Bioorganic & Medicinal Chemistry Letters, Vol.1, No.8, pp. 391-394, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

CHEMO-ENZYMATIC SYNTHESIS OF A BRANCHING DECASACCHARIDE FRAGMENT OF THE CAPSULAR POLYSACCHARIDE OF TYPE III GROUP B *STREPTOCOCCUS*[1,*]

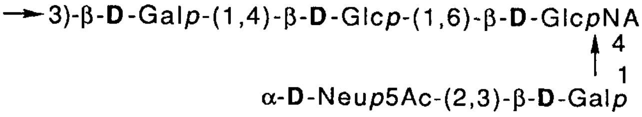
V. Pozsgay[a,2*], J. Gaudino[b], J. C. Paulson[b,3], H. J. Jennings[a*]

[a]Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada, K1A 0R6
[b]Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, California, 90024

(*Received* 26 *June* 1991)

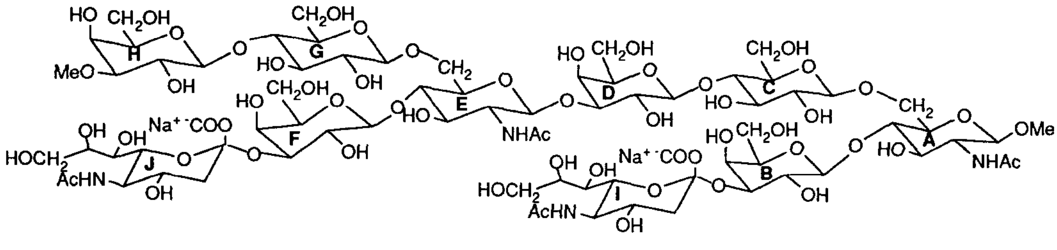
Abstract *A combined, chemical and enzymatic synthesis is reported of a decasaccharide corresponding to two contiguous repeating units of the type-specific, capsular polysaccharide of the human pathogenic Type III group B Streptococcus.*

Type III Group B *Streptococcus* (GBS) causes more than 60 % of all group B streptococcal infections in humans and is a major causative organism of bacterial meningitis and sepsis in newborn infants.[4,5] The potentials of its specific, capsular polysaccharide[6] (**1**) as an immunogen have been demonstrated[7] and the role of this polysaccharide as a virulence factor in bacterial infections has been established.[8] As part of our program aimed at developing *diagnostics*[9] for and *vaccines*[7] against Group B streptococcal infections in humans we have been investigating the structural factors governing the binding of the native, capsular polysaccharide of the Type III GBS with its homologous antibodies. This interaction could not be inhibited by pentasaccharide repeating units of the native polysaccharide prepared either by enzymatic degradation[6] or by synthesis[10] which can be interpreted by the failure of such, relatively small fragments to assume a particular conformational feature inherent in the native polysaccharide. Here we describe the synthesis of decasaccharide methyl glycoside **2** for use as an extended chain-length probe for further mapping the specificities of the antibody combining site at the molecular level.



$$\begin{array}{r} \rightarrow 3)\text{-}\beta\text{-}\mathbf{D}\text{-Gal}p\text{-}(1,4)\text{-}\beta\text{-}\mathbf{D}\text{-Glc}p\text{-}(1,6)\text{-}\beta\text{-}\mathbf{D}\text{-Glc}p\text{NAc-}(1\text{-} \qquad \\ \uparrow 4 \quad \\ 1 \quad \\ \alpha\text{-}\mathbf{D}\text{-Neu}p\text{5Ac-}(2,3)\text{-}\beta\text{-}\mathbf{D}\text{-Gal}p \qquad\quad \end{array}$$

1

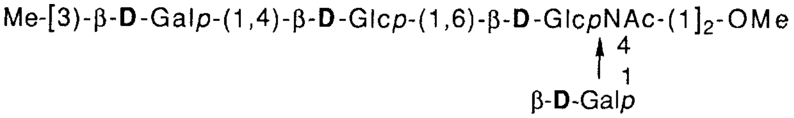


2

---

*NRCC No: 31960

The synthesis of **2** is based on preparation by chemical methods of asialo octasaccharide **3** followed by bio-synthetic attachment of N-acetyl-neuraminic acid to HO-3 of the side-chain galactose residues. The synthesis of compound **3** relied on a [4+4] block-synthetic approach: two tetrasaccharides corresponding to the Eastern and Western half, respectively, were prepared and were combined to provide a protected derivative of **3**.

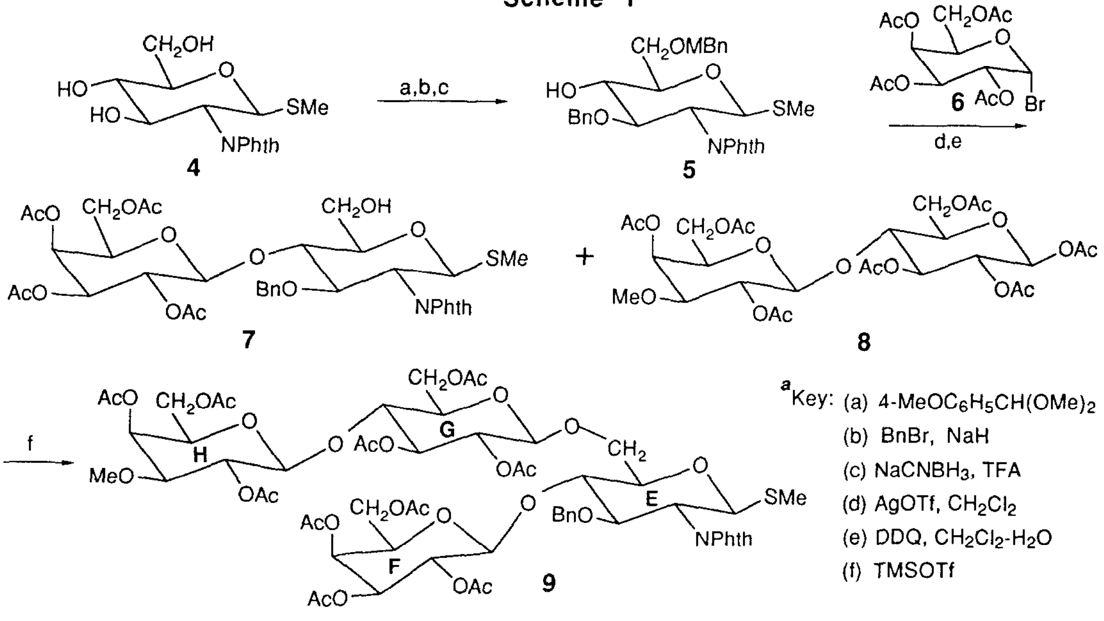


$$\text{Me-[3)-}\beta\text{-}\mathbf{D}\text{-Gal}p\text{-(1,4)-}\beta\text{-}\mathbf{D}\text{-Glc}p\text{-(1,6)-}\beta\text{-}\mathbf{D}\text{-}\underset{\substack{\uparrow 4\\ 1\\ \beta\text{-}\mathbf{D}\text{-Gal}p}}{\text{Glc}p\text{NAc}}\text{-(1]}_2\text{-OMe}$$

3

The synthesis of the Western half tetrasaccharide [HG(F)E][11] is shown in Scheme 1. Thioglycoside **4** was routinely converted into hetero-protected alcohol **5** (66%) which was condensed with acetobromo-galactose (**6**) followed by selective removal of the temporary, 4-methoxybenzyl protecting group to provide thioglycoside alcohol **7** (48%) coupling of which with the known lactose donor **8**[10] under catalysis by TMSOTf gave tetrasaccharide **9** (81%). The persistent, methyl protecting group at position 3 in residue H in **9** was introduced with the intent to prevent sialylation at this site. It is of interest to note that attempted glycosylation of related alcohols using Koenigs-Knorr type protocols was found[12] to lead mostly to orthoesters and not to glycosides and this difficulty of introducing a glycosyl residue at the primary hydroxyl group of a 4-O-glycosylated glucosamine derivative induced us to abandon our initial attempt for the octasaccharide synthesis which relied on the use of a [6+2] block scheme.

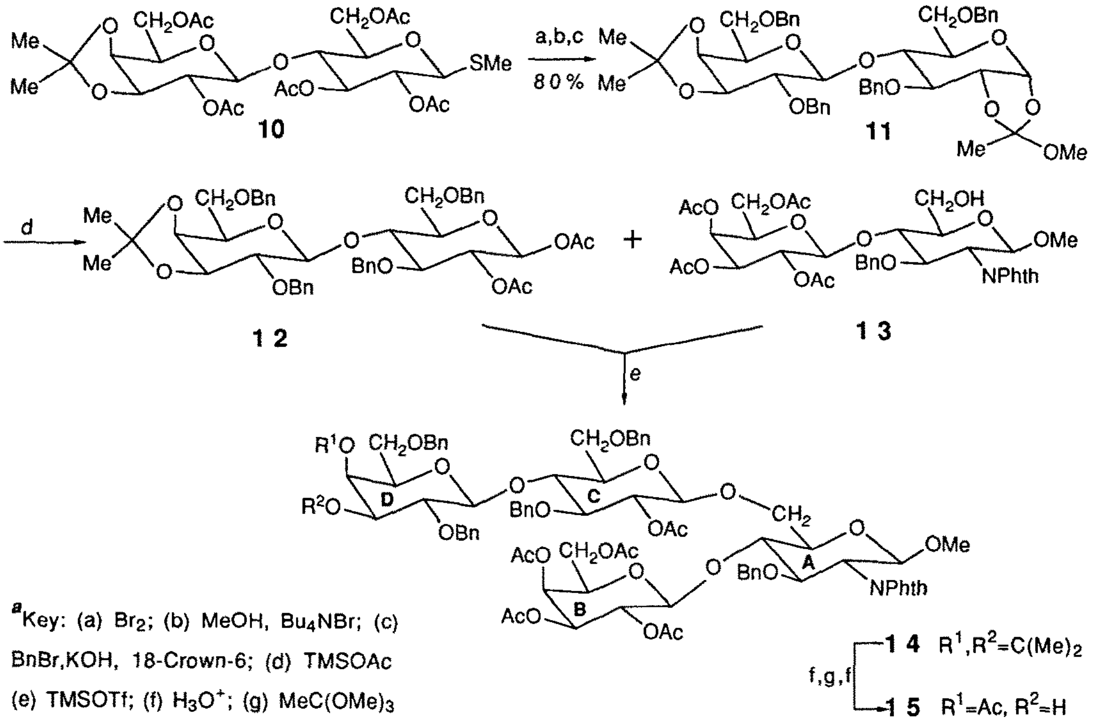
Scheme 1[a]



[a]Key: (a) 4-$MeOC_6H_5CH(OMe)_2$
(b) BnBr, NaH
(c) $NaCNBH_3$, TFA
(d) AgOTf, CH_2Cl_2
(e) DDQ, CH_2Cl_2-H_2O
(f) TMSOTf

The interchain lactose synthon (disaccharide D-C) was prepared from thiolactoside **10**[13] (Scheme 2). A crucial step in this synthetic sequence was the stereoselective opening[13] of the orthoester ring in compound **11** by the mild reagent trimethylsilyl acetate to provide 1,2-*trans*-diacetate **12** (80%) which was coupled to disaccharide **13** obtained in analogy to the corresponding S-glycoside **7**[14] to afford tetrasaccharide **14** (57%).

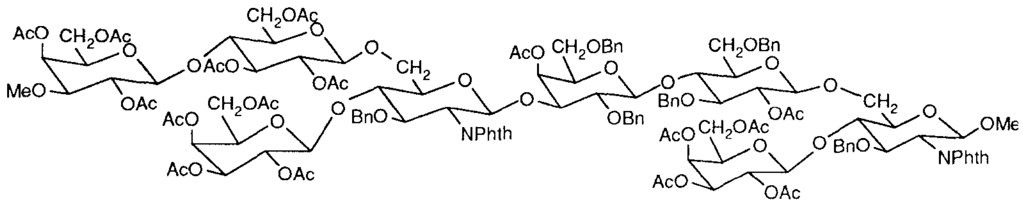
Scheme 2^a



^{a}Key: (a) Br_2; (b) MeOH, Bu_4NBr; (c) BnBr,KOH, 18-Crown-6; (d) TMSOAc (e) TMSOTf; (f) H_3O^+; (g) $MeC(OMe)_3$

Hydrolytic removal of the acetal group from compound **14** followed by a two-step, regioselective O-acetylation protocol[15] by way of a cyclic orthoester led to the Eastern half tetrasaccharide **15** (quantitative yield) as glycosyl acceptor. Tetrasaccharides **9** and **15** were combined under MeOTf catalysis[16] to give octasaccharide **16** in a highly stereoselective manner in 69 % yield. Routine deblocking of compound **16** and N-acetylation afforded octasaccharide methyl glycoside **3**[17] in 40 % yield.

We found the branching octasaccharide **3** to be an excellent substrate for Gal-β-1,3(4)-GlcNAc-α-2,3-sialyltransferase (EC 2.4.99.5) isolated from rat liver,[18] which transfered Neu5Ac from CMP-Neu5Ac to HO-3 of *both* side-chain galactose residues to provide decasaccharide **2** in a near quantitative yield on 10 mg scale. The structure of **2** was confirmed by 500 MHz,^{1}H-NMR spectroscopy and by mass spectral data.[19,20]



16

References and Notes

1. Synthetic Oligosaccharides Related to Group B Streptococcal Polysaccharides 6. Part 5 see Ref. 10.
2. Current address: National Institutes of Health, NICHD and NIDDK, Building 8, Room B1A-23, Bethesda, MD 20892, USA.
3. Current address: Cytel Corp., 11099 N. Torrey Pines Rd., La Jolla, CA 92037.
4. Baker, C.J.; Kasper, D.L. *Rev. Infect. Dis.* **1985**, *7*, 458.
5. Jennings, H.J. *Curr. Topics Microbiol. Imunol.* **1990**, *150*, 97.
6. Wessels, M.R.; Pozsgay, V.; Kasper, D.; Jennings, H.J. *J. Biol. Chem.* **1987**, *262*, 8262.
7. Wessels, M.R.; Paoletti, L.C.; Kasper, D.L.; DiFabio, J.L.; Michon, F.; Holme, K.; Jennings, H.J. *J. Clin. Invest.* **1990**, *86*, 1428.
8. Kasper, D.L.; Wessels, M.R.; Rubens, C.E.; Levy, N.J.; Pozsgay, V.; Jennings, H.J. in *Bacteria-Host Cell Interactions*, Horowitz, M.A. (Ed.), Alan L. Liss, Inc., New York, **1988**, 171.
9. Michon, F.; Chalifour, R.; Feldman, R.; Wessels, M.; Kasper, D.L.; Gamian, A.; Pozsgay, V.; Jennings, H.J. *Infect. Immun.* **1991**, *59*, 1690.
10. Pozsgay, V.; Brisson, J.-R.; Jennings, H.J.; Allen, S.; Paulson, J.C. *J. Org. Chem.* **1991**, *56*, 3377.
11. For designation of the individual glycose residues see formulae **2**, **9**, and **15**.
12. Pozsgay, V.; Brisson, J.-R.; Jennings, H.J. *Carbohydr. Res.* **1990**, *205*, 133.
13. Pozsgay, V.; Jennings, H.J. *Synthesis* **1990**, 724.
14. Pozsgay, V. in *Carbohydrates, Synthetic Methods and Applications in Medicinal Chemistry*, H. Ogura, A. Hasegawa, T. Suami (Eds.) In press.
15. King, J.F.; Albutt, A.D. *Can. J. Chem.* **1970**, *48*, 1754.
16. Lonn, H. *Carbohydr. Res.* **1985**, *139*, 105.
17. Partial spectral data: (a) ^{1}H-NMR (In D_2O, 500 MHz, 305K): 2.033, 2.036 (2 $C\underline{H}_3CO$); 3.445, 3.504 (2 $C\underline{H}_3O$); 3.920 (dd, 2H, *J*=3.6 Hz, H-4$_B$ and H-4$_F$). 4.159 (d, 1H, *J*=3.5 Hz, H-4$_D$); 4.213 (d, 1H, *J*=3.5 Hz, H=4$_H$); 4.288 (m, 2H, H-6$_A$ and H-6$_E$); 4.439 (d, 1H, *J*= 7.9 Hz, H-1$_D$); 4.467 and 4.472 (2d, 2 x1H, *J*=7.9 and 8.0 Hz, H-1$_A$ and H-1$_H$); 4.529 and 4.533 (2d, 2x1H, *J*=7.8 and 7.7 Hz, H-1$_B$ and H-1$_F$); 4.548 and 4.554 (2d, 2 x1H, *J*=7.9 and 8.0 Hz, H-1$_C$ and H-1$_G$; 4.719 (d, 1H, *J*=8 Hz, H-1$_E$). (b) ^{13}C-NMR (In D_2O, 125 MHz, 305K): 22.98 ($\underline{C}H_3CO$); 55.81 and 56.00 (2 $\underline{C}H_3O$); 78.50 and 78.58 (C-4$_A$ and C-4$_E$); 79.04 and 79.24 (C-4$_C$ and C-4$_G$); 82.46 (C-3$_H$); 82.95 (C-3$_D$), 102 85 (C-1$_A$); 103.20, 103.41, 103.54 (2x), 103.62, 103.68, and 103.77 (C-1$_B$, C-1$_C$, C-1$_D$, C-1$_E$, C-1$_F$, C-1$_G$, and C-1$_H$). (c) FAB-MS: 1424 $[M+H]^+$.
18. Sabesan, S.; Paulson, J.C. *J. Am. Chem. Soc.* **1986**, *108*, 2068, and references therein.
19. Partial spectral data: (a) ^{1}H-NMR (In D_2O, 500 MHz, 300K): 1.815 (t, 2H, *J*=12 Hz, H-3(ax)$_I$ and H-3(ax)$_J$); 2.033, (3H), 2.040 (3H), 2.042 (6H) (4 $C\underline{H}_3CO$); 2.765 (dd, 2H, *J*=12 Hz and 4.6 Hz, H-3(eq)$_I$ and H-3(eq)$_J$); 3.454 and 3.509 (2 $C\underline{H}_3O$); 4.167 (d, 1H, *J*=3.7 Hz, H-4$_D$); 4.215 (d, 1H *J*=3.5 Hz, H-4$_H$); 4.31 (m, 2H, H-6$_A$ and H-6$_E$); 4.444 (d, 1H, *J*=7.8 Hz, H-1$_D$); 4.470 and 4.472 (2d, 2 x 1H, *J*=8.4 Hz and 7.6 Hz, H-1$_A$ and H-1$_H$); 4.550 and 4.559 (2d, 2 x 1H, *J*=7.9 Hz, H-1$_C$ and H-1$_G$); 4.624 (d, 2H, *J*=7.8 Hz, H-1$_B$ and H-1$_F$); 4.722 (d, 1H, *J*=8.3 Hz, H-1$_E$). (b) FAB MS: 2007 $[M+H]^+$, 2045 $[M+K]^+$.
20. Detailed NMR analysis as well as immunological studies of compounds **2** and **3** are in progress.