Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2941–2946

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

AN EFFICIENT SYNTHESIS OF TWO MONOSULFATED TRISACCHARIDES WITH THE Galβ1,3GlcNAcβ1,3Galβ-*O*-Allyl BACKBONE

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Received 26 July 1999; accepted 7 September 1999

Abstract: The GlcNAcβ(1→3) Gal linked disaccharide **7** was synthesized as key building blocks for the construction of target monosulfated trisaccharides **1** and **2** using oxazoline **3** as glycosyl donor promoted by $BF_3{\cdot}Et_2O$

Localization of sulfate groups to defined positions in glycoproteins suggests a physiological role for these groups. The major structural motif of mucins synthesized by a highly metastatic colon carcinoma cell line has been identified as a sulfated Lewis^x determinant.[1] Mucin from a non-secretor, chronic bronchitis patient contained sulfated oligosaccharides of core 1, 2 or 4 structures, while that from secretor individuals with cystic fibrosis involved core 2 structures only.[2, 3] Such differences in the localization of sulfate in mucin oligosaccharide chains may have clinical relevance. We reported earlier that human colon Gal: 3-O-sulfotransferase can utilize only the type 1 sulfated disaccharide compound, Galβ1,3(6-O-sulfo)GlcNAcβ-O-Allyl as an acceptor.[4] Interestingly, sulfated oligosaccharide with the 3-O-sulfoGalβ1,3GlcNAcβ1,3Galβ1,3GalNAcα- structure was identified in chronic bronchitis mucin.[2] We here describe the synthesis of 3"-O-sulfo and 6'-O-sulfo derivatives of Galβ1,3GlcNAcβ1,3Galβ-O-Allyl for specificity studies of glycosyltransferases and sulfotransferases. Further preparation of acrylamide copolymers may potentially provide inhibitors of selectin binding.

Chemistry

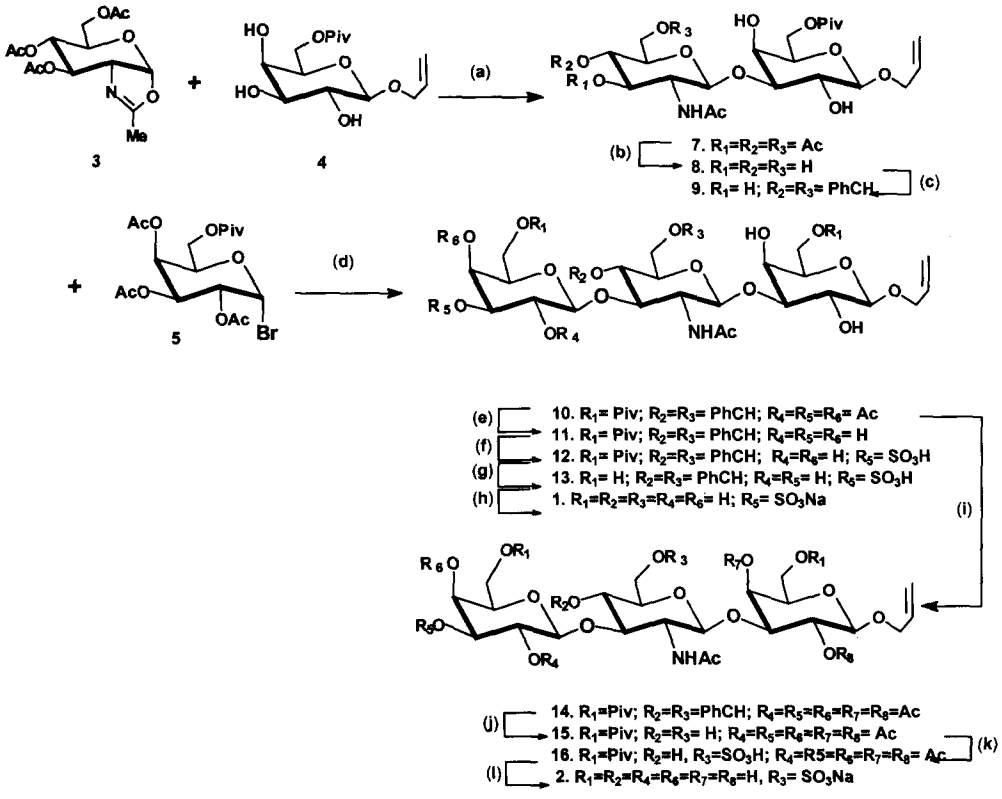
Synthesis of two monosulfated trisaccharides ($3SO_3Na$)Gal (β1→3)GlcNAcβ(1→3)Galβ-O Allyl 1 and Galβ (1→3)($6SO_3Na$)GlcNAcβ (1→3)Galβ-O Allyl 2: The synthesis of these two compounds involved the use of the key intermediates: 2-methyl (3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-*d*-glucopyrano) [2,1-*d*]-2-oxazoline **3**, allyl 6-*O*-trimethylacetyl-β-*d*-galactopyranosyl bromide **5**. The original oxazoline

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00507-7

procedure, together with its improved variations, was instrumental in the synthesis of 1,2-transglycosides.[5] It has the added advantage of preserving the integrity of the N-acetylated form of the sugar moiety during glycosylation. However, this procedure is practically ineffective for glycosylation of relatively unreactive hydroxyl groups, even under strong acid catalysis. This fact was successfully exploited in the present synthesis, and no protection was required at HO-2 and HO-4 of the galactoside acceptor. In the present investigation, glycosylation of acceptor **4** with oxazoline **3** in the presence of excess trimethylsilyl trifluoromethanesulfonate (TMSOTf),[6] at different reaction temperatures (room temperature to boiling dichloromethane) and for extended reaction times (4 to 5 days) was far from satisfactory. A discouragingly complex mixture was obtained, and the desired disaccharide **7** was obtained in only 27% yield, together with traces of the β(1→2) linked isomer. By contrast, when acceptor **4** was allowed to react with donor **3** at room temperature for 3 to 4 days in dry dichloromethane and in the presence of BF_3·Et_2O,[7] a cleaner reaction mixture was obtained, and the desired disaccharide **7** was the only major product isolated in reasonable yield. The structure of the β(1→3) linked disaccharide **7** was confirmed by ^{1}H NMR, ^{13}C NMR, and FABMS. *O*-Deacetylation of disaccharide **7** in MeOH-CH_2Cl_2 (1:1) with 1 M sodium methoxide solution at -10 °C to -5 °C for 20 min provided compound **8** in 85 to 90% yield. The 4,6-*O*-benzylidenated disaccharide acceptor **9** was obtained in 85% yield by treatment of compound **8** with α,α-dimethoxy toluene at room temperature in dry acetonitrile and in the presence of *p*-TsOH. Condensation of **9** with protected galactosyl bromide **5** (1.2 to 1.5 equiv) in the presence of $Hg(CN)_2$-$HgBr_2$ afforded the desired β(1→3) linked trisaccharide **10** as the major product in 65% yield. Interestingly, when excess of the protected galactosyl bromide **5** (2.2 equiv) was used, a β (1→2) linked trisaccharide was also obtained. *O*-Deacetylation of trisaccharide **10**, exactly as described for **7** (to give **8**), afforded **11**, which was selectively monosulfated with SO_3-pyridine complex in dry pyridine at 0 to 5 °C to provide the 3''-sulfo compound **12** as the major product.[8, 9] Deprotection of **12** then gave the target monsulfated trisaccharide **1**. The structure of **1** was confirmed by 2 D ^{1}H-^{1}H DQF-COSY, 2 D ROESY spectroscopy, ^{13}CNMR, and FABMS.[10] Acetylation of trisaccharide **10**, followed by cleavage of the benzylidene acetal of the resulting **14** with 60% HOAc at 60 to 65 °C, furnished diol **15**, which was selectively monosulfated as described for **11** (to give **12**) to provide the 6-*O*-sulfo-derivative **16**.

The target compound **2** was obtained by systematic deprotection of compound **16** as shown in Scheme 1. The structure of **2** was confirmed by 2 D ^{1}H-^{1}H DQF-COSY, 2 D ROESY spectroscopy, ^{13}CNMR, and FABMS.[10]

Scheme 1



Reagents and conditions:

(a) TMSOTf/CH_2Cl_2-4 AMS, refluxing, 4 - 5 days, yield 25-30% or $BF_3.OEt_2/CH_2Cl_2$-4AMS, rt, 3-4 days, yield 45-50%.

(b) CH_3ONa-CH_3OH/CH_3OH-CH_2Cl_2(1:1), pH 10, -10 °C to - 5 °C, 20 min, then, H^+-resin, yield 85-90%.

(c) $PhCH(OMe)_2$/*p*-TsOH, CH_3CN, rt, 40-60 min, yield 85%.

(d) **5** (1.2-1.5 equiv) $Hg(CN)_2$-$HgBr_2$, $ClCH_2CH_2Cl$, 4 AMS, 40 - 45 °C, 18 h, yield 65-70%.

(e) CH_3ONa-CH_3OH(1N)/CH_3OH-CH_2Cl_2(1:1), pH 10, -10 °C to -5 °C, 20min, yield 90%.

(f) SO_3·Pyridine/pyridine (1.2 equiv), 0 to 5 °C 5-6h, yield 80-85%.

(g) CH_3ONa-CH_3OH, rt, 1-2 h, yield 100%.

(h) 60% HOAc-H_2O, 60-65 °C, 1.5 h, yield 100%.

(i) Ac_2O/pyridine-DMAP, rt, 12 h, yield 95%

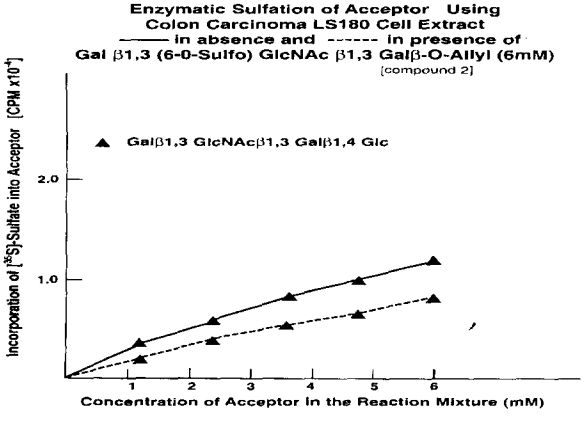
(j) 60% HOAc, 60-65°C, 0.5 h, yield 100%.

(k) SO_3·Pyridine/Pyridine(1.2 equiv), 0-5 °C, 5-6 h, yield 80-85%.

(l) CH_3ONa-CH_3OH, rt, 1-2 h, yield 100%, then, Na^+-resin, 3-4 h.

Biochemical Study

Galβ1,3(6-O-sulfo)GlcNAcβ1,3Galβ-O-Allyl 2 as an efficient acceptor for Gal: 3-O-sulfotransferases: The compounds, Galβ1,4GlcNAcβ-*O*-Allyl, Galβ1,3GlcNAcβ1,3Galβ-*O*-Allyl, and Galβ1,3(6-*O*-sulfo)GlcNAcβ1,3Galβ-*O*-Allyl (compound **2**), and Galβ1,3GlcNAcβ1,3Galβ1,4Glc were examined as acceptors for Gal: 3-*O*-sulfotransferase from human carcinoma cells *LS180* and found to be 25%, 21%, 55%, and 68%, respectively, as compared to the mucin core 2 acceptor, Galβ1,4GlcNAcβ1,6[3-*O*-MeGalβ1,3]GalNAcα-*O*-Benzyl. The disulfated product arising from compound **2** was quantitated by a TLC method since it is not elutable from the Dowex-1-column following conditions employed for the assay of monosulfated products. The acceptor efficiency of compound **2** can be easily demonstrated by competitive inhibition using the Dowex-1-Cl methodology, as illustrated in the figure.



In conclusion, the aforedescribed procedures enabled us to obtain the desired trisaccharides **1** and **2**. Structure **1** functions as a reference compound, and **2** as an efficient acceptor, as well as a useful competitive inhibitor, in studying Gal: 3-*O*-sulfotransferases. These compounds will be further employed for examining the specificity of α(1,4)-fucosyltransferases. Those results will be published elsewhere.

Acknowlegements: We thank Dr. Abbas for helpful discussions pertaining to synthetic chemistry. This work was supported by Grants No. CA-63218, CA-35329 and in part by P30 CA-16056, awarded by the National Cancer Institute.

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10. The structure assignments of compound **1** and **2** were based on 2-D ^{1}H-^{1}H DQF-COSY and 2-D ROESY spectroscopy: **1.** ^{1}H NMR (CD_3OD, 600 MHz) δ 6.20–6.00 (m, 1 H, -C**H**=C), 5.50–5.40 (dd, 1 H, C=C**H**), 5.30–5.20 (dd, 1 H, -C=C**H**), 4.80 (d, 1 H, *J* = 8.4 Hz, H-1'), 4.50 (d, 1 H, *J* = 7.3 Hz, H-1"), 4.48–4.40 (dd, 1 H, OC**H**-C=C), 4.48–4.32 (d, 1 H, *J* = 7.0 Hz, H-1, Gal), 4.25–4.20 (dd, 1 H, H-3" sulfated position), 4.25–4.15 (dd, 1 H, OC**H**C=C), 4.10 (d, 1 H, *J* = 3.2 Hz, H-4), 3.93–3.90 (dd, 1 H), 3.90–3.71

(m, 6 H, H-2', H-3', H-2", H-6a'), 3.70–3.60 (m, 3 H, H-2, H-6a, H-3), 3.60–3.50 (dt, 2 H, H-5, H-5"), 3.40–3.35 (m, 1 H, H-5'), 1.98 (s, 3H, AC); ^{13}C NMR (CD_3OD, 100.6 Hz) δ 172.8 (C=O), 136.3 (C=), 117.8 (C=), 105.5, 104.4, 104.0, 85.1, 84.1. 82.2, 77.9, 77.2, 76.6, 72.1, 71.5, 71.1, 70.7, 70.5, 68.9, 63.0, 62.9, 62.7, 56.7, 23.7 (NAc). **2**. FABMS (positive ion mode) for $C_{23}H_{38}O_{19}NSNa$ (*m/z*): 688.7 (M^++Na), ^{1}H NMR (CD_3OD, 600 MHz) δ 6.00 (m, 1 H, C**H**=), 5.40–5.28 (dq, 1 H, C**H**=), 5.20–5.18 (dq, 1 H, C**H**=), 4.75–4.73 (d, 1 H, *J* = 8.5Hz, H-1), 4.46–4.36 (m, 2 H, H-6b', OC**H**C=), 4.36–4.32 (d, 1 H, *J* = 7.5 Hz, H-1"), 4.32–4.28 (d, 1 H, *J* = 6.8 Hz, H-1), 4.20–4.08 (m, 3 H, H-6a', OC**H**C=), 3.88–3.68 (m, 7 H, H-2', H-4, H-4"), 3.68–3.30 (m, 8 H, H-2, H-2", H-3", H-3), 1.99 (s, 3 H, Ac); ^{13}C NMR (CD_3OD,100.6 Hz) δ 172.5, 136.0, 117.8, 105.5, 104.0, 103.8, 84.6, 84.1, 77.2, 76.2, 75.5, 74.8, 72.5, 71.6, 71.0, 70.8, 70.4, 70.0, 68.5, 62.9, 62.5, 56.5, 23.4.