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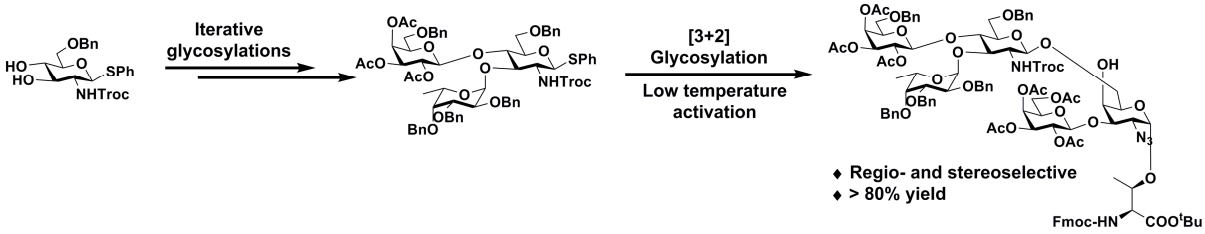

Synthesis of Lewis^X-*O*-Core-1 threonine: A building block for *O*-linked Lewis^X glycopeptides

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Abstract

Lewis X (Le^X) is a branched trisaccharide Galβ1→4(Fucα1→3)GlcNAc that is expressed on many cell surface glycoproteins and plays critical roles in innate and adaptive immune responses. However, efficient synthesis of glycopeptides bearing Le^X remains a major limitation for structure-function studies of the Le^X determinant. Here we report a total synthesis of a Le^X pentasaccharide **1** using a regioselective 1-benzenesulfinyl piperidine/triflic anhydride promoted [3+2] glycosylation. The presence of an Fmoc-threonine amino acid facilitates incorporation of the pentasaccharide in solid phase peptide synthesis, providing a route to diverse O-linked Le^X glycopeptides. The described approach is broadly applicable to the synthesis of a variety of complex glycopeptides containing O-linked Le^X or sialyl Lewis^X (sLe^X).

Introduction

The structural diversity of glycoproteins underlies a variety of molecular recognition events, which are critical to many biological functions, including innate and adaptive immune responses. Lewis^X (Le^X) is a branched trisaccharide Galβ1→4(Fucα1→3)GlcNAc that is present on a variety of cell surface glycoproteins, including CD15,[1–5] carcinoembryonic antigen-related cell adhesion molecule (CEACAM),[6–9] and stage specific embryonic antigen-1 (SSEA-1).[3,4,10–13] Glycoproteins expressing Le^X have been identified as early mediators of the immune response against invading pathogens,[6,14,15] and are pivotal in immunomodulation,[6,16–19] regulation of the growth and angiogenesis of tumor cells,[1,4] embryogenesis of the central nervous system[3,10,11] and promotion of the formation of a spermatozoa reservoir and subsequent release from the oviduct.[20–22] DC-SIGN (dendritic cell specific ICAM grabbing non-integrin) is a counter-receptor to many Le^X bearing glycoproteins, including CEACAM-1, Mac-1 and ICAM3, as well as unidentified Le^X glycoproteins on invading pathogens and neutrophils.[2,7,9,23–27] In turn, the interaction of dendritic cells and neutrophils enables antigen-presenting cells to stimulate T cells and modulate adaptive immunity. Despite the critical role of DC-SIGN in the recognition of invading pathogens, human immunodeficiency virus (HIV), hepatitis C virus, and *Mycobacterium tuberculosis*, also use DC-SIGN to escape the immune response.[17,28,29] A recent study has also demonstrated that terminal Le^X on cell surface glycoproteins regulates neutrophil chemotaxis, transepithelial migration, and post-migratory function.[19] Hence, blocking the carbohydrate binding site of DC-SIGN may provide a strategy to impede the immunological evasion of a number of infectious pathogens.[14,30–32] Moreover, blocking the interaction of Le^X glycoproteins with their respective counter receptors holds therapeutic potential for many diseases, including rheumatoid arthritis, inflammatory bowel diseases, metabolic syndrome, cancer, HIV, as well as many other chronic disorders of innate immunity. Le^X-antigens (CD15) are also expressed on cancer stem cells and may be potentially used to develop antitumor vaccines.

In nature, glycans are either linked to proteins or lipids and their functions are increasingly best understood in the context of its aglycone component. Thus, Le^X containing glycans linked to model glycopeptides provide an important tool to study proteins that may interact with Le^X in context of the aglycone moiety. An important challenge, however, in the generation of high affinity glycopeptide ligands is the lack of efficient and scalable chemical synthetic schemes. For example, undesired regio- and stereoselectivity during key glycosylation steps have limited the development of an efficient chemical synthesis of P-selectin glycoprotein ligand-1 (PSGL-1) analogues.[33,34] Additionally, the chemical incorporation of *N*-acetylglucosamine remains a significant impediment due to the formation of inert oxazoline during the activation step[35,36] A common strategy to bypass oxazoline formation is to employ a phthalimide protecting group, which has been used for the synthesis of Le^X containing analogues in other reports.[37–45] However, the conditions for the removal of the phthalimide protecting group are incompatible with an Fmoc group, essential to generating a glycopeptide. Moreover, the fucopyranosyl glycosidic linkage in Le^X is highly acid-sensitive and any modification involving a stronger acid results in degradation of the fucosyl residue.[46] Thus, there is great need to develop efficient schemes for the facile synthesis of Le^X bearing *O*-glycans. While glycopeptides containing Le^X are responsible for diverse biological functions, structural and conformational information of these glycans in participating molecular interactions are poorly understood. Hence, it is critical to establish a synthetic platform for glycopeptides containing Le^X. Lack of facile synthesis of complex O-glycans still impedes the generation glycopeptide despite seminal contributions by research groups including Danishefsky, Wong, and Kunz.[46–50]

The mucin type T-antigen (Galβ1→3GalNAcα-*O*-Ser/Thr) is one of the most abundant O-glycan motifs of many complex glycoproteins and is the precursor for the synthesis of the branched core 2 O-glycan on which Le^X can be found within a pentasaccharide (Le^X-C2-*O*-pentasaccharide). The availability of a Le^X-C2-*O*-pentasaccharide-bearing threonine amino

acid **1** (**Fig. 1**) will be a very useful precursor towards the synthesis of a variety of glycopeptides for biological studies. In this report, we describe the synthesis of C2-O-Le^X pentasaccharide **1** as a glycopeptide building block by a convergent [3+2] glycosylation between a Le^X donor **2** and a Core-1 diol acceptor **3**.

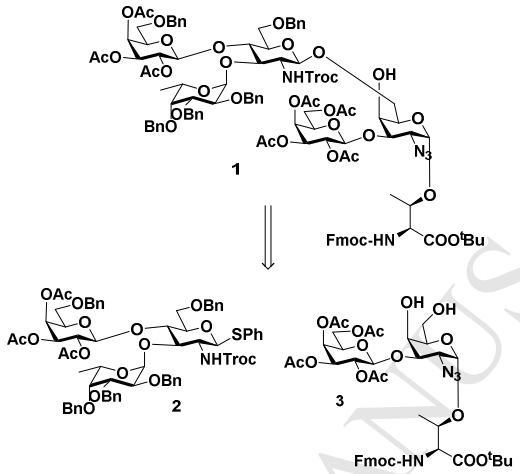


Figure 1. Retrosynthesis of C2-*O*-Le^X pentasaccharide.

Results and Discussion

Galactosyl thioglycoside donor **8**, fucosyl imidate donor **13**, and Troc-protected glucosamine diol acceptor **19** were chosen as suitable building blocks for Le^X donor **2** (**Fig. 2**). Orthogonality of the protecting groups and ease of synthesis provided the rationale for selecting building blocks **8**, **9**, and **15**. In particular, the NHTroc protecting group was chosen as it displays β-selectivity and its deprotection conditions are compatible with NHFmoc chemistry.[51]

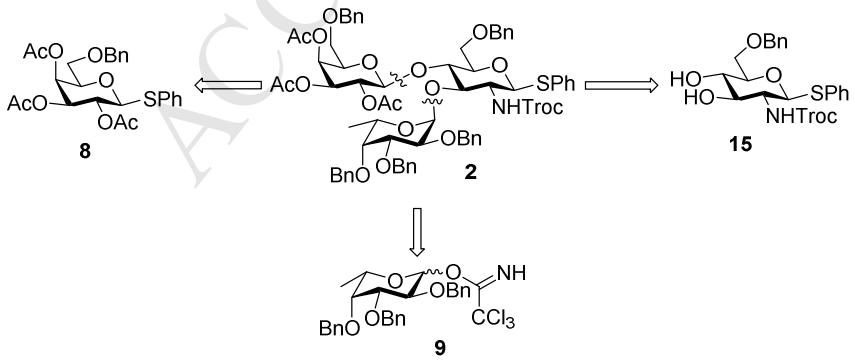
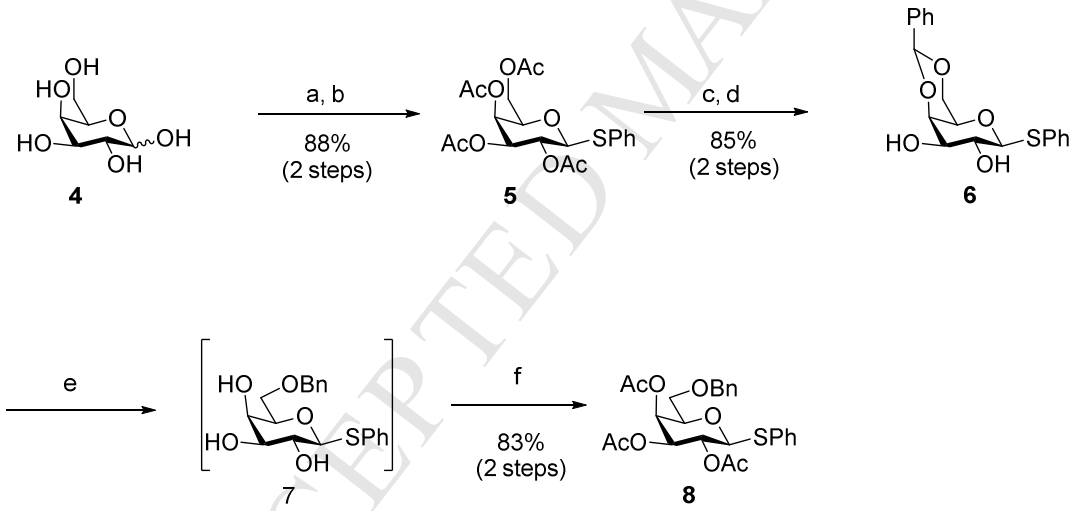


Figure 2. Building blocks for Le^X donor **2**.

The synthesis of **8** commenced from commercially available D-galactose **4**. As detailed elsewhere, D-galactose **4** was converted to galactose thioglycoside **5** by initial acetylation using pyridine-acetic anhydride and catalytic DMAP, followed by substitution of the anomeric OAc using BF_3OEt_2, PhSH.[52] Deacetylation of **5** under Zémplen conditions afforded the intermediate tetraol, which was converted *in situ* using $PhCH(OMe)_2$ and catalytic camphor sulfonic acid to the 4,6-*O*-benzylidine acetal protected **6**.[53] Regioselective cleavage was carried out using triethylsilane and trifluoroacetic acid to afford the 6-*O*-benzyl protected galacoside donor **7**. The free hydroxyl in **7** was subsequently acetylated using acetic anhydride and pyridine to obtain donor **8** (**Scheme 1**).[53] Of note, TES/TFA was used in the regioselective ring opening of **6**, which lead to a significantly higher yield, as compared to previously reported approaches that have used $BH_3{\cdot}NMe_3$-$AlCl_3$.

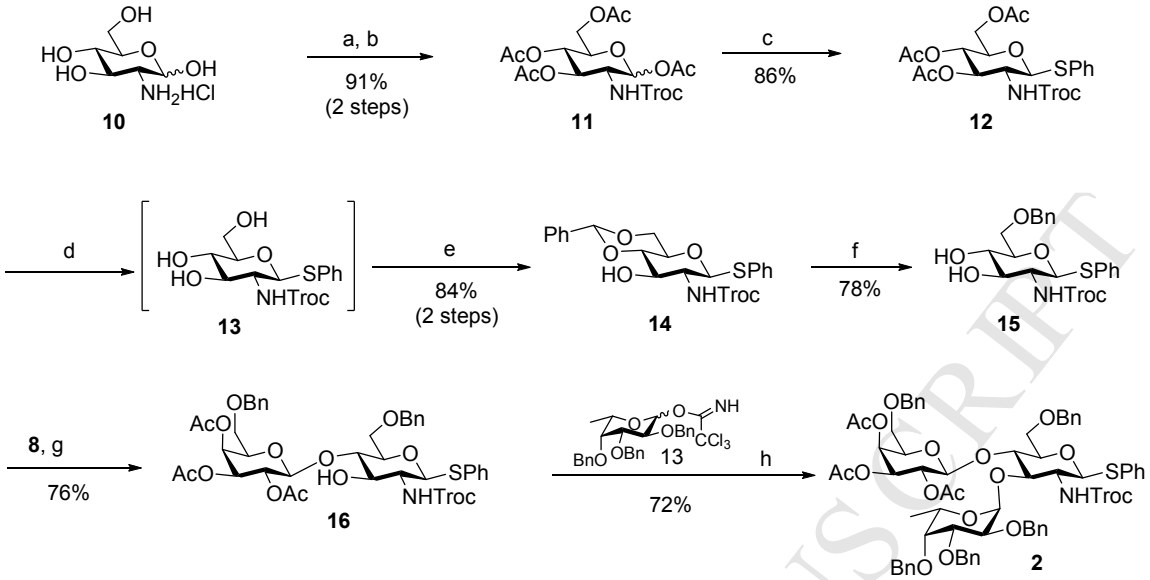


Scheme 1. Synthesis of galactose donor **5**: a) Py, Ac_2O, DMAP(cat): b) PhSH, BF_3 OEt_2, CH_2Cl_2, rt, 12h: c) 0.2 (N) NaOMe, MeOH, 5h: d) $PhCH(OMe)_2$, cat CSA, CH_3CN, 80°C: e) TES, TFA, CH_2Cl_2, RT 3h: f) Py, Ac_2O, DMAP(cat), rt, 3h: TES = triethylsilane, TFA = trifluoroacetic acid, DMAP = 4-dimethylaminopyridine.

D-Glucosamine was selectively protected using TrocCl and sodium bicarbonate as an initial step to synthesize acceptor **15**. The Troc protected glucosamine was then converted to tetracetylated D-glucosamine **11** by pyridine and acetic anhydride. Compound **11** was then converted to thioglycoside **12** by treatment with BF_3-OEt_2 and PhSH. Deacetylation under

Zémplen conditions afforded triol **17**, which was converted *in situ* to a 4,6-O-benzylidene acetal **14** by $PhCH(OMe)_2$ and catalytic CSA.[54] Regioselective ring opening of **14** afforded acceptor **15**.

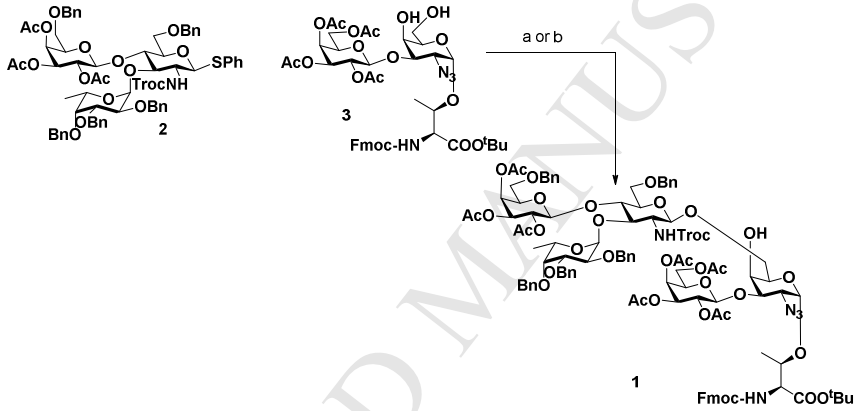
The approach of selecting either galactosylation or fucosylation of diol **15** was influenced by prior studies by Ellervik, Cao, and colleagues.[37,53] While the galactosylation of a similar diol acceptor resulted in a (1→4) linked glycan, exclusively, fucosylation was associated with a 3.6:1 ratio favoring the (1→3) linked glycan. Hence, the synthesis of thioglycoside **2** was initiated by assembling Galβ(1→4)-GalNAc **16** using iterative glycosylation. Preactivation of thioglycoside donor **8** at -70°C using BSP in the presence of triflic anhydride, followed by the addition of acceptor **15** afforded thioglycoside **16** in 76% yield (**Scheme 3**). It was imperative to quench the excess promoter with 1-octene before addition of the acceptor in the reaction mixture.[55] This low temperature pre-activation strategy enabled selective activation of thioglycoside in **8** without activation of thioglycoside in **15**. As a consequence, byproducts arising from oligomerization of **15** were minimized and overall yield of the desired disaccharide **16** improved. The outcome of the glycosylation reaction between donor **8** and acceptor **15** was in agreement with previous reports[53,56] and afforded the β(1→4) linked product, which was confirmed by COSY and HSQC NMR spectra (Supporting Information). Specifically, the coupling constant of the anomeric proton (4.50 ppm, J = 8.0 Hz) and the downfield ^{13}C chemical shift (78.0 ppm) of glucosamine C4 were consistent with β(1→4) linkage formation. The resulting disaccharide **16** was readily fucosylated using fucosyl imidate donor **9**[57] and activated by TMS-OTf catalyst at -20°C to obtain the Le^X thioglycoside **2** in 72% yield. Sterically crowded 3-OH group of glucosamine is known to be a poor acceptor and excess fucose donor was required to drive the reaction to completion.[58] The α(1→3) linkage was confirmed by COSY and HSQC NMR spectra, as demonstrated by the coupling constant of the fucosyl anomeric proton (5.15 ppm, J = 3.6 Hz).



Scheme 3. Synthesis of sLe^X thioglycoside: a) TrocCl, $NaHCO_3$, H_2O, 0°C: b) Py, Ac_2O, DMAP(cat), c) PhSH, BF_3 OEt_2, CH_2Cl_2, 12h, rt: d) 0.1 (N) NaOMe, MeOH, 2h: e) $PhCH(OMe)_2$, cat CSA, CH_3CN: f) TES, TFA, CH_2Cl_2, RT 3h: g) BSP, Tf_2O, octene, 4 Å MS, CH_2Cl_2, -60°C, 1h: h) TMS-OTf, 4 Å MS, CH_2Cl_2, -20°C, 1h: CSA = (1S)-(+)-10-camphorsulfonic acid, BSP = 1-benzeneylsulfinylpiperidine, MS = molecular sieves.

We performed a [3+2] glycosylation between the Le^X thioglycoside and the Core-1 diol[33] using 1-benzenesulfinylpiperidine (BSP), Tf_2O promoter[59], and 2,4,6-tri-*tert*-butylpyrimidine TTBP at -60°C. Unfortunately, the yield was poor and a significant amount of hydrolyzed donor was recovered (**Table 1**, entry 1). With the presumption that the reaction temperature was too low for such bulky donor and acceptor, we explored promoters suitable for activation at higher temperature. Dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST)[60] was found to be compatible with room temperature activation. Unfortunately, the yield continued to be poor and a new regioisomer was formed, as observed in ^{1}H NMR (**Table 1**, entry 2). Furthermore, de-fucosylated tetrasaccharide was observed by MALDI-TOF, highlighting the acid lability of the highly armed fucopyranoside. As a consequence, we investigated the NIS/TfOH[61,62] promoter and performed the glycosylation at a lower temperature. While yield improved, regioselectivity and cleavage of fucose, though minimized, continued to be problematic (**Table 1**, entry 3). Similar reaction conditions with a different solvent system (DCM-toluene) did not improve the regioselectivity. While unexpected, we have previously observed the formation of a similar 1→4

linkage.[33] We speculate that the intermediate oxazolinium ion reacts with the 6-OH group via the exocyclic carbon and upon intramolecular rearrangement forms the 1→4 linked product. It was apparent that the stability of the fucose and regioselectivity were both dependent on reaction temperature. Therefore, we attempted the glycosylation reaction at -40°C using NIS/TfOH activation with improved yield and regioselectivity. Anticipating that the presence of base might facilitate the formation of an inert oxazoline, we explored glycosylation BSP-Tf_2O activation without TTBP and observed optimal yield with only a single regioisomer (**Table 1**, entry 6).



Scheme 4. [3+2] Glycosylation: a) BSP, Tf_2O, 4 Å MS, CH_2Cl_2, -60°C, 1h, 84%: b) NIS, TfOH, 4 Å MS, CH_2Cl_2, -40°C, 1h, 81%.

Table 1

Entry	Promoter	Temperature	Solvent	Yield	Selectivity*
1	BSP, TTBP, Tf_2O	- 60°C	DCM	48%	20:1
2	DMTST	r.t.	DCM	40%	3:1
3	NIS, TfOH	0°C	DCM	54%	4:1
4	NIS, TfOH	0°C	DCM/Toluene(3:1)	52%	4:1
5	NIS, TfOH	- 40°C	DCM	81%	10:1
6	BSP, Tf_2O	- 60°C	DCM	84%	20:1

*ratio between 1→6 and 1→4 linked regioisomer

Structural assignment of the final pentasaccharide was made by a combination of 1-D (^{1}H and ^{13}C) and 2-D (COSY, HSQC, and HMBC) NMR spectroscopy (Supporting information). ^{13}C NMR spectra of the Core-1 diol and pentasaccharide **1** revealed a significant downfield shift of C6 of GalN_3Thr, while C4 was unchanged indicating the formation of 1→6 linkage. Furthermore, HMBC correlation between H6 protons of GalN_3 and C1 of GlucNAc confirmed the formation of 1→6 linkage. Additionally, a large coupling constant of GlcNHTroc H1 (4.62 ppm, *J* = 10.2 Hz) confirmed the formation of the β-linkage.

In conclusion, we were successful in synthesizing a protected Le^X bearing Core-1-*O*-threonine pentasaccharide, and the anomeric linkage was assigned by 2-D NMR. Major synthetic hurdles involving efficient development of Le^X and regio- and enantioselective [3+2] glycosylation were resolved. The synthetic methodology described herein can serve as a strategy to afford various glycoamino acid analogues in high yield. Furthermore, the protected C2-O-Le^X pentasaccharide **1** will serve as an important building block in future efforts directed at the synthesis of a variety of target glycoproteins to probe the biological roles of Le^X in the context of diverse aglycone peptide sequences.

3. Experimental

3.1 General

All reactions were performed under inert atmosphere of nitrogen or argon, unless otherwise noted. D-Galactose, D-glucosamine hydrochloride, and L-fucose were purchased from Carbosynth LLC, CA. All other reagents were purchased from commercial sources and used directly. All solvents were dried and distilled following standard protocols. All glycosylation reactions were performed in oven dried round bottom flasks. Proton nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectra were recorded with a Varian 400-MHz spectrometer. High Resolution Mass spectra (HRMS) were acquired using an Orbitrap Lumos MS (Thermo Fisher Scientific, CA). Thin layer chromatography (TLC) was performed on silica gel matrix,

with a 254 nm fluorescent indicator, and flash column chromatography purification performed on Silica Gel 60 (Sigma-Aldrich Corporation, WI).

3.4. Phenyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-1-thio- β-D-galactopyranoside (8)

TFA (5.3 mL, 68 mmol) and triethylsilane (10.9 mL, 68.0 mmol) were added to a solution of galactose diol **6** (4.68 gm, 13.0 mmol) in CH_2Cl_2 (50 mL) at 0°C and stirred at room temperature for 3 h. The reaction was monitored by TLC and upon completion of the reaction, concentrated *in vacuo*. The crude residue was dissolved in pyridine (50 mL), followed by the addition of Ac_2O (30 mL) and DMAP (245 mg, 2.0 mmol), which was stirred at room temperature for 12 h. The reaction mixture was concentrated and dissolved in EtOAc (100 mL), washed with satd aq NaHCO3 (2 x 50 mL), brine (100 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo.* The crude residue was purified by column chromatography over silica gel using 30% EtOAc in hexanes to obtain a white solid (5.4 gm, 85%). The NMR spectra for **8** matched those previously reported.[53] ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.52-7.49 (2H, m), 7.33-7.26 (8H, m), 5.49 (1H, dd, *J* 0.8, 3.2 Hz), 5.23 (1H, t, *J* 10.0 Hz), 5.05 (1H, dd, *J* 3.2, 10.0 Hz), 4.73 (1H, d, *J* 10.0 Hz), 4.54 (1H, d, *J* 11.6 Hz), 4.42 (1H, d, *J* 11.6 Hz), 3.89 (1H, td, *J* 1.2, 6.0 Hz), 3.60 (1H, dd, *J* 6.0, 9.6 Hz), 3.50 (1H, dd, *J* 6.4, 9.6 Hz), 2.08 (3H, s), 2.03 (3H, s), 1.97 (3H, s).

3.8. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranoside (11)

To a solution of D-glucosamine hydrochloride (20 g, 92.8 mmol) in H_2O (250 mL) satd aq $NaHCO_3$ (250 mL) and 2,2,2-trichloroethyl chloroformate (14.05 mL, 102 mmol) were added and stirred vigorously for 18 h at room temperature. The reaction mixture was filtered through a fritted funnel and the white solid was dried under vacuum overnight. A solution of the solid in pyridine (100 mL) was cooled to 0°C, to which Ac_2O (100 mL) was added slowly, followed by DMAP (500 mg, 41 mmol). The solution was warmed to room temperature and stirred for 12 h. The solution was then diluted with CH_2Cl_2 (200 mL), washed with 1M HCl (3 x 100 mL), satd aq

$NaHCO_3$ (2 x 100 mL), brine (100 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*, to afford glucosamine **11** as a white foam (44.3 g, 91%). The NMR spectra for **11** matched those previously reported.[54] ^{1}H NMR (400 MHz; $CDCl_3$) δ: 6.23 (1H, d, *J* 3.4 Hz), 5.28 (1H, dd, *J* 10.7, 9.6 Hz), 5.20 (1H, t, *J* 9.8 Hz), 5.14 (1H, d, *J* 9.3 Hz, NHTroc), 4.82 (1H, d, *J* 12.1 Hz), 4.62 (1H, d, *J* 12.1 Hz), 4.28 (1H, dd, *J* 12.4, 4.0 Hz), 4.20 (1H, ddd, *J* 10.7, 9.4, 3.8 Hz), 4.06 (1H, dd, *J* 12.5, 2.4 Hz), 4.06-4.01 (1H, m), 2.20 (3H, s), 2.09 (3H, s), 2.04 (6H, 2xs).

3.9. Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (12)

Glucosamine **11** (14.5 g, 27.8 mmol) was dissolved in anhydrous CH_2Cl_2 (150 mL) and cooled to 0°C. Thiophenol (6.8 mL, 66 mmol) and BF_3.OEt_2 (6.6 mL, 55.4 mmol) were added and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted in CH_2Cl_2 (100 mL), washed with water, aqueous $NaHCO_3$ (saturated) (2 x 100 mL), dried over $MgSO_4$, filtered, and concentrated. Purification by column chromatography over silica gel using 30% EtOAc in hexanes as eluent afforded thioglycoside **12** (13.7 gm, 86 %) as a white foam. The NMR spectra for **12** matched those previously reported.[54] ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.55-7.48 (2H, m), 7.35-7.28 (3H, m), 5.32 (1H, d, *J* 9.2 Hz), 5.28 (1H, t, *J* 9.8 Hz), 5.02 (1H, t, *J* 9.7 Hz), 4.86 (1H, d, *J* 10.3 Hz), 4.79 (1H, d, *J* 12.0 Hz), 4.72 (1H, d, *J* 12.0 Hz), 4.23 (1H, dd, *J* 12.4, 5.3 Hz), 4.16 (1H, dd, *J* 12.4, 2.5 Hz), 3.77-3.65 (2H, m), 2.07 (3H, s), 2.00 (3H, s),1.99 (3H, s).

3.10. Phenyl 4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonyl-amino)-β-D-glucopyranoside (14)

Glucosamine thioglycoside **12** (5.2 gm, 9.1 mmol) was dissolved in MeOH (100 mL) and cooled to 0°C. A freshly prepared 0.2 (N) solution of NaOMe in MeOH (100 µL) was added dropwise and the reaction mixture stirred for 5h at room temperature. The reaction was then quenched with acetic acid (300 µL) before concentrating in *vacuo*. The resulting deacetylated

crude was dissolved in MeCN (100 mL), after which $PhCH(OMe)_2$ (5.5 mL, 36.7 mmol) and CSA (464 mg, 2.0 mmol) were added and the reaction mixture stirred for 4h at 80°C. The reaction was subsequently quenched by the addition of Et_3N (2 mL), concentrated *in vacuo*, and purified by column chromatography over silica gel using 30% EtOAc in hexanes to obtain **14** as a white solid (4.1 gm, 84 %). The NMR spectra for **14** matched those previously reported.[54] ^1H NMR (400 MHz; $CDCl_3$) δ 7.53-7.45 (4H, m), 7.40-7.30 (6H, m), 5.54 (1H, s), 5.29 (1H, d, *J* 7.5 Hz, 1H), 4.91 (1H, d, *J* 10.2 Hz), 4.82 (1H, d, *J* 12.0 Hz), 4.71 (1H, d, *J* 12.0 Hz), 4.37 (1H, dd, *J* 10.6, 4.6 Hz), 4.08-3.96 (1H, m), 3.79 (1H, t, J 10.3 Hz), 3.57-3.40 (3H, m), 2.90 (1H br s).

3.11 Phenyl 6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonyl-amino)-β-D-glucopyranoside (15)

Trifluoroacetic acid (1.42 mL, 18.5 mmol) was added to a solution of **14** (2 gm, 3.7 mmol) and trimethylsilane (2.95 mL, 18.5 mmol) in CH_2Cl_2 (30 mL) at 0°C. The reaction mixture was then warmed to room temperature and stirred for 3 h. The reaction mixture was subsequently diluted in chloroform (20 mL), washed with water, satd aq $NaHCO_3$ (2 x 20 mL), dried over $MgSO_4$, filtered and concentrated. Purification was performed by column chromatography over silica gel using 40% EtOAc in hexanes as eluent, which afforded thioglycoside **15** as a white foam (1.54 gm, 78%). $[\alpha]_D^{24}$ 28.8 (C = 1.0, $CHCl_3$), R_f 0.42 (hexane/EtOAc, 1:1). ^1H NMR (400 MHz; $CDCl_3$) δ 7.49-7.30 (10H, m, 2 Ph), 5.64 (1H, d, *J* = 5.2 Hz, NHTroc), 5.58 (1H, d, *J* = 7.6 Hz, H-1), 4.78 (1H, d, *J* = 12.0 Hz, Troc-CH_2), 4.71 (1H, d, *J* = 12.0 Hz, Troc-CH_2), 4.62 (1H, d, *J* = 11.8 Hz, $PhCH_2$), 4.55(1H, d, *J* = 11.8 Hz, $PhCH_2$), 4.31 (1H, m, H-4), 4.12 (1H, m, H-2), 3.82 (1H, dd, *J* = 4.8, 10.8 Hz, H-6), 3.75 (1H, dd, *J* = 4.4, 10.0 Hz, H-6), 3.71-3.64 (2H, m, H-3, H-5), 3.34 (1H, br s, OH), 3.33 (1H, br s, OH). ^{13}C NMR (100.5 Hz, $CDCl_3$) δ 155.7, 137.6, 136.6, 131.9, 130.1, 129.1, 128.5, 128.4, 127.9, 127.8, 88.8 (C-1), 74.8, 73.7 (C-5), 73.1 (C-3), 72.8, 71.1 (C-4), 69.9 (C-6), 55.5 (C-2), ESIHRMS m/z calcd for $C_{22}H_{24}Cl_3NO_6SNa$ $[M + Na]^+$ 558.0288, found 558.0276.

3.12 Phenyl 3,4,6-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl-(1→4)-6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonyl-amino)-β-D-glucopyranoside (20)

A mixture of phenyl thiogalactopyranoside **8** (244 mg, 0.5 mmol), BSP (126 mg, 0.6 mmol), and 4 Å molecular sieves (300 mg) was dried under vacuum for 0.5 h. Freshly distilled CH_2Cl_2 (10 mL) was added, and the reaction mixture stirred for 0.5 h at room temperature, then cooled to -65°C and stirred for an additional 5 min before the addition of Tf_2O (120 µL, 0.64 mmol). The reaction mixture was stirred for 30 min at −65 to −60°C and then was cooled to −78°C and stirred for 5 min before 1-octene (0.6 mL, 4 mmol) was added and stirring continued for an additional 5 min. A solution of thioglucopyranoside acceptor **15** (288 mg, 0.54 mmol) in CH_2Cl_2 (5 mL) was added dropwise, and the reaction mixture stirred for 1.5 h at -60°C before it was quenched with Et_3N (0.5 mL) and warmed to room temperature. The reaction mixture was filtered through Celite, concentrated, and purified by chromatography over silica gel using 30% EtOAc in hexanes as eluent to afford disaccharide **16** as a white foam (348 mg, 76 %). $[\alpha]_D^{24}$ -54.2 (C = 1.0, $CHCl_3$), R_f 0.34 (hexane/EtOAc, 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53-7.22 (15H, m, 3 Ph), 5.36 (1H, d, *J* 2.8 Hz, H-4'), 5.19 (1H, s, NHTroc), 5.15 (1H, dd, *J* 3.8, 10.6 Hz, H-2'), 4.95 (1H, dd, *J* 3.6, 10.4 Hz, H-3'), 4.92 (1H, d, *J* 10.0 Hz, H-1), 4.80 (1H, d, *J* 11.8 Hz, $PhCH_2$), 4.71 (1H, d, *J* 12.0 Hz, $PhCH_2$), 4.65 (1H, d, *J* 11.8 Hz, $PhCH_2$), 4.52 (1H, t, *J* 11.8 Hz, $TrocCH_2$), 4.50 (1H, d, *J* 8.0 Hz, H-1'), 4.39 (1H, d, *J* 12.4 Hz, $TrocCH_2$), 4.27 (1H, s, OH), 3.89 (1H, t, *J* 8.0 Hz, H-3), 3.84 (1H, t, *J* 6.4 Hz, H-5'), 3.73-3.61 (3H, m, H-6, H-4), 3.54-3.48 (1H, t, *J* 7.2 Hz, H-5, H-6'), 3.46-3.39 (2H, m, H-2, H-6'), 2.05 (3H, s, CH_3), 1.98 (3H, s, CH_3), 1.97(3H, s, CH_3); ^{13}C NMR (100.5 Hz, $CDCl_3$) δ 170.0, 169.9, 169.2, 153.9, 138.0, 137.0, 132.7, 132.5, 128.9, 128.5, 128.4, 128.0, 128.9, 128.8, 128.7, 101.2 (C-1'), 86.0 (C-1), 80.9 (C-5), 78.0 (C-4), 74.5 (C-3), 73.6 ($PhCH_2$), 73.3 (C-5'), 72.4 (NHTroc), 70.9 (C-3'), 69.0 (C-2'), 68.2 (C-6), 67.3 (C-6'), 67.2 (C-4'), 56.6 (C-2), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3); ESIHRMS m/z calcd for $C_{41}H_{46}Cl_3NO_{14}SNa$ $[M + Na]^+$ 936.1597, found 936.1599.

3.13 Phenyl 3,4,6-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-α-(1→3)]-6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonyl-amino)-β-D-glucopyranoside (2)

Trichloroacetimidate **9**[57] (404 mg, 0.7 mmol), disaccharide acceptor **26** (340 mg, 0.37 mmol), and 4 Å freshly activated molecular sieves (300 mg) were stirred in freshly distilled CH_2Cl_2 (10 mL) for 1 h at -30°C. TMSOTf (18.4 µL, 0.1 mmol) was added dropwise and the reaction mixture stirred for 1 h at -15°C. Progress of reaction was monitored by TLC and upon consumption of the acceptor, the reaction mixture was quenched with diisopropylethylamine (1 mL). The reaction mixture was filtered through Celite, concentrated and purified by chromatography over silica gel with 35% EtOAc in hexanes to obtain Le^X trisaccharide **2** (354 mg, 72 %) as a white foam. $[\alpha]_D^{24}$ -28.4 (C = 1.0, $CHCl_3$), R_f 0.42 (hexane/EtOAc, 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.18 (m, 30H, 5 Bn, Ph), 5.49 (1H, d, *J* 7.2Hz, TrocNH), 5.39 (1H, d, *J* 3.2Hz, H-4'), 5.21 (1H, d, *J* 10.0 Hz, H-1), 5.15 (1H, d, *J* 3.6 Hz, H-1''), 5.01 (1H, dd, *J* 8.0, 10.0 Hz, H-2'), 4.96 (1H, d, *J* 11.8 Hz, $PhCH_2$), 4.84 (1H, d *J* 11.4 Hz, $PhCH_2$), 4.84 (1H, d, *J* 11.4 Hz, $PhCH_2$), 4.75-4.69 (5H, m), 4.66 (1H, d, *J* 11.6 Hz, $PhCH_2$), 4.66 (1H, d, *J* 11.6 Hz, $PhCH_2$), 4.64 (1H d, *J* 7.2 Hz, H-1'), 4.57 (1H, d, *J* 6.4 Hz, H-5''), 4.49 (1H, d, *J* 12.0 Hz, $PhCH_2$), 4.43 (1H, d, *J* 12.4 Hz, $TrocCH_2$), 4.27 (1H, d, *J* 12.4 Hz, $TrocCH_2$), 4.17 (1H, t, *J* 8.8 Hz, H-3), 4.10 (1H, dd, *J* 4.7, 10.2 Hz, H-2''), 3.92 (1H, t, *J* 8.8 Hz, H-4), 3.89 (1H, dd, *J* 2.4, 11.2 Hz, H-3''), 3.82 (1H, dd, *J* 3.0, 11.4 Hz, H-6'), 3.76 (1H, dd, *J* 1.8, 11.0 Hz, H-6'), 3.58 (1H, s, H-4''), 3.56-3.50 (2H, m, H-5, H-6), 3.42 (1H, d, *J* 8.8 Hz, H-5'), 3.32 (1H, dd, *J* 5.2, 13.6 Hz, H-6), 3.30 (1H, m, H-2), 1.96 (6H, s, $2CH_3$), 1.81 (3H, s, CH_3), 1.19 (3H, d, *J* 6.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.8, 169.7, 168.9, 153.5, 138.7, 138.6, 138.4, 137.8, 137.4, 133.1, 132.3, 128.9, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.1, 99.7 (C-1'), 99.0 (C-1'), 95.4 (Troc-CCl_3), 84.6 (C-1), 80.3 (C-4), 79.3, 75.5 (C-3), 74.5 (C-3'), 74.3 (C-4''), 74.1 (C-5), 73.5, 73.2, 72.8, 71.4, 71.1 (C-2'), 69.1, 67.9 (C-4'), 67.3, 66.6 (C-5''), 66.4,

57.8 (C-2), 20.7, 20.6, 20.5, 16.8 (C-6''); ESIHRMS m/z calcd for $C_{68}H_{75}Cl_3NO_{18}SNa$ $[M + Na]^+$ 1352.3584, found 1352.3583.

3.14 *N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonyl-amino)-β-D-glucopyranosyl]-(1→6)-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-*O*-2-azido-2-deoxy- α-D-galactopyranosyl]-L-threonine *tert*-butyl ester (1)

3.14.1 Method A: Le^X thioglycoside **2** (340 mg, 0.26 mmol), Core-1 diol **3** (357 mg, 0.38 mmol), and 4 Å freshly activated molecular sieves (200 mg) were stirred in freshly distilled CH_2Cl_2 (8 mL) for 1 h at room temperature. The reaction mixture was cooled to -60°C before adding BSP (75 mg, 0.36 mmol) and Tf_2O (60 µL, 0.36 mmol). Progress of the reaction was monitored by TLC, and upon consumption of the donor, the reaction mixture was quenched with diisopropylethylamine (2 mL). The reaction mixture was then filtered through Celite, concentrated and purified by chromatography over silica gel with 60% EtOAc in hexanes to obtain pentasaccharide **1** as a white foam (442 mg, 81%).

3.14.2 Method B: Le^X thioglycoside **2** (340 mg, 0.26 mmol), Core-1 diol **3** (357 mg, 0.38 mmol), and 4 Å freshly activated molecular sieves (200 mg) were stirred in freshly distilled CH_2Cl_2 (8 mL) for 1 h at room temperature. The reaction mixture was cooled to -40°C before adding NIS (137 mg, 0.61 mmol) and TfOH (4.4 µL, 0.05 mmol). Progress of the reaction was monitored by TLC and upon consumption of the donor, the reaction mixture was quenched with diisopropylethylamine (2 mL). The reaction mixture was then filtered through Celite, concentrated and purified by chromatography over silica gel with 60% EtOAc in hexanes to obtain pentasaccharide **1** as a white foam (459 mg 84%). $[\alpha]_D^{24}$ 23.8 (C = 1.0, $CHCl_3$), R_f 0.28 (hexane/EtOAc, 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (2H, d, *J* 7.6 Hz Fmoc H), 7.65 (2H, d, *J* 7.2 Hz, Fmoc H), 7.40 (2H, dt, *J* 7.6 Hz, 2.4 Hz, Fmoc H), 7.36-7.11 (27H, m, 5Bn, Fmoc), 5.64

(1H, d, *J* 9.2 Hz, FmocNH), 5.48 (1H, d, *J* 6.4 Hz, TrocNH), 5.41 (1H, d, *J* 3.2 Hz, H-4'), 5.37 (1H, d, *J* 3.6 Hz, H4''''), 5.29 (1H, dd, *J* 8.0 Hz, 10.4 Hz, H-2'), 5.15 (1H, br s, H-1''''), 5.04 (1H, dd, *J* 3.4 Hz, 6.6 Hz, H-3'), 5.00-4.92 (3H, m, H-1, H-2, $PhCH_2$), 4.86 (1H, d, *J* 9.2 Hz, H-1''), 4.82-4.60 (12H, m, H-3'''', H-1''', $5PhCH_2$), 4.54 (2H, d, *J* 7.6 Hz, H-1'''',H-5'''), 4.49 (1H, dd, J 6.0, 8.8 Hz, H-6), 4.47-4.40 (3H, m, $FmocCHCH_2$, $TrocCH_2$), 4.38 (1H, d, *J* 6.4 Hz, ThrH-2), 4.31-4.24 (4H, m, H-6, $TrocCH_2$, Thr H-1, FmocCH), 4.16-4.07 (5H, m, H-3'', H-2''', 2H-6', H-5'), 3.99-3.86 (4H, m, H-5, H-3'', H-3''', H-4'', H-5', H-4), 3.76 (1H, dd, *J* 3.4, 10.2 Hz, H-6a'), 3,71 (1H, s), 3.67 (1H, dd *J* 2.0, 10.0 Hz, H-6b'), 3.58 (1H, d, *J* 1.6 Hz, h-4'''), 3.53-3.47 (3H, m, H-6a'''', H-2, H-5''''), 3.39 (1H, d, *J* 7.2 Hz, H-5''), 3.32 (1H, t, *J* 10.2 Hz, H-6b''';), 3.16 (1H, q, *J* 8.4 Hz, H-2''), 2.14 (3H, s, $OCOCH_3$), 2.11 (3H, s, $OCOCH_3$), 2.01 (3H, s, $OCOCH_3$), 1.99 (3H, s, $OCOCH_3$), 1.95 (3H, s, $OCOCH_3$), 1.94 (3H, s, $OCOCH_3$), 1.79 (3H, s, $OCOCH_3$), 1.51 (9H, s, O^tBu), 1.25 (3H, d, J 6.0 Hz, Thr-CH_3), 1.17 (3H, d, J 6.4 Hz, Fuc-CH_3); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.7, 169.5, 169.1, 168.8, 156.8, 153.5, 143.8, 141.3, 138.6, 138.3, 137.4, 128.7, 128.5, 128.2, 128.0, 127.5, 127.1, 125.2, 119.9, 101.9 (C-1''''), 100.1 (C-1'), 99.9 (C-1''), 99.7 (C-1), , 97.8(C-1'''), 95.5 (Troc-CCl_3), 82.9, 80.2, 76.7 (C-5'''') 74.8 (C-3''), 74.6 (C-5''), 74.3 (C-5'), 74.0, 73.6, 73.3, 73.0, 71.4 (C-3'), 71.0 (C-3''''), 70.7, 69.1 (C-6), 68.4 (C-2''''), 68.0 (C-4''''), 67.4 (C-6'), 66.8 (C-4'), 66.6 (C-6''), 66.4 (C-6''''), 61.3, 59.4, 58.9 (C-2), 58.6 (C-2''), 47.1 (FmocCH), 29.7, 28.0, 20.6, 19.1, 16.7 (C-6'''); ESIHRMS m/z calcd for $C_{105}H_{122}Cl_3N_5O_{36}Na$ $[M + Na]^+$ 2156.6827, found 2156.6367.

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Author contribution

M.Y.R.S., V.R.K., and E.L.C. jointly conceived the project, and directed the chemistry. M.Y.R.S. was responsible for execution of the chemistry. All authors contributed to the preparation of the manuscript.

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Highlights:

- Synthesis of Lewis X thioglycoside by iterative glycosylation.
- Regio- and stereoselective [3+2] glycosylation of the Le^X donor to the Core 1 diol acceptor.
- Synthesis of $Lewis^X$-*O*-Core-1 threonine pentasaccharide with an Fmoc-Thr handle to access a wide array of Le^X based glycopeptide analogues.