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SYNTHESIS OF Gal-β-(1→4)-6-O-SO_3Na-GlcNAc-β-(1→6)-Man-α-OR AS A PART OF gp 120

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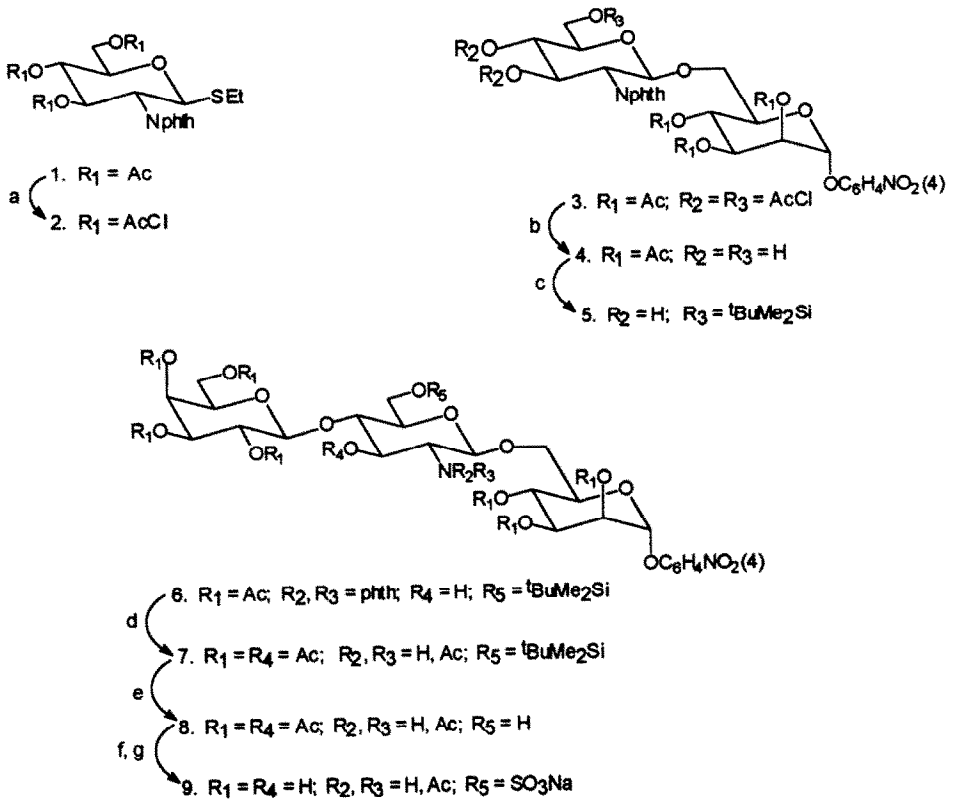
Abstract: A stereoselective synthesis of Gal-β-(1→4)-6-O-SO_3Na-GlcNAc-β-(1→6)-Man-α-OR (as a part of gp 120) containing an anomeric p-nitrophenyl group was accomplished through the use of ethyl 3,4,6-tri-O-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside.

The major envelope glycoprotein (gp 120) of human immunodeficiency virus (HIV) mediates the binding of the virus to CD_4 receptors[1-4], thus, becoming a causative agent for human AIDS[5,6]. This gp 120 glycoprotein has been shown to contain numerous N-linked oligosaccharides of both high mannose and complex type[7-12]. Recently, Cummings et al.[13] reported the presence of a NeuAcα-(2→3/6)-Galβ-(1→4)-6-O-SO_3GlcNAcβ-moiety in the complex type N-linked oligosaccharide structure of gp 120. This finding has prompted us to develop the chemical synthesis of Gal-β-(1→4)-6-O-SO_3Na-GlcNAc-β-(1→6)-Manα-$OC_6H_4NO_2$(4) [9] and Gal-β-(1→4)-6-O-SO_3Na-GlcNAc-β-(1→6)-Manα-OMe [14] to utilize in examining the biosynthesis of the NeuAc α-(2→3/6)Galβ-(1→4)-6-OSO_3NaGlcNAcβ-moiety in gp 120. The p-nitrophenyl derivative 9 can be employed in immunological studies after reduction of its nitro group and subsequent covalently linking to protein.

Ethyl 3,4,6-tri-O-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside [2] was employed as the key glycosyl donor for the synthesis of compound 9 containing p-nitrophenyl aglycon. The thioglycoside 2 was prepared in 2 steps from ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside [1][14]. Compound 1 after de-O-acetylation with methanolic sodium methoxide, was treated with chloroacetic anhydride/$NaHCO_3$/DMF[15] to furnish compound 2 in 66% yield; $[\alpha]_D$ +23° (c 1.3, $CHCl_3$); ^{1}H-N.M.R. ($CDCl_3$): δ 7.69 (m, 4 H, arom.), 4.08, 3.96, 3.78 (each s, 6 H, 3xOCH_2Cl), 2.59 (q, 2 H, SCH_2), 1.21 (t, 3 H, $-CH_3$). Glycosylation of 2 (1.56 g; 2.7 mmol) with p-nitrophenyl 2,3,4-tri-O-acetyl-α-D-mannopyranoside (1.43 g, 3.2 mmol) in



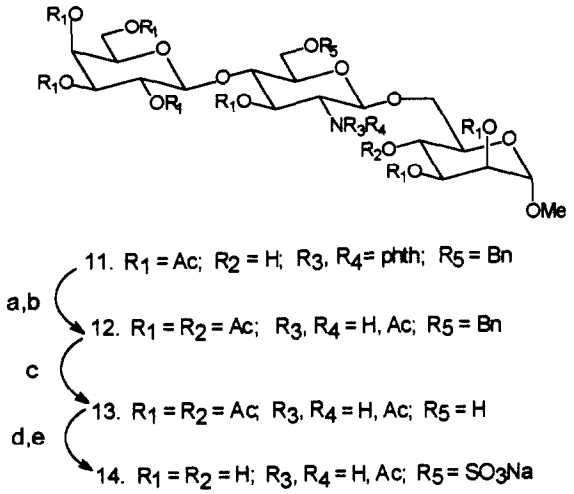
Reagents: a, chloroacetic anhydride/$NaHCO_3$/DMF; b, thiourea/pyridine/ethanol; c, tert-butyldimethylchlorosilane/imidazole/DMF; d, AgOTf/Cp_2ZrCl_2/2,6-(di-tert-butyl)-4-methylpyridine; e, pyridinium p-toluenesulfonate/ethanol; f, SO_3-pyridine complex/DMF; g, MeOH/MeONa.

SCHEME I

the presence of N-iodosuccinimide (1.5 g, 2.7 mmol) and triflic acid[16] (0.3 ml in 25 ml CH_2Cl_2) gave exclusively the β-linked disaccharide **3** in 83% yield; $[\alpha]_D$ +59° (c 1.2, $CHCl_3$); ^{1}H-N.M.R. ($CDCl_3$): δ 8.19 (d, 2 H, arom.), 7.87-7.74 (m, 4 H, arom.), 7.09 (d, 2 H, arom.), 4.02, 4.01 and 3.84 (each s, 6 H, 3xOCH_2Cl), 2.18, 1.95 and 1.91 (each

s, 9 H, 3xOAc); ^{13}C-N.M.R. ($CDCl_3$): δ 98.08 (C-1'), 95.94 (C-1), 65.98 (C-6), 63.33 (C-6'), 54.15 (C-2'), 40.76, 40.44 and 40.27 (3xCH_2Cl), 20.94, 20.80 and 20.66 (3xOAc). De-O-chloroacetylation[17] of **3** was found to proceed smoothly in a thiourea/pyridine/ethanol system to give the triol intermediate **4** in 63% yield; $[\alpha]_D$ +53° (c 1.0, $CHCl_3$): ^{1}H-N.M.R. ($CDCl_3$): δ 8.16 (d, 2 H, arom.), 7.82-7.70 (m, 4 H, arom.), 7.10 (d, 2 H, arom.), 2.20, 1.95 and 1.91 (each s, 9 H, 3xOAc); ^{13}C-N.M.R.: δ 98.31 (C-1'), 95.86 (C-1), 66.36 (C-6), 61.78 (C-6'), 56.48 (C-2'), 20.95, 20.82 and 20.77 (3xOAc). Treatment of **4** with tert-butylchlorodimethylsilane[18] in N,N-dimethylformamide, in the presence of imidazole gave in 68% yield the 6-O-tert-butyldimethylsilyl derivative **5** as an amorphous solid; $[\alpha]_D$ +42° (c 1.3, $CHCl_3$); ^{1}H-N.M.R. ($CDCl_3$): δ 8.13 (d, 2 H, arom.), 7.82-7.69 (m, 4 H, arom.), 7.08 (d, 2 H, arom.), 2.17, 1.94 and 1.89 (each s, 9 H, 3xOAc), 0.90 (s, 9 H, CMe_3), 0.1 and 0.09 (each s, 6 H, $SiMe_2$); ^{13}C-N.M.R.: δ 98.14 (C-1'), 95.85 (C-1), 74.65 (C-3'), 73.98 (C-4'), 65.87 (C-6), 64.65 (C-6'), 55.75 (C-2'). Glycosylation of diol **5** with 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl fluoride under Mikaiyama and Suzuki[19] conditions (Scheme I), AgOTf/zirconocene dichloride/2,6-(di-tert-butyl)-4-methylpyridine in methylene chloride, afforded a major β-(1→4) linked trisaccharide **6** in 81% yield; $[\alpha]_D$ +50° (c 1.1, $CHCl_3$); ^{1}H-N.M.R. ($CDCl_3$): δ 8.10 (d, 2 H, arom.), 7.74-7.71 (m, 4 H, arom.), 7.07 (d, 2 H, arom.), 4.58 (d, 1 H, H-1"), 2.16-1.86 (cluster of s, 21 H, 7xOAc), 0.89 (s, 9 H, CMe_3), 0.89 and 0.6 (each s, 6 H, $SiMe_2$); ^{13}C-N.M.R.: δ101.74 (C-1"), 97.69 (C-1'), 95.99 (C-1), 77.32 (C-4'), 74.79 (C-3'), 66.78 (C-6), 65.95 (C-6'), 61.47 (C-6"), 55.68 (C-2'). Conversion of **6** into **8** was carried out in 3 steps [1. hydrazine hydrate/ethanol (phthalimido group removal), 2. pyridine/acetic anhydride (N- and O- acetylation), 3. pyridinium p-toluenesulfonate/ethanol[20] (tert-butyldimethylsilyl group removal)]; 59% yield; $[\alpha]_D$ +37° (c 1.2, $CHCl_3$); ^{1}H-N.M.R. ($CDCl_3$): δ 8.21 (d, 2 H, arom.), 7.19 (d, 2 H, arom.), 2.25-1.96 (cluster of s, 27 H, 8xOAc and NAc); ^{13}C-N.M.R.: δ 102.21 (C-1"), 100.99 (C-1'), 95.23 (C-1), 77.31 (C-4'), 66.71 (C-6), 60.80 (C-6"), 60.39 (C-6'), 53.45 (C-2'). Reaction of **8** with five molar equivalents of sulfur trioxide-pyridine complex in N,N-dimethylformamide followed by de-O-acetylation with methanolic sodium methoxide produced **9** as its sodium salt after treatment with Na^+ resin; $[\alpha]_D$ +38° (c 1.3, H_2O); ^{1}H-N.M.R. (D_2O): δ 8.34 (d, 2 H, arom.), 7.34 (d, 2 H, arom.), 2.05 (s, 3 H, NAc); ^{13}C-N.M.R.: δ 101.70 (C-1"), 100.41 (C-1'), 96.76 (C-1), 77.05 (C-4'), 67.55 (C-6), 65.88 (C-6'), 59.89 (C-6"), 54.00 (C-2'), m/z 745.0 $[M-Na]^-$ 790.7 $(M+Na)^+$.

For the synthesis of compound **14**, our interest was directed towards the use of ethyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-3-O-acetyl-6-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**10**) as the glycosyl donor[21].



Reagents: a, hydrazine hydrate/ethanol; b, pyridine/acetic anhydride; c, 10% Pd-C/H_2; d, SO_3-pyridine complex/DMF; e, MeOH/MeONa.

SCHEME II

Similarly, N-iodosuccinimide/triflic acid catalyzed glycosylation of methyl 2,3-di-O-acetyl-α-D-mannopyranoside (Scheme II), as described for the preparation of compound **3**, with donor **10** gave compound **11**.[22] Conventional transformation of **11** into **12** was achieved in two steps as described earlier in the preparation of **7** from **6**. The removal of benzyl group in compound **7** was achieved by hydrogenolysis using 10% Pd/C in 95% aqueous ethanol to afford compound **13**. Sulfation of compound **13** with five molar equivalents of sulfur trioxide/ pyridine complex followed by de-O-acetylation with methanolic sodium methoxide afforded compound **14** as an amorphous solid. $[\alpha]_D$ +6° (H_2O, C 1.0); ^{1}H-N.M.R. (D_2O): δ 4.51 (d, 1 H, H-1'), 4.47 (d, 1 H, H-1"), 3.46 (s, 3 H,

OMe), 2.10 (s, 3 H, NAc); ^{13}C-N.M.R.: δ101.51 (C-1"), 100.79 (C-1'), 99.72 (C-1), 67.62 (C-6'), 65.32 (C-6), 60.01 (C-6"), 53.65 (OMe), 21.21 (NAc); m/z: 684.0 $(M+Na)^+$, 638.2 $(M-Na)^-$, 660.2 $(M-H)^-$.

N-Acetyllactosamine (Galß-(1→4)-GlcNAc) and the oligosaccharides containing this sequence have been frequently used as acceptors for α-2,3-sialyltransferases. Our recent finding showed that our synthetic compound, Galß-(1→4)-6-O-SO_3NaGlcNAcß-(1→6)-Manα-OR, can act as an acceptor for α-2,3-sialyltransferase to give NeuAcα-(2→3)-Gal-ß-(1→4)-6-O-SO_3GlcNAcß-(1→6)-Man-α-OR which occurs as a part of gp 120. The detailed biochemical investigation will be published elsewhere. It should be pointed out that the availability of these synthetic sulfated as reference compounds will facilitate the identification of 6-O-sulfotransferase product when the corresponding unsulfated trisaccharide, Galß-(1→4)-GlcNAcß-(1→6)-Man-α-OR, is used as an acceptor for this enzyme.

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22. Data for compounds are given below: values of $[\alpha]_D$ are measured at 25° ±3° for solution a. $CHCl_3$ b. H_2O; All compounds gave satisfactory elemental analysis; Compound **7** $[\alpha]_D$ +33° (c.1, a); ^{1}H-N.M.R. ($CDCl_3$): δ 7.19 (d, 2 H, arom.), 4.64 (d, 1 H, H-1"), 2.25-1.90 (cluster of s, 27 H, 8xOAc and NAc), 0.92 (s, 9 H, CMe_3), 0.09 and 0.07 (each s, 6 H, $SiMe_2$); ^{13}C-N.M.R.: δ 102.51 (C-1"), 100.34 (C-1'), 95.36 (C-1), 77.32 (C-4'), 66.87 (C-6), 65.55 (C-6'), 61.03 (C-6"), 53.30 (C-2'). Compound **11** $[\alpha]_D$ +32° (c 1.0, a); ^{1}H-N.M.R. ($CDCl_3$): δ 7.89-7.71 (m, 4 H, arom.), 7.27 (s, 5 H, arom.), 3.34 (s, 3 H, OMe), 2.17-1.93 (cluster of s, 21 H, 7xOAc); Compound **12** $[\alpha]_D$ +10° (c 0.8, a); ^{1}H-N.M.R. ($CDCl_3$): δ 7.19 (s, 5 H, arom.); 3.31 (s, 3 H, OMe), 2.12-1.69 (cluster of s, 27 H, 8xOAc and NAc); Compound **13** $[\alpha]_D$ +7° (c 0.6, a); ^{1}H-N.M.R. ($CDCl_3$): δ 3.34 (s, 3 H, OMe), 2.13-1.70 (cluster of s, 27 H, 8xOAc and NAc).
23. This publication is Part 93 of Synthetic Studies in Carbohydrates. For Part 92, see Khan, S.; Matta, K.L. Submitted to Carbohydr. Res. 1994.

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