Glycobiology

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Chemoenzymatic Synthesis of DSGb5 and Sialylated Globo-series Glycans

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Abstract: *Sialic-acid-binding, immunoglobulin-type lectin-7 (Siglec-7) is present on the surface of natural killer cells. Siglec-7 shows preference for disialylated glycans, including α(2,8)-α(2,3)-disialic acids or internally branched α(2,6)-NeuAc, such as disialosylglobopentaose (DSGb5). Herein, DSGb5 was synthesized by a one-pot multiple enzyme method from Gb5 by α2,3-sialylation (with PmST1) followed by α2,6-sialylation (with Psp2,6ST) in 23% overall yield. DSGb5 was also chemoenzymatically synthesized. The protection of the non-reducing-end galactose of Gb5 as 3,4-O-acetonide, 3,4-O-benzylidene, and 4,6-O-benzylidene derivatives provided DSGb5 in overall yields of 26%, 12%, and 19%, respectively. Gb3, Gb4, and Gb5 were enzymatically sialylated to afford a range of globo-glycans. Surprisingly, DSGb5 shows a low affinity for Siglec-7 in a glycan microarray binding affinity assay. Among the synthesized globo-series glycans, α6α3DSGb4 shows the highest binding affinity for Siglec-7.*

Globosides, stage-specific embryonic antigen-4 (SSEA4, MSGb5, or α3SGb5 in Scheme 1) and disialosylglobopentaosylceramide (DSGb5–Cer), are expressed in relatively large amounts on the surface of cancer cells, including renal cancer cells (RCC), and they are associated with cancer proliferation and metastasis.[1] Therefore, globosides have been used for cancer detection[2] and vaccine development.[3] The structure of DSGb5-Cer was determined in 1994,[4] and it is an interesting target since its total synthesis has not been reported. The high affinity of DSGb5-Cer for Siglec-7 (sialic acid-binding immunoglobulin-type lectin-7) was reported in 2011.[5] Siglec-7 is a lectin present on the surface of natural killer (NK) cells as an inhibitory receptor of NK cell cytotoxicity.[6] Later, glycans with α2,8-α2,3-disialic acid or internally branched α2,6-NeuAc were shown to be potent ligands for Siglec-7 in competitive binding assays.[7] In nature, NK cell cytotoxicity is modulated by *cis* interactions between Siglec-7 and endogenous sialoconjugates on the cell surface.[6] By treating NK cells with sialidase to remove NeuAc and disrupt the *cis* interactions, the unmasked Siglec-7 can interact with DSGb5-Cer on the surface of the human renal cancer cell line ACHN, decreasing the cytotoxicity of the NK cells and promoting the metastasis and invasion of RCC.[8] Obtaining a sufficient amount of pure DSGb5 is key to elucidating its mechanism of action and to further develop cancer immunotherapies. Clarifying the binding activities of sialylated globo-series glycans and the effect of the NeuAc position on their interactions with Siglec-7 is also of great interest.

The enzymatic and chemoenzymatic syntheses of globo-series glycans, such as Gb5, SSEA-4, and Globo H, have been reported by several research groups,[9–11] but no method has been reported for the synthesis of DSGb5. DSGb5 has a unique glycan structure with two NeuAcs as α2,3- and α2,6-linkages on the nonreducing-end galactose (Gal) and *N*-acetylgalactosamine (GalNAc), respectively, of Gb5 (Figure 1). Thus, to achieve the enzymatic synthesis of DSGb5, a suitable synthetic route to build up the glycan structures with available enzymes, mainly sialyltransferases, must be designed. To construct the α2,3-sialyl linkage, we attempted to use two different α2,3-sialyltransferases, CstI and PmST1, which have been widely used in the enzymatic synthesis of α2,3-sialosides.[12] In this study, bacterial glycosyltransferases were investigated since the recombinant human enzyme ST6GalNAc is not available in a bacterial expression system. A sequential, one-pot, multienzyme (OPME)[13] method was utilized to synthesize DSGb5 and sialylated globo-series glycans.[14] To achieve our goals, two issues needed to be solved: 1) the order of the α2,3-sialylation and α2,6-sialylation and 2) the site-selectivity of the α2,6-sialylation for the internal GalNAc of Gb5.

Most previous studies[11,15] on the sialylation substrates have focused on lacto- and ganglio-series glycans, not globo-series glycans. Recently, Meng et al. showed the α2,6-sialylation of a NeuAcα2,3Galβ1,3GalNAc trisaccharide using α2,6-sialyltransferase from *Photobacterium damselae* (Pd2,6ST), which preferentially installed the NeuAc moiety at the nonreducing-end Gal rather than at the reducing GalNAc end.[16] Moreover, lactone formation between the carboxylic group of NeuAc and the 4-hydroxyl group on the neighboring Gal provided steric hindrance, which enhanced the selectivity of the sialylation for the inner GalNAc and improved the yield of the desired NeuAcα2,6GalNAc structure from 29% to 47%. In contrast, Ding et al. showed that α2,6-sialyltransferase from *Photobacterium* sp. JH-ISH-224 (Psp2,6ST) is highly reactive in the α2,6-sialylation at GalNAc with an α Fmoc–Ser or azide linker.[17] Inspired by these studies, the

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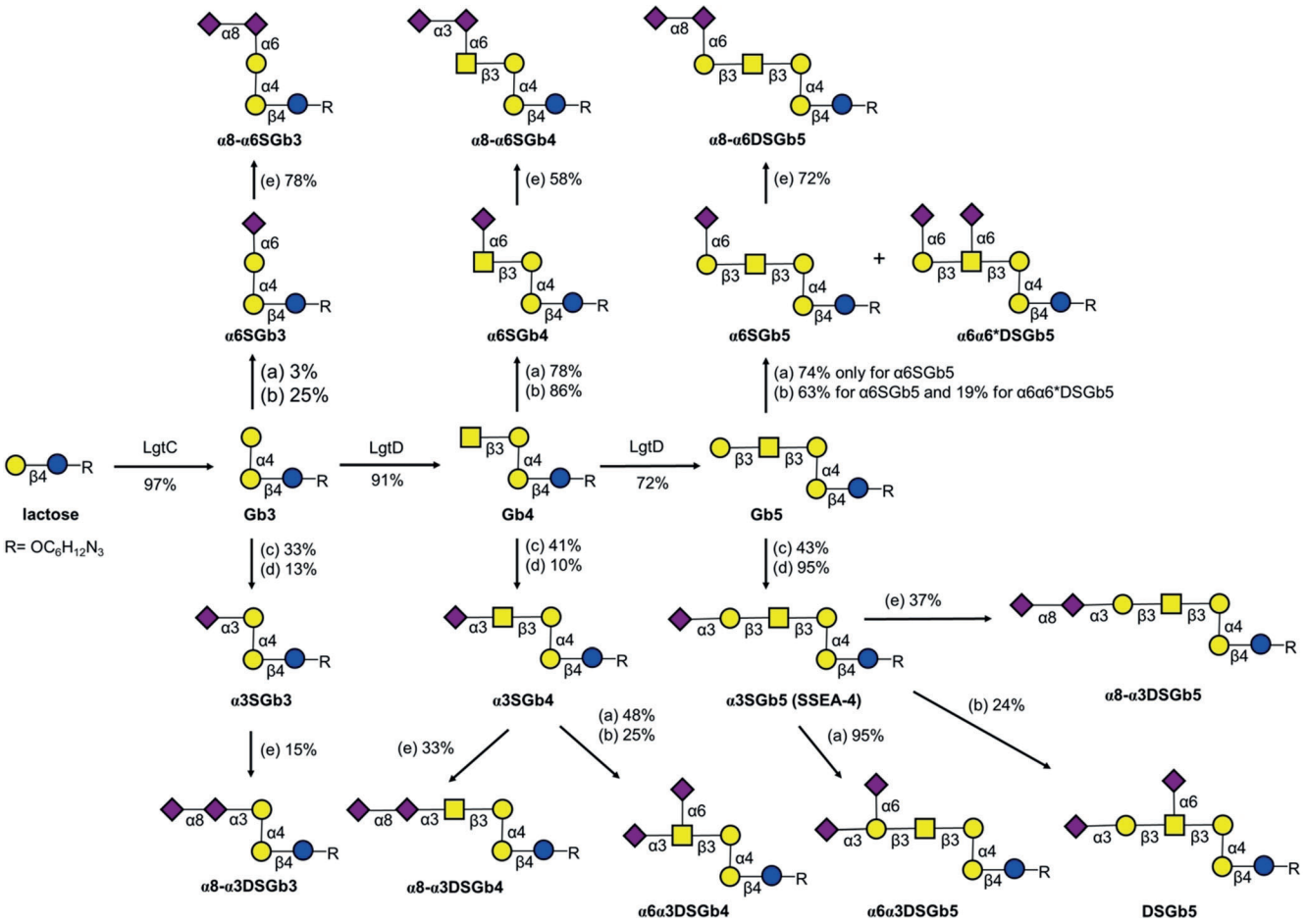


Scheme 1. Schematic of the enzymatic synthesis of DSGb5 and globo-series sialosides. Enzymes and conditions: a) Pd2,6ST, pH 8.5, 37 °C; b) Psp2,6ST, pH 8.0, RT; c) CstI, pH 7.5, 37 °C; d) PmST1, pH 8.5, 37 °C; and e) CstII, pH 7.5, 37 °C with corresponding sugar nucleotide donor.

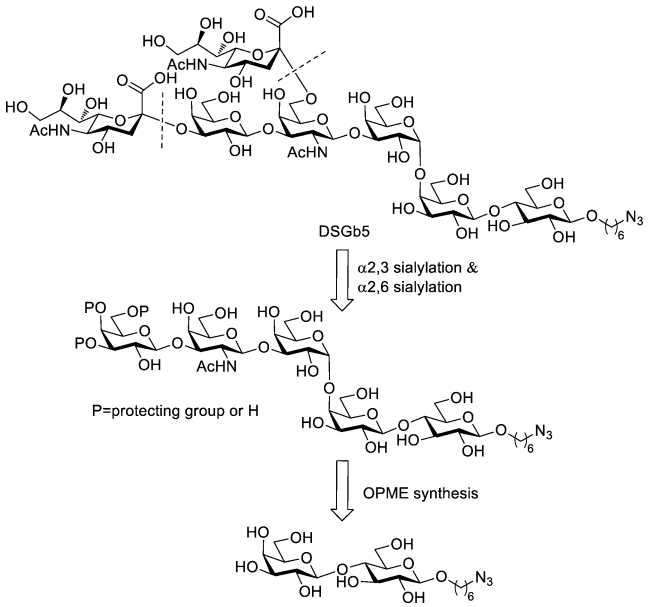


Figure 1. The retrosynthetic analysis of DSGb5.

regioselectivities of Pd2,6ST and Psp2,6ST in the installation of NeuAc on globo-series glycans were investigated. Moreover, an α2,8-sialyl linkage was constructed using CstII.[11,15c,18]

Herein, two strategies were investigated for the synthesis of DSGb5, namely, an enzymatic method and a chemoenzymatic method. In the enzymatic approach, four sialyltransferases, CstI, PmST1, Pd2,6ST, and Psp2,6ST, were investigated to determine their substrate preferences for globo-series-glycan acceptors and the reaction order of the α2,3- and α2,6-sialylations to provide higher yields of the corresponding branched disialyl glycans. In the chemoenzymatic approach, the nonreducing-end Gal of Gb5 was selectively protected to achieve enzymatic sialylation at GalNAc of Gb5.

Both the enzymatic[9,10] and chemoenzymatic[11,19] strategies were explored in the syntheses of DSGb5 and various sialylated globo-series glycans. As shown in Figure 1, the retrosynthetic analysis of DSGb5 indicated that the two NeuAcs can be assembled by enzymatic sialylations of Gb5. Thus, the most critical step in the synthesis of DSGb5 is the installation of an α2,6-NeuAc at the internal GalNAc. However, the α2,6-NeuAc sialylation could be achieved before or after the α2,3-sialylation of Gb5. Furthermore, the azido linker was utilized for the further fabrication of a glycan microarray by Cu^{I}-catalyzed alkyne-azide cycloaddition (CuAAC).[20]

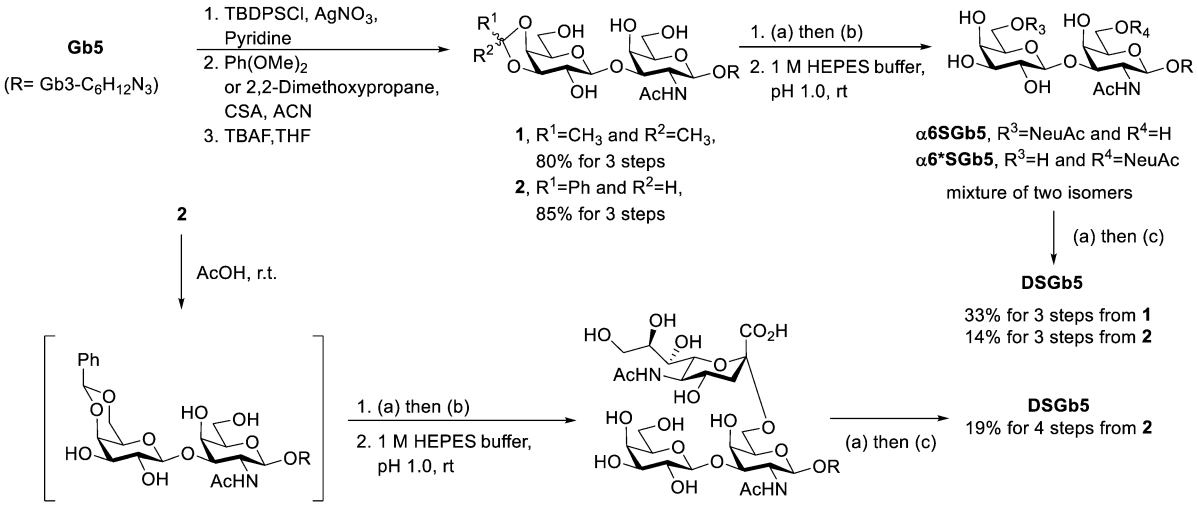
The globo-series glycans (Gb3, Gb4, and Gb5) were prepared by an OPME method using kinases (MtGalK and NahK)[15a,21] for the production of sugar 1-phosphates, AtUSP to generate UDP-sugar nucleotides,[11,22] and glycosyltransferases (LgtC and LgtD) to construct glycosidic bonds.[9,10] Both CstI and PmST1 were used to assemble an α2,3-NeuAc moiety at the nonreducing-Gal/GalNAc end of Gb3, Gb4, and Gb5, respectively. As shown in Scheme 1 and Table S1 in the Supporting Information, extending the sugar chain resulted in higher yields of the products; yields of 33%, 41%, and 43% were obtained in the production of α3SGb3, α3SGb4, and α3SGb5 by CstI, respectively. However, Gb3 and Gb4 were poor substrates for PmST1. Neither α2,3-sialyltransferase preferentially formed the α-linkage to the nonreducing Gal/GalNAc, and this reaction was especially challenging with PmST1.[12a] Surprisingly, when the sugar chain was extended to Gb5, it was a better acceptor for PmST1 than for Cst1, as indicated by the 95% yield of α3SGb5. It should be noted that α3SGb5 (SSEA4) was previously synthesized.[10,11]

Similarly, α2,6-sialyltransferases were applied for the sialylations of Gb3, Gb4, and Gb5. Gb3 is not a good acceptor of either Pd2,6ST or Psp2,6ST, and provided α6Gb3 in yields of 3% and 25%, respectively, which is consistent with previous observations that α-galactosides are not well tolerated by Pd2,6ST.[23] Although Psp2,6ST favors the α-linkage of *N*-acetylgalactosides,[17] α-galactoside Gb3 is not a good substrate. In contrast, Gb4 is a good substrate for both Pd2,6ST and Psp2,6ST with yields of 78% and 86%, respectively, indicating that an α1,4-linkage on an internal galactose has a limited effect on α2,6-sialylations. Due to the low yields in the syntheses of α6Gb3 by Pd2,6ST and Psp2,6ST, the NeuAc of α6SGb4 was at the C6-OH of terminal GalNAc. Surprisingly, sialylation of Gb5 by Pd2,6ST yielded only one monosialylated product (74% yield), while Psp2,6ST produced 63% of the monosialylated product and 19% of the disialylated product (α6α6*DSGb5), and the monosialylated products showed identical ^{1}H NMR spectra. Thus, the preferential α2,6-monosialylation site on Gb5 is unclear. Previous studies showed that the presence of C6-NeuAc at the nonreducing-end Gal blocks the C3 site of Gal for sialylation by PmST1.[24] Thus, PmST1 was used for the α2,3-sialylation of α6SGb4 and α6SGb5. None of the reactions provided detectable products, confirming that the NeuAc moiety is located at the C6-OH of the nonreducing GalNAc and Gal ends, respectively. Furthermore, the generation of α6α6*DSGb5 by Psp2,6ST indicated that Psp2,6ST offers broader acceptor tolerance than Pd2,6ST. Thus, the second NeuAc of disialylated product α6α6*DSGb5 was installed at the C6-OH of GalNAc, demonstrating that Psp2,6ST can potentially catalyze the α2,6-sialylation of an internal GalNAc, which inspired us to design an enzymatic strategy for the syntheses of DSGb5, as shown in Scheme 1.

Moreover, to understand whether the presence of an α2,3-NeuAc moiety affects the α2,6-sialylation, α3SGb3, α3SGb4, and α3SGb5 were sialylated by Pd2,6ST and Psp2,6ST. Neither Pd2,6ST nor Psp2,6ST could recognize α3SGb3 as a substrate, while α3SGb4 was sialylated to give α6α3DSGb4 in yields of 48% and 25% with Pd2,6ST and Psp2,6ST, respectively, indicating that Psp2,6ST is more sensitive to the presence of an α2,3-NeuAc moiety. However, the Pd2,6ST- and Psp2,6ST-catalyzed α2,6-sialylations of α3SGb5 afforded different products, as indicated by their distinct ^{1}H NMR spectra. Because the Gb3 acceptor is not a good substrate for either Pd2,6ST or Psp2,6ST, as revealed in the above studies, we assigned the product obtained from the Pd2,6ST-catalyzed α2,6-sialylation of α3SGb5 as α6α3DSGb5 (95% yield), and the product of the Psp2,6ST-catalyzed reaction was DSGb5 (24% yield). However, these structures require further confirmation, as discussed below.

Due to linear α2,8–α2,3-disialosides being the preferential ligands for Siglec-7, α3SGb3, α3SGb4, and α3SGb5 were further sialylated by CstII to afford α2,8–α2,3-disialyl compounds with yields of 15%, 33%, and 37%, respectively. Furthermore, to investigate the difference between the affinities of α2,8–α2,3-disialyl and α2,8–α2,6-disialyl compounds for Siglec-7, compounds α6SGb3, α6SGb4, and α6SGb5 were also sialylated by CstII to provide α8-α6DSGb3, α8-α6DSGb4, and α8α6DSGb5 in yields of 78%, 58%, and 72%, respectively. These results reveal that CstII is less sensitive to the inner-core structures of α2,6-sialylated glycans; however, it was less reactive towards α2,3-sialylated globo-series glycans.

To further confirm the structure of DSGb5 obtained by enzymatic synthesis, we designed chemoenzymatic synthetic routes by blocking the C6-OH of the nonreducing-end Gal to enhance the selectivity of the α2,6-sialylation at GalNAc,[16] as shown in Scheme 2. Initially, the lactonization of the carboxyl acid of NeuAc with the hydroxyl group of Gal in α3SGb5 was performed under acidic conditions to give inseparable isomers (the carboxyl acid group reacts with C3-OH and C4-OH of Gal to form lactones). However, the lactone isomers were spontaneously hydrolyzed at pH 7.0 and pH 8.5 under α2,6-sialylation conditions, affording α6α3DSGb5. To increase the steric hinderance, the C3 and C4 hydroxyl groups of the nonreducing-end Gal of Gb5 were protected as acetonide or benzylidene groups by three-step manipulations (TBDPSCl for primary alcohol protection followed by acetal formation and TBDPS deprotection) to give **1** and **2** in overall yields of 80% and 85%, respectively. Then, Psp2,6ST was used for the α2,6-sialylation. However, the reaction efficiency was significantly decreased in comparison with that of Gb5 based on TLC analysis after 14-24 h of incubation. The mixture was partially purified and then treated with HEPES buffer (pH 1.0) to remove the acetonide or benzylidene protecting group. Without purification, the mixture was α2,3-sialylated by PmST1 to give unreacted α6SGb5, α3SGb5, and DSGb5. The total yields of DSGb5 from Gb5 over the six-step procedures involving acetonide and benzylidene protection were 26% and 12%, respectively. To further improve the efficiency of the α2,6-sialylation of GalNAc in Gb5, the 3,4-benzylidene acetal of **2** was migrated to afford the 4,6 benzylidene acetal intermediate by treatment with acetic acid. Thus, the C6-OH of the nonreducing-end Gal was protected. Similarly, without purification of the migration products from the preceding step, DSGb5 was obtained, however, in 19% yield over four steps from Gb5. Notably, if Psp2,6ST was replaced with Pd2,6ST in the above α2,6-



Scheme 2. Chemoenzymatic synthesis of DSGb5.

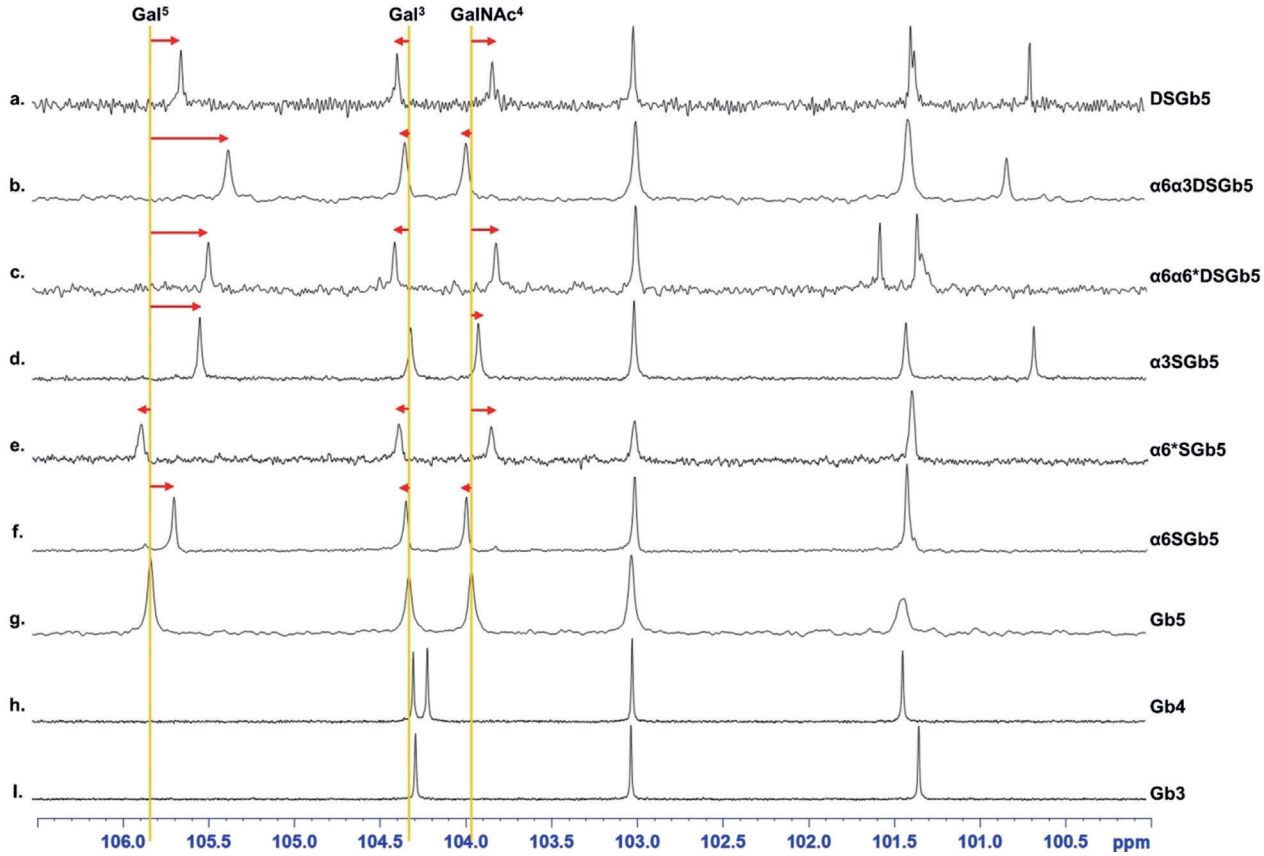


Figure 2. The ^{13}C NMR spectra in the chemical-shift range of the anomeric carbons of Gb3, Gb4, Gb5, α6SGb5, α6*SGb5, α6α3DSGb5, α6α6*DSGb5, and DSGb5.

sialylation of Gb5 derivatives, no new sialylated products were observed by TLC.

As shown in Figure 2, the ^{13}C NMR spectra in the chemical shift range of the anomeric carbons of Gb3, Gb4, Gb5, mono-sialylated Gb5 and disialylated Gb5 were compared (the chemical shifts are shown in Table S2 in the Supporting Information). Gal^3, $GalNAc^4$, and Gal^5 were used to represent the anomeric centers of the inner Gal, GalNAc, and nonreducing-end Gal of Gb5, respectively. The signals of Gal^5 shifted upfield upon α2,6- or α2,3-sialylation, especially

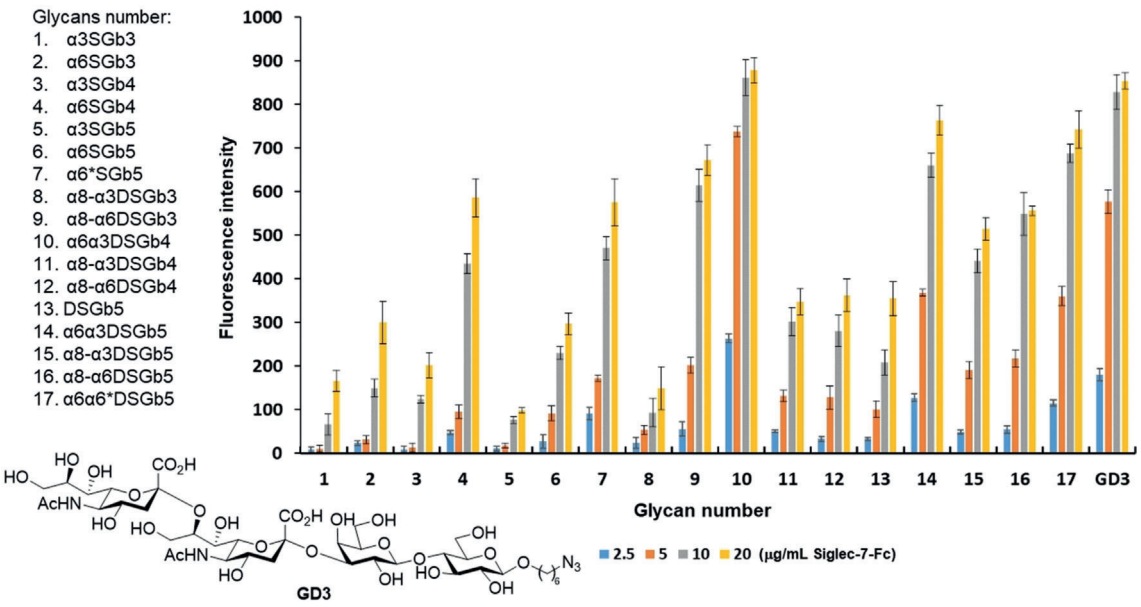


Figure 3. Binding affinities of Siglec-7–Fc with sialylated globo-series glycans and GD3.

for α6α3DSGb5, while those of Gal[3] shifted downfield upon sialylation on Gb5. Furthermore, α2,6-sialylation on GalNAc of Gb5 caused an upfield shift of the signal of GalNAc[4]. Due to the sensitivity of the anomeric ^{13}C chemical shifts of GalNAc to sialylation, the α2,6-sialylation sites of DSGb5 and α6α3DSGb5 can be easily distinguished.

Previous studies indicated that disialoglycolipids, including GD2, GD3, GT1b, disialyl lactotetraose (DSLc4), GalNAc-disialyl Lc4 (GalNAcDSLc4), and DSGb5, show high affinities for Siglec-7.[5] To investigate the binding affinity of sialoglycans for Siglec-7, the synthesized sialoglobo-series glycans were used to fabricate a glycan microarray. The glycans were first immobilized on an alkyne-modified glass slide by CuAAC (see the Supporting Information for details), the slide was then treated with various concentrations of Siglec-7 fused to the fragment crystallizable region of an antibody (Siglec-7–Fc), and the binding was visualized by staining with human IgG-Cy3. GD3[18b] was used as a positive control because of its well-known high affinity for Siglec-7.[7] As shown in Figure 3, the concentration of Siglec-7-Fc at 20 μg mL^{-1} displays a clear binding trend. Among monosialosides, α2,6-sialylated glycans, especially at GalNAc, show higher affinities than α2,3-sialylated glycans.[5] Both α6SGb4 and α6*SGb5 exhibit higher affinity than DSGb5. Moreover, all of the disialosides except α8-α3DSGb3 provide equal or better affinity than DSGb5, and the branched disialosides exhibit higher affinity than the linear disialosides. However, among the disialylated Gb5 derivatives, DSGb5 displays the lowest affinity to Siglec-7-Fc. Overall, of the compounds tested in this study, α6α3DSGb4 shows the highest binding affinity for Siglec-7-Fc. Unexpectedly, the affinity of DSGb5 for Siglec-7-Fc is only about half of that of GD3. However, this unexpected result may be caused by the different linker in this structure in comparison with the naturally occurring ceramide-conjugated glycans and the multivalent interactions in natural systems.

In summary, we have achieved the first total synthesis of DSGb5 by enzymatic and chemoenzymatic approaches. A small library of globo-series sialosides was built, and the preferred substrates of α2,3-sialyltransferases (PmST1 and CstI) and α2,6-sialyltransferases (Psp2,6ST and Pd2,6ST) were investigated. We found that Psp2,6ST tolerates a broader range of acceptors than Pd2,6ST for globo-series glycans. By applying a straightforward one-pot enzymatic strategy, DSGb5 was successfully synthesized from Gb5 on a milligram scale in an overall yield of 23%, while the chemoenzymatic methods gave 19% and 26% yields, respectively. The synthesized glycans were fabricated as a glycan microarray to facilitate the screening of their binding affinities with Siglec-7-Fc. The binding affinity of DSGb5 for Siglec-7-Fc was lower than expected, which does not agree with previous reports.[7,8] Thus, the ceramide and the presence of multivalency of DSGb5 on the cell surface may play important roles in the interaction with Siglec-7, but this hypothesis requires further confirmation. Among the synthesized sialylated globo-series glycans, α6α3DSGb4 showed the highest binding affinity for Siglec-7-Fc.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: enzymatic synthesis · DSGb5 · microarrays · sialic acid · siglec-7 lectin

[1] R. Kannagi, B.-H. Cai, H.-C. Huang, C.-C. Chao, K. Sakuma in *Ganglosides, Methods and Protocols* (Eds.: J. S. Sonnino, A. Prinetti), Humana Press, New York, **2019**, pp. 143 – 172.
[2] S. K. Cheung, P. K. Chuang, et al., *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 960 – 965.
[3] R. D. Astronomo, D. R. Burton, *Nat. Rev. Drug Discovery* **2010**, *9*, 308 – 324.
[4] S. Saito, S. B. Levery, M. E. Salyan, R. I. Goldberg, S.-i. Hakomori, *J. Biol. Chem.* **1994**, *269*, 5644 – 5652.
[5] A. Ito, K. Handa, D. A. Withers, M. Satoh, S.-i. Hakomori, *FEBS Lett.* **2001**, *504*, 82 – 86.
[6] G. Nicoll, T. Avril, K. Lock, K. Furukawa, N. Bovin, P. R. Crocker, *Eur. J. Immunol.* **2003**, *33*, 1642 – 1648.
[7] T. Yamaji, T. Teranishi, M. S. Alphey, P. R. Crocker, Y. Hashimoto, *J. Biol. Chem.* **2002**, *277*, 6324 – 6332.
[8] Y. Kawasaki, A. Ito, D. A. Withers, T. Taima, N. Kakoi, S. Saito, Y. Arai, *Glycobiology* **2010**, *20*, 1373 – 1379.
[9] a) D. M. Su, H. Eguchi, W. Yi, L. Li, P. G. Wang, C. Xia, *Org. Lett.* **2008**, *10*, 1009 – 1012; b) C. D. Hunter, T. Guo, G. Daskhan, M. R. Richards, C. W. Cairo, *Chem. Rev.* **2018**, *118*, 8188 – 8241.
[10] T.-I. Tsai, H.-Y. Lee, S.-H. Chang, C.-H. Wang, Y.-C. Tu, Y.-C. Lin, D.-R. Hwang, C.-Y. Wu, C.-H. Wong, *J. Am. Chem. Soc.* **2013**, *135*, 14831 – 14839.
[11] H. Yu, Y. Li, J. Zeng, V. Thon, D. M. Nguyen, T. Ly, H. Y. Kuang, A. Ngo, X. Chen, *J. Org. Chem.* **2016**, *81*, 10809 – 10824.
[12] a) H. Yu, H. Chokhawala, R. Karpel, H. Yu, B. Wu, J. Zhang, Y. Zhang, Q. Jia, X. Chen, *J. Am. Chem. Soc.* **2005**, *127*, 17618 – 17619; b) C.-C. Yu, S. G. Withers, *Adv. Synth. Catal.* **2015**, *357*, 1633 – 1654.
[13] X. Chen, A. Varki, *ACS Chem. Biol.* **2010**, *5*, 163 – 176.
[14] M. Senda, et al., *Biochem. J.* **2007**, *402*, 459 – 470.
[15] a) W.-T. Chien, C.-F. Liang, C.-C. Yu, C.-H. Lin, S.-P. Li, I. Primadona, Y.-J. Chen, K. K. T. Mong, C.-C. Lin, *Chem. Commun.* **2014**, *50*, 5786 – 5789; b) A. R. Prudden, L. Liu, C. J. Capicciotti, M. A. Wolfert, S. Wang, Z. Gao, L. Meng, K. W. Moremen, G.-J. Boons, *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 6954 – 6959; c) O. Blixt, et al., *Carbohydr. Res.* **2005**, *340*, 1963 – 1972; d) C. M. Nycholat, et al., *J. Am. Chem. Soc.* **2013**, *135*, 18280 – 18283.
[16] X. Meng, W. Yao, J. Cheng, X. Zhang, L. Jin, H. Yu, X. Chen, F. Wang, H. Cao, *J. Am. Chem. Soc.* **2014**, *136*, 5205 – 5208.
[17] L. Ding, H. Yu, K. Lau, Y. Li, S. Muthana, J. Wang, X. Chen, *Chem. Commun.* **2011**, *47*, 8691 – 8693.
[18] a) M. Gilbert, J. R. Brisson, M. F. Karwaski, J. Michniewicz, A. M. Cunningham, Y. Wu, N. M. Young, W. W. Wakarchuk, *J. Biol. Chem.* **2000**, *275*, 3896 – 3906; b) T.-C. Kuan, et al., *Chem. Eur. J.* **2017**, *23*, 6876 – 6887; c) H. Yu, J. Cheng, L. Ding, Z. Khedri, Y. Chen, S. Chin, K. Lau, V. K. Tiwari, X. Chen, *J. Am. Chem. Soc.* **2009**, *131*, 18467 – 18477.
[19] H. Yu, X. Chen, *Org. Biomol. Chem.* **2016**, *14*, 2809 – 2818.
[20] a) M. C. Bryan, et al., *J. Am. Chem. Soc.* **2004**, *126*, 8640 – 8641; b) G. Qin, et al., *J. Am. Chem. Soc.* **2010**, *132*, 16432 – 16441.
[21] S.-P. Li, et al., *Adv. Synth. Catal.* **2014**, *356*, 3199 – 3213.
[22] J. Liu, et al., *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3764 – 3768.
[23] H. Yu, S. Huang, H. Chokhawala, M. Sun, H. Zheng, X. Chen, *Angew. Chem. Int. Ed.* **2006**, *45*, 3938 – 3944; *Angew. Chem.* **2006**, *118*, 4042 – 4048.
[24] H. Yu, X. Chen, *Org. Lett.* **2006**, *8*, 2393 – 2396.

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Communications

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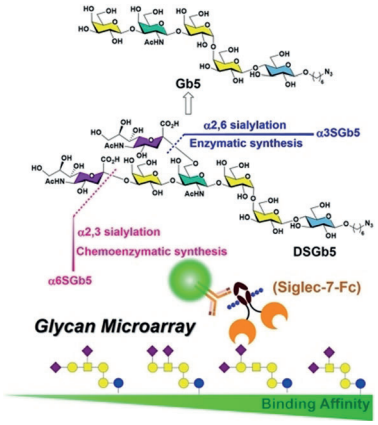
Chemoenzymatic Synthesis of DSGb5 and Sialylated Globo-series Glycans



Sialyl-globo-series glycans, including DSGb5 and sialylglobosides, have been prepared using both a one-pot multiple enzyme synthesis method and chemoenzymatic synthesis. The resulting glycans were screened for binding to sialic-acid-binding, immunoglobulin-type lectin-7 (siglet-7) using a glycan-microarray-based binding assay.