefin), 5.15 (d, 1H, $J_{1,2}$ = 3.4 Hz, GalH-1), 5.14 (d, $J_{1,2}$ = 3.1 Hz, GlcH-1), 4.15 (d, $J_{3,4}$ = 3.0 Hz, GalH-4), 3.36 (dd, 1H, $J_{2,3}$ = 6.7 Hz, $J_{3,4}$ = 9.0 Hz, GlcH-4), 1.40−3.50 (dd × 2 and m × 4, 12H, 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.

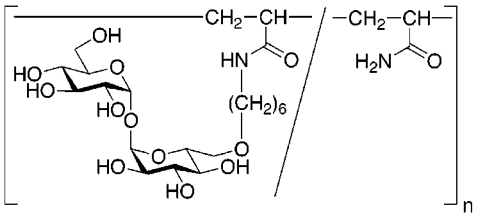
(9) Richardson, A. C.; Tarelli, E. *J. Chem. Soc. C* **1971**, 22, 3733−3735.



Gb_2 copolymer **8**
$M_n = 4.4 \times 10^5$
Mol fraction of the sugar unit = 0.21



α-Gal copolymer **9**
$M_n = 4.4 \times 10^5$
Mol fraction of the sugar unit =0.21



trehalose copolymer **10**
$M_n = 3.5 \times 10^5$
Mol fraction of the sugar unit = 0.19

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

and *p*-nitrophenyl α-D-galactoside[10] 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[12] that a monomeric methyl globotriaoside (Gb_3) has a very low binding affinity to Stx-I ($K_a < 1.0 \times 10^3$ M^{-1}). On the other hand, activity was observed clearly for copolymers due to a cluster effect (Table 1).[13] The galacto-trehalose copolymer **7** showed blocking activity for both Stx-I (MIC = ca. 60 μM) and Stx-II (ca. 240 μM). Though the activity was slightly lower than that of the natural Gb_2 copolymer **8** (MIC = 40 μM for Stx-I and 160 μM for Stx-II), the activity was apparently higher than that of the α-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 α-galactoside moiety is essential for the Stx binding, and the α-glucoside moiety in **1b**, similarly to the β-galactoside moiety in **2**, plays an important role as a supplementary binding site.

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

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

[a] Minimum inhibition concentration.[13] [b] Relative activity to that of Gb_3 copolymer. [c] Not determined.

Stx-I neutralization assay using HeLa cells was performed for the binding-active copolymers **7**−**9**. Under conditions in which the Gb_3/acrylamide copolymer[10] (10 μM) 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 α(1−1)-linkage as a promising substitute for the globosyl α(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_2 and Gb_3 globosides, galacto-trehalose may integrate the binding affinity significantly by additionally introducing a β-glucoside moiety or other related units. Such efforts are in progress in our group and will be reported elsewhere. Moreover, the idea of applying α- (or even β-) (1−1)-linked sugars, instead of natural and labile glycosyl linkages, will open a promising pathway leading to sugar-based therapeutic agents.

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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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(10) Dohi, H.; Nishida, Y.; Mizuno, M.; Shinkai, M.; Kobayashi, T.; Takeda, T.; Uzawa, H.; Kobayashi, K. *Bioorg. Med. Chem.* **1999**, *7*, 2053−2062.
(11) Kawagishi, H.; Yamawaki, M.; Isobe, S.; Usui, T.; Kimura, A.; Chiba, S. *J. Biol. Chem.* **1994**, *269*, 1375−1379.
(12) St. Hilaire, P. M.; Boyd, M. K.; Toone, E. J. *Biochemistry* **1994**, *33*, 14452−14463.
(13) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327.