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Syntheses of fluorine-containing mucin core 2/core 6 structures using novel fluorinated glucosaminyl donors

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ABSTRACT

Syntheses of fluorinated mucin core 6 disaccharides and core 2 trisaccharides modified at the C-3 or C-4 position of the pertinent glucosamine residue required for mechanistic study of glycosyltransferases and sulfotransferases involved in the biosynthesis of *O*-glycans are reported. Novel fluorinated glucosaminyl donors were synthesized from 2-naphthylmethyl β-D-*N*-acetylglucosamine (β-*O*-NAP-GlcNAc) via double inversion of the C-3 or C-4 configuration. A one-step β-alkylation of GlcNAc was reported for the first time to afford β-*O*-NAP-GlcNAc in high yield, which constitutes the cornerstone of the synthetic strategy based on NAP-glycosides in oligosaccharides synthesis.

1. Introduction

Glycosyltransferases, sulfotransferases, and sialyltransferases are widely implicated in regulating the construction of oligosaccharides, polysaccharides, and glycoconjugates in various cells and tissues.[1] Any undesirable alteration in their basic structure results in various fundamental changes in their roles in specific cell signaling and recognition events of biological processes,[2] such as chronic inflammation, cancer metastasis, cartilage formation, and hormone regulation. Thus, it became imperative to study the mechanistic pathway of these enzyme families in order to understand their role in various biological processes. Modifications to the basic enzyme acceptor structures by introduction of fluoro groups can result in highly specific acceptors or inhibitors for these individual enzymes. Consequently, this might help in exploring their catalytic mechanism and in development of therapeutics for curing the medical complications that occur due to their malfunction. In this context, several laboratories are engaged in the synthesis of fluoro substituted saccharides and their application as probes to investigate various enzymes involved in carbohydrate metabolism has been reported.[3] It is now unambiguously accepted that fluorinated carbohydrates play an important role in investigating carbohydrates–enzyme and carbohydrate–selectin interactions. For instance, an efficient route for the synthesis of UDP-5-F-GlcNAc has been reported via epoxide fluoridolysis by Hartman and Coward.[4] UDP-5-F-GlcNAc was found to behave as a β-Gal-T acceptor but competed with UDP-GlcNAc for binding to *N*-acetyl glucosaminylphosphotransferase. Feng et al.[5] reported the chemo-enzymatic synthesis of UDP-4-F-GlcNAc and UDP-4-F-GalNAc, which would serve as potential candidates for novel anticancer drugs when acting as glycosyltransferase inhibitors. Blood group A and B glycosyltransferases were shown to act on C-6 fluoro substituted Gal whereas a C-3 fluoro substituent on Gal created competitive inhibitors for both enzymes.[3j] Berkin et al.[6] accomplished the synthesis of 4-F-GlcNAc and 4-F-GalNAc, which were evaluated as inhibitors of hepatic glycosaminoglycan biosynthesis. 3-Fluoro and 4-fluoro analogs of D-glucose were found to be higher affinity substrates than D-glucose for aldolase reductase while 2-fluoro and 4-fluoro analogs of D-glucitol were inactive substrates for sorbitol dehydrogenase.[3h] Esko et al. reported a series of synthetic acetylated analogs of disaccharide peracetylated GlcNAcβ1-3Galβ-*O*-naphthalenemethanol containing –H, –F, $-N_3$, $-NH_2$, or $-OCH_3$ instead of the hydroxyl groups at C-3′ and C-4′ positions of the terminal *N*-acetylglucosamine residue. These compounds were found to reduce the formation of the glycan sialyl Lewis X in tumor cells. In addition, the reduction of sLe^x by the 4′-deoxy analog also results in diminished experimental tumor metastasis by Lewis lung carcinoma in vivo.[7]

Interest in the synthesis of fluorine modified acceptors is also augmented due to the application of the fully acetylated derivatives of various fluoro sugars as specific decoys/primers for glycosyltransferases. Endogenous carboxyesterases present in the cells remove the acetyl groups from these acetylated fluoro sugars. The resulting deacetyalted derivatives can act as acceptors for the

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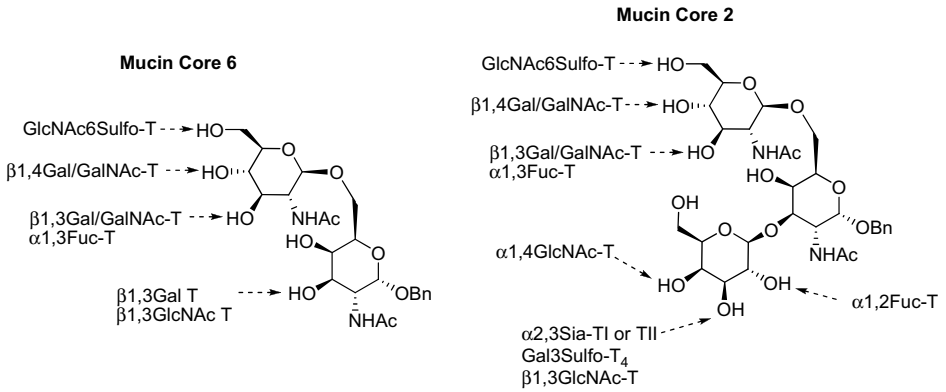

Figure 1. Glycosyltransferases and sulfotransferases acting on the mucin core 6 disaccharide and core 2 trisaccharide.

enzymes involved in the biosynthetic pathways of glycans. The anti-tumor activities of acetylated hexosamines were studied by Sharma et al.[8] and Thomas et al.[9] Matta et al. demonstrated the in vivo inhibition of ovarian tumor cell surface glycoprotein synthesis by peracetylated 3-F-GlcNAc and 4-F-GlcNAc.[10] Dimitroff et al.[3f,11] and Zollner et al.[12] introduced a new strategy to impede the selectin ligand production using peracetylated 4-F-GlcNAc as a glycosylation inhibitor. 4-F-GlcNAc was directly incorporated into native cutaneous lymphocyte associated antigen (CLA) expressed on human T cells, indicating direct inhibition of poly-*N*-acetyllactosamine elongation and selectin-binding determinants on PSGL-1 *O*-glycans. These observations establish a potential treatment approach for targeting pathologic lymphocyte trafficking to skin and indicate that 4-F-GlcNAc may be a promising agent for treatment of dermal tropism associated with malignancies and inflammatory disorder.

In addition to the exhaustive research carried out by other groups on fluoro analogs of carbohydrates for understanding the enzyme machinery, over the years our laboratory has been involved in the synthesis and examination of various fluoro acceptors for their specificity for these enzymes.[9,10,13] In this regard, we first reported the synthesis of UDP-6-F-GlcNAc and UDP-4-F-GalNAc used for the biological studies of GalNAc/GlcNAc transferases and phosphatetransferases.[13g] In this direction, the first synthesis of fluorinated mucin core 6 structure (GlcNAcβ1, 6GalNAcα-OR) using oxazoline as a 4-F-GlcNAc glycosyl donor was also reported.[9] Recently, we described the first effective syntheses and biochemical evaluation of fluorinated core 2-branched oligosaccharide analogs modified at the C-3 or C-4 position of the pertinent galactose.[13a,b] In continuation of our search for a better fluoro acceptor for various enzymes involved in biosynthesis of *O*-glycans, we report herein a straightforward syntheses of fluorinated mucin core 6 disaccharides and core 2 trisaccharides [GlcNAc-beta-1,6-(Gal-beta-1,3)-GalNAc-alpha-OR] modified at the C-3 or C-4 position of the glucosamine residue using a novel fluorinated glucosaminyl donor. These compounds would be used for the investigation of glycosyltransferases and sulfotransferases to determine the influence of enzyme activities resulting from structural modifications (Fig. 1).[14]

2. Results and discussion

2.1. Retrosynthetic analysis of fluorinated mucin core 2 and core 6 oligosaccharides

Figure 2 outlines the retrosynthetic analysis of fluorinated mucin core 2 and core 6 structures. Retrosynthetic scission of indicated bond in mucin core 6 disaccharides **1**, **2** and core 2 trisaccharides **3**, **4** provides fluorinated glucosaminyl donors **13**, **18** and glycosyl acceptors **19**, **24** as potential precursors. It was projected that C-3 or C-4 fluorinated glucosamine **13** and **18**, equipped with anomeric trichloroacetimidate function, could serve as the glycosyl donor in a Schmidt coupling reaction[15] with the primary hydroxyl group of the diol acceptor **19** and **24**. Based on the previous reports,[16] it was expected that the above coupling reaction should progress smoothly with the formation of the desired crucial β-glycosidic bond guided by the C-2 carbamate (*N*-Troc in **13** or *N*-Phth in **18**) functionality.

2.2. Retrosynthetic analysis of fluorinated glucosaminyl donors 13 and 18

The retrosynthetic analysis of key synthetic intermediates **13** and **18** is outlined in Figure 3. The 3-fluoro and 4-fluoro glucosaminyl donors could be synthesized from the same starting material, namely β-*O*-(2-naphthylmethyl)-GlcNAc **6** via double inversion of the C-3 or C-4 configuration. Use of NAP (2-naphthylmethyl) group as anomeric protection proved to be highly suitable for our purpose owing to its stability under a wide range of reaction conditions required for hydroxyl differentiation, amino protection and fluorination. Also, its chemoselective removal under DDQ oxidative conditions as required in the later part of our synthetic strategy is an added advantage.[17] The choice of a temporary protecting group for nitrogen is crucial to both fluorination and β-glycosylation. During the course of the synthesis of 4-deoxy-4-fluoro donor **13**, it was expected that the fluorination would advance smoothly in the presence of C-2 *N*-acetyl group,[5,6] whereas in the case of 3-deoxy-

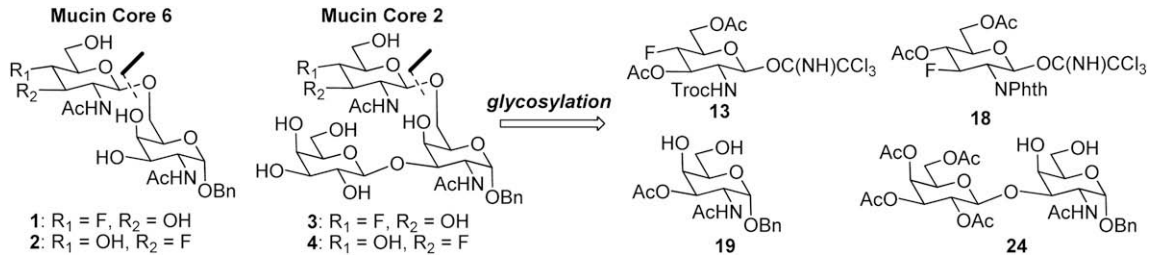

Figure 2. Retrosynthetic analysis of fluorinated mucin core 2 and core 6 structures.