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Syntheses of 6-Sulfo Sialyl Lewis X Glycans Corresponding to the L-Selectin Ligand "Sulfoadhesin"

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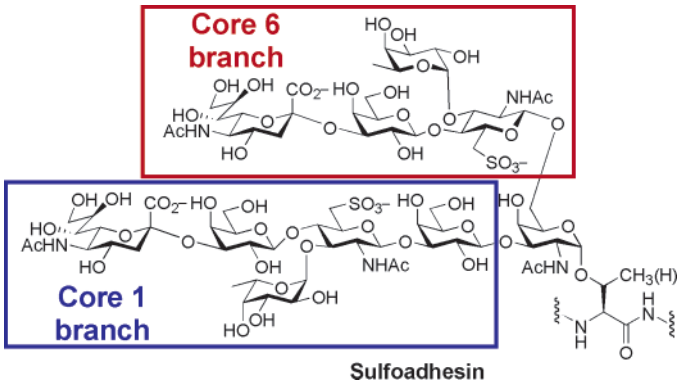
ABSTRACT



Divergent syntheses of sulfated sialyl Lewis X oligosaccharides corresponding to the core 1 and core 6 branches of the L-selectin ligand are reported. These synthetic targets incorporate a selectively protected serine residue at the reducing terminus, providing a functional handle for further conjugation.

The selectins are a family of C-type lectins that mediate the early stages of leukocyte homing to sites of inflammation.[1] E- and P-selectin are induced on endothelial cells upon cytokine stimulation from underlying inflamed tissue.[2] L-Selectin is constitutively expressed on leukocytes and is involved in interactions with high endothelial venule (HEV)-like blood vessels that develop at sites of chronic inflammation.[3] The interactions of the three selectins with their cognate glycoprotein ligands are necessary for the adhesion of leukocytes to the endothelium and their subsequent extravasation into the tissue.

A common carbohydrate motif shared by many selectin ligands is the sialyl Lewis X tetrasaccharide (sLe^x).[4] It has been shown further that 6-sulfo-sLe^x is the primary recognition epitope for L-selectin and that anti 6-sulfo-sLe^x antibodies bind ligands expressed on HEV, thus inhibiting L-selectin binding.[5] "Sulfoadhesin" represents a family of glycan structures found on the glycoprotein ligands for L-selectin (Figure 1). This family consists of a core α-GalNAc residue that is elaborated with one or more 6-sulfo-sLe^x capping groups. An extended carbohydrate chain elaborated from the 3-OH of the core GalNAc residue defines the core 1 branch. Likewise, the core 6 branch is defined by elaboration from the 6-OH of the same GalNAc residue.[6] Both the core 6 and core 1 branches bearing the 6-sulfo-sLe^x epitope have been shown to be involved in L-selectin binding.[7] However, the

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(2) Lowe, J. B. *Immunol. Rev.* **2002**, *186*, 19−36.
(3) Rosen, S. D. *Am. J. Pathol.* **1999**, *155*, 1013−1020.
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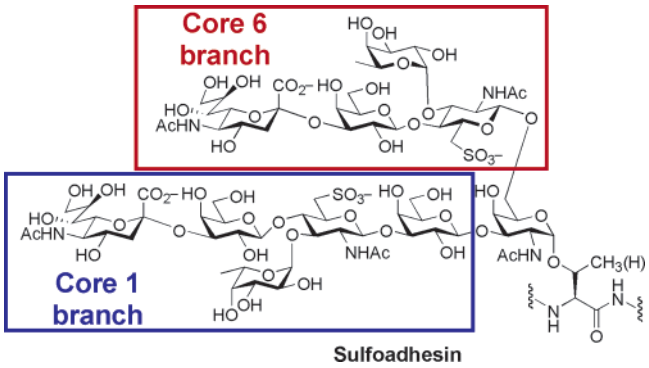


Figure 1. Structure of sulfoadhesin with core 1 and core 6 branches highlighted.

specific contribution of each branch to L-selectin binding has not been directly measured.

To evaluate the individual contributions of the sulfoadhesin branches to L-selectin binding, the synthesis of 6-O-sulfated oligosaccharides corresponding to core 1 (**1**) and core 6 (**2**) branches and their nonsulfated counterparts (**3**, **4**) was undertaken (Figure 2). Although sulfated oligosaccharides

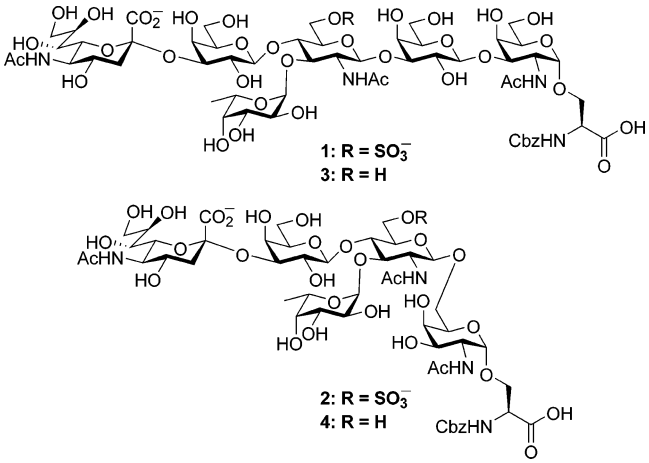
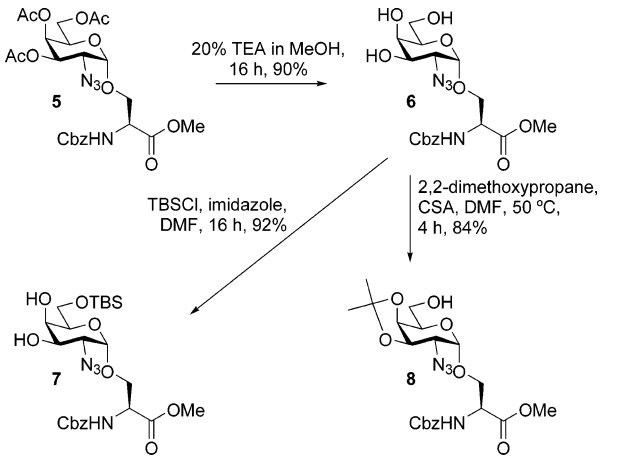


Figure 2. Synthetic targets corresponding to core 1 (**1**) and core 6 (**2**) branches and their nonsulfated counterparts (**3** and **4**, respectively).

similar to **1** and **2** have been synthesized previously by other groups,[8] the incorporation of reactive functional groups at their reducing termini has not been reported. Such a reactive handle is critical for the conjugation of the carbohydrate epitopes to any carrier of interest and thereby increases the scope of biological studies that can be performed with these compounds. A selectively protected serine residue was introduced at the reducing terminus as an aglycone. Furthermore, because serine is located at the reducing terminus in natural oligosaccharides of this type, compounds **1**−**4** are biologically relevant.

The synthesis was initiated with compound **5**,[9] which was deprotected with 20% triethylamine (TEA) in MeOH to provide triol **6**, an intermediate common to all structures (Scheme 1). Triol **6** was then divergently converted to **7** or

Scheme 1. Synthesis of α-GalNAc-Serine



8 for the synthesis of the core 1 and core 6 structures, respectively. The use of an *N*-phthalimido group was deemed to be incompatible with the protecting groups of the serine residue. Therefore, the 2-*N*-phthalimido (NPht) group of **9**[10] was replaced with a 2-*N*-2′,2′,2′-trichloroethylcarbonyl (NHTroc) group to provide **10** in a three-step procedure (Scheme 2).

To maximize the efficiency of the synthesis of hexasaccharides **1** and **3**, a route was developed that incorporated two key regioselective glycosylation reactions. Known compound **11**[11] was reacted with **7** using *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) as promoters to give the disaccharide in good yield with complete regioselectivity (Scheme 2). The acetyl esters of this disaccharide were then removed to provide **12**. Compound **12** was condensed with **10** under identical conditions to yield tetrasaccharide **13**, again with complete regioselectivity and in excellent yield for this type of unprecedented transformation.

Over the next three steps, **13** was converted to **17** using standard protecting group manipulations (Scheme 3). First, the 6-*O*-TBS and 4,6-*O*-benzylidene groups were removed

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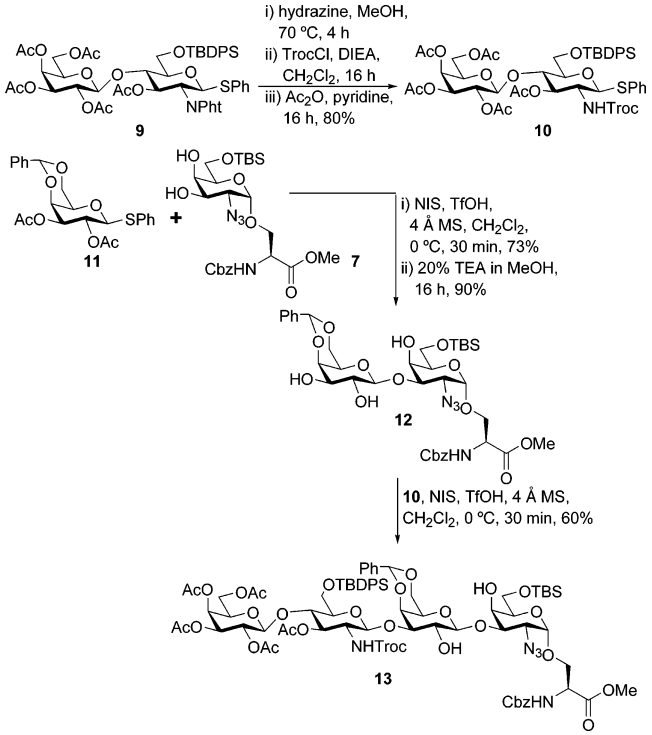
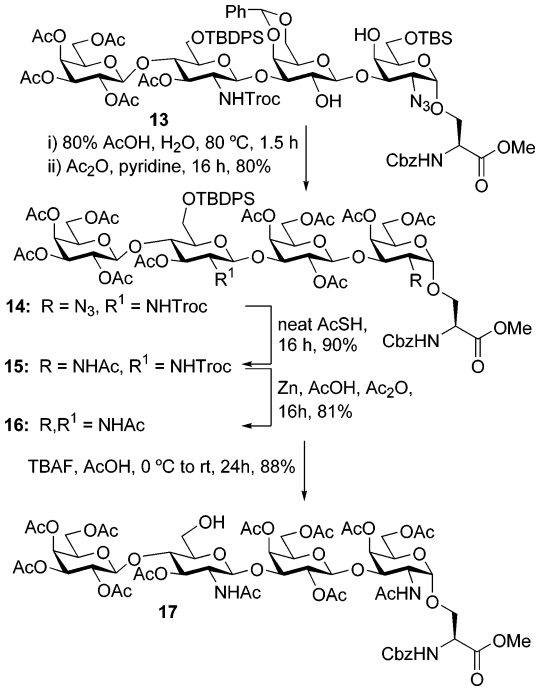
(11) de Pouilly, P.; Chenede, A.; Mallet, J.-M.; Sinay, P. *Bull. Soc. Chim. Fr.* **1993**, *130*, 256−265.

Scheme 2. Synthesis of Core 1 Tetrasaccharide

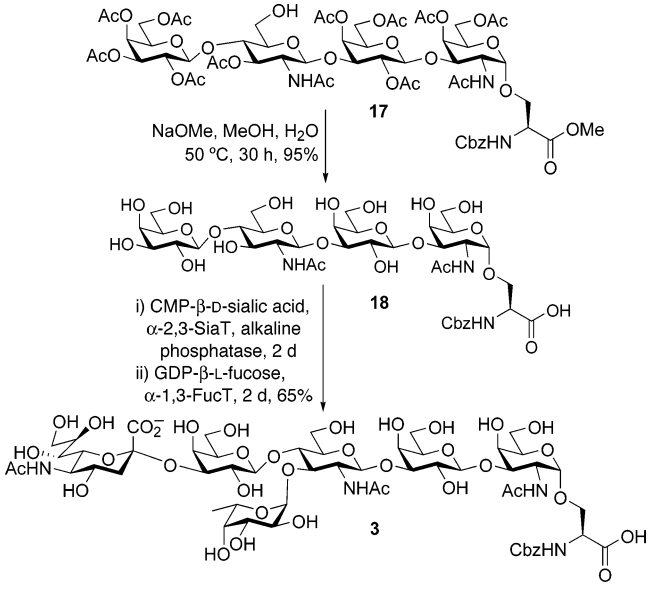


with acid. All the free hydroxyl groups were then protected as acetyl esters to give **14**. The azide of **14** was reductively acetylated using neat AcSH, and this was followed by removal of the NHTroc group with Zn and subsequent acetylation of the unmasked amine to provide **15** and **16**, respectively. The 6-*O*-TBDPS group of **16** was then removed

Scheme 3. Synthesis of Core 1 Continued



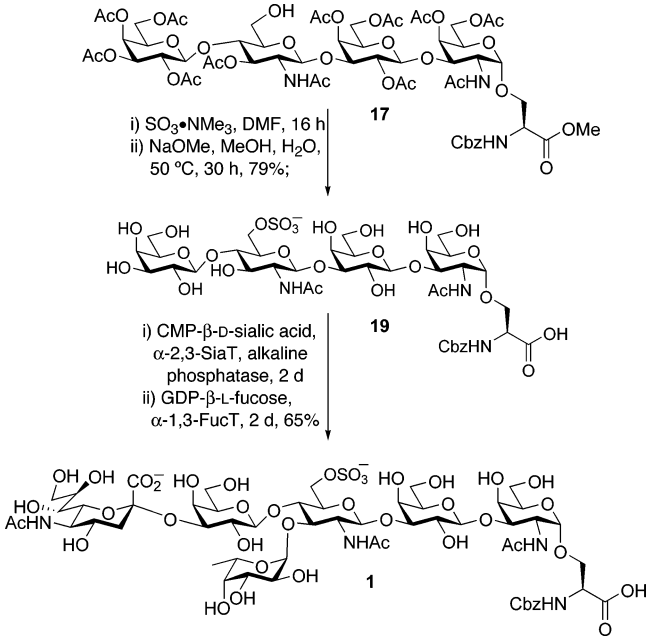
Scheme 4. Completion of Compound **3**



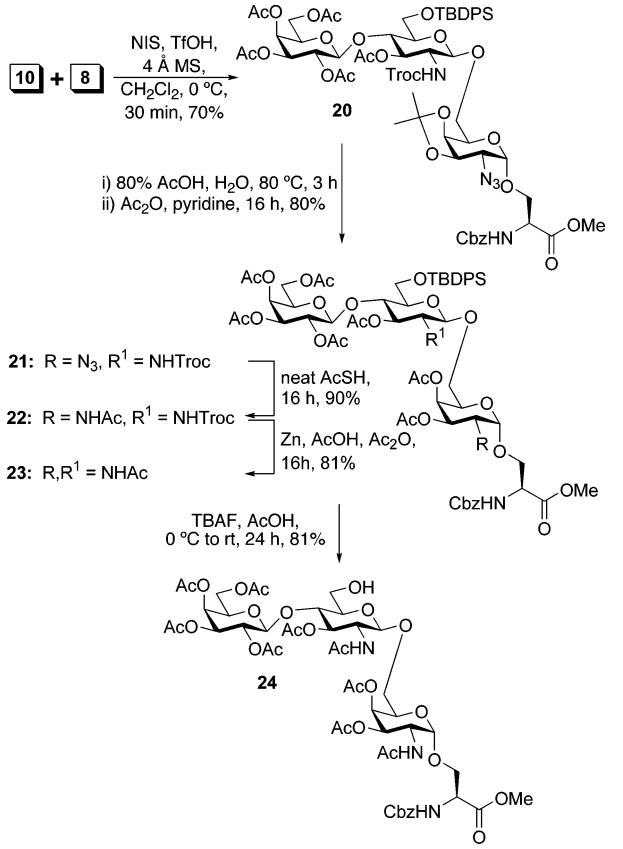
to provide the selectively protected compound **17**, a key intermediate, needed for selective sulfation. Indeed, global deprotection of all the ester functionality in **17** with NaOMe in MeOH/H_2O gave **18** (Scheme 4), whereas sulfation followed by deprotection yielded **19** (Scheme 5). Enzymatic sialylation of the tetrasaccharide intermediates using a recombinant α-2,3-sialyltransferase (α-2,3-SiaT) and *β*-CMP-D-sialic acid as the donor, followed by fucosylation with an α-1,3-fucosyltransferase (α-1,3-FucT) and *β*-GDP-L-fucose, gave the desired compounds **1** and **3** in excellent yield.[12]

For the synthesis of compounds **2** and **4**, compound **10** was condensed with **8** using NIS/TfOH to give **20** (Scheme

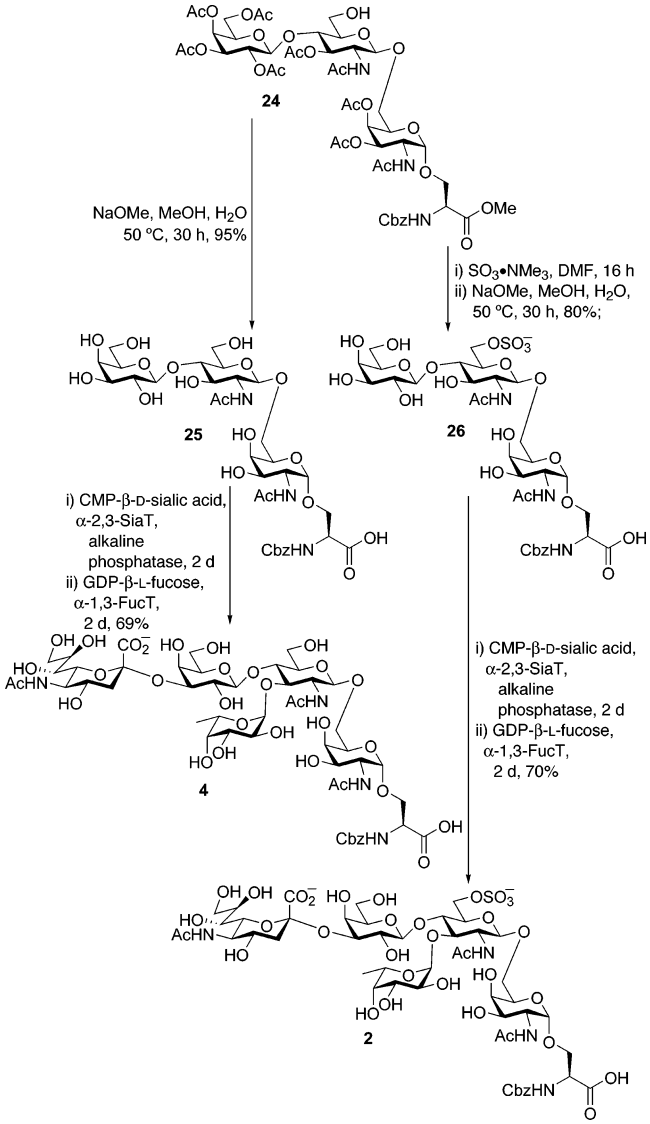
Scheme 5. Completion of Compound **1**



Scheme 6. Synthesis of Core 6



Scheme 7. Completion of Compounds **2** and **4**



6). Protecting group manipulations similar to those used to generate **17** were used to convert **20** to **23** over three steps. Just as in the case of **17**, the 6-*O*-TBDPS group of **23** was removed to provide trisaccharide **24**.

Compound **24** was then either fully deprotected or subjected to sulfation/deprotection conditions to provide **25** and **26**, respectively. Elaboration of these trisaccharides by enzymatic sialylation and fucosylation procedures gave core 6 pentasaccharides **2** and **4**, again in excellent yield (Scheme 7).

In conclusion, syntheses of 6-sulfo-sLe^x glycans corresponding to the branches of the L-selectin ligand sulfoadhesin were completed. Both core 1 and core 6 oligosaccharides were synthesized in a divergent fashion from common intermediates. Furthermore, the synthesis of core 1 involved two novel regioselective glycosylations. This allowed for minimal protecting group manipulations and maximal efficency.

Supporting Information Available: Synthetic procedures and spectral data of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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