Systematic Chemoenzymatic Synthesis of *O*-Sulfated Sialyl Lewis x Antigens

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Abstract

O-Sulfated sialyl Lewis x antigens play important roles in nature. However, due to their structural complexity, they are not readily accessible by either chemical or enzymatic synthetic processes. Taking advantage of a bacterial sialyltransferase mutant that can catalyze the transfer of different sialic acid forms from the corresponding sugar nucleotide donors to Lewis x antigens which are fucosylated glycans as well as an efficient one-pot multienzyme (OPME) sialylation system, O-sulfated sialyl Lewis x antigens containing different sialic acid forms and O-sulfation at different locations were systematically synthesized by chemoenzymatic methods.

Introduction

O-Sulfated sialyl Lewis x structures play important roles in immune regulation, inflammation, and cancer metastasis.[1] For example, 6-*O*-sulfo-sialyl Lewis x [6–*O*-sulfo-sLe[x] (**1**), Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAc6SβOR] with an *O*-sulfate group at the carbon-6 of the *N*-acetylglucosamine (GlcNAc) residue (Fig. 1) is a well known ligand for L-selectin, a C-type (Ca^{2+}-dependent) carbonhydrate-binding protein (lectin) expressed broadly on most leukocytes in the blood.[1, 2] The interaction of 6-*O*-sulfo-sLe[x] (**1**) and L-selectin plays critical roles in lymphocyte homing to the peripheral lymph nodes[2] and in chronic inflammation.[3] It has also been shown that human sialic acid-binding immunoglobulin-like lectin[4] Siglec-9 binds strongly[5, 6] to 6-*O*-sulfo-sLe[x] but the biological importance of this interaction is less well understood.

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On the other hand, 6'-O-sulfo-sialyl Lewis x [6'-O-sulfo-sLe^x (**2**), Neu5Acα2–3Gal6Sβ1–4(Fucα1–3)GlcNAcβOR] with an O-sulfate group at the carbon-6 of the galactose (Gal) residue (Fig. 1),[7] in addition to 6'-O-sulfo-sialyl-N-acetyllactosamine (6'-O-sulfo-sLacNAc, Neu5Acα2–3Gal6Sβ1–4GlcNAcβOR),[8] was shown by glycan microarray studies to be a preferred glycan ligand for Siglec-8 and for its paralog mouse Siglec-F.[9] Siglec-8 is expressed on human allergic inflammatory cells including eosinophils, mast cells, and basophils.[5, 10] Reducing the number of eosinophiles, such as by soluble 6'-O-sulfo-sLe^x synthetic polymer induced apoptosis,[11] has been suggested as an approach for asthma therapies.[12] Furthermore, 6'-O-sulfo-sLe^x (**2**), in addition to 6'-O-sulfo-sLacNAc and 6'-O-sulfo-sialyl-lacto-N-neotetraose (6'-O-sulfo-sLNnT, Neu5Acα2–3Gal6Sβ1–4GlcNAcβ1–3Galβ1–4GlcβOR), was shown to bind to langerin,[13] a C-type (Ca^{2+}-dependent) lectin specific to Langerhans cells (immature antigen-presenting specific T cell immunity initiating dendritic cells of epidermis and mucosal tissues).[14]

Although less efficient than Neu5Acα2–8Neu5Acα2–3LacNAc, both 6-O-sulfo-sLe^x (**1**) and 6'-O-sulfo-sLe^x (**2**) bound to human Siglec-7 moderately.[5] Both are presented in glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1), an L-selectin ligand,[15] with 6'-O-sulfo-sLe^x (**2**) as the major sulfated form.[16–18] Gal-6-O-sulfotransferase and GlcNAc-6-O-sulfotransferase have been found to synergistically produce L-selectin ligands. This indicates either the potential synergistic involvement of both 6-O-sulfo-sLe^x (**1**) and 6'-O-sulfo-sLe^x (**2**) or 6',6-di-O-sulfo-sLe^x (**3**) (Fig. 1) with O-sulfate groups at both Gal and GlcNAc residues in sLe^x in L-selectin-binding.[19] Human Siglec-7 and –8 have also been shown to bind stronger to 6',6-di-O-sulfo-sLe^x (**3**) than its mono-O-sulfated derivative (**1**) or (**2**) while mouse Siglec-F has been shown to bound similarly strongly to 6',6-di-O-sulfo-sLe^x (**3**) and 6'-O-sulfo-sLe^x (**2**).[6]

The biological importance of O-sulfated sLe^x structures make them attractive synthetic targets. However, the structures of these compounds are relatively complex and include synthetically challenging α2–3-linked sialic acid which suffers from low stereoselectivity and high 2,3-elimination rate in chemical synthesis[20–22] and acid labile O-sulfate group.[23, 24] Chemically[20, 25, 26] or chemoenzymatically[27] synthesized Neu5Acα2–3Gal building blocks have been used as effective synthons for constructing more complex sialosides including sLe^x and 6-O-sulfo-sLe^x (**1**).[20] Several examples of chemical[22, 28] or chemoenzymatic[29] synthesis of 6-O-sulfo-sLe^x (**1**) as well as chemical synthesis of 6'-O-sulfo-sLe^x (**2**)[22, 30, 31] and 6',6-di-O-sulfo-sLe^x (**3**)[32] have been reported. All these examples are, however, limited to compounds with the most abundant sialic acid form, N-acetylneuraminic acid (Neu5Ac). Despite the presence of more than 50 different sialic acid forms identified in nature,[33, 34] O-sulfated sLe^x containing a sialic acid form other than Neu5Ac has not been synthesized.

We report here the development of efficient chemoenzymatic methods for systematic synthesis of O-sulfated sLe^x containing different sialic acid forms. The methods are demonstrated for representative examples of 6'-O-sulfo-sLe^x (**1**), 6-O-sulfo-sLe^x (**2**), and/or 6',6-di-O-sulfo-sLe^x (**3**) containing the most abundant Neu5Ac form and N-glycolylneuraminic acid (Neu5Gc), a sialic acid form commonly found in mammals other than human but can be incorporated into the human glycome from dietary sources.[35]

One efficient approach for the synthesis of O-sulfated sLe^x with different sialic acid forms would be by direct sialylation of O-sulfated Le^x using one-pot multienzyme (OPME) sialylation systems[36] containing an α2–3-sialyltransferase, a CMP-sialic acid synthetase (CSS),[37] with or without a sialic acid aldolase.[38] Such an approach has been successfully demonstrated for direct sialylation of non-sulfated Le^x for the synthesis of sLe^x containing a diverse array of naturally occurring and non-natural sialic acid forms using OPME systems containing a recombinant viral α2–3-sialyltransferase vST3Gal-I[39] or a bacterial multifunctional sialyltransferase mutant, *Pasteurella multocida* α2–3-sialyltransferase 1 (PmST1) M144D.[40] The latter with a high expression level (98 mg L-1 culture, >1000-fold higher than that of vST3Gal-I) and high promiscuity in tolerating different modification on the sialic acid in the substrates is a superior choice for the synthesis.[40] However, it was not clear whether O-sulfated Le^x structures could be used by PmST1 M144D as suitable acceptors in OPME sialylation process to produce desired O-sulfated sLe^x with different sialic acid forms.

Results and discussion

Synthesis of *O*-sulfated disaccharides and *O*-sulfated Le^x

In order to obtain O-sulfated Le^x as potential acceptor substrates for PmST1 M144D, enzyme-catalyzed α1–3-fucosylation of the corresponding O-sulfated disaccharides was test as a potential strategy. A one-pot three-enzyme (OP3E) α1–3-fucosylation system (Scheme 1)[39, 41] containing *Bacteroides fragilis* bifunctional L-fucokinase/GDP-fucose pyrophosphorylase (BfFKP),[42] *Pasteurella multocida* inorganic pyrophosphorylase (PmPpA),[43] and *Helicobacter pylori* α1–3-fucosyltransferase (Hp1–3FT 66 or Hp3FT) was used for this purpose. The O-sulfated disaccharides tested were 6-O-sulfo-LacNAcβProN$_3$ (**4**) (Scheme 1), 6'-O-sulfo-LacNAcβProN$_3$ (**5**), and 6,6'-di-O-sulfo-LacNAcβProN$_3$ (**6**) (Figure 2). LacNAcβProN$_3$[43] without any O-sulfate groups was used as a positive control.

6-O-Sulfo-LacNAcβProN$_3$ (**4**) was synthesized from 6-O-sulfo-GlcNAcβProN$_3$ (**7**)[43] using an improved OPME galactosyl activation and transfer system (Scheme 1) containing *Streptococcus pneumoniae* TIGR4 galactokinase (SpGalK),[44] *Bifidobacterium longum* UDP-sugar pyrophosphorylase (BLUSP),[45] PmPpA, and a *Helicobacter pylori* β1–4-galactosyltransferase (Hp1–4GalT or Hp4GalT).[43] The EcGalK, BLUSP, and PmPpA allowed *in situ* formation of the donor substrate of Hp4GalT, uridine 5'-diphosphate-galactose (UDP-Gal), from monosaccharide galactose (Gal).[45] It was previously shown that Hp4GalT, but not *Neisseria meningitidis* β1–4-galactosyltransferase (NmLgtB), was able to use 6-O-sulfated GlcNAc and derivatives as acceptor substrates for the synthesis of β1–4-linked galactosides.[43] The activity of Hp4GalT in synthesizing 6-O-sulfo-LacNAcβProN$_3$ (**4**) was confirmed again here using the improved OPME approach.[45, 46] An excellent 89% yield was obtained, compared favourably to the previous Hp4GalT-dependent OPME β1–4-galactosylation approach (70% yield) which used *Escherichia coli* K-12 glucose-1-P uridylyltransferase (EcGalU), *Escherichia coli* UDP-galactose-4-epimerase (EcGalE), and PmPpA to produce UDP-Gal *in situ* from glucose-1-phosphate.[43] 6'-O-Sulfo-LacNAcβProN$_3$ (**5**) and 6,6'-di-O-sulfo-LacNAcβProN$_3$ (**6**) (Scheme 2) were chemically synthesized (see supporting information).

Among three O-sulfated disaccharides tested, only 6-O-sulfo-LacNAcβProN$_3$ (**4**) was a suitable acceptor for Hp3FT to produce the desired 6-O-sulfo-Le^{x}βProN$_3$ (**8**). In contrast, 6'-O-sulfo-LacNAcβProN$_3$ (**5**) and 6,6'-di-O-sulfo-LacNAcβProN$_3$ (**6**) were not used efficiently by Hp3FT for the synthesis of the corresponding O-sulfated Le^x derivatives. With the positive outcome in small scale reactions for fucosylation of 6-O-sulfo-LacNAcβProN$_3$ (**4**), the preparative-scale synthesis of 6-O-sulfo-Le^{x}βProN$_3$ (**8**) was carried out using the OP3E α1–3-fucosyl activation and transfer system (Scheme 1). A yield of 70% was obtained. The combined sequential OPME β1–4-galactosylation and OPME α1–3-fucosylation (Scheme 1) was an effective approach for obtaining 6-O-sulfo-Le^{x}βProN$_3$ (**8**) from a simple monosaccharide derivative 6-O-sulfo-GlcNAcβProN$_3$ (**7**) in an overall yield of 62%.

As Hp3FT was not able to use 6'-O-sulfo-LacNAcβProN$_3$ (**5**) nor 6,6'-di-O-sulfo-LacNAcβProN$_3$ (**6**) efficiently as acceptors for fucosylation to obtain the desired Le^x trisaccharides, the target trisaccharides 6'-O-sulfo-Le^{x}βProNH$_2$ (**9**) and 6,6'-di-O-sulfo-Le^{x}βProNH$_2$ (**10**) were chemically synthesized (Scheme 2) from monosaccharide synthons **11**, **12**,[27] **13**, and **14**.[27] Notable features of the synthetic strategy include: (a) application of an efficient general protection strategy[47] for the synthesis of two trisaccharides (i.e. similar protecting groups were used in the synthesis and same reagents were used for their removal); (b) use of similar thioglycosides derivatives as glycosyl donors in all glycosylations; (c) high regio and stereo selectivity in product formation; (d) one step removal of benzyl ethers and reduction of azido group using 20% Pd(OH)$_2$/C (the Pearlman's catalyst) and H$_2$.[48] More specifically, for the synthesis of **9** and **10**, two N-phthalimide glucosamine derivatives **11** and **12** selectively protected at C6 with benzyl and *tert*-butyldiphenylsilyl ether (TBDPS), respectively, were coupled stereoselectively with thioglycoside donor **13** selectively protected with TBDPS at C6, in the presence of N-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[49] in dichloromethane. Disaccharide derivatives **15** and **16** were obtained in 72% and 78% yields, respectively. The bulky N-phthalimido protecting group in acceptors **11** and **12** provides steric hindrance to the neighboring C-3 hydroxyl group and decreases the reactivity of the C-3 hydroxyl group. Therefore, glycosylation occurs regioselectively at C-4 hydroxyl group.[27] Initial attempts to glycosylate acceptor **15** and **16** in dichloromethane with 1.2 equivalents of thiophenyl fucoside **14** produced trisaccharides of alpha and beta mixtures. In contrast, stereospecific formation of trisaccharides was achieved when a mixed solvent of diethylether and dichloromethane (1:1)[50, 51] was employed. Reaction of acceptors **15** and **16** with 1.2 equivalents of fucosyl donor **14** produced compounds **17** and **18** in 68% and 65% yields, respectively. Compounds **17** and **18** were then subjected to a series of synthetic transformations involving (a) conversion of N-phthaloyl group to acetamido group by removing phthaloyl group using ethylenediamine followed by N- and O-acetylation using acetic anhydride and pyridine; (b) HF-pyridine-mediated selective removal of TBDPS group;[52] (c) O-sulfation of the primary hydroxyl group by SO$_3$·pyridine complex;[52, 53] (d) deacetylation by NaOMe in MeOH;[54] and (e) hydrogenation using Pd(OH)$_2$/C and H$_2$[55] to obtain desired 6'-O-sulfo-Le^{x}βProNH$_2$ (**9**) and 6,6'-di-O-sulfo-Le^{x}βProNH$_2$ (**10**).

Enzymatic synthesis of *O*-sulfated sLe^x

With chemoenzymatically synthesized 6-*O*-sulfo-Le^{x}βProN$_3$ (**8**) as well as chemically synthesized 6'-*O*-sulfo-Le^{x}βProNH$_2$ (**9**) and 6,6'-di-*O*-sulfo-Le^{x}βProNH$_2$ (**10**) in hand, a one-pot two-enzyme (OP2E) sialylation system (Scheme 3) was used to test the tolerance of PmST1 M144D[40] in using these *O*-sulfated Le^x compounds as potential acceptor substrates. PmST1 M144D was previously engineered by protein crystal structure-assisted design. It has 20-fold reduced CMP-sialic acid (donor) hydrolysis activity and significantly (5588-fold) decreased α2–3-sialidase activity of the wild-type enzyme. It was used efficiently in a one-pot three-enzyme (OP3E) sialylation systems for the synthesis of non-sulfated sLe^x tetrasaccharides containing diverse sialic acid forms from Le^x.[40] To our delight, PmST1 M144D also tolerated *O*-sulfated Le^x containing *O*-sulfate at C-6, C-6', or both. In addition to *N*-acetylneuraminic acid (Neu5Ac), *N*-acetylneuraminic acid (Neu5Gc) was also successfully introduced to compounds **8–10**. *O*-Sulfated sLe^x tetrasaccharides 6-*O*-sulfo-Neu5Acα2–3Le^{x}βProN$_3$ (**1a**, 80 mg, 85%); 6-*O*-sulfo-Neu5Gcα2–3Le^{x}βProN$_3$ (**1b**, 22 mg, 47%), 6'-*O*-sulfo-Neu5Acα2–3Le^{x}βProNH$_2$ (**2a**, 75 mg, 82%); 6'-*O*-sulfo-Neu5Acα2–3Le^{x}βProNH$_2$ (**2b**, 45 mg, 60%), 6,6'-di-*O*-sulfo-Neu5Acα2–3Le^{x}βProNH$_2$ (**3a**, 42 mg, 64%), and 6,6'-di-*O*-sulfo-Neu5Gcα2–3Le^{x}βProNH$_2$ (**3b**, 40 mg, 38%) were successfully obtained by this highly efficient one-pot two-enzyme system containing *Neisseria meningitidis* CMP-sialic acid (NmCSS)[37] and PmST1 M144D[40] from the corresponding acceptors **8–10** and Neu5Ac or Neu5Gc, respectively. In general, Neu5Gc was used less efficiently by the OPME sialylation system, leading to lower yields for **1b–3b** (38–60%) compared to their Neu5Ac-counterparts **1a–3a** (64–85%). O-Sulfated sLe^x glycans with a propyl amine aglycone (compounds **2a**, **3a**, **2b**, **3b**) were found to be more challenging for column purification compared to the ones with a propyl azide aglycone (compounds **1a** and **1b**). When a desired sialic acid is readily available such as the case presented here, a one-pot two-enzyme (OP2E) system is sufficient. When only the 6-carbon precursors of the desired sialic acid forms are available, the one-pot three-enzyme (OP3E) sialylation system including an aldolase in addition to NmCSS and PmST1 M144D[40] should be used.

Conclusions

In conclusion, we have successfully developed an efficient chemoenzymatic method for systematic synthesis of synthetically challenging *O*-sulfated sLe^x (**1a–3a** and **1b–3b**) containing different sialic acid forms (Neu5Ac or Neu5Gc) by direct sialylation of the corresponding *O*-sulfated Le^x structures **8–10** using an efficient one-pot two-enzyme (OP2E) system containing NmCSS and PmST1 M144D. The method can be extended to the synthesis of *O*-sulfated sLe^x structures containing other sialic acid forms. We have also shown here, a relatively complex trisaccharide 6-*O*-sulfo-Le^{x}βProN$_3$ (**8**) can be efficiently produced from a simple monosaccharide derivative 6-*O*-sulfo-GlcNAcβProN$_3$ (**7**) by a sequential OPME β1–4-galactosylation and OPME α1–3-fucosylation process. PmST1 M144D has been demonstrated to be a powerful catalyst not only for synthesizing non-sulfated sLe^x structures as shown previously,[40] but also for producing biologically important but difficult-to-obtain *O*-sulfated sLe^x.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Rosen SD. Annu. Rev. Immunol. 2004; 22:129. [PubMed: 15032576]

2. Kawashima H, Fukuda M. Ann. N. Y. Acad. Sci. 2012; 1253:112. [PubMed: 22288521]

3. Kobayashi M, Lee H, Nakayama J, Fukuda M. Current drug metabolism. 2009; 10:29. [PubMed: 19149511]

4. Macauley MS, Crocker PR, Paulson JC. Nature reviews. Immunology. 2014; 14:653.

5. Kiwamoto T, Kawasaki N, Paulson JC, Bochner BS. Pharmacol. Ther. 2012; 135:327. [PubMed: 22749793]

6. Campanero-Rhodes MA, Childs RA, Kiso M, Komba S, Le Narvor C, Warren J, Otto D, Crocker PR, Feizi T. Biochem. Biophys. Res. Commun. 2006; 344:1141. [PubMed: 16647038]

7. Bochner BS, Alvarez RA, Mehta P, Bovin NV, Blixt O, White JR, Schnaar RL. J. Biol. Chem. 2005; 280:4307. [PubMed: 15563466]

8. Kiwamoto T, Brummet ME, Wu F, Motari MG, Smith DF, Schnaar RL, Zhu Z, Bochner BS. J. Allergy Clin. Immunol. 2014; 133:240. [PubMed: 23830412]

9. Tateno H, Crocker PR, Paulson JC. Glycobiology. 2005; 15:1125. [PubMed: 15972893]

10. Bochner BS. Clin. Exp. Allergy. 2009; 39:317. [PubMed: 19178537]

11. Hudson SA, Bovin NV, Schnaar RL, Crocker PR, Bochner BS. J. Pharmacol. Exp. Ther. 2009; 330:608. [PubMed: 19458105]

12. Kiwamoto T, Katoh T, Evans CM, Janssen WJ, Brummet ME, Hudson SA, Zhu Z, Tiemeyer M, Bochner BS. J. Allergy Clin. Immunol. 2015; 135:1329. [PubMed: 25497369]

13. Galustian C, Park CG, Chai W, Kiso M, Bruening SA, Kang YS, Steinman RM, Feizi T. Int. Immunol. 2004; 16:853. [PubMed: 15136555]

14. Valladeau J, Ravel O, Dezutter-Dambuyant C, Moore K, Kleijmeer M, Liu Y, Duvert-Frances V, Vincent C, Schmitt D, Davoust J, Caux C, Lebecque S, Saeland S. Immunity. 2000; 12:71. [PubMed: 10661407]

15. Lasky LA, Singer MS, Dowbenko D, Imai Y, Henzel WJ, Grimley C, Fennie C, Gillett N, Watson SR, Rosen SD. Cell. 1992; 69:927. [PubMed: 1376638]

16. Hemmerich S, Rosen SD. Biochemistry. 1994; 33:4830. [PubMed: 8161542]

17. Hemmerich S, Leffler H, Rosen SD. J. Biol. Chem. 1995; 270:12035. [PubMed: 7538131]

18. Hemmerich S, Bertozzi CR, Leffler H, Rosen SD. Biochemistry. 1994; 33:4820. [PubMed: 7512827]

19. Bistrup A, Bhakta S, Lee JK, Belov YY, Gunn MD, Zuo FR, Huang CC, Kannagi R, Rosen SD, Hemmerich S. J. Cell. Biol. 1999; 145:899. [PubMed: 10330415]

20. Pazynina GV, Sablina MA, Tuzikov AB, Chinarev AA, Bovin NV. Mendeleev Commun. 2003; 13:245.

21. Lai CH, Hahm HS, Liang CF, Seeberger PH. Beilstein J. Org. Chem. 2015; 11:617. [PubMed: 26124863]

22. Misra AK, Ding Y, Lowe JB, Hindsgaul O. Bioorg. Med. Chem. Lett. 2000; 10:1505. [PubMed: 10915037]

23. Liang A, Thakkar JN, Desai UR. J. Pharm. Sci. 2010; 99:1207. [PubMed: 19711446]

24. Al-Horani RA, Desai UR. Tetrahedron. 2010; 66:2907. [PubMed: 20689724]

25. Krock L, Esposito D, Castagner B, Wang C-C, Bindschadler P, Seeberger PH. Chem. Sci. 2012; 3:1617.

26. Esposito D, Hurevich M, Castagner B, Wang CC, Seeberger PH. Beilstein J. Org. Chem. 2012; 8:1601. [PubMed: 23209492]

27. Cao H, Huang S, Cheng J, Li Y, Muthana S, Son B, Chen X. Carbohydr. Res. 2008; 343:2863. [PubMed: 18639240]

28. Komba S, Galustian C, Ishida H, Feizi T, Kannagi R, Kiso M. Angew. Chem. Int. Ed. 1999; 38:1131.

29. Pratt MR, Bertozzi CR. Org. Lett. 2004; 6:2345. [PubMed: 15228275]

30. Komba S, Ishida H, Kiso M, Hasegawa A. Bioorg. Med. Chem. 1996; 4:1833. [PubMed: 9007268]

31. Jain RK, Vig R, Rampal R, Chandrasekaran EV, Matta KL. J. Am. Chem. Soc. 1994; 116:12123.

32. Komba S, Ishida H, Kiso M, Hasegawa A. Carbohydr. Res. 1996; 285:C1. [PubMed: 9011371]

33. Chen X, Varki A. ACS Chem. Biol. 2010; 5:163. [PubMed: 20020717]

34. Angata T, Varki A. Chem. Rev. 2002; 102:439. [PubMed: 11841250]

35. Varki A. Am. J. Phys. Anthropol. 2001; Suppl 33:54.

36. Yu H, Chokhawala HA, Huang S, Chen X. Nat. Protoc. 2006; 1:2485. [PubMed: 17406495]

37. Yu H, Yu H, Karpel R, Chen X. Bioorg. Med. Chem. 2004; 12:6427. [PubMed: 15556760]

38. Li Y, Yu H, Cao H, Lau K, Muthana S, Tiwari VK, Son B, Chen X. Appl. Microbiol. Biotechnol. 2008; 79:963. [PubMed: 18521592]

39. Sugiarto G, Lau K, Yu H, Vuong S, Thon V, Li Y, Huang S, Chen X. Glycobiology. 2011; 21:387. [PubMed: 20978012]

40. Sugiarto G, Lau K, Qu J, Li Y, Lim S, Mu S, Ames JB, Fisher AJ, Chen X. ACS Chem. Biol. 2012; 7:1232. [PubMed: 22583967]

41. Yu H, Lau K, Li Y, Sugiarto G, Chen X. Curr. Protoc. Chem. Biol. 2012; 4:233. [PubMed: 25000293]

42. Yi W, Liu X, Li Y, Li J, Xia C, Zhou G, Zhang W, Zhao W, Chen X, Wang PG. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:4207. [PubMed: 19251666]

43. Lau K, Thon V, Yu H, Ding L, Chen Y, Muthana MM, Wong D, Huang R, Chen X. Chem. Commun. 2010; 46:6066.

44. Chen M, Chen LL, Zou Y, Xue M, Liang M, Jin L, Guan WY, Shen J, Wang W, Wang L, Liu J, Wang PG. Carbohydr. Res. 2011; 346:2421. [PubMed: 21903203]

45. Chen X, Fang JW, Zhang JB, Liu ZY, Shao J, Kowal P, Andreana P, Wang PG. J. Am. Chem. Soc. 2001; 123:2081. [PubMed: 11456841]

46. Yu H, Lau K, Thon V, Autran CA, Jantscher-Krenn E, Xue M, Li Y, Sugiarto G, Qu J, Mu S, Ding L, Bode L, Chen X. Angew. Chem. Int. Ed. Engl. 2014; 53:6687. [PubMed: 24848971]

47. Miranda LP, Meldal M. Angew. Chem. Int. Ed. Engl. 2001; 113:3767.

48. Corey EJ, Link JO. J. Am. Chem. Soc. 1992; 114:1906.

49. Veeneman GH, van Leeuwen SH, van Boom JH. Tetrahedron Lett. 1990; 31:1331.

50. Ghosh T, Santra A, Misra AK. Rsc. Adv. 2014; 4:54.

51. Si A, Misra AK. ChemistryOpen. 2016; 5:47–50. [PubMed: 27308211]

52. Yang B, Yoshida K, Yin Z, Dai H, Kavunja H, El-Dakdouki MH, Sungsuwan S, Dulaney SB, Huang X. Angew. Chem. Int. Ed. Engl. 2012; 51:10185. [PubMed: 22961711]

53. Santra A, Guchhait G, Misra AK. Green Chemistry. 2011; 13:1345.

54. Wang, Z. Comprehensive Organic Name Reactions and Reagents. John Wiley & Sons, Inc.; 2010.
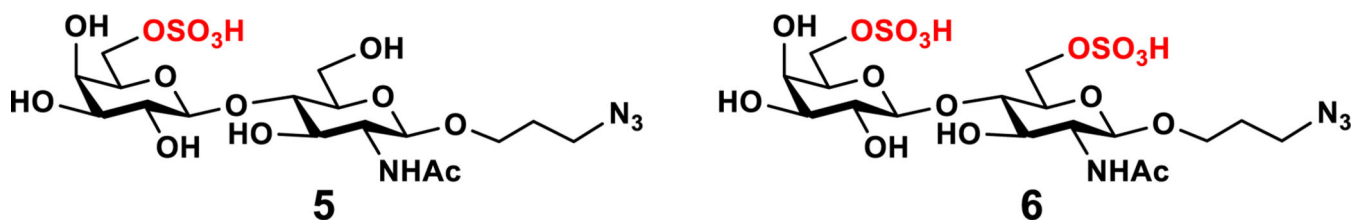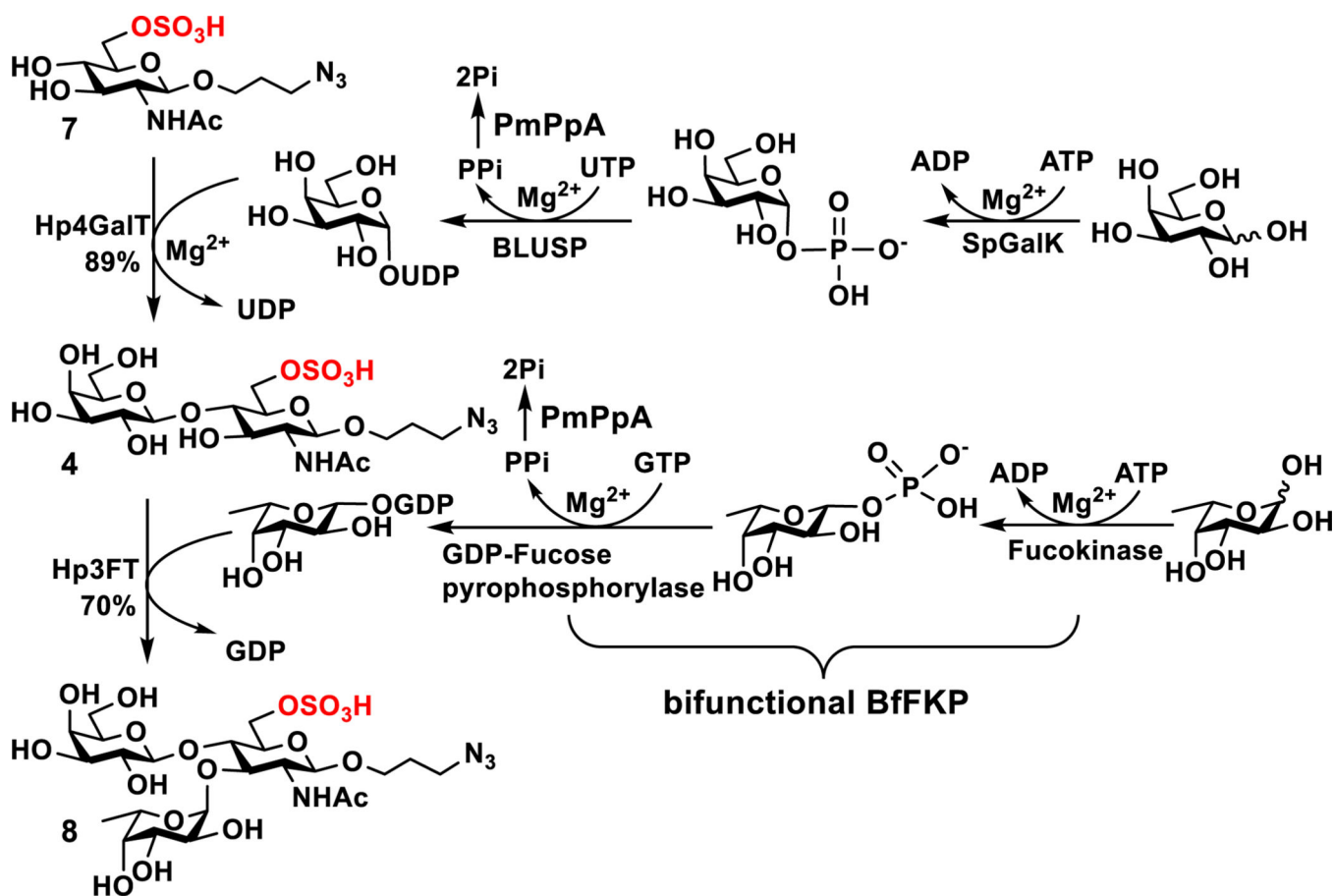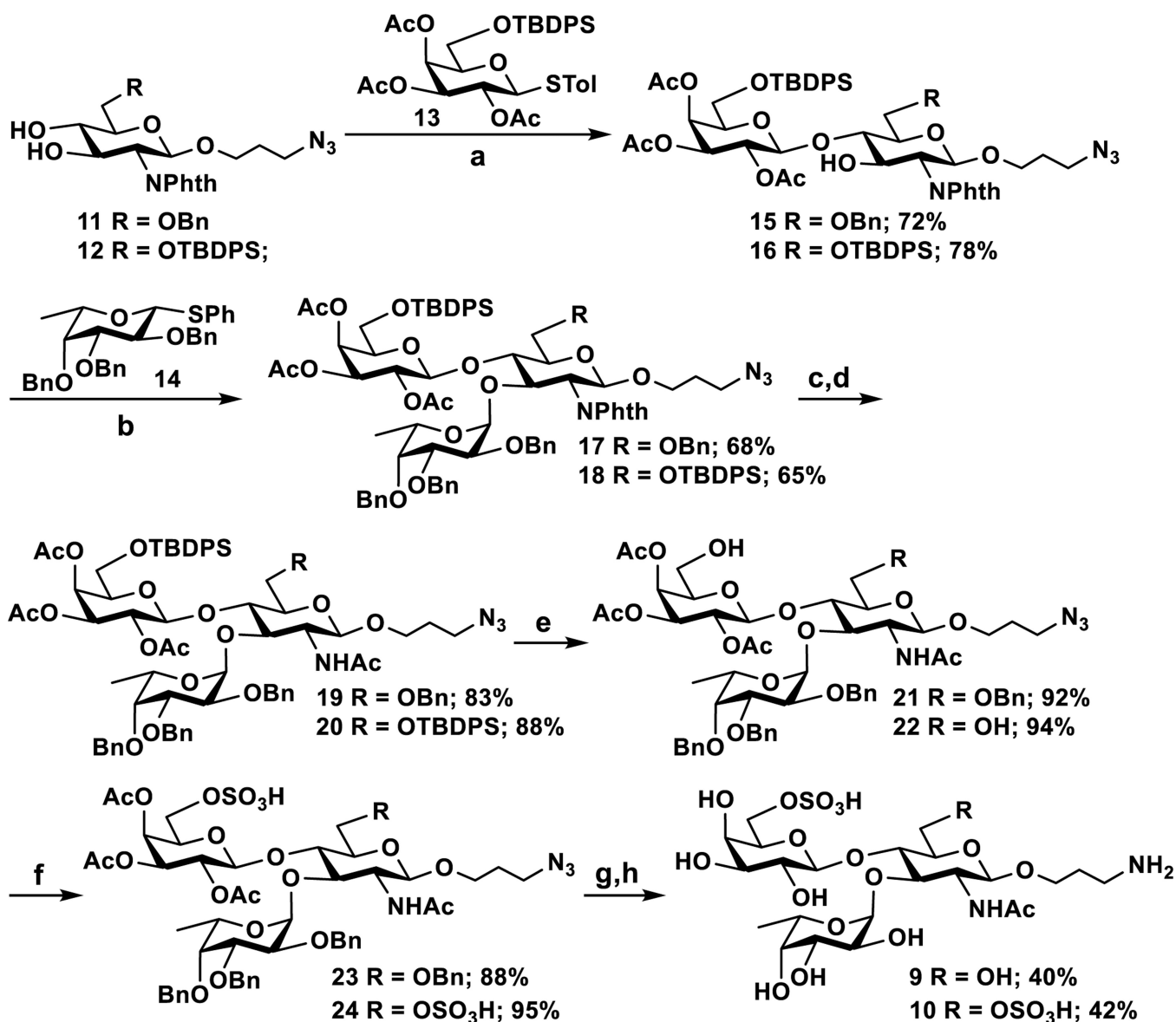
55. Pearlman WM. Tetrahedron Lett. 1967:1663.

Fig. 1.
Structures of *O*-sulfated sialyl Lewis x including 6-*O*-sulfo-sLe^x (**1**), 6'-*O*-sulfo-sLe^x (**2**), and 6', 6-di-*O*-sulfo-sLe^x (**3**).

Fig. 2.
Structures of chemically synthesized 6'-O-sulfo-LacNAcβProN$_3$ (5) and 6,6'-di-O-sulfo-LacNAcβProN$_3$ (6).

Scheme 1.
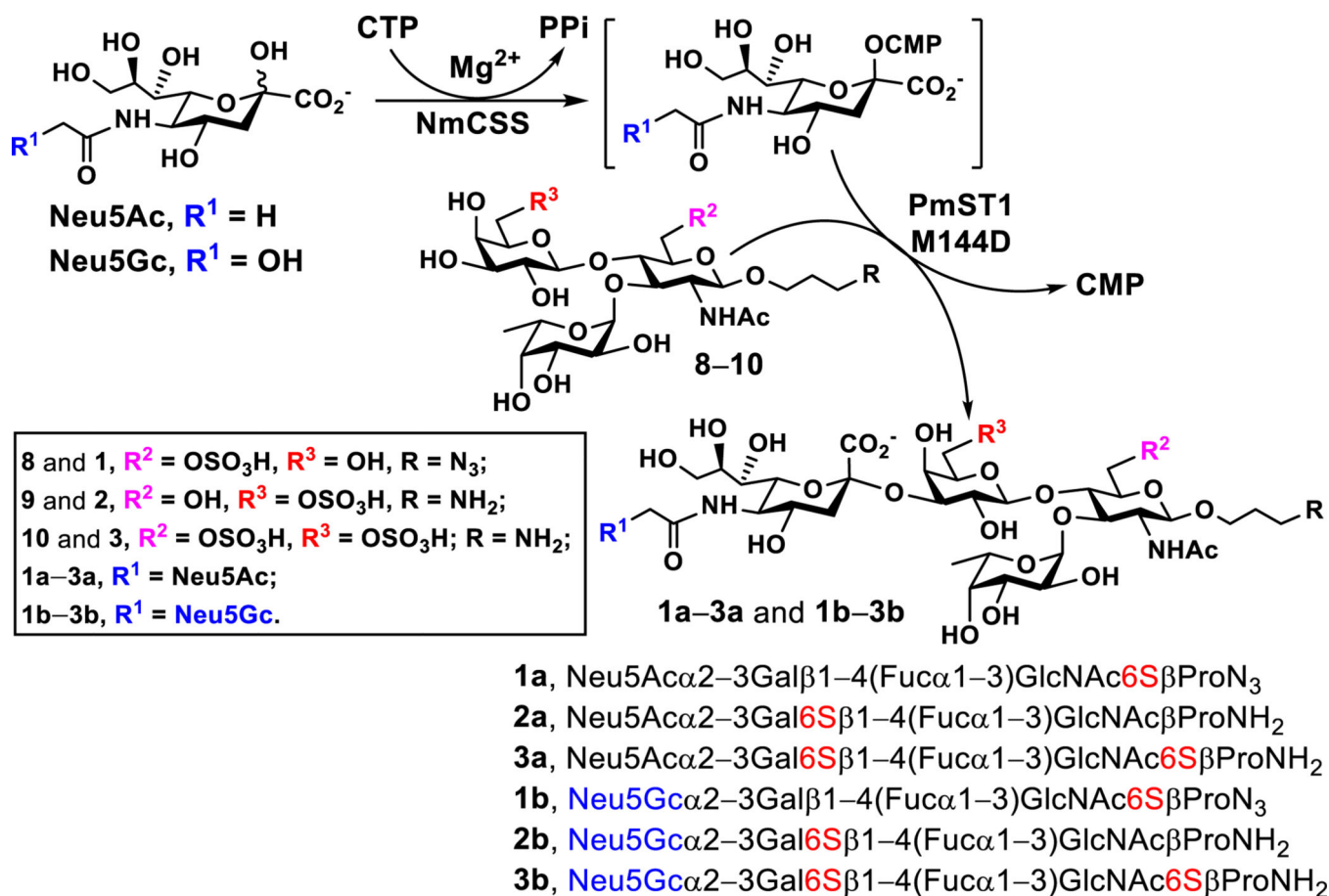Sequential OPME synthesis of 6-O-sulfo-Le^{x}βProN$_3$ (8) from 6-O-sulfo-GlcNAcβProN$_3$ (7) by OPME β1–4-galactosyl activation and transfer system for the formation of 6-O-sulfo-LacNAcβProN$_3$ (4) followed by OPME α1–3-fucosyl activation and transfer system for the formation of 6-O-sulfo-Le^{x}βProN$_3$ (8). Enzymes and abbreviations: SpGalK, *Streptococcus pneumoniae* TIGR4 galactokinase;[44] BLUSP, *Bifidobacterium longum* UDP-sugar pyrophosphorylase;[45] PmPpA, *Pasteurella multocida* inorganic pyrophosphorylase;[43] Hp4GalT, Helicobacter pylori β1–4-galactosyltransferase;[43] BfFKP, *Bacteroides fragilis* bifunctional L-fucokinase/GDP-fucose pyrophosphorylase;[42] and Hp3FT, *Helicobacter pylori* α1–3-fucosyltransferase.[39, 41]

Scheme 2.

Chemical synthesis of 6'-*O*-sulfo-Le^{x}βProNH$_2$ (**9**) and 6,6'-di-*O*-sulfo-Le^{x}βProNH$_2$ (**10**).
Reagents and conditions: a) *N*-iodosuccinimide (NIS), TMSOTf, MS 4 Å, CH$_2$Cl$_2$, −40 °C,
30 min; b) *N*-iodosuccinimide (NIS), TMSOTf, MS 4 Å, CH$_2$Cl$_2$-Et$_2$O (1:1), −18 °C, 45
min; c) H$_2$N(CH$_2$)$_2$NH$_2$, *n*-BuOH, 90 °C, 8 h; d) pyridine, Ac$_2$O, r.t., 10 h; e) HF·pyridine,
0 °C to r.t., overnight; f) SO$_3$·pyridine, pyridine, 0 °C to r.t.; g) 0.1 M NaOMe, MeOH, r.t., 3
h; h) Pd(OH)$_2$/C, H$_2$, CH$_3$OH, 48 h.

Scheme 3.
PmST1 M144D-mediated one-pot two-enzyme (OP2E) sialylation of *O*-sulfo analogues of Lewis[x]. Yields obtained for *O*-sulfated sLe[x] tetrasaccharides: **1a**, 85%; **1b**, 47%; **2a**, 82%; **2b**, 60%; **3a**, 64%; **3b**, 38%. Enzymes and abbreviations: NmCSS, *Neisseria meningitidis* CMP-sialic acid;[37] PmST1 M144D, *Pasteurella multocida* α2–3-sialyltransferase 1 (PmST1) M144D mutant.[40]