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Total synthesis of a sialyl Lewis^x derivative for the diagnosis of cancer

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ABSTRACT

The total synthesis of aminoethyl glycoside of sialyl Lewis^x (sLe^x) is described. A galactose donor was condensed with a diol of glucosamine to afford regioselectively a β1,4 linked disaccharide, which was further stereoselectively fucosylated to provide a protected Lewis^x trisaccharide. After chemical modification, the trisaccharide was sialylated to give regio- and stereoselectively an azidoethyl glycoside of sLe^x. Finally, deprotection and azide reduction afforded the target compound. This compound will be coupled with protein and then be used to conduct further preclinical studies for the diagnosis of cancer.

1. Introduction

Metastatic spread is a major complication of cancer responsible for the majority of deaths. A key adhesion molecule associated with the capture of metastatic cells is E-selectin that is located on the surface of endothelial cells.[1,2] It is noteworthy that E-selectin expression is often enhanced at the site proximal to or directly at tumor metastases.[3]

Several E-selectin ligands have been identified on tumor cells, including PSG-1, ESL-1, Death receptor-3, and CD44, which contain sialyl Lewis^x (sLe^x) or sialyl Lewis^a (sLe^a) structure as the recognition motif.[4] SLe^x (NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc, Fig. 1) was discovered in 1990 as glycosphingolipids and glycoproteins that play a significant role in the adhesion between tumor cells and blood endothelial cells in metastases, as well as the adhesion of leucocytes to vascular wall in inflammation.[5–8] From then on, several synthetic methodologies (chemical and enzymatic) toward this tetrasaccharide have emerged in the literature despite synthetic challenges about regio- and stereoselectivity, the acid instability of the fucoside bond, and side reactions during sialylation.[9–13] Crystal structure analysis of E-selectin binding sLe^x displayed interactions between several amino acids coordinated to Ca^{2+} and fucose OHs at C-2 and C-3. Galactose OH at C-6 and neuraminic acid COOH are also involved in the binding events.[14]

The objective of our study was to search a kind of molecular probe that is able to target E-selectin through sLe^x moiety and recognize metastatic spot by imaging. The efficient synthesis of aminoethyl glycoside of sLe^x (**1**) (Fig. 2) as the targeting part is described here in detail. As an unreported sLe^x derivative, it was efficiently prepared from four building blocks through only seven steps with 18% total yield in our group. In addition, Le^x trisaccharide is the terminal moiety of numerous cell surface glycoproteins and glycolipids, correlated with selectin-mediated cell-cell recognition and adhesion processes,[15] thus aminoethyl glycoside of Le^x was also synthesized (**2**) as control (Fig. 2).

2. Results and discussion

For synthesis of the targets **1** and **2**, compounds **3**–**6** were chosen as building blocks (Fig. 3). They were prepared respectively from D-galactose,[10,16,17] D-glucosamine,[18] L-fucose[19–21] and *N*-acetyl neuraminic acid.[22,23] Then, they were condensed by glycosylation reaction to form desired trisaccharide and tetrasaccharide.

As illustrated in Scheme 1, the donor 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-D-galactopyranosyl fluoride **3a** was firstly designed to couple with the acceptor **4**, unfortunately, the designed disaccharide **7a** was not obtained despite changing reaction conditions (time, temperature, donor equivalent, solvent, and promoter), probably due to the disarmed effects and the weak reactivity (fluorine) of the donor.[24–26] Compound **3a** was then replaced by the trichloroacetimidate **3b**, which was coupled with **4** in CH_2Cl_2 using trimethylsilyl triflate (TMSOTf) as promoter to give regio- and

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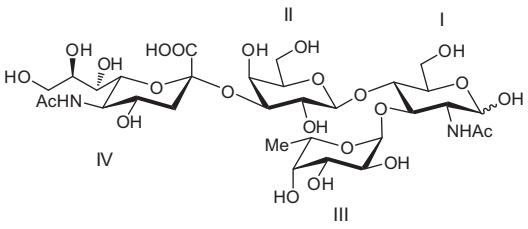

Figure 1. Structure of sLe^x.

stereoselectively β1,4 linked disaccharide **7a** in 77% yield. Such a selective behavior has been observed in our previous work.[27]

The ^{1}H NMR spectrum of **7a** displayed the presence of H-3^I of the glucosamine residue at δ 4.49 (dt, $J_{2,3}$, $J_{3,4}$ 10.4 Hz, $J_{3,OH}$ 1.8 Hz), indicating the position of the newly formed glycosidic linkage in the disaccharide **7a** to be at OH-4 of the acceptor **4**. This regioselectivity was further confirmed from the ^{1}H NMR spectrum of its acetylated derivative **7b**, which showed a deshielded signal for H-3^I at δ 5.71 (dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 8.9 Hz). Its stereochemistry was identified to be the desired β anomer on the basis of the H-1^{II}, H-2^{II} coupling constant ($J_{1,2}$ 7.9 Hz).

In order to construct the trisaccharide **8**, 2,3,4-tri-*O*-benzyl-L-fucopyranosyl fluoride (**5b**), synthesized from ethyl 2,3,4-tri-*O*-benzyl-1-thio-L-fucopyranoside **5a** (Scheme 2),[19] was used for the fucosylation of the disaccharide **7a** according to our previous work.[28] During preparation, it was observed that the reaction should be conducted at very low temperature (−90 °C), especially when the amount of **5a** was increased (more than 0.2 g). In addition, the reaction could be completed in only 10 min (Table 1).

Coupling of **5b** and **7a** in toluene/CH_2Cl_2 using silver triflate (AgOTf) and stannous chloride ($SnCl_2$) as promoters gave the trisaccharide **8** in 80% yield (Scheme 2).[28–30] The newly generated glycosidic linkage was confirmed to be α based on the low value of the Fuc H-1^{III}, H-2^{III} coupling constant ($J_{1,2}$ 3.5 Hz).

Subsequently, 2-azidoethanol, which was readily prepared from 2-bromoethanol,[31] was glycosylated with the trisaccharide **8** in the presence of *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) at −15 °C, giving compound **9** in 90% yield, as shown in Scheme 3. Treatment of compound **9** with hydrazine and water in boiling ethanol for 4 h, followed by selective N-acetylation in a mild condition for another 4 h, provided compound **10** in 88% overall yield (Scheme 3).[32]

The last building block **6** was then coupled with compound **10** in CH_2Cl_2/CH_3CN using AgOTf, benzenesulfenyl chloride (PhSCl), and di-*tert*-butylpyridine (DTBP) as promoters to give α-product in 42% yield (Scheme 4).[23,33,34] Compound **10** could not completely react, which was quite close to the product on analytical thin-layer chromatography (TLC) in all tested eluants. It needed to be purified on silica gel column for two times with different eluants and then on Sephadex column (LH20) for one time to get the pure product **11a**. Fortunately, 44% of compound **10** could be recovered from the reaction.

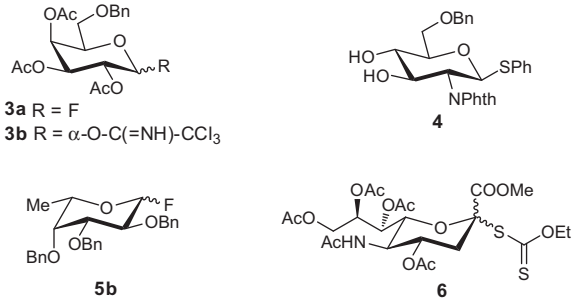

Figure 3. Key building blocks for synthesis of targets **1** and **2**.

According to our previous work, the sialylation was supposed to be regioselective at OH-3 of the acceptor **10**. This regioselectivity was confirmed from the ^{1}H NMR spectrum of its acetylated derivative **11b**. The ^{1}H NMR spectrum of **11a** displayed the presence of H-2^{II} and H-4^{II} of the galactose residue at δ 3.77–3.58, and the ^{1}H NMR spectrum of **11b** showed a deshielded signal at δ 4.95 for H-2^{II}, and another deshielded signal at δ 5.06 for H-4^{II}. Its stereochemistry was determined to be α on the basis of the chemical shift of H-3$^{IV}_e$ at δ 2.68, which was larger for α-glycosides (in the approximate range of 2.72 ± 0.05 ppm) than for β-anomers (in the approximate range of 2.32 ± 0.08 ppm).[35] In addition, the appearance of the NeuAc H-4 at ~4.8 ppm and the $J_{NeuAc7,8}$ >7.0 Hz also confirmed this stereochemistry.[36]

The acetyl groups of compound **11a** on hydroxyls were subsequently removed by reaction with NaOMe/MeOH. After adding several drops of water, compound **12** was obtained in 89% yield (Scheme 4).

We then performed reduction of azide and debenzylation of hydroxyl groups in order to get the targets **1** and **2** from compounds **12** and **10**, respectively. Surprisingly, the simultaneous debenzylation and azide reduction failed through conventional catalytic hydrogenation in MeOH. Instead, the intermediates **13a** and **13b** were generated, and they were quite unstable. No improvement was observed when Pd/C equivalent, hydrogen pressure, and reaction time were increased or acid (hydrochloric acid, acetic acid, formic acid) was added. On the basis of the structural feature of target compounds: solubility in water, toxicity of amine to Pd/C, and instability of fucoside bond to strong acid, the reaction condition was further improved. Finally, target compounds were obtained quantitatively when reaction was conducted with 1.5 equivalent of Pd/C in MeOH–H_2O–AcOH (3:1:0.5) or in THF–H_2O–AcOH (4:2:1) under an atmosphere of hydrogen (see Scheme 5).[37,38]

The compounds **1** and **2** were fully characterized by ^{1}H and ^{13}C NMR, as well as HRMS.

3. Conclusions

We have developed a concise total synthesis of aminoethyl glycoside of sLe^x with high overall yield. The disaccharide was successfully prepared through a highly regio- and stereoselective

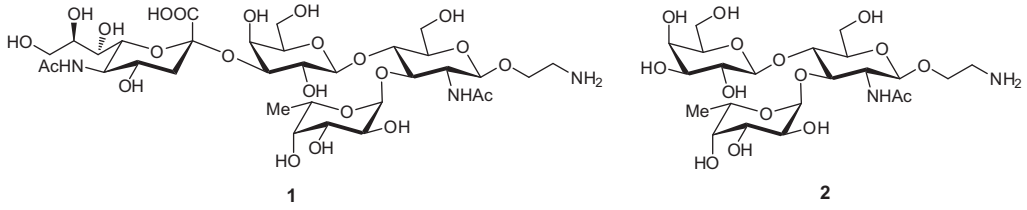

Figure 2. Aminoethyl glycoside of sLe^x **1** and aminoethyl glycoside of Le^x **2**.