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A Highly Convergent Synthesis of a Hexasaccharide Derived from the Oligosaccharide of Group B Type III *Streptococcus*

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Abstract: We have developed a novel glycosylation strategy which enabled a highly convergent assembly of a hexasaccharide derived from group B type III *Streptococcus*.

Group B type III *Streptococcus* is a major cause of bacterial meningitis in new-born infants.[1] Mortality and morbidity rates from these infections continue to be substantial and a method to prevent the illnesses caused by this pathogen is needed. Active immunisation of new-borns is not practical, since most cases occur within the first month of life. Therefore, it has been proposed[2,3] that the target population for the polysaccharide vaccination would be pregnant mothers deficient in antibodies specific for the native, type III group B *Streptococcus* polysaccharides. The feasibility of this approach is supported by studies of Baker *et al.*[4] in which purified group B *Streptococcus* capsular polysaccharide was administered to women during the third trimester of pregnancy. Among women with low or undetectable pre-existing levels of specific antibodies, 57% developed a rise in antibody titre. However, the low response rate and the absence of T-lymphocyte stimulation, are major drawbacks of this type of vaccination. It is to be expected that the immunogenicity of the oligosaccharide may be improved by conjugation to a carrier protein.[5] A synthetic oligosaccharide that contains an artificial spacer will be very suitable to make such a conjugate in a controlled and reproducible manner.[6]

The minimum structure of an oligosaccharide required for inducing the production of a population of antibodies involved in the protection of humans against group B type III *Streptococcus* infection is depicted in **Figure 1**.[7]

Figure 1

D-Gal*p*-(1-4)-β-D-Glc*p*-(1-6)-β-D-GlcNAc*p*-(1-3)-β-D-Gal*p*-(1-4)-β-D-Glc*p*

4
↑
1
β-D-Gal*p*
3
↑
2
α-D-NeuNAc*p*

(I)

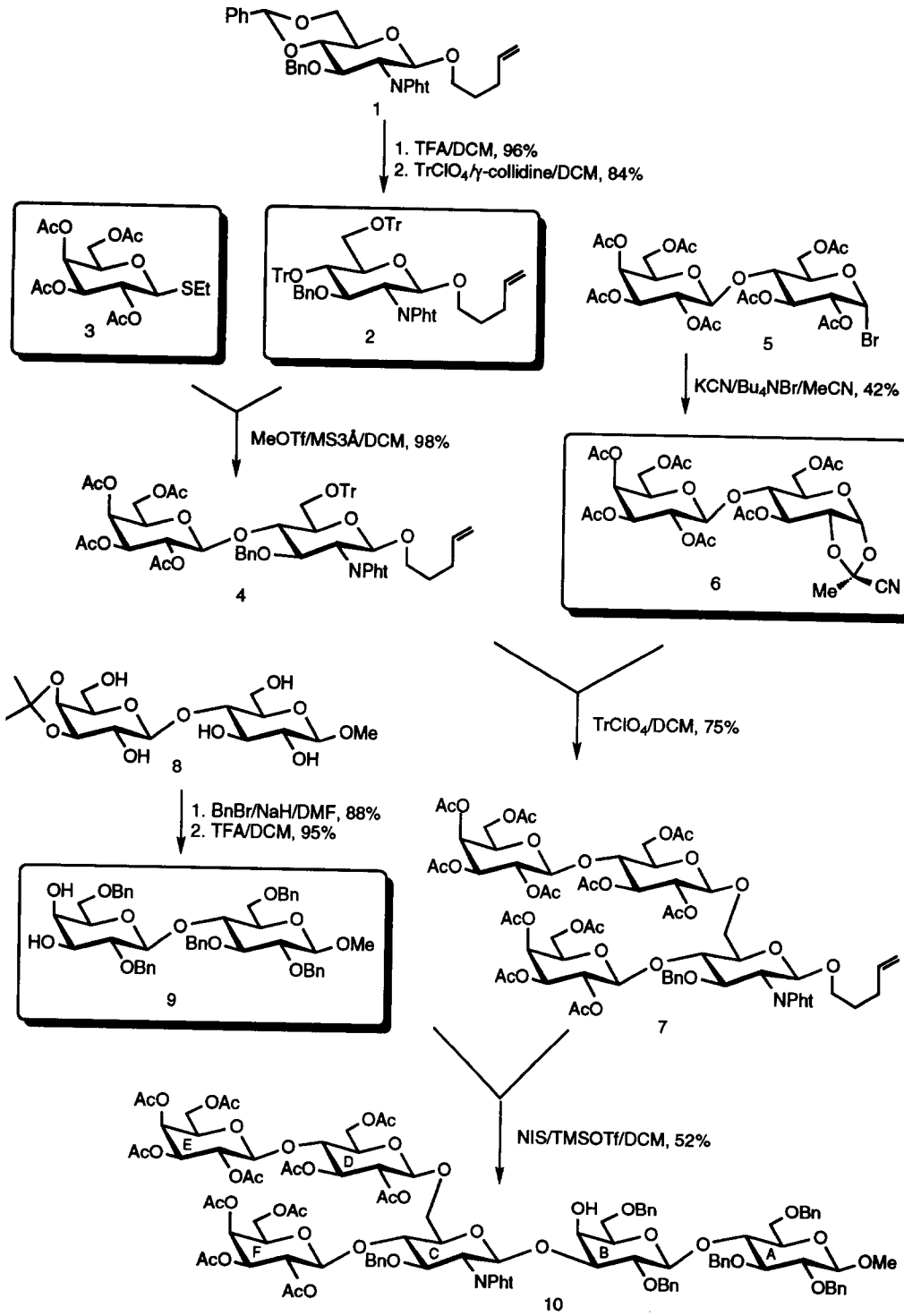
We report here a highly convergent synthesis of a hexasaccharide derived from group B type III *Streptococcus* oligosaccharide. Enzymatic sialylation of this oligosaccharide may give a the heptameric oligomer **I** that will be valuable for vaccination studies and to develop an ELISA assay.

The preparation of complex oligosaccharides like **I** requires a highly convergent strategy.[8] In such a glycosylation strategy, most of the synthetic effort should be directed towards the preparation of the glycosyl donor and acceptor building blocks which can be assembled into an oligomer involving a minimal number of synthetic steps. Each reaction step should proceed with high stereoselectivity and in high yield. Ideally, the anomeric substituent of the saccharide building blocks should be sufficiently stable to withstand protecting group manipulations (*i.e.* act as a protecting group), but also have an adequate reactivity to permit its use as a glycosyl donor (*i.e.* act as a leaving group). Thioglycosides[9] and n-pentenyl glycosides[10] possess these features.

The hexasaccharide **10** was assembled form the building blocks **2**, **3**, **6** and **9** and the designed strategy was based on the above mentioned considerations. Furthermore, some other important strategic aspects were used for the synthetic plan. The cheaply available disaccharide "lactose" was utilised for the preparation of the building blocks **6** and **9** as the use of this starting material reduces the number of glycosylation steps to be performed. Trityl ethers are convenient glycosyl acceptors and it has been shown that secondary trityl ethers are in general much more reactive than primary trityl ether.[11] These features permitted first galactosylation of the trityl ether at C-4 of **2** followed by lactosylation of the trityl at the C-6 position. Finally, the different reactivities of n-pentenyl and thioglycosides mean that thioglycosides can be activated in the presence of a n-pentenyl glycoside. These combination of chemical features facilitate the assembly of **10** in a convergent manner form the building blocks **2**, **3**, **6** and **9** without a single protecting group manipulation.

Thus, key building block **2** was prepared from the known[12] pentenyl glycoside **1** by acid mediated cleavage of the benzylidene acetal followed by ditritylation with trityl perchlorate. Coupling of thioglycoside **3** with di-*O*-trityl thioglycosyl acceptor **2** in the presence of MeOTf[13,14] proceeded with absolute regio- and stereoselectivity and disaccharide **4** was obtained in a quantitative yield. To prevent cleavage of the secondary trityl ether, the reaction was performed in the presence of a relatively large amount of activated molecular sieves (3Å). The preclusion of hydrolysis is very important since we observed that the analogous saccharide having a free 4-hydroxyl group could not be glycosylated confirming that tritylation of a secondary positions activates a hydroxyl for glycosylation.[11] Next, the less reactive primary trityl ether of **4** was glycosylated with the cyanoethylidine derivative **6**, having been prepared by treatment of aceto bromo lactose **5** with potassium cyanide, in the presence of a catalytic amount of trityl perchlorate, to furnish tetrasaccharide **7** in a good yield.[15] It was attempted to use ethyl per-*O*-acetyl thiolactoside instead of **6** as the glycosyl donor. However, this coupling was only modestly successful when MeOTf was used as the promoter in the absence of molecular sieves. Probably, under these glycosylation conditions, the primary trityl ether is cleaved by the acidic nature of the reaction and the resulting alcohol is glycosylated. This proposed reaction path is supported by the fact that in the presence of molecular sieves (acid scavenger) no glycosylation occured and only destruction of the thioglycosyl donor was observed.

The pentenyl moiety of tetrasaccharide **7** has remained intact throughout the above discussed synthetic steps leaving it available to serve as an efficient anomeric leaving group.[10] Thus, coupling of **7** with **9**[16] in the presence of NIS/TMSOTf gave the requisite hexasaccharide **10**[17] in an acceptable yield. Compound **10** was deprotected by base mediated cleavage of the acetyl groups followed hydrazinolysis of the phthalamido moiety and acetylation of the revealed amino group and finally catalytic hydrogenation of the benzyl ethers. The *N*-acetyl neuraminic acid of **I** may now be introduced by the employment of a NAcNeu transferase.[18]


1
1. TFA/DCM, 96%
2. TrClO4/γ-collidine/DCM, 84%
3
2
5
KCN/Bu4NBr/MeCN, 42%
MeOTf/MS3Å/DCM, 98%
4
6
8
TrClO4/DCM, 75%
1. BnBr/NaH/DMF, 88%
2. TFA/DCM, 95%
9
7
NIS/TMSOTf/DCM, 52%
10

Scheme 1

In conclusion, we have reported a highly convergent synthesis of a hexasaccharide derived from the group B Type III *Streptococcus* polysaccharide. It is to be expected that the strategic principles used here will be important for the assembly of other oligosaccharides. A similar hexasaccharide will be prepared that contains an artificial spacer at the anomeric centre for selective conjugation to carrier proteins.

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17. Analytical data of compound **10**.

	H-1	$J_{1,2}$	H-2	$J_{2,3}$	H-3	$J_{3,4}$	H-4	$J_{4,5}$	H-5	$J_{5,6a}$, $J_{5,6b}$, $J_{6a,6b}$	H-6a, 6b
A	4.12	8.0	3.27	9.0	3.40	9.0	3.80	10.0	3.02	5.0, 10.0, 10.0	3.43-3.50
B	4.26	7.5	3.72	9.5	3.38	2.5	3.97	2.0	3.55	4.0, 8.0, 9.0	3.92-3.98
C	5.33	8.0	4.18	8.0	4.27	–	3.34	–	3.46	–	3.79-3.90
D	4.74	8.0	5.19	10.0	4.94	8.0	3.82	8.0	4.56	–	4.05-4.15
E	4.46	8.0	5.09	10.0	4.90	2.5	5.34	1.5	3.46	–	3.90-4.15
F	4.44	8.0	4.95	10.0	5.17	2.5	5.34	1.5	3.47	–	3.90-4.15

Additional ^{1}H NMR data 400MHz ($CDCl_3$): 6.75-7.50 (m, 34H, aromatic), 4.75, 4.72, 4,41, 4.33, 4.32, 4.25 (6 dd, 12H, J^2 12.0, 6 CH_2), 3.45 (s, 3H, CH_3), 2.98 (bs, 1H, OH), 2.16, 2.14, 2.08, 2.07, 2.07, 2.06, 2.04, 2.00, 1.97, 1.95, 1.89 (11s, 33H, 11 $COCH_3$).

Fab Mass: Found 2158.749887. Calc. 2158.752303; $C_{109}H_{125}NO_{43}Na$.

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