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MIMICS OF THE STRUCTURAL ELEMENTS OF TYPE III GROUP B *STREPTOCOCCUS* CAPSULAR POLYSACCHARIDE. PART III: TWO REPEATING UNITS (OCTASACCHARIDE) WITH (*S*)-1-CARBOXYETHYL GROUPS REPLACING SIALIC ACIDS[1]

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Abstract: A chemical synthesis of octasaccharide **1** is described in which (*S*)-1-carboxyethyl groups replace sialic acid residues to yield a mimic of two repeating units of the type III group B *Streptococcus* capsular polysaccharide. © 1997 Elsevier Science Ltd. All rights reserved.

The immunodominant conformational epitope of the type III group B *Streptococcus* capsular polysaccharide (GBSP III) is dependent on the presence of its terminal sialic acid residues.[2] Although partially oxidised GBSP III, in which both C-8 and C-9 had been removed from the exocyclic chain of terminal sialic acid residues, still bound to homologous antibodies,[3] neither carboxyl-reduced nor desialylated GBSP III were able to bind.[2] Thus, it was concluded that although the entire sialic acid molecule was not required for the formation of the conformational epitope at least its carboxylate groups were essential.[2,3] In order to further investigate the possibility that the carboxylate groups alone could fulfil the role of sialic acid, oligosaccharide probes were

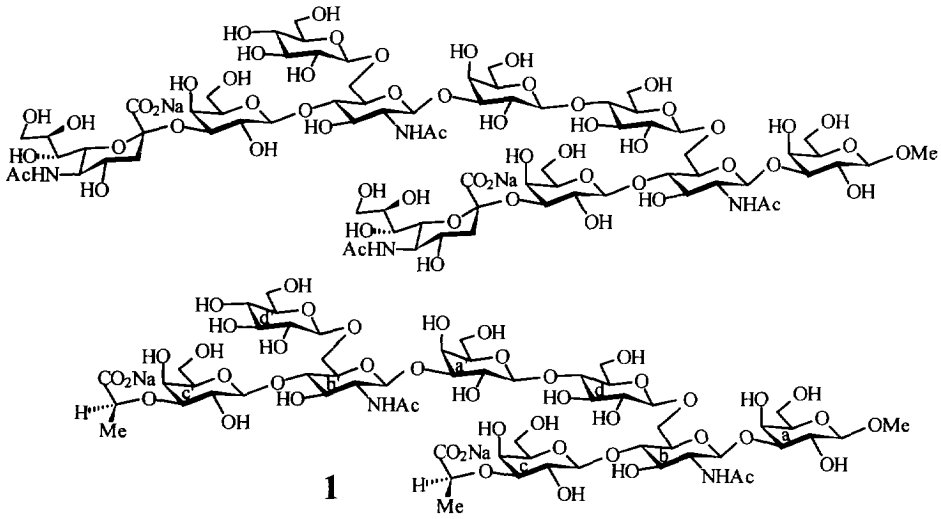
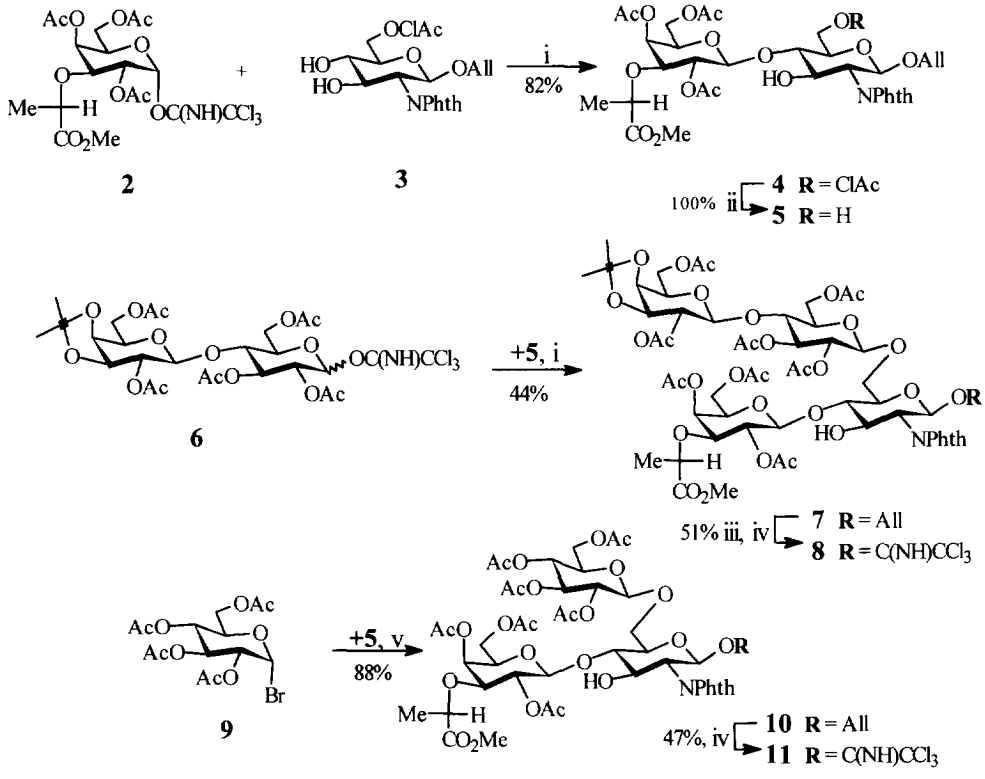


Figure 1. Two repeating units (methyl glycoside) of GBSP III and its mimic **1**.

synthesised in which sialic acid was replaced by lactic acid-ether anion groups. These groups, when introduced at the 3-*O*-position of the terminal galactose residues of desialylated GBSP III, put carboxylate groups in the same position as they are in the GBSP III (see Figure 1). Following the synthesis of carboxylate-containing penta- and hexa-oligosaccharides,[4] here we describe the synthesis of octasaccharide methyl glycoside **1**, a mimic of previously synthesised two repeating units (methyl glycoside) of the GBSP III.[5] This will be useful probe for further antibody-binding studies to further define the nature of the conformational epitope of GBSP III.

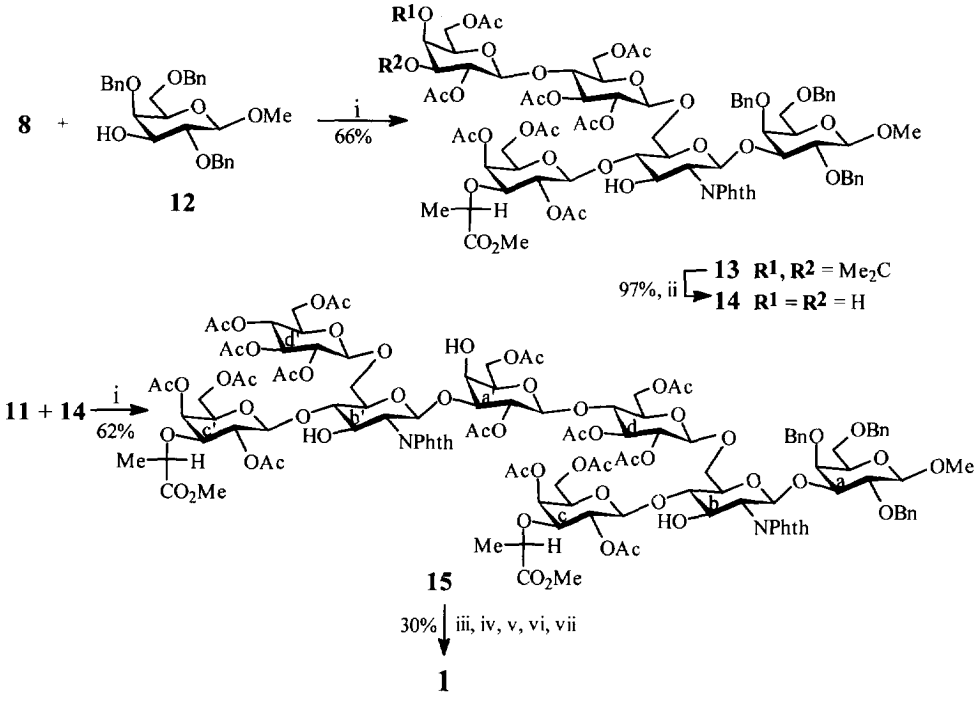


Scheme 1. Reagents and conditions: (I) TMS triflate (0.25 equiv) 4 Å MS in CH_2Cl_2, -45 °C, 2 h; (ii) thiourea/2,6-lutidine in MeOH:CH_2Cl_2 (1:1), overnight; (iii) $PdCl_2$ in MeOH:H_2O (6:1), 2 h; (iv) K_2CO_3/Cl_3CCN in CH_2Cl_2, 4 h; (v) $HgCN_2$ in $C_6H_5CH_3$:CH_3NO_2 (1:1), 50 °C, overnight.

Syntheses of two glycosyl donors, **8** and **11**, are illustrated in Scheme 1. Condensation of **2**[4a] and **3**, which was prepared in 54% yield from allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside[6] by 6-*O*-chloroacetylation with $(ClCH_2CO)_2O$/2,6-lutidine in CH_3CN, afforded **4**. Removal of 6-*O*-ClAc gave **5**, which in turn was coupled with lactosyl donor **6**[4b] to furnish **7**. The allyl group was then removed by treatment with $PdCl_2$ in aq MeOH,[7] followed by trichloroacetimidation[8] to give **8** (δ_H: 6.439 ppm, H-1, $J_{1,2}$ = 9.0 Hz; C=NH, 8.657 ppm; δ_C: 93.60, 100.40, 100.72, 102.03 ppm, 4 x C-1). Similarly, after glycosylation of **5** with **9** the

trisaccharide **10** was transformed into **11** (δ_H: 6.265 ppm, H-1, $J_{1,2}$ = 9.1 Hz; C=NH, 8.650 ppm; δ_C: 93.58, 101.81, 102.04 ppm, 3 x C-1).

A reaction pattern of [4 + 1 + 3] was followed (see Scheme 2) in the synthesis of **15**. Tetrasaccharide donor **8** reacted with **12**[9] in the presence of TMS triflate afforded pentasaccharide **13** (δ_H: 5.535 ppm, H-1^b, $J_{1,2}$ = 8.4 Hz; δ_C: 98.69, 100.87, 101.15, 101.87, 104.96 ppm, 5 x C-1). The isopropylidene group was removed to yield **14**, which was followed by condensation with **11** under same condition to obtain the protected octasaccharide **15**.[10]



Scheme 2. Reagents and conditions: (i) TMS triflate (0.25 equiv) 4 Å MS in CH_2Cl_2, -45 °C, 2 h; (ii) 90% CF_3CO_2H:CH_2Cl_2 (1:10), 0 °C, 1 h; (iii) H_2/Pd-C in MeOH:CH_3CO_2H (8:2), overnight; (iv) 0.1 N NaOH:MeOH (1:10), overnight; (v) $NH_2NH_2H_2O$ in 95% EtOH, reflux, 16 h; (vi) Ac_2O in MeOH:H_2O (9:1), overnight; (vii) 0.1 N NaOH, 4 h.

The deprotection procedure used in the transformation of **15** into **1**[11] included five steps: (1) removal of benzyl groups by hydrogenation; (2) de-*O*-acetylation and hydrolysis of methyl ester by saponification; (3) removal of *N*-phthalimido groups; (4) *N*-acetylation, and finally (5) treatment with 0.1 N NaOH to hydrolyse the lactones formed during *N*-acetylation. We previously reported a hydrazide by-product was formed in the process of de-*N*-phthalimidation and *N*-acetylation [4] due to the reaction of mixed anhydride (formed by acetic anhydride and lactic acid) with remaining hydrazine. This by-product could be eliminated by removal of

hydrazine prior to the *N*-acetylation, which was achieved by passing the solution through a Sephadex G-10 column. The final product was purified by passage through a Bio-gel P-2 column using water as eluent.

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1. This is NRCC publication No.39537. For Part I and II see reference 4.
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10. Partial spectral data for **15**: ^{1}H NMR ($CDCl_3$, 500 MHz, 300 K) δ_H 1.297, 1.305 (2d, 3H each, 2 x *Me*$CHCO_2Me$, J = 6.8 Hz), 1.428, 1.772, 1.779, 1.955, 1.992, 1.996, 2.019, 2.040, 2.052, 2.063, 2.090(3), 2.098, 2.107 (15 x OAc) 3.343 (s, 3H, OMe, aglycon), 3.685, 3.690 (2s, 3H each, 2 x CO_2*Me*), 5.301 (d, 1H, H-1$^{b'}$, $J_{1,2}$ = 8.5 Hz), 5.465, 5.473 (2d, 1H each, H-4^c and H-4$^{c'}$, $J_{3,4}$ = 3.8 Hz), 5.514 (d, 1H, H-1^b, $J_{1,2}$ = 8.4 Hz) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz, 300K) δ_C 98.55, 98.64, 100.79, 101.07, 101.41, 101.78(2), 104.93 ppm.
11. Partial spectral data for **1**: ^{1}H NMR (D_2O, 500 MHz, 295 K) δ_H 1.389 (d, 6H, 2 x *Me*$CHCO_2Me$, J = 6.8 Hz), 2.029, 2.038 (2 x NAc) 3.564 (s, 3H, OMe, aglycon), 4.065 (m, 2H, 2 x MeC*H*CO_2Me), 4.299 (d, 1H, H-1^a, $J_{1,2}$ = 7.6 Hz), 4.440 (d, 1H, H-1$^{a'}$, $J_{1,2}$ = 7.9 Hz); 4.526 (d, 1H, H-1$^{d'}$, $J_{1,2}$ = 8.0 Hz), 4.553 (d, 3H, H-1^c, H-1$^{c'}$, and H-1^d, $J_{1,2}$ = 7.8 Hz); 4.716 (d, 1H, H-1$^{b'}$, $J_{1,2}$ = 8.2 Hz), 4.725 (d, 1H, H-1^b, $J_{1,2}$ = 8.2 Hz) ppm; ^{13}C NMR (D_2O, 125 MHz, 295 K) δ_C 19.46 (2 x *Me*$CHCO_2$), 22.98 (2 x CH_3CON), 57.99 (OMe), 103.36 (4C, C-1^c, 1$^{c'}$, 1^d, 1$^{d'}$), 103.57 (2C, C-1^b, 1$^{b'}$), 103.74 (C-1$^{a'}$), 104.70 (C-1^a) ppm (the resonances of anomeric carbons were assigned based on ^{13}C-^{1}H heteronuclear correlation); ESMS for $C_{59}H_{96}N_2O_{45}Na_2$ (1599.38): 1598.34 $[M-H]^+$, 1555.27 $[M-2Na+2H]^+$ (positive); 1577.28 $[M-Na+H]^-$, 1555.06 $[M-2Na+2H]^-$ (negative).

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