Synthesis of Ganglioside Epitopes for Oligosaccharide Specific Immunoadsorption Therapy of Guillain–Barré Syndrome

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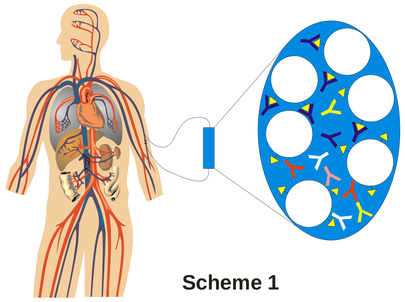
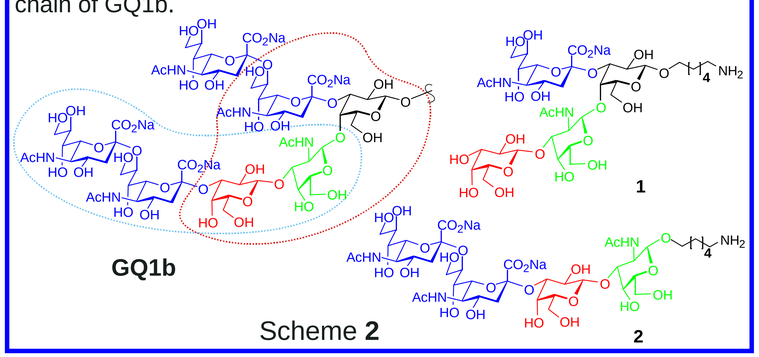
Introduction

Campylobacter jejuni is a Gram-negative bacteria, causing gastroenteritis in human. The most striking feature of *C. jejuni* lipopolysaccharides is the presence of epitopes that resemble human gangliosides. Consequently a *C. jejuni* infection frequently lead to Guillain-Barré Syndrome (GBS) which is characterized by attack of the body's immune system on the peripheral nervous system, usually the myelin sheaths, resulting in an inability of nerves to transmit signals to muscles. In grave cases, a total paralysis occurs. The pathophysiologic mechanism of GBS is directly related to *C. jejuni* infection because the immune responses directed against the lipopolysaccharide components produce antibodies that cross-react with human gangliosides such as GD_3, GQ_{1b} and GM_2 etc. Therefore, oligosaccharide inhibitors or structural mimics of these highly complex carbohydrate epitopes have the potential for therapy, either as soluble blocking ligands administered systemically, or as immuno-affinity ligands for use as extracorporeal immunoadsorbents. (Scheme **1**).



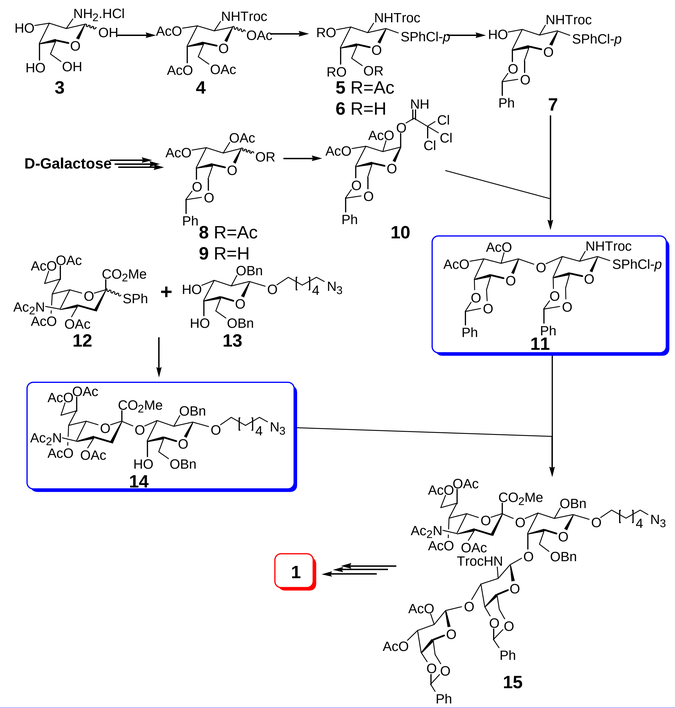
Scheme 1

In the study of sera of patients with Miller-Fisher Syndrome (MFS, a variant of GBS), our research showed that the antibodies can almost always bind to GQ1b (Scheme 2) which contains a disialoside epitope [αNeuNAc(2→8)αNeuNAc]. Previously, we have synthesized two trisaccharide epitopes that are related to GD_3 and GM_2 gangliosides. As a continuation of present project, we wish to report the synthesis of another two tetrasaccharide epitopes (**1** and **2**) that will be used for immuno-absorbance studies. The epitope **1** relates to ganglioside GM_1 while epitope **2** relates to one branch of the side chain of GQ1b.

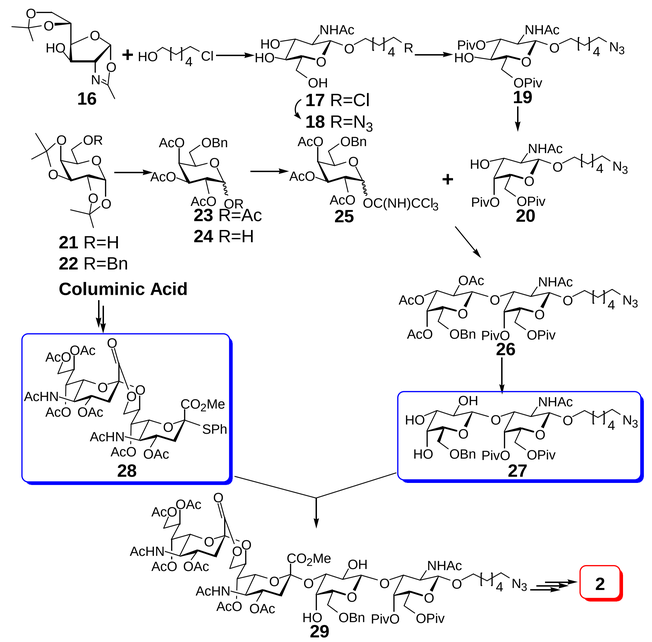


Scheme **2**

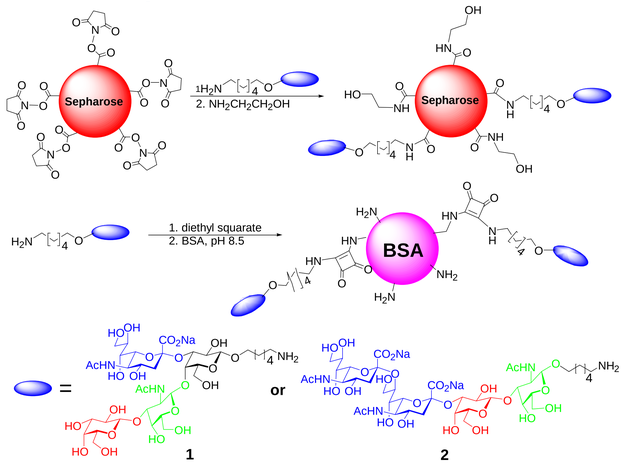
Scheme 3 Synthesis of GM1 Epitope



Scheme 4 Synthesis of 'GQ1b' Epitope



Scheme 5 Preparation of Immunoadsorbents



Results and Discussion

The tetrasaccharides **1** and **2** were prepared by following schemes **3** and **4**. In both cases, a 2+2 strategy was adopted. The thioglycoside donor **11** was obtained in 62% yield and sialylation of **13** using **12** afforded **14** in 64% yield; *N*-iodosuccinimide/TfOH mediated glycosylation between disaccharides **11** and **14** yielded the desired tetrasaccharide **15** in 23% yield. The disaccharide acceptor **27** was prepared by reacting imidate **25** with alcohol **20** in 51% yield; as reported before, the disialoside donor **28** was prepared by hydrolysis of colominic acid via an improved procedure in 33% yield over 4 steps. Glycosylation of triol **27** using **28** afforded the desired tetrasaccharide **29** in poor yield (<20%). Both compounds **1** and **2** were obtained after multi-deprotection steps.

The BSA conjugates of tethered epitopes **1** and **2** were also prepared using squarate as the cross linking reagent (Scheme **5**). Using 15 times excess of oligosaccharide/BSA ratio, the corresponding glycoconjugates bearing 4-6 epitopes per BSA were obtained. These conjugates were used for ELISA assay.

For tetrasaccharide **1**, affinity columns were prepared using the commercially available *N*-hydroxysuccimide activated sepharose gel (Scheme **5**), in order to evaluate its ability to bind to antibodies present in the serum of patients with GBS and MFS. The solid matrix contains ~0.82 mmol per mL of dry gel. The tetrasaccharide **2** will be coupled to the sepharose gel in the near future.

References

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2. Andersen, S.M. *et al Org. Biomol. Chem.*, 2004, **2**, 1199-1212.

Acknowledgements

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