Synthesis of Thio-Linked Gangliosides for Use as Immunogens

Jamie R. Rich, and David R. Bundle*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada,T6G 2G2

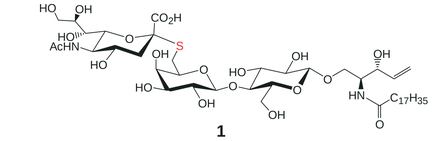
INTRODUCTION

Over-expression of glycolipid antigens on the surface of cancerous cells make these structures attractive targets for use in the active immunotherapy of tumors.[1] 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* lability 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.[2] Although S-linked oligosaccharides are more flexible than their *O*-linked counterparts, the geometry about the glycosidic linkage remains similar.[3] 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_3 (**2**) and GM_2 (**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.

References: 1) Ragupathi, G. (1996) *Cancer Immunol. Immunother.*, **43**, 152-157. 2) Wilson, J.C., Kiefel, M.J., Angus, D.I., von Itzstein, M. (1999) *Org. Lett.*, **3**, 443-446 3) Geyer, A., Hummel, G., Eisele, T., Reinhardt, S., Schmidt, R.R. (1996) *Chem. Eur. J.*, **2**, 981-988. Aguilera, B., Jiménez-Barbero, J., Fernández-Mayoralas, A. (1998) *Carbohydr. Res.*, **308**, 19-52.



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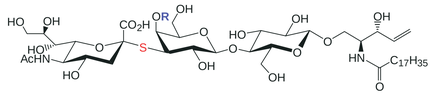


2 (R = H)
3 (R = βGalNAc)

SYNTHESIS

A. Pseudo-ganglioside 1

Reaction of diol **4** with trifluoromethane-sulphonic anhydride in pyridine and dichloromethane gave triflate **5** in 81% yield. *In situ* generation of the 2-thiolate from Neu5Ac thioglycoside **6,** and subsequent displacement of the triflate afforded the 2,6-thio-linked trisaccharide **7** stereoselectively and in high yield. The azidospingosine analog **8** was obtained in 73% yield after acetylation, anomeric deblocking, generation of the glycosyl trichloroacetimidate, and boron trifluoride promoted glycosylation of the azido-alcohol. The protected glycolipid **9** was generated by reduction of the azide followed by *N*-acylation. 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.

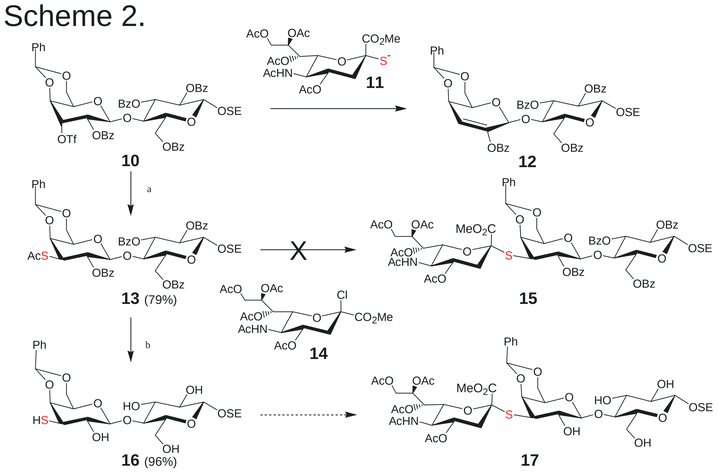
Scheme 1. Synthesis of Unnatural Glycolipid **1**

Reagents and Conditions: a) Tf_2O (1.1 eq.), pyridine, CH_2Cl_2, -20°C b) Me_2NH (10 eq.), **6** (1.2 eq.), DMF c) Ac_2O, pyridine d) TFA/toluene (2:1) e) Cl_3CCN, DBU, CH_2Cl_2 f) (2S,3R)-2-Azido-3-*O*-benzoyl-4-penten-ol (0.8 eq.), BF_3Et_2O (2.8 eq.), CH_2Cl_2, Drierite g) (i) PPh_3 (2 eq.), pyridine/H_2O (10:1), 50°C (ii) *N*-hydroxy-succinimidyl octadecnoate (5 eq.), 50°C h) MeOH, Na, then add H_2O, reflux.

B. Progress Towards GM_2/GM_3 Analogues 2 and 3

Attempted displacement of triflate **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 thioacetate 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 deacylation 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.



Reagents and Conditions: a) KSAc, DMF,50°C b) Na, MeOH, toluene, reflux

Summary

Synthesis of ganglioside analogue **1** containing an α-(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_3 and GM_2 analogues.

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

CSC 2002
Vancouver, B.C.
June 1 - 5, 2002

Financial support was provided by a research grant from the Canadian Institutes for Health Research and postgraduate studentships from the Alberta Heritage Foundation for Medical Research and the Natural Sciences and Engineering Research Council of Canada