Synthesis of Thio-Linked Gangliosides for Use as Immunogens

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INTRODUCTION

Over-expression of glycolipid antigens on the surface of cancerous cells make these structures attractive targets for use in the active immunotherapy of tumors. Ganglioside glycolipids are constituents of conjugate vaccines currently in clinical trials. Although there are numerous potential reasons for poor immunogenicity of ganglioside vaccine preparations, we hypothesize that one cause may be the in vivo liability of terminal N-acetyl neuraminic acid (Neu5Ac) residues. If valid, this hypothesis could be tested by evaluating the immunogenicity of glycolipid analogues that incorporate hydrolytically resistant Neu5Ac residues in ganglioside conjugate vaccines.

Glycolipid analogues in which the non-reducing terminal glycosidic oxygen is replaced by sulfur have been shown to resist enzymatic degradation by exoglycosidases. Although S-linked oligosaccharides are more flexible than their O-linked counterparts, the geometry about the glycosidic linkage remains similar. Vaccines containing a thio-linked Neu5Ac residue will be compared with those containing natural O-linked ganglioside epitopes.

Herein we describe the synthesis of an unnatural thioglycolipid (1), and progress towards the synthesis of analogues of the tumor associated glycolipid antigens GM \(_2\) (2) and GM \(_3\) (3). These structures contain a truncated ceramide aglycon, functionalized to allow conjugation to a carrier protein. As an initial proof of concept, antibodies will be raised against 1-3 and tested for cross reactivity with the corresponding O-linked compounds.

SYNTHESIS

A. Pseudo-ganglioside 1

Reaction of diol 4 with trifluoromethane-sulphonic anhydride in pyridine and dichloromethane gave trflate 5 in 81% yield. In situ generation of the 2-thiolate from Neu5Ac thioglycoside 6, and subsequent displacement of the trflate afforded the 2,6-thio-linked trisaccharide 7 stereoselectively and in high yield. The azidosphingosine analog 8 was obtained in 73% yield after acetylation, anomeric deblocking, generation of the glycosyl trichloroacetimidate, and boron trifluoride promoted glucosylation of the azido-alcohol. The protected glycolipid 9 was generated by reduction of the azide followed by N-acetylation. Transesterification followed by saponification of the methyl ester afforded the target compound 1. The terminal olefin in 1 is suitable for coupling to protein following, for example, reaction with cysteamine.

Scheme 1. Synthesis of Unnatural Glycolipid 1

B. Progress Towards GM\(_2\)/GM\(_3\) Analogues 2 and 3

Attempts to displace of trflate 10 (derived from lactose in 11 steps), with 11 under a variety of conditions yielded elimination product 12 (see Scheme 2). Reaction of 10 with potassium thiocetate in dimethyl formamide afforded 13 in good yield. It was expected that deprotection of the 3'-thioacetate would provide the 3'-thiolate, suitable for coupling with 14 to give 15 under basic conditions. However, selective deacetylation proved difficult. Instead, compound 16 was isolated after transesterification in NaOMe/MeOH/toluene at reflux. Reaction of 16 with 14 under basic conditions should afford 17, a key intermediate in the synthesis of both GM\(_2\) and GM\(_3\) analogues 2 and 3.

Scheme 2.

Summary

Synthesis of ganglioside analogue 1 containing an \(\alpha-(2,6)\) thio-linked N-acetyl neuraminic acid residue has been completed. This molecule is suitable for elaboration and conjugation to a carrier protein via its terminal olefin.

Progress towards 2 and 3 is outlined. Reaction of 16 with 14 under basic conditions should afford trisaccharide 17, a central intermediate for both the GM\(_2\) and GM\(_3\) analogues.

Antibodies will be raised against 1-3 and tested for cross-reactivity with the O-linked compounds.

References:

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