Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 13, pp. 1389-1394, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00229-4

A CONVENIENT SYNTHESIS OF SULFATED AND SIALYL OLIGOSACCHARIDES AS POTENTIAL LIGANDS FOR CD22 ADHESION PROTEIN

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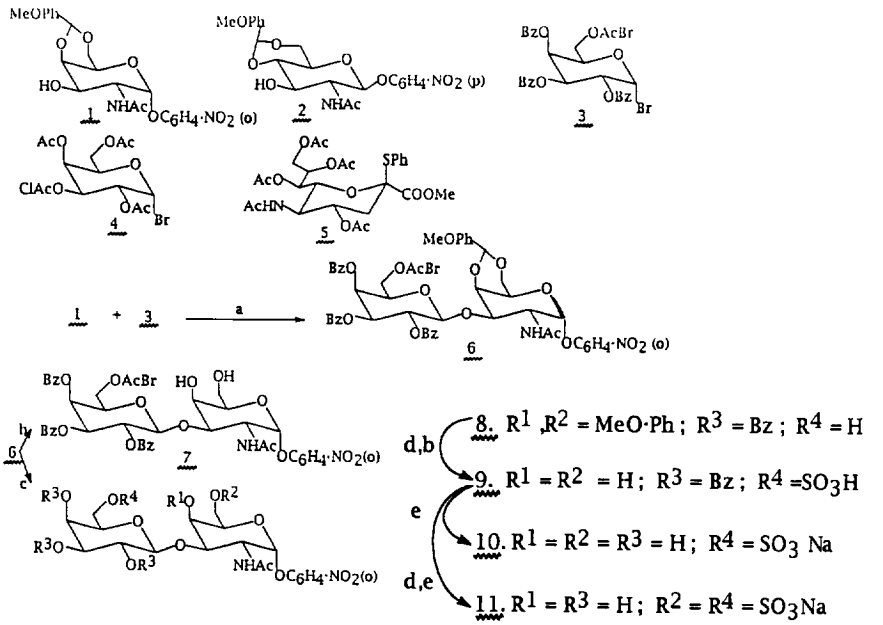
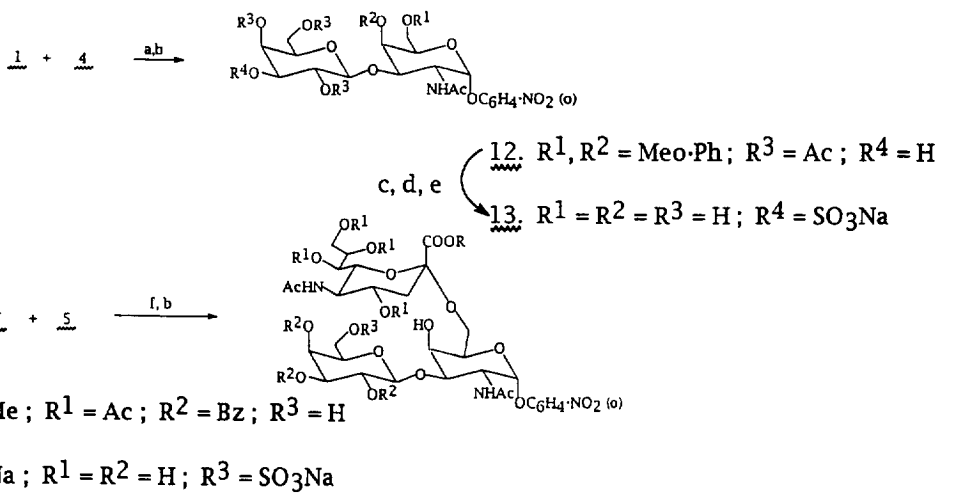
Abstract: Stereoselective syntheses of 6-O-SO_3Na-Gal-ß-(1→3)-GalNAc-α-OR (**10**), 6-O-SO_3Na-Gal-ß-(1→3)-6-O-SO_3Na-GalNAc-α-OR (**11**), 3-O-SO_3Na-Gal-ß-(1→3)GalNAc-α-OR (**13**), 6-O-SO_3Na-Gal-ß-(1→3)[NeuAc-α-(2→6)]-GalNAc-α-OR (**15**), Gal-ß-(1→3)[NeuAc-α-(2→6)]-GlcNAc-ß-OR (**18**) and 6-O-SO_3Na-Gal-ß-(1→3)[NeuAc-α-(2→6)]-GlcNAc-ß-OR (**19**) were accomplished through utilization of two key glycosyl donors, namely, 2,3,4-tri-O-benzoyl-6-O-bromoacetyl-α-D-galactopyranosyl bromide (**3**) and 2,4,6-tri-O-acetyl-3-O-chloroacetyl-α-D-galactopyranosyl bromide (**4**). The synthesized compounds were evaluated as ligands for CD22 adhesion protein.

In the last few years we are witnessing a surge of interest in the study of cell adhesion proteins some of which are shown to interact with carbohydrate structures.[1] Selectins are a class of three carbohydrate binding Ca^+ dependent membrane lectins and are designated as P, E and L selectin. These are cell adhesion proteins which bind carbohydrate determinants comprised of sialyl and/or sulfated $Lewis^x$ and $Lewis^a$ structures.[2] CD22 is another cell adhesion protein which is found predominantly on IgM^+IgD^+ ß cells.[3] It binds to oligosaccharides containing the NeuAcα2→6Galß1→4Glc/GlcNAc sequence and does not bind to α2→3 linked sialic acid moieties.[3f] In order to further examine the specificity of this lectin we have synthesized a series of NeuAcα2→6-linked compounds. We have also synthesized oligosaccharides incorporating the sulfate group at selected positions for similar studies.

Synthesis of 6-O-SO_3Na-Gal-ß-(1→3)-GalNAc-α-O-$C_6H_4NO_2$ (10), 6-O-SO_3Na-Gal-ß-(1→3)-6-O-SO_3Na-GalNAcα-O-$C_6H_4NO_2$ (11), 3-O-SO_3Na-Gal-ß-(1→3)-GalNAc-α-O-$C_6H_4NO_2$ (13) and 6-O-SO_3Na-Gal-ß-(1→3)[NeuAcα-(2→6)]GalNAc-α-O-$C_6H_4NO_2$ (15). A common intermediate, namely, 2-nitrophenyl O-(2,3,4-tri-O-benzoyl-6-O-bromoacetyl-ß-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-O-(4-methoxybenzylidene)-2-deoxy-α-D-galactopyranoside (**6**) was utilized for the synthesis of our target compounds. Glycosylation of **1**[4] with bromide **3**[5] under



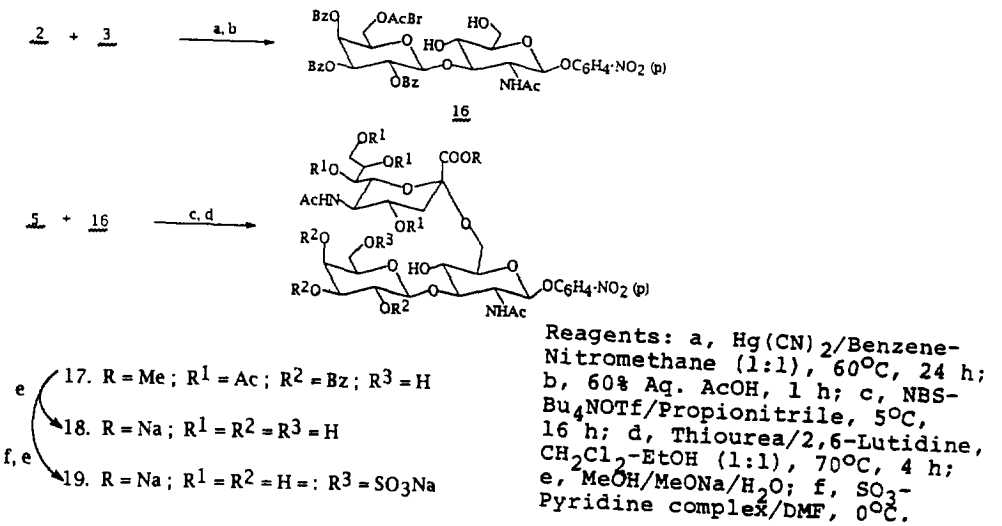
Reagents: a, $Hg(CN)_2$/Benzene-Nitromethane (1:1), 60°C, 24 h.
b, 60% Aq. 70°C, AcOH, 1 h; c, Thiourea/Pyridine/
Ethanol, 70°C, 2 h; d, SO_3-Pyridine/DMF; e, MeOH-MeONa.



Reagents: a, $Hg(CN)_2$/Benzene-Nitromethane (1:1), 60°C, 24 h.
b, Thiourea/2,6-Lutidine, CH_2Cl_2-EtOH (1:1), 70°C,
4 h; c, SO_3-Pyridine complex/DMF; d, 60% Aq. AcOH,
60°C, 1 h; e, MeOH-MeONa, H_2O; f, NBS-Bu_4NOTf/
Propionitrile, 5°C, 16 h.

Helfrich's condition [$Hg(CN)_2$] afforded the fully protected disaccharide **6** in 76% yield. Hydrolysis of **6** with 60% aqueous acetic acid provided the desired diol **7** in 69% yield. De-O-bromoacetylation of **6** was found to proceed smoothly in a thiourea-pyridine-ethanol[6] condition to give **8** in 68% yield. Sulfation of **8** with SO_3-pyridine complex[7] in DMF followed by the removal of the acetal group (to give intermediate **9**), then removal of O-benzoyl protection followed treatment with IR-120 (Na^+) resin gave **10** as an amorphous sodium salt. Similarly, sulfation of intermediate **9**, as described for the preparation of **10** (from **8**), afforded the di-O-sulfo derivative **11** as an amorphous sodium salt. Reaction of **1** with bromide **4**[8] followed by the removal of chloroacetyl group provided **12** in 37% yield. Compound **12** was converted into the 3-O-sulfo derivative **13** following a reaction sequence similar to that described for the synthesis of **10** from **8**. Glycosylation of **7** with the sialic acid donor **5**[9] under N-bromosuccinimide-tetrabutylammonium triflate[10] catalyzed condition in propionitrile followed by the removal of bromoacetyl provided **14** in 44% yield. Sulfation of **14** with SO_3-pyridine complex and de-O-acetylation with methanolic sodium methoxide followed by the addition of water to the reaction mixture to hydrolyze the methyl ester to the sodium salt of the acid provided compound **15** in 39% yield. The structural assignment of **10**, **11**, **13** and **15** was confirmed by ^{13}C-NMR and FAB mass spectroscopy.[11]

Synthesis of Gal-β-(1→3)-[NeuAc-α-2→6]-GlcNAc-β-$C_6H_4NO_2$ (18) and 6-O-SO_3Na-Gal-β-(1→3)-[NeuAc-α-2→6]-GlcNAc-β-$C_6H_4NO_2$ (19). A similar reaction sequence was enlisted for the synthesis of **16** from **2** as that described for the preparation of **6** from **1** followed by removal of the 4-methoxybenzylidene group with 60% aqueous acetic acid. Glycosylation of **16** with **5** followed by bromoacetyl removal provided **17** in 37% yield. Compound **17** was converted to **18** and **19** employing a similar reaction sequence to that described for the preparation of **15** from **14** in 75% yield. Structural confirmation of **18** and **19** was accomplished through NMR and FAB mass spectroscopy.[11] In a collaborative study these compounds were examined for binding with CD22 in an ELISA competition assay where relative inhibitory



concentrations (RIC) were determined against that of NeuAcα2→6Galβ1→4Glc.[3f] We found that sulfation did not affect the binding of sialylated compounds, the sulfate group does not substitute for sialic acid and that employment of the nitrophenyl aglycon improves RIC considerably.

Acknowledgment: This investigation[12] was supported by grant No. CA35329 awarded by the National Cancer Institute.

References and Notes:

1. (a) Varki, A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7390; (b) Lasky, L.A. *Science* **1992**, *258*, 964; (c) Bevilacqua, M.P.; Nelson, R.M.J. *Clin. Invest.* **1993**, *91*, 379; (d) Gallatin, W.M.; Weismann, I.L.; Butcher, E.C. *Nature* **1983**, *303*, 30.
2. (a) Lowe, J.B.; Stoolman, L.M.; Nair, R.P.; Larsen, R.D.; Berhend, T.L.; Marks, R.M. *Cell* **1990**, *63*, 475; (b) Berg, E.L.; Robinson, M.K.; Mansson, O.; Butcher, E.C.; Magnani, J.L. *J. Biol. Chem.* **1991**, *266*, 14869; (c) Tyrrell, D.; James, P.; Rao, N.; Foxall, C.; Abbas, S.; Dasgupta, F.; Nashed, M.; Hasegawa, A.; Kiso, M.; Asa, D.; Kidd, J.; Brandley, B.K. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10372; (d) Yuen, C.-T.; Lawson, A.M.; Chai, W.; Larkin, M.; Stoll, M.S.; Stuart, A.C.; Sullivan, F.X.; Ahern, T.J.; Feizi, T. *Biochemistry* **1992**, *31*, 9126; (e) Yuen, C.-T.; Betowska, K.; O'Brien, J.; Stoll, M.S.; Lemoine, R.; Lubineau, A.; Kiso, M.; Hasegawa, A.; Bockovich, N.J.;

Nicolaou, K.C.; Feizi, T. *J. Biol. Chem.* **1994**, *269*, 1595.

3. (a) Dorken, B.; Moldenhauer, G.; Pezzutto, A.; Schwartz, R.; Feller, A.; Kiesel, S.; Nadler, L.M. *J. Immunol.* **1986**, *136*, 4470; (b) Law, C.-L.; Sidorenko, S.P.; Clark, E.A. *Immunol. Today* **1994**, *15*, 442; (c) Pezzutto, A.; Dorken, B.; Moldenhauer, G.; Clark, E.A. *J. Immunol.* **1987**, *138*, 98; (d) Boue, D.R.; Lebien, T.W. *J. Immunol.* **1988**, *140*, 192; (e) Boue, D.R.; Lebien, T.W. *Blood* **1988**, *71*, 1480; (f) Powell, L.D.; Jain, R.K.; Matta, K.L.; Sabesan, S.; Varki, A. *J. Biol. Chem.* **1995**, *270*, 7523.
4. Matta, K.L.; Rana, S.S.; Abbas, S.A. *Carbohydr. Res.* **1984**, *131*, 265.
5. Kovac, P.; Glaudemans, C.P.J.; Guo, W.; Wong, T.C. *Carbohydr. Res.* **1985**, *140*, 299.
6. Bertolini, M.; Glaudemans, C.P.J. *Carbohydr. Res.* **1970**, *15*, 263.
7. Whistler, R.L.; Spencer, W.W.; BeMiller, J.N. *Methods Carbohydr. Chem.* **1963**, *2*, 298.
8. Jain, R.K., Matta, K.L. XVIIth International Carbohydrate Symposium, 1994, July 17-22, Ottawa, Canada, pg. 443.
9. Marra, S.; Sinay, P. *Carbohydr. Res.* **1989**, *187*, 35.
10. Fukase, K.; Hasuoka, A.; Kusumoto, S. *Tetrahedron Lett.* **1993**, *34*, 2187.
11. Data for compounds are given below as follows: compound no., yield (%). Values of $[\alpha]_D$ measured at 25° ± 3° for solution a. $CHCl_3$, b. H_2O. ^1H- and ^{13}C-NMR are taken for solution c. $CDCl_3$, d. D_2O. **6** (76) +148° (c 0.6, a), ^1H-NMR (c): δ 5.95 (d, J = 3.3 Hz, H-1), 5.22 (d, J = 7.7 Hz, H-1'), 3.79 (s, CH_2Br), 3.78 (s, OMe), 1.46 (s, NAc); ^{13}C-NMR: δ 101.73 (C-1'), 100.81 (CH-O), 98.71 (C-1), 64.58 (C-6), 64.12 (C-6'), 25.18 (OAcBr); **7** (69), +182° (c 1.3, a), ^1H-NMR (c): δ 5.91 (d, J = 3.4 Hz, H-1), 5.17 (d, J = 7.9 Hz, H-1'), 3.85 (s, CH_2Br); ^{13}C-NMR: δ 102.17 (C-1'), 98.38 (C-1), 63.89 (C-6'), 62.29 (C-6), 25.27 (OAcBr), **8** (68), +197° (c 1.0,a), ^1H-NMR (c): δ 5.93 (d, J = 3.3 Hz, H-1), 5.42 (s, CH-O), 3.78 (s, OMe); ^{13}C-NMR: δ 100.78 (C-1'), 100.01 (CH-O), 98.76 (C-1), 64.56 (C-6), 61.21 (C-6'); **10** (62), +117° (c 0.8, b), ^1H-NMR (d): δ 5.90 (d, J = 3.7 Hz,

H-1), 4.65 (d, J = 7.8 Hz, H-1'), 2.08 (s, NAc); ^{13}C-NMR: δ 103.49 (C-1'), 96.11 (C-1), 66.40 (C-6'), 60.22 (C-6); m/z: 607.3 $(M+H)^+$, 629.3 $(M+Na)^+$, 583.4 $(M-Na)^-$; **11** (57); +80° (c 1.0, b), ^{1}H-NMR (d): δ 5.86 (d, J = 3.7 Hz, H-1), 4.66 (d, J = 7.7 Hz, H-1'), 2.10 (s, NAc); ^{13}C-NMR: δ 103.47 (C-1'), 96.43 (C-1), 67.02 (C-6), 66.01 (C-6'); m/z: 731.1 $(M+Na)^+$, 685.2 $(M-Na)^-$; **12** (37), +84° (c 0.8, a), ^{1}H-NMR (c): δ 5.93 (d, J = 3.3 Hz, H-1), 5.52 (s, CH-O), 5.34 (d, J = 3.1 Hz, H-4'), 5.01 (d, J = 8.1 Hz, H-1'), 2.20, 2.13, 2.05 and 1.99 (each s, 3 x OAc and NAc); **13** (43), +125° (c 0.8, b), ^{1}H-NMR (d): δ 5.93 (d, J = 3.6 Hz, H-1), 2.07 (s, NAc); ^{13}C-NMR: δ 103.27 (C-1'), 95.97 (C-1), 79.22 (C-3'), 76.39 (C-3); **14** (44), +91° (c 0.4, a), ^{1}H-NMR (c): δ 5.87 (d, J = 3.3 Hz, H-1), 5.09 (d, J = 7.9 Hz, H-1'), 3.72 (s, OMe), 2.56 (dd, J = 4.7 Hz, H-3''e), 1.49 (t, J = 12.1 Hz, H-3''a); **15** (39), +56° (c 1.0, b), ^{1}H-NMR (d): δ 5.79 (d, J = 3.7 Hz, H-1), 4.65 (d, J = 7.8 Hz, H-1'), 2.68 (dd, J = 4.5 Hz, H-3''e), 2.10 and 2.08 (each s, 2 x NAc), 1.59 (t, J = 12.1 Hz, H-3''a); m/z: 896 $(M-Na)^-$, 919.8 $(M+H)^+$, 941.7 $(M+Na)^+$; **16** (92), +105° (c 1.6, a), ^{1}H-NMR (a): δ 5.37 (d, J = 8.2 Hz, H-1), 5.09 (d, J = 7.9 Hz, H-1'), 3.95 (s, CH_2Br), 1.32 (s, NAc); **17** (37), +65° (c 0.6, a), ^{1}H-NMR (c): δ 5.76 (d, J = 7.9 Hz, H-1), 5.49 (d, J = 7.3 Hz, H-1'), 3.70 (s, OMe), 2.58 (dd, J = 5.2 Hz, H-3''e), 1.51 (t, J = 12.1 Hz, H-3''a); **18** (79), -30° (c 0.5, b), ^{1}H-NMR (d): δ 5.33 (d, J = 8.5 Hz, H-1), 4.51 (d, J = 7.7 Hz, H-1), 2.74 (dd, J = 4.6 Hz, H-3''e), 1.68 (t, J = 12.2 Hz, H-3''a); ^{13}C-NMR: δ 102.44 (C-1'), 99.09 (C-1), 97.31 (C-2''), 80.46 (C-3), 67.24 (C-6), 61.60 (C-9''), 60.02 (C-6'); **19** (75); -13° (c 0.8, b), ^{1}H-NMR (d): δ 5.34 (d, J = 8.5 Hz, H-1), 4.48 (d, J = 7.8 Hz, H-1'), 2.75 (dd, J = 4.6 Hz, H-3''e), 1.68 (t, J = 12.2 Hz, H-3''a); ^{13}C-NMR: δ 102.25 (C-1'), 99.07 (C-1), 97.32 (C-2''), 80.11 (C-3), 67.23 (C-6), 67.20 (C-6'), 61.59 (C-9'').

12. This publication is part 102 of Synthetic Studies in Carbohydrates. Part 101, see Jain, R.K.; Vig, R.; Locke, R.D.; Mohammad, A.; Matta, K.L. *J. Org. Chem.* Submitted (**1995**).

(*Received in USA* 31 *March* 1995; *accepted* 23 *May* 1995)