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S-Linked Ganglioside Analogues for Use in Conjugate Vaccines

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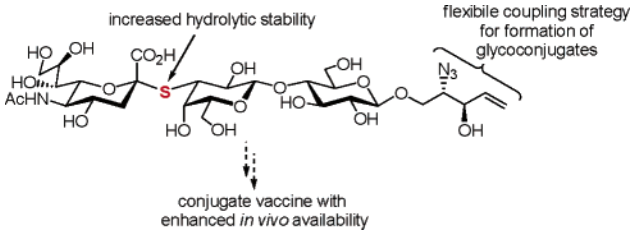
ABSTRACT



Glycosidase resistant thioglycoside precursors of the melanoma-associated ganglioside GM_2 have been synthesized starting from lactose. Syntheses of several analogues of ganglioside GM_3 and a positional isomer have been developed. These compounds contain thio-linked sialic acid residues and a modified ceramide aglycon functionalized for coupling to proteins, surfaces, or matrices. The hydrolytic stability of these oligosaccharides enhances the immunogenicity of the corresponding conjugate vaccines by ensuring their integrity in the acidic compartments of antigen processing cells.

The overexpression of glycolipid and glycoprotein structures on the surface of cancerous cells makes these structures attractive targets for use in the active immunotherapy of tumors.[1] Gangliosides are a class of *N*-acetyl neuraminic acid (Neu5Ac) containing glycolipid molecules that are expressed at greatly elevated concentrations in melanomas and other cancers of neuroectodermal origin.[2] Low levels of immune response to vaccines containing naturally occurring ganglioside antigens have thus far proven prohibitive to the development of a viable anticancer vaccine.[1b,c] There is therefore a need for improved design of ganglioside-based melanoma vaccines that have the capability to induce a strong humoral and if possible also a cell-mediated immune response, since recent preclinical and clinical studies with poorly immunogenic semisynthetic GM_2 vaccines linked to KLH enjoyed only limited success.

Glycosidase-resistant thioglycoside analogues of the melanoma-associated ganglioside GM_2 are attractive candidates as components of improved vaccines since their much slower rates of either chemical or enzymatic hydrolysis[3] would render these antigens more persistent. Conformational studies of *S*-linked oligosaccharides (thiooligosaccharides) suggest that although the flexibility about the anomeric linkage is increased for these molecules, they sample similar conformational space to their natural *O*-linked counterparts.[4] We propose, based on preliminary immunochemical evidence,[5] that the inclusion of thiooligosaccharides in conjugate vaccine

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preparations will result in the production of antibodies, which cross-react with gangliosides on the tumor cell surface. Owing to their metabolic stability *S*-linked gangliosides should serve as antigens with extended in vivo stability, and thus they are one part of our strategy to enhance the immunogenicity of carbohydrate-based anticancer vaccine preparations.[6]

Ganglioside GM_3 (**1**) is the most commonly expressed ganglioside structure in human cells, and is a biosynthetic precursor to tumor-associated gangliosides of higher complexity such as GM_2 and GD_3. We report here the synthesis of GM_3 analogues **2** and **3** as well as their positional isomer

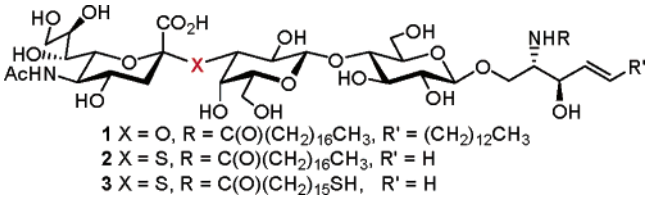


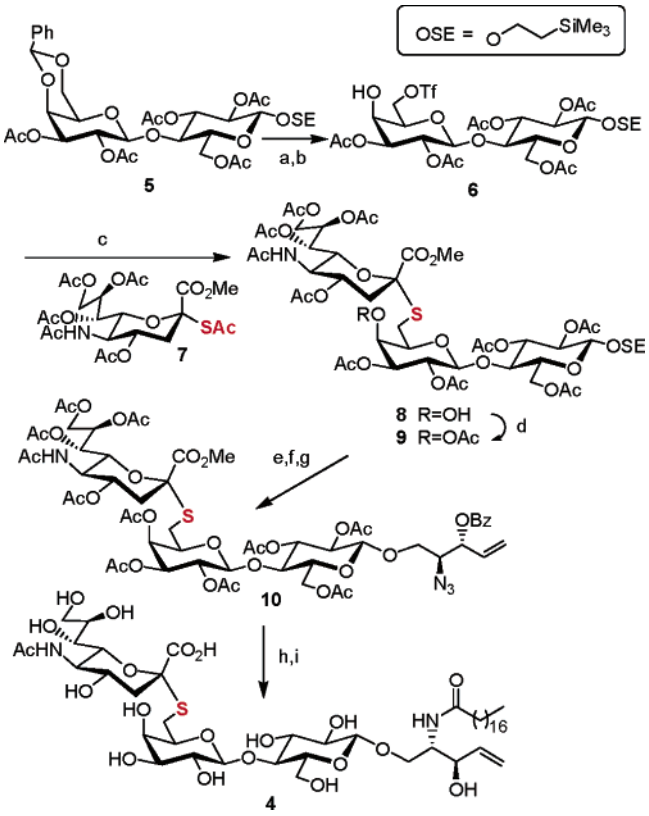
Figure 1. GM_3 (**1**) and GM_3 analogues **2** and **3**.

4 (Scheme 1) for all of which the terminal Neu5Ac residue is attached via a thioglycosidic linkage. These structures bear a modified ceramide aglycon that has been designed to facilitate coupling of the neoglycolipids to carrier protein while maintaining the stereochemical integrity and relevant structural features found in naturally occurring ceramides.[7] GM_3 analogues **2** and **3** and their precursors are poised for chemical and enzymatic elaboration into analogues of more complex gangliosides.

Before embarking on a synthesis of a sulfur-containing GM_3 oligosaccharide we sought to identify suitable chemistries for the assembly of our target. To this end, we envisioned the construction of an isomer (**4**) that would serve as an appropriate model system. It was expected that the formation of the thioglycosidic linkage at the primary position would proceed most readily. The synthesis of an α 2−6 linked thiooligosaccharide is outlined in Scheme 1.

Hydrolysis of the benzylidene acetal **5**[8] afforded the crystalline diol, which after selective sulfonylation of the primary alcohol yielded triflate **6**. Displacement of the triflate with a Neu5Ac thiolate, generated in situ from thioacetate **7**[9] by treatment with diethylamine,[10] yielded the trisaccharide in 86% yield.[11] Acetylation of this mixture followed by deprotection[8] of the anomeric position yielded the hemi-

Scheme 1 [a]



[a] Reagents and conditions: (a) 80% AcOH, 50 °C, 9 h, 86%; (b) Tf_2O, pyridine, DCM, −25 °C, 1 h, 72%; (c) Et_2NH, **7**, DMF, −25 °C, 2 h, 86%; (d) Ac_2O, pyridine, rt, 12 h, 96%; (e) TFA/toluene (1:1), rt, 5 h; (f) Cl_3CCN, DBU, DCM, 0 °C, 5 h; (g) (2*S*,3*R*)-2-azido-3-*O*-benzoyl-4-pentene-1,3-diol, BF_3Et_2O, Drierite, −10 to 20 °C, 2.5 h, 71%; (h) (i) PPh_3, pyridine/H_2O, 50 °C, 4 h, (ii) *N*-(octadecanoyloxy)succinimide, 50 °C, 5 h, 88%; (i) (i) NaOMe, MeOH, rt, 24 h, (ii) NaOH, rt, 24 h, 84%.

acetal.[12] Conversion to the trichloroacetimidate[13] and coupling with (2*S*,3*R*)-2-azido-*O*-benzoyl-4-pentene-1,3-diol[7] afforded the glycoside **10**. This aglycon represents a truncated version of azidosphingosine reported by Schmidt et al.[14] and was designed to allow (a) *N*-acylation to form ceramide-type structures and (b) opportunities for coupling of the glycolipid to protein via either the amine or olefin. The azide in **10** was reduced with triphenylphosphine, and the amine acylated. Removal of the acetates by transesterification and saponification of the remaining methyl ester gave thiosialoside **4**.

(6) Metabolically stable glycosides have recently been proposed for use in vaccines: (a) Kuberan, B.; Sikkander, S. A.; Tomiyama, H.; Linhardt, R. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 2073−2075. (b) Bousquet, E.; Spadaro, A.; Pappalardo, M. S.; Bernardini, R.; Romeo, R.; Panza, L.; Ronsisvalle, G. *J. Carbohydr. Chem.* **2000**, *19*, 527−541.

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(9) Hasegawa, A.; Nakamura, J.; Kiso, M. *J. Carbohydr. Chem.* **1986**, *5*, 11−19.

(10) Bennett, S.; Von Itzstein, M.; Kiefel, M. J. *Carbohydr. Res.* **1994**, *259*, 293−299.

(11) Alternatively, **8** could be obtained directly from the diol without purification of the sulfonate ester. Interestingly, some acetyl transfer to the 4′-OH (presumably originating from sulfur) was also observed in this case and **9** (7%) accompanied the formation of **8** (73%).

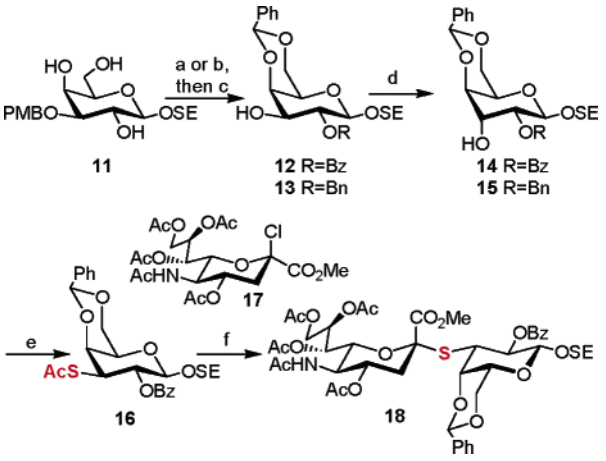
(12) Hasegawa, A.; Morita, M.; Ito, Y.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1990**, *9*, 369−392.

(13) (a) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed.* **1980**, *19*, 731−732. (b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21−123.

(14) (a) Schmidt, R. R.; Zimmermann, P. *Tetrahedron Lett.* **1986**, *27*, 481−484. (b) Schmidt, R. R.; Zimmermann, P. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 725−726. (c) Zimmermann, P.; Bommer, R.; Bar, T.; Schmidt, R. R. *J. Carbohydr. Chem.* **1988**, *7*, 435−452.

There are several reports of the synthesis of sulfur-containing Neu5Ac(α2−3)Gal linkages in the literature.[15] Owing to the apparent difficulties encountered in the installation of sulfur at this position,[15d,16] we began with synthesis of the disaccharide (Neu5Ac-*S*-(α2−3)Gal) rather than the trisaccharide. The protected thiodisaccharide **18** was obtained as outlined in Scheme 2. Galactoside **11**[17] was

Scheme 2 [a]

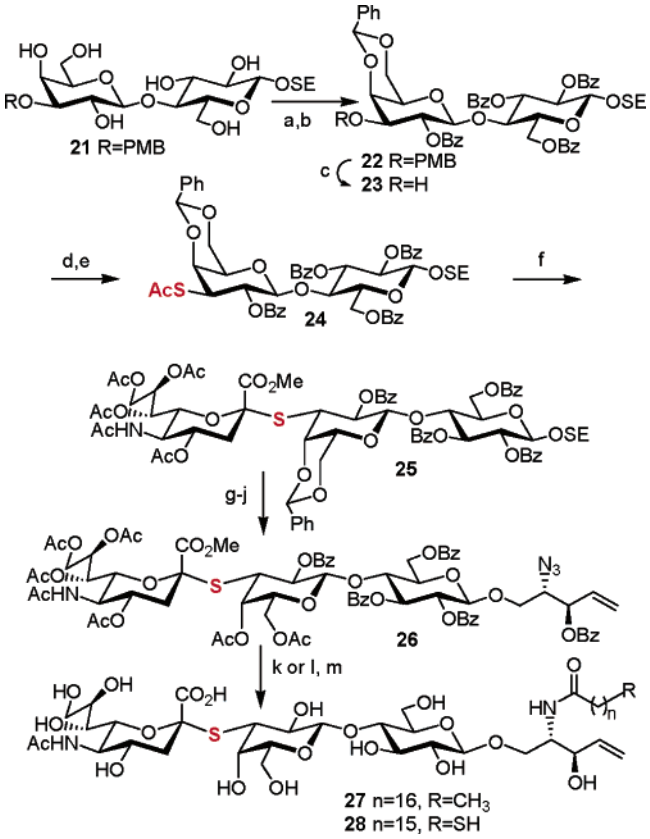


[a] Reagents and conditions: (a) for **12**: (i) benzaldehyde dimethyl acetal, *p*-TSA, MeCN, rt, 25 min, (ii) BzCl, pyridine, rt, 12 h, 76%; (b) for **13**: (i) benzaldehyde dimethyl acetal, *p*-TSA, MeCN, rt, 25 min, (ii) NaH, BnBr, rt, 3 h, 71%; (c) CAN, MeCN/H_2O (19:1), rt, 30 min, 85% (**12**), 77% (**13**); (d) (i) Tf_2O, pyridine, DCM, −20 °C, 1 h, (ii) $Bu_4N^{+-}NO_2$, MeCN, rt, 12 h, 72% (**14**), 40% (**15**); (e) (i) Tf_2O, pyridine, DCM, −20 °C to rt, 50 min, (ii) KSAc, DMF, 60 °C, 12 h, 79%; (f) (i) hydrazinium acetate, DMF, rt, 90 min, (ii) **17**, NaH, DMF, rt, 12 h, 67%.

transformed to the 4,6-*O*-benzylidene acetal and the remaining hydroxyl protected as a benzoate ester or benzyl ether. Removal of the *p*-methoxybenzyl ether yielded **12**[18] or **13**, which after triflation could be converted to the gulosides **14** (72%) or **15** (40%) by displacement with tetrabutylammonium nitrite.[19] Installation of the sulfur atom was effected in 79% yield by treatment of the triflate formed from alcohol **14** with potassium thioacetate. The benzyl-protected guloside proved to be a poor substrate for the triflation−inversion sequence and thus its further elaboration to the disaccharide was abandoned. Removal of the thioester with hydrazine acetate provided the thiol, which following treatment with sodium hydride underwent reaction with **17** to afford the disaccharide **18**.

The synthesis of a 3′-thiolactoside (Scheme 3) for the construction of the GM_3 trisaccharide followed closely the

Scheme 3 [a]



[a] Reagents and conditions: (a) benzaldehyde dimethyl acetal, DMF, *p*-TSA, rt, 13 h; (b) BzCl, pyridine, rt, 12 h, 70%; (c) CAN, MeCN/H_2O, rt, 40 min, 86%; (d) (i) Tf_2O, pyridine, DCM, −40 °C, 70 min, (ii) $Bu_4N^{+-}NO_2$, DMF, rt, 14 h, 91%; (e) (i) Tf_2O, pyridine, DCM, −65 °C to rt over 30 min, then rt for 1 h, (ii) KSAc, DMF, 70 °C, 1 h, 79%; (f) hydrazinium acetate, DMF, rt, 1.5 h, then **17**, NaH, DMF, Kryptofix-21, rt, 12 h, 65%; (g) 80% AcOH, 45 °C, 48 h, 81%; (h) Ac_2O, pyridine, rt, 12 h, 95%; (i) toluene/TFA, rt, 2 h, 96%; (j) (i) Cl_3CCN, DBU, DCM, rt, 1.5 h, (ii) (2*S*,3*R*)-2-azido-3-*O*-benzoyl-4-pentene-1,3-diol, BF_3Et_2O, Drierite, −15 °C, 24 h, 80%; (k) (i) PPh_3, pyridine/H_2O, 40 °C, 7 h, (ii) *N*-(octadecanoyloxy)succinimide, 40 °C, 12 h, 71%; (l) (i) PPh_3, pyridine/H_2O, 45 °C, 3 h, (ii) *N*-(*S*-acetyl-16-mercaptohexadecanoyloxy)succinimide, 45 °C, 5 h, 74%; (m) (i) NaOMe, MeOH, rt, 24 h, (ii) NaOH, rt, 24 h, 76% for **27**, 82% for **28**.

route identified for assembly of the thiodisaccharide. 3′-*O*-*p*-Methoxybenzyl lactoside **21**[20] underwent benzylidene acetal formation followed by benzoylation to give **22**. Following selective deprotection of the 3′-hydroxyl, triflation of the alcohol and displacement with nitrite gave the axial epimer. A second triflation−inversion sequence yielded thioacetate **24**.[21] After reaction of the thiolate with Neu5Ac

(15) (a) Kanie, O.; Nakamura, J.; Itoh, Y.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1987**, *6*, 117−128. (b) Eisele, T.; Schmidt, R. R. *Liebigs Ann. Recl.* **1997**, 865−872. (c) Wilson, J. C.; Kiefel, M. J.; Angus, D. I.; von Itzstein, M. *Org. Lett.* **1999**, *1*, 443−446. (d) Turnbull, W. B.; Field, R. A. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1859−1866.

(16) Marcaurelle, L. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2001**, *123*, 1587−1595.

(17) Hasegawa, A.; Ando, T.; Kameyama, A.; Kiso, M. *J. Carbohydr. Chem.* **1992**, *11*, 645−658.

(18) Previous synthesis: Lowary, T. L.; Eichler, E.; Bundle, D. R. *J. Org. Chem.* **1995**, *60*, 7316−7327.

(19) (a) Lattrell, R.; Lohaus, G. *Justus Leibigs Ann. Chem.* **1974**, 901−920. (b) Albert, R.; Dax, K.; Link, R. W.; Stutz, A. E. *Carbohydr. Res.* **1983**, *118*, C5−C6.

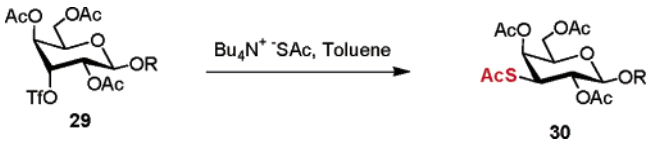
(20) Hotta, K.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1994**, *13*, 175−191.

(21) Efforts to employ the anomeric thiolate of sialic acid as a nucleophile (see Scheme 1) to produce disaccharide **18** or trisaccharide **25** met with failure, only 2,3-elimination products were obtained.

glycosyl chloride **17**, the benzylidene acetal was replaced by acetates, and the trisaccharide was converted to its hemiacetal. Formation of the trichloroacetimidate and coupling with (2*S*,3*R*)-2-azido-3-*O*-benzoyl-4-pentene-1,3-diol[7] afforded the benzoylated azido alcohol **26**. At this stage, azide reduction, *N*-acylation with an activated octadecanoate, and deprotection gave a GM_3 analogue suitable for further elaboration. For example, derivatives such as **27** will undergo radical addition of cysteamine to the terminal olefin.[22] Alternatively, **26** could be converted to **28**, which bears a thiol at the *N*-acyl chain terminus.[23] These two strategies allow for coupling of the thioganglioside analogue to carrier protein via either the sphingosine base or hydrocarbon portions of the aglycon.

A recent report[24] describing the first synthesis of a 3′-thio lactoside from lactose suggests that a 4,6-*O*-benzylidene acetal is important for successful *gulo* to *galacto* interconversions with KSAc and confirms our concurrent findings. In our hands, only cyclic acetal (benzylidene or isopropylidene)-protected (ga)lactosides undergo smooth inversion with KSAc. To circumvent this protecting group requirement we have demonstrated that tetrabutylammonium thioacetate can be employed as a nucleophile when esters are used as protecting groups as shown for the conversion of **29** to **30** in Scheme 4.[25] However, we found that acetal-

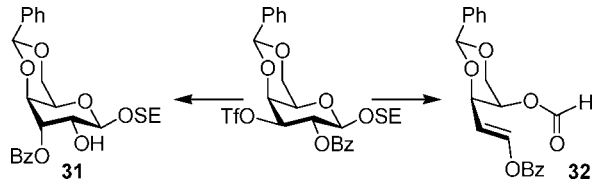
Scheme 4



protected 3-thio(ga)lactosides are superior nucleophiles for reaction with **17** when compared to 3-thio-4,6-diesters.

The conversion of *galacto*-configured alcohols such as **12** and **23** to the corresponding *gulo* alcohols via displacement with nitrite ion also bears consideration. We and others[24] have found that inversion of configuration at C-3 of both galactosides and gulosides precludes the use of a benzyl protecting group at O-2. While the benzoate ester proves superior in this regard, careful temperature optimization and control are often required to realize acceptable yields. We

Scheme 5



have observed two side reactions as outlined in Scheme 5. Acyl migration was frequently observed during the synthesis of **14** and the analogous disaccharide, and could be suppressed by conducting the reaction at lower temperatures. While acyl migration (see **31)** appears to be a general feature[26] of similarly protected galactosides, a second product that we have tentatively assigned as **32**[27] was observed less frequently, and only with the substrate shown in Scheme 5. We are currently investigating the basis and scope of this reaction.

In conclusion, we have developed syntheses of several novel thioglycoside analogues of the ganglioside GM_3. These compounds are functionalized so as to allow for their facile incorporation into carbohydrate−protein conjugates for use as conjugate vaccines. GM_3-type molecules **27** and **28** will be elaborated by chemoenzymatic means to analogues of higher gangliosides such as GM_2, also for inclusion in antimelanoma vaccine preparations. Similarly, intermediates such as **18** and **25** will serve as advanced synthetic precursors to analogues of gangliosides GM_2, GM_1, and GD1a. It is expected that the enhanced metabolic stability of these antigens will render them more immunogenic and that antibodies raised in this manner will cross react with their naturally occurring *O*-glycosides.

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Supporting Information Available: Experimental procedures and ^{1}H NMR and other spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) Liakatos, A.; Kiefel, M. J.; von Itzstein, M. *Org. Lett.* **2003**, *5*, 4365−4368.
(25) Rich, J. R.; Szpacenko, A.; Palcic, M. M.; Bundle, D. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 613−615.
(26) This reaction has been observed for β-octyl, β-allyl, and β-8-methoxycarbonyloctyl galactosides and a variety of lactosides.
(27) The proposed structural assignment of **32** is based on analysis of IR, HRMS, and ^{1}H and ^{13}C NMR data, including TROESY spectra.