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Synthesis of a Sialic Acid α(2–3) Galactose Building Block and Its Use in a Linear Synthesis of Sialyl Lewis X

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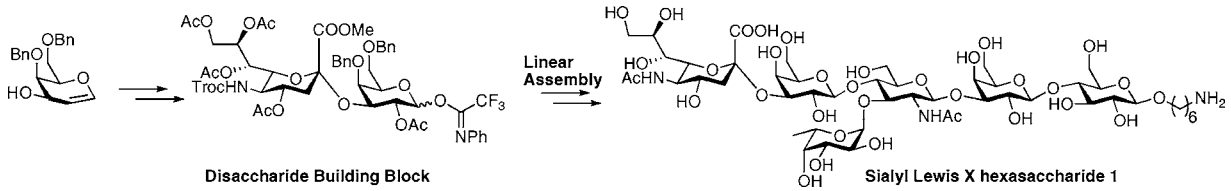
ABSTRACT



The ubiquity of the sialic acid α(2–3) galactose linkage in oligosaccharides of biological relevance necessitates a building block for the incorporation of this motif into oligosaccharides prepared by modular synthesis. The linear synthesis of the sialyl Lewis X tumor-associated antigen (1) has been accomplished in good yield using a sialic acid α(2–3) galactose disaccharide building block. The disaccharide building block was synthesized efficiently from readily available galactal by a high-yielding and selective sialylation reaction.

N-Acetylneuraminic acid (Neu5Ac) is the most abundant member of the sialic acid family in mammals.[1,2] In humans, Neu5Ac is mostly linked to either the C3- or C6-position of galactose or to the C6-position of *N*-acetylgalactosamine and glucosamine. Typically, Neu5Ac is located at the nonreducing terminus of glycolipids and glycoproteins and thus is important for interactions with proteins. The tumor-associated antigen sialyl Lewis X (SLx) **1** contains a sialic acid α(2−3) galactose moiety and has been implicated in inflammation and cancer metastasis.[3]

Because sialylation by chemical means remains a challenge, enzymatic glycosylations are often used to construct sialylated oligosaccharides.[4] Glycosylations with Neu5Ac building blocks, especially on the C3-position of galactose, often result in low yields and anomeric mixtures due to the hindered anomeric position of sialic acid and the lack of a participating group on the neighboring C3.[5] Given the ubiquity of terminal α2,3-linked sialyl galactose-capped oligosaccharides, we sought to find a disaccharide building block that would give access to these complex carbohydrates by modular solution or solid-phase synthesis. A suitable building block should be high yielding and selective in glycosylation reactions with a complex oligosaccharide partner and should be amenable to assembly on the solid phase. Here, we report the efficient synthesis of the sialic acid α(2−3) galactose building block **11** and its use in a linear synthesis of sialyl Lewis X.[6]

Sialic acid glycosylating agents carrying amine protecting groups such as carbamate,[7,8] *N*-TFA,[9] azide,[10] and imide[11,12]

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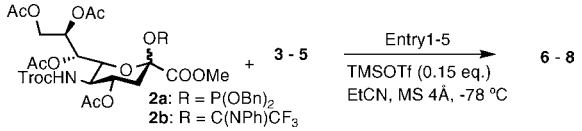
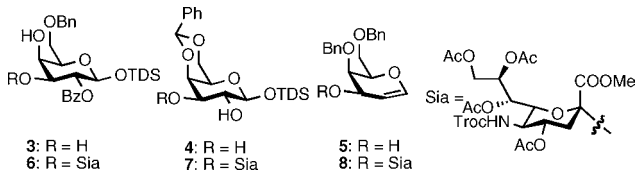
have shown improved yields and selectivities in glycosylations. In contrast, few good nucleophiles have been reported.[7b] We began our disaccharide building block synthesis by evaluating different nucleophiles in sialylation reactions (Table 1). Glycals are excellent precursors for the preparation

Table 1. Glycosylation of Galactose Nucleophiles **3**−**5** with Sialic Acid Building Block **2**



entry	building block (equiv)	nucleophile (equiv)	yield of α-anomer (%)	α/β ratio[a]
1	**2a** (1.5)	**3** (1.0)	**6**: 44	3:1
2	**2b** (1.0)	**4** (1.5)	**7**: 56	8:1
3	**2a** (1.0)	**5** (1.5)	**8**: 76	9.5:1
4	**2a** (1.2)	**5** (1.0)	**8**: 80	11:1
5	**2a** (1.5)	**5** (1.0)	**8**: 86	11:1

[a] Determined by ^{1}H NMR after size-exclusion chromatography.

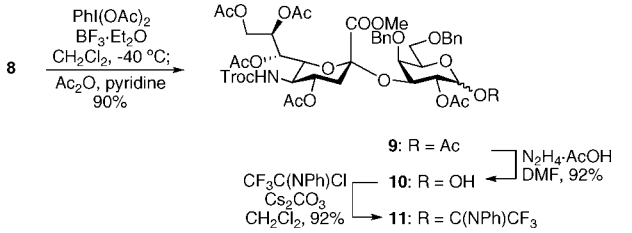


of glycosylating agents.[13] Galactal **5** is an attractive nucleophile in the sialylation reaction and would subsequently be equipped with an anomeric leaving group. We reasoned that galactal may be well suited for coupling with sialic acid because the hydroxyl group on the C3-position is sterically less hindered, due to the absence of a C2-hydroxyl. In addition, the C3-hydroxyl group is more nucleophilic than a typical galactose C3-hydroxyl group, due to its allylic nature. Few reports on the use of galactals as nucleophiles in sialylation reactions have been published.[14] This is mainly due to the instability of the olefin under the activation conditions of the common sialyl thioglycoside building blocks. We employed phosphite[15] or *N*-phenyl trifluoroacetimidate[11] leaving groups on *N*-Troc-protected building blocks for the potent reactivity and accessibility of different sialic acid derivatives such as *N*-acetyl, *N*-glycolyl, and free amine upon *N*-Troc deprotection.

Sialic acid building blocks **2a** and **2b** were activated with TMSOTf (0.15 equiv) in propionitrile[16] at −78 °C (Table 1). In the case of diol acceptors **3** and **4**, desired disaccharides **6** and **7** were obtained in moderate yield (entries 1 and 2, Table 1). In contrast, galactal **5** showed a remarkable improvement in both yield and stereoselectivity (entries 3−5, Table 1). The use of 1.5 equiv of galactal **5** with donor **2a** provided disaccharide **8** with good selectivity (α/β = 9.5:1, entry 3, Table 1). The α-anomer was isolated in 76% yield. The use of 1.5 equiv of donor **2a** provided **8** in 86% yield with the best selectivity (α/β = 11:1, entry 5, Table 1). The use of *N*-phenyl trifluoroacetimidate **2b** gave only low yields of the desired product due to decomposition of galactal.

Next, the galactal portion of disaccharide **8** was equipped with a suitable anomeric leaving group (Scheme 1). Disaccharide **8** was treated with $PhI(OAc)_2$ and a catalytic amount of $BF_3{\cdot}Et_2O$,[13a] followed by complete acetylation to produce diacetate **9** with high selectivity in one pot.[17] The anomeric acetate was cleaved with hydrazine acetate to afford hemiacetal **10**, followed by introduction of the anomeric *N*-phenyl trifluoroacetimidate to furnish building block **11** in good yield.[18,19]

Scheme 1. Transformation of **8** into Sialyl Galactose Building Block **11**



To demonstrate the viability of disaccharide building block **11**, SLx hexasaccharide **16** was synthesized (Scheme 2). Trisaccharide **12**,[20] equipped with a protected amine-containing linker for further conjugation, was glycosylated using building block **11**. The desired pentasaccharide **13** was obtained as a single anomer in excellent yield. Deprotection of the levulinoyl group using hydrazine monohydrate in

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(17) Treatment with $PhI(OAc)_2$ and $BF_3{\cdot}Et_2O$ produced a mixture of 2-OH and OAc.

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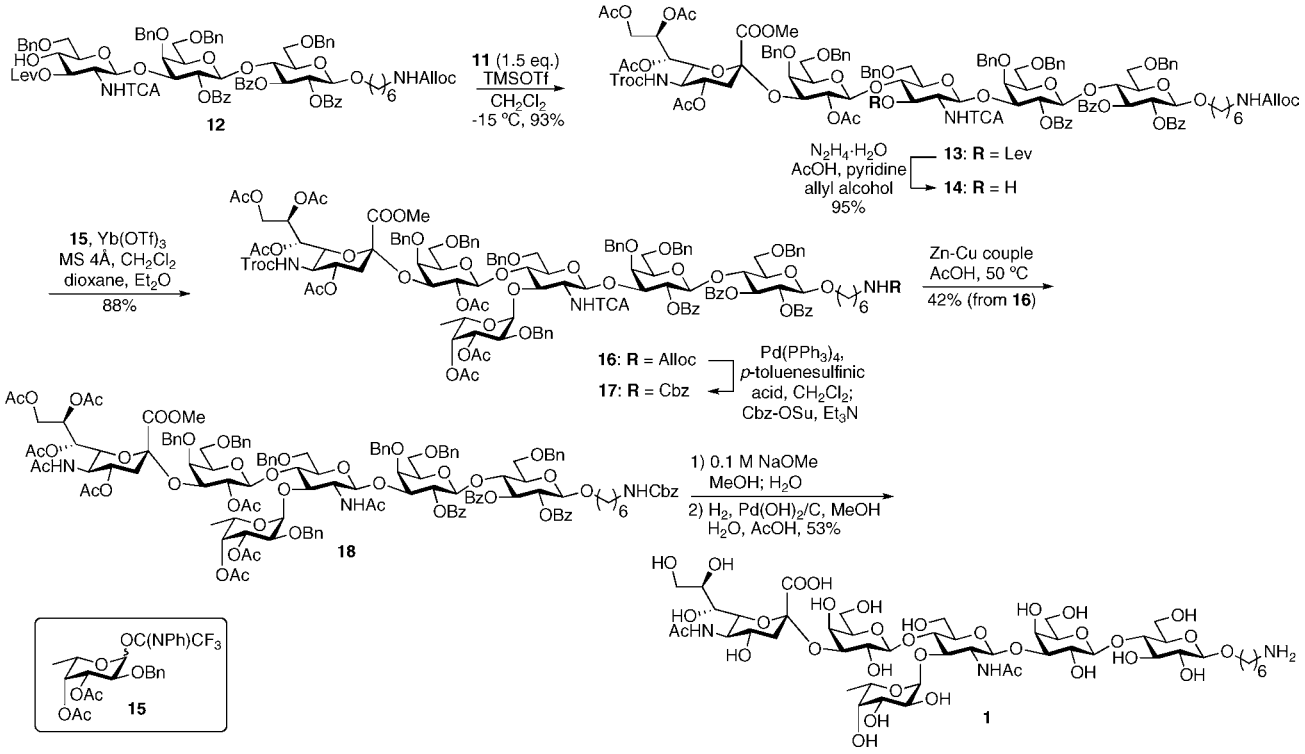
(19) The phosphate sialyl galactose building block obtained in moderate yield by the one-pot method described in ref 13b gave poor yield in the subsequent glycosylation of trisaccharide **12**.

(20) Hanashima, S.; Seeberger, P. H., submitted.

Scheme 2. Linear Synthesis of Sialyl Lewis X Hexasaccharide **1**



AcOH/pyridine in the presence of allyl alcohol[21] set the stage for a final fucosylation. Pentasaccharide **14** was glycosylated with fucose building block **15** in 88% yield using $Yb(OTf)_3$[22] as a mild activating agent to avoid the formation of byproducts derived from bisfucosylation.

Global deprotection of hexasaccharide **16** required several steps. Initially, the Alloc group was exchanged to a Cbz group followed by treatment with a Zn−Cu couple to remove the Troc group, accompanied by reduction of the trichloroacetamide (TCA), to afford **18** upon acetylation. Finally, treatment of **18** with sodium methoxide, followed by addition of water to hydrolyze the methyl ester and hydrogenolysis with 20% $Pd(OH)_2/C$, gave the desired deprotected SLx **1** ready for conjugation or printing on microarrays via the amine linker.[23]

In summary, we developed an efficient strategy to synthesize a sialic acid α(2−3) galactose building block by combining galactal nucleophile **5** and phosphite sialylating agent **2a**. The galactal moiety was readily oxidized and functionalized to afford *N*-phenyl trifluoroacetimidyl glycoside **11**. This new disaccharide building block performed well in the glycosylation of a trisaccharide, during the synthesis of SLx hexasaccharide, ready for biological applications. This disaccharide building block is currently used for the efficient chemical synthesis of other complex sialylated oligosaccharides in solution and on solid support.

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

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(21) The allyl alcohol prevents the reduction of the Alloc olefin under the reaction conditions.

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