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Oligosaccharide fragments of the type III group B streptococcal polysaccharide derived from *S. pneumoniae* type 14 capsular polysaccharide by a chemoenzymatic method[1]

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Abstract

Partial N-deacetylation of the GlcNAc residues in *S. pneumoniae* type 14 capsular polysaccharide (Pn14-PS) backbone was achieved by treatment with base, and the product was subsequently enzymatically sialylated at the 3-*O*-positions of the terminal galactose residues. The resultant, partially N-deacetylated type III Group B *streptococcus* capsular polysaccharide (GBSIII-PS) was subjected to nitrous acid deamination, which resulted in the degradation of GBSIII-PS polysaccharide into oligosaccharides containing increasing numbers of the identical repeating units. The oligosaccharides were then separated by passage through a Superdex 30 column and characterized by ESIMS and NMR spectroscopic analysis.

Keywords: Type III group B *streptococcus*; Capsular polysaccharide; Oligosaccharides

1. Introduction

The immunodominant epitope of GBSIII-PS is conformational in nature [1–3] and has recently been proposed to be exclusively located on extended helical domains of the polysaccharide [4]. Both polyclonal [5] and monoclonal [6] antibodies bind to two repeating unit decasaccharides but not to a pentasaccharide single repeating unit. To fully reveal the nature of this conformational epitope in terms of its overall structural requirements and its role in the antigenic and immunogenic properties of GBSIII-PS, oligosaccharides with a longer chain length are essential. Attempts to prepare such oligomer repeating units of GBSIII-PS by enzymatic degradation using endo-β-D-galactosidase have proven to be difficult because of the extremely low overall yields obtained and a tendency for the smaller oligomers (≤2RU) to predominate [7]. Although the chemoenzymatic synthesis of two repeating units has been achieved in this laboratory [8,9], the synthesis of larger oligosaccharides is a

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daunting task. These difficulties prompted us to search for a more efficient method, preferably one having the added advantage of being able to conjugate the oligomers directly to protein carriers. Therefore, a novel approach employing a combination of chemical and enzymatic procedures was developed for the preparation of these oligosaccharide repeating units using the readily available Pn14-PS as the starting material.

2. Results and discussion

Deamination of glucosamine with nitrous acid has been systematically investigated and reviewed [10]. Although side reactions have been observed, the product of 1,2-rearrangement (2,5-anhydromannose) was always dominant. This method was used to break down the native Pn14-PS into smaller saccharides, and advantage was taken of the aldehyde group in the terminal 2,5-anhydromannose for further conjugation of the saccharides to a protein carrier by reductive amination [11]. The successful application of the same method to prepare the fragments of GBSIII-PS depended on two critical factors: firstly, being able to fully sialylate the partially N-deacetylated Pn14-PS, and secondly, on the stability of the sialic acid residues of the resultant partially N-deacetylated GBSIII-PS to the acid conditions (pH 4.5) required for deamination.

The Pn14-PS was treated with aqueous sodium hydroxide to achieve a desired degree of N-deacetylation, which was measured by integrating the H-2 signal of the resulting glucosamine ($\delta_H \cong 2.9$ ppm) in the ^{1}H NMR spectrum of the partially N-deacetylated Pn14-PS. Sodium borohydride was added to the alkaline solution prior to heating to reduce the terminal aldehyde group of the polysaccharide and thus avoid alkaline degradation. The partially N-deacetylated Pn14-PS was enzymatically sialylated with CMP-NeuAc synthetase and α-(2→3)-sialyltransferase [12,13] to afford partially N-deacetylated GBSIII-PS. The product was analyzed by ^{1}H NMR spectroscopy, which indicated that sialylation was complete. The glucosamine residues in the partially N-deacetylated GBSIII-PS on treatment with nitrous acid were converted to terminal 2,5-anhydromannoses, thus introducing a free terminal aldehyde group in each of the fragments (Fig. 1). These oligomers, namely **1**, **2**, **3**, **4** and **5**, were separated by a Superdex 30 gel-filtration column in an overall yield about 50%. The elution profile is shown in Fig. 2. Oligosaccharides **1**, **2**, **3**, **4** and **5** as obtained from peaks in the column eluate were characterized by electrospray ionization mass spectroscopy (ESIMS) in both positive- and negative-ion modes. The analytical data are summarized in Table 1 and are in agreement with the calculated molecular weights. Interestingly, a peak with molecular weight plus methanol (M + MeOH) was also observed in the negative mode, but not in positive mode except in the case of oligosaccharide **1**. This product can be accounted for by the formation of a hemiacetal between the terminal aldehyde group and the methanol solvent used in the ESIMS analysis. Similarly the ^{1}H NMR spectra of the oligosaccharides exhibited no signal indicating the presence of a free aldehyde group, and only the hydrated form (doublet at δ_H 5.09 ppm) was observed. When the aldehyde group in the molecule was reduced, only the molecular ion (M) was observed under the same ESIMS analytical conditions. A fragment peak (*m/z* 470) in negative-ion ESIMS was always observed, which corresponds to α-NeuAc-(2→3)-Gal disaccharide of the side chain.

Table 1
ESIMS analysis of GBS III oligosaccharide repeating units

Oligosaccharide	Obtained ion (*m/z*)		Molecular weight	
	Positive mode	Negative mode	Observed[a]	Calculated[b]
1	940.4 $(M+H)^+$, 994.4 $(M+MeOH+Na)^+$	970.3 $(M+MeOH-H)^-$	939.4 (939.3)	939.8
2	961.5 $(M+2H)^{2+}$	975.0 $(M+MeOH-2H)^{2-}$	1921.0 (1920.0)	1920.7
2-red		960.7 $(M-2H)^{2-}$	(1923.4)	1922.7
3	968.4 $(M+3H)^{3+}$, 1451.7 $(M+2H)^{2+}$	976.8 $(M+MeOH-3H)^{3-}$	2901.7 (2901.4)	2901.6
3-red		966.9 $(M-3H)^{3-}$, 1450.9 $(M-2H)^{2-}$	(2903.7)	2903.6
4	1295.2 $(M+3H)^{3+}$	977.9 $(M+MeOH-4H)^{4-}$	3882.6 (3883.6)	3882.5
4-red		969.9 $(M-4H)^{4-}$, 1293.7 $(M-3H)^{3-}$	(3884.8)	3884.5
5-red		971.9 $(M-5H)^{5-}$, 1215.1 $(M-4H)^{4-}$	(4864.6)	4865.4

[a] Observed in positive-ion mode and negative-ion mode (in parentheses), respectively.
[b] Average mass units were used for calculation of molecular weight values.

A sixth peak was also present in the column eluate profile (Fig. 2) corresponding to oligosaccharide **6**. However, **6-red** obtained by reduction of **6** was shown by ESIMS also to contain larger oligomers. Other small extraneous peaks were detectable in the eluate profile, which probably represent oligosaccharides lacking a sialic acid residue. This was confirmed by ESIMS, when the peak between oligosaccharides **1** and **2** was shown, after sodium borohydride reduction, to have the same molecular weight of **2-red** minus a sialic acid residue [*m/z* 1630.4 $(M-H)^-$ in negative-ion mode].

The ^1H NMR spectra of aldehyde-reduced oligosaccharides (**2-red**, **3-red** and **4-red**) were recorded

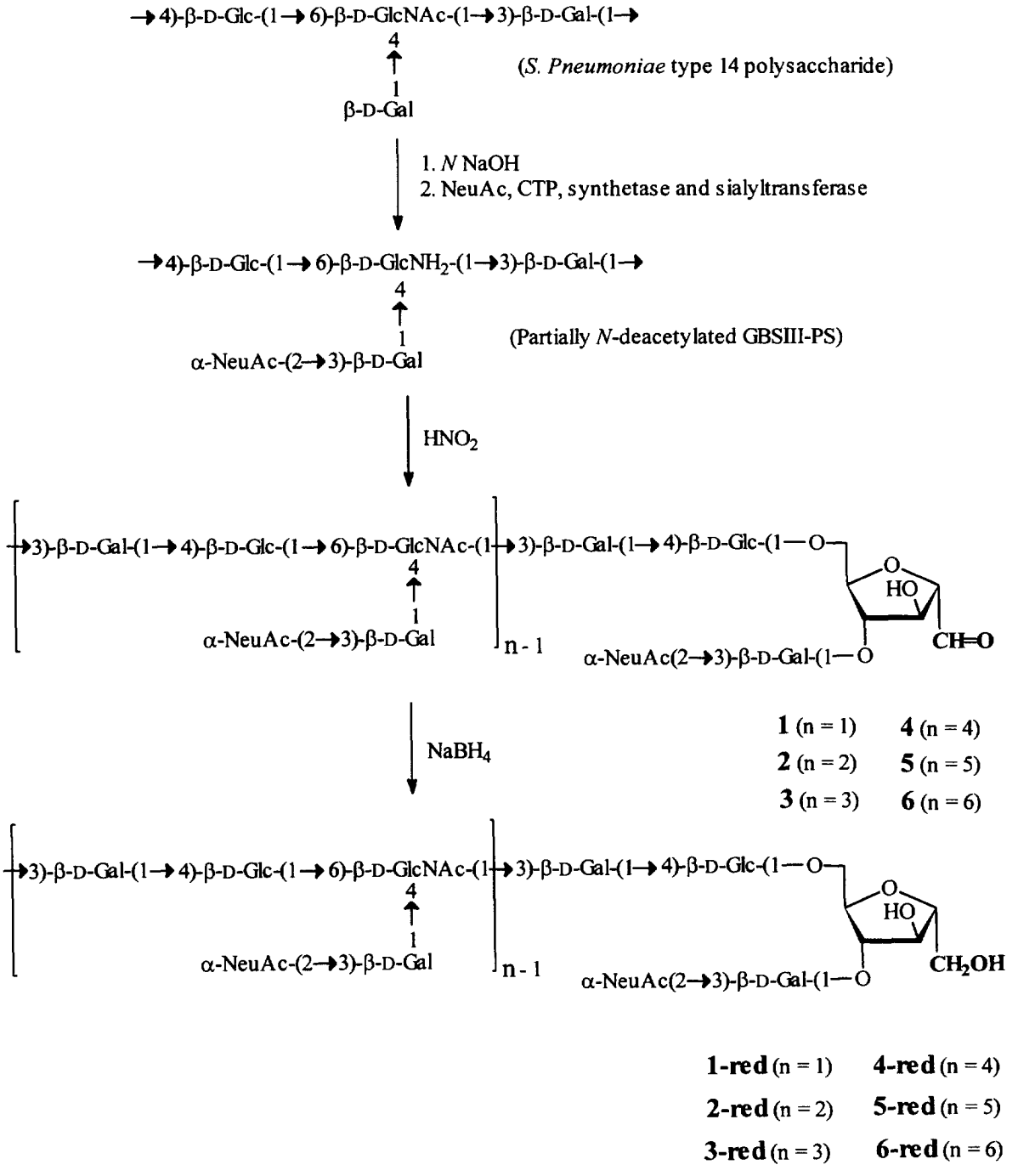

Fig. 1. Oligosaccharides **1–6** derived by controlled N-deacetylation, enzymatic sialylation, and depolymerization with nitrous acid deamination from Pn14-PS. A terminal aldehyde was generated and could be conjugated with amino groups of proteins. Oligosaccharides **1-red–6-red** were obtained accordingly by reduction of aldehyde group.

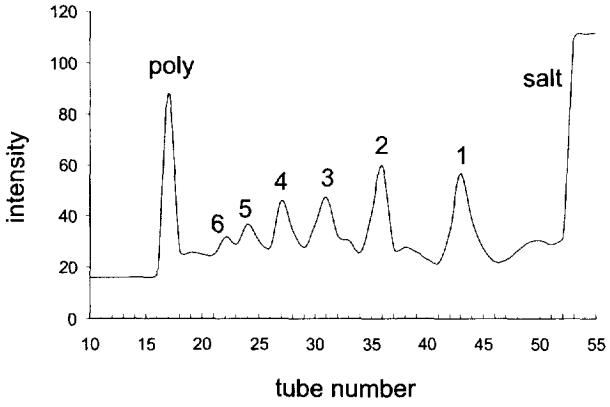

Fig. 2. Superdex 30 elution profile of depolymerized products recorded by differential refractometer; oligosaccharides **1–6** were separated using PBS buffer as eluent (7.5 mL per tube).

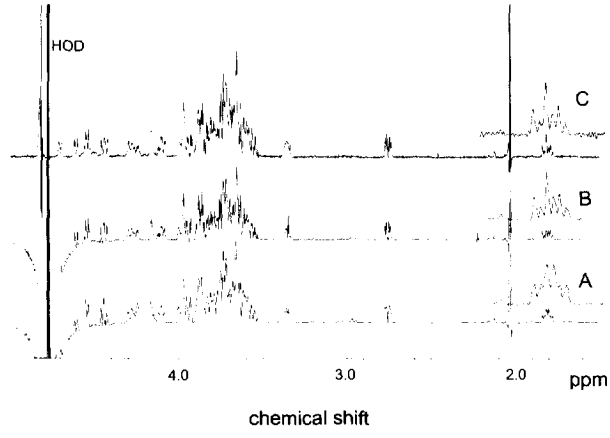

Fig. 3. 500 MHz ^{1}H NMR spectra of oligosaccharides **2-red** (A), **3-red** (B), and **4-red** (C) recorded in D_2O at 300 K.

and are shown in Fig. 3. The correct ratio of sialic acid to the other residues was observed. The chemical shifts of the anomeric protons of oligosacchardes at δ_H 4.71 ppm for GlcNAc, 4.61 ppm for Gal on side chain, 4.54 ppm for Glc, and 4.45 ppm for Gal were in agreement to those observed in GBSIII-PS [4,5]. Two different triplets for the H-3a (δ_H 1.80 and 1.81 ppm) of the two consecutive sialic acid residues in **2-red** were observed probably because the two sialic acid residues in **2-red** are exposed to different environments. This is due to the α-NeuAc-(1→3)-Gal side chain attached to 2,5-anhydromannitol. However, the H-3a protons of additional NeuAc residues in the spectra of **3-red** and **4-red** overlap at δ_H 1.81 as the chain length is extended. The above oligosaccharides were subsequently conjugated to protein carriers to be used as immunogens and/or antibody screening reagents in ELISA and surface plasmon resonance studies.

The evaluation of the immunological properties of the oligosaccharides and their neoglycoconjugates will be reported in a future publication.

3. Experimental

General methods.—Pn14-PS was purchased from American Type Culture Collection, Rockville, MD. α-(2→3)-Sialyltransferase was provided by Dr. J. C. Paulson of Cytel Corporation, and the sialic acid-CMP synthetase was prepared by Drs. W. Wakarchuk and M. Gilbert. ^{1}H NMR spectra were recorded at 500 MHz in D_2O with a Bruker AMX 500 instrument at 300 K. Chemical shifts (δ) are given relative to the signal of Me_4Si. ESIMS were performed with QUATTRO API-300LC/MS/MS (MICROMASS). Samples were dissolved in H_2O, and analysis was done by injection of 5 μL of sample into a flow of MeOH (15 μL/min). All chemicals were purchased from Aldrich Chemical Co. and used without further purification.

Partially N-deacetylated Pn14-PS.—A solution of Pn14-PS (200 mg) in N NaOH (10 mL) containing 1% NaBH4 was kept at 80 °C for 1 h. Upon cooling the solution was dialyzed against distilled water overnight and lyophilized to yield partially N-deacetylated Pn14-PS (120 mg, 60%). The degree of N-deacetylation is about 40%, which was measured by ^{1}H NMR spectroscopy according to the integration of H-2 of $GlcNH_2$ at δ_H 2.90 ppm.

Partially N-deacetylated GBSIII-PS.—To a solution of partially N-deacetylated Pn14-PS(100 mg), NeuAc (50 mg), and CTP (75 mg) in water (6 mL) were added M $MnCl_2$ (0.2 mL), $MgCl_2 \bullet 6H_2O$ (5 mg), and the pH was adjusted to pH 7.5 by the addition of N sodium cacodylate. To the above solution were added 1% HSA (150 μL), inorganic pyrophosphatase (50 U), sialic acid-CMP synthetase (10 U), and α-(2→3)-sialyltransferase (0.5 U). Again the pH was adjusted to 7.5 using N sodium cacodylate solution. After 24 h at 37 °C additional NeuAc (20 mg) and CTP (30 mg) were added, and the mixture was incubated for another 48 h. The mixture was dialyzed against distilled water for 48 h and lyophilized to yield partially N-deacetylated GBSIII-PS (105 mg).

Depolymerization and separation.—To a solution of partially N-deacetylated GBSIII-PS (75 mg) prepared above in 5% $NaNO_2$ (4 mL) was added 25% acetic acid at 0 °C until pH 4.4–4.5. The mixture was kept at 4 °C overnight to achieve the

depolymerization. The solution was directly loaded onto a Superdex 30 gel-filtration column (2.6×80 cm) and eluted with PBS buffer (0.33 mM, 5 mM NaCl, pH 7.1). The fractions were collected and lyophilized. Removal of salt by the passage through a Sephadex G-10 column afforded **1** (14 mg, 18.7%), **2** (9 mg, 12.0%), **3** (7 mg, 9.3%), **4** (4 mg, 5.3%), **5** (3 mg, 4.0%) and impure **6** (2 mg, 2.7%), respectively. Treatment of these oligomers with $NaBH_4$ in aqueous solution afforded **1-red**, **2-red**, **3-red**, **4-red**, **5-red** and **6-red**, accordingly.

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References

[1] H.J. Jennings, E. Katzenellenbogen, C. Lugowski, F. Michon, R. Roy, and D.L. Kasper, *Pure Appl. Chem.*, 56 (1984) 893–905.

[2] H.J. Jennings, C. Lugowski, and D.L. Kasper, *Biochemistry*, 20 (1981) 4511–4518.

[3] M.R. Wessels, A. Munoz, and D.L. Kasper, *Proc. Natl. Acad. Sci. U. S. A.*, 84 (1987) 9170–9174.

[4] J.-R. Brisson, S. Uhrinova, R.J. Woods, M. van der Zwan, H.C. Jarrell, L.C. Paoletti, D.L. Kasper, and H.J. Jennings, *Biochemistry*, 36 (1997) 3278–3292.

[5] M.R. Wessels, V. Pozsgay, D.L. Kasper, and H.J. Jennings, *J. Biol. Chem.*, 262 (1987) 8262–8267.

[6] W. Zou, R. MacKenzie, L. Thérien, T. Hirama, Q. Yang, M.A. Gidney, and H.J. Jennings, to be published.

[7] L.C. Paoletti and K.D. Johnson, *J. Chromatography, A*, 705 (1995) 363–368.

[8] V. Pozsgay, J. Gaudino, J.C. Paulson, and H.J. Jennings, *Bioorg. Med. Chem. Lett.*, 1 (1991) 391–394.

[9] W. Zou, J.-R. Brisson, Q.-L. Yang, M. van der Zwan, and H.J. Jennings, *Carbohydr. Res.*, 295 (1996) 209–228.

[10] D. Horton and J.D. Wander, *Amino Sugars*, in W. Pigman and D. Horton (Eds.), *The Carbohydrates: Chemistry and Biochemistry*, 2nd ed., Vol. IB, 1980, pp. 702–710, and references cited therein.

[11] C.A. Laferriere, R.K. Sood, J.-M. deMuys, F. Michon, and H.J. Jennings, *Infect. Immun.*, 66 (1998) 2441–2446.

[12] S. Sabesan and J.C. Paulson, *J. Am. Chem. Soc.*, 108 (1986) 2068–2080.

[13] R.L. Halcomb, H. Huang, and C.-H. Wong, *J. Am. Chem. Soc.*, 116 (1994) 11315–11322.