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Synthesis of a novel glycosphingolipid from the millipede, *Parafontaria laminata armigera*, and the assembly of its carbohydrate moiety into multivalent structures

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Abstract—A novel glycosphingolipid, β-D-Man*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*-(1→1)-Cer, found in the millipede, *Parafontaria laminata armigera*, and multivalent derivatives of its carbohydrate moiety were synthesized. As the key step, the target glycolipid (**1**) was obtained through an inversion reaction at the 2-position of a β-glucopyranoside residue yielding a β-mannopyranoside. In addition, the synthesis of fluorescently labeled trimer and tetramer glycoconjugates (**2**, **3**) was achieved by iterative amide bond formation using a monomer unit (**24**).

Keywords: Glycosphingolipids; *Parafontaria laminata armigera*; Intermolecular nucleophile approach; Glycocluster

1. Introduction

In our continuing studies to elucidate the biological function of glycoconjugates, we have synthesized novel glycosphingolipids found in various invertebrates[1–6] that do not have gangliosides. A number of glycosphingolipids from a variety of invertebrate origins and related analogues have been synthesized and their influence on proliferation has been examined using mouse melanoma B16 cells. In these studies, the glycolipid, β-D-Man*p*-(1→4)-[α-Fuc*p*-(1→3)]-β-D-Glc*p*-(1→1)-Cer, which has been isolated from the millipede *Parafontaria laminata armigera*, has been observed to inhibit significantly cell proliferation (details will be reported elsewhere).

In a previous paper,[2] we reported the synthesis of this glycosphingolipid. The key reaction in that synthesis was a two-step glycosylation. The first step was the formation of an orthoester from two sugar moieties and the second step was the reductive cleavage of one of the orthoester C–O bonds leading to β-selective mannosylation. This earlier paper was the first report on the total synthesis of β-D-Man*p*-(1→4)-[α-Fuc*p*-(1→3)]-β-D-Glc*p*-(1→1)-Cer by this method. Among β-mannopyranoside syntheses, one approach is the so called intermolecular nucleophiles approach,[7–10] in which an inversion reaction at the 2-position of β-glucopuranoside yields a β-mannopyranoside. The strategy, which provides good yields, relies upon creating a highly reactive leaving group at the position that is to be inverted and then treating it with a strong nucleophile. The nucleophile causes S_N2 displacement and epimerizes the position on the carbohydrate ring. We report here the application of this approach for the synthesis of glycosphingolipid **1**.

It is known that oligosaccharide chains generally interact with their protein receptors in a multivalent fashion to overcome the inherently low affinity of monovalent carbohydrate–protein interactions. Therefore, the construction of a clustered glycoconjugates is an important subject in glycoscience.[11,12] For this reason, we have used a new method to synthesize new peptidic glycoclusters and glycodendrons (**2**, **3**), which consist of a β-alanine derivative linked to the sugar moiety of **1** (Fig. 1).[13,14]

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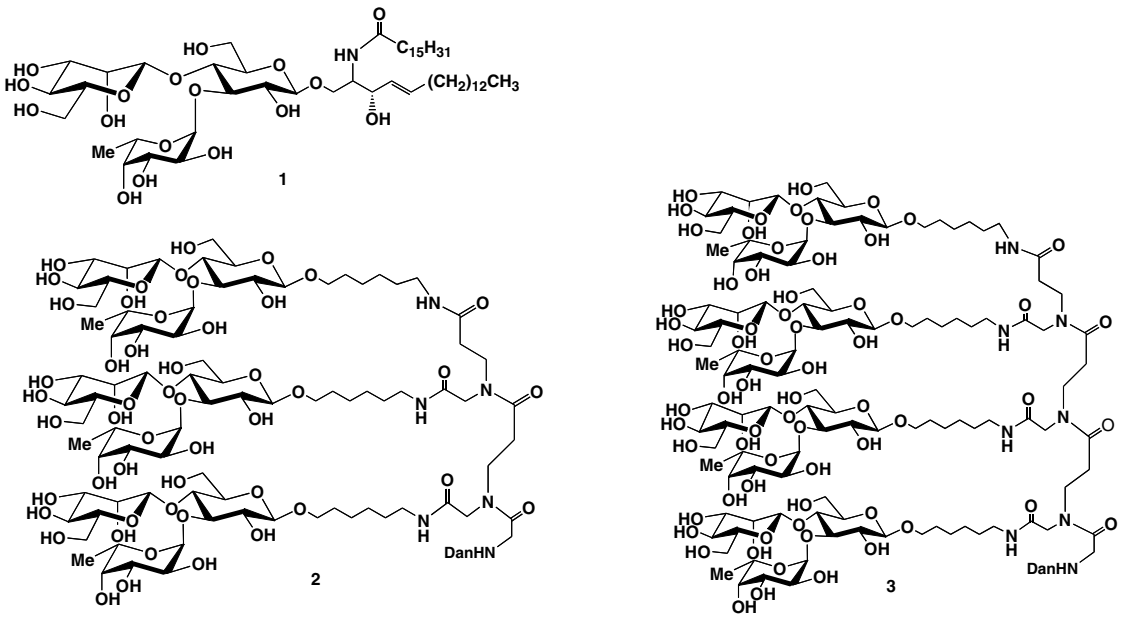


Figure 1. Structure of target glycoconjugates.

2. Results and discussion

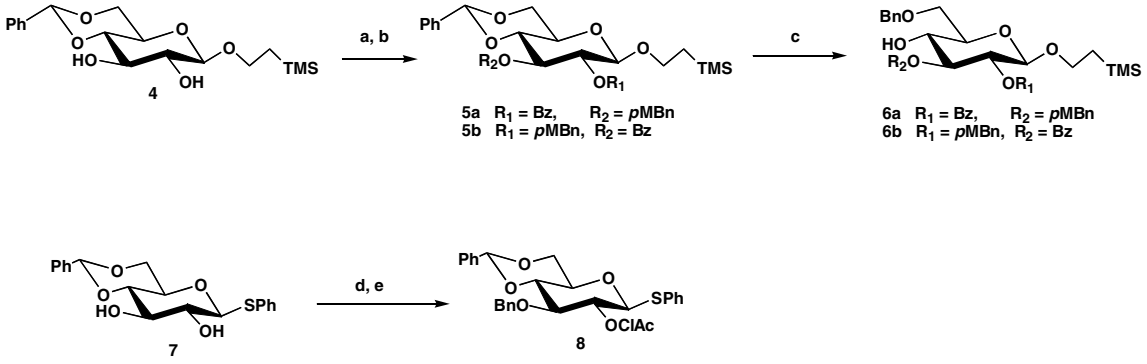
2.1. Syntheses of monosaccharide derivatives

Syntheses of the glucopyranose building blocks **6a** and **8** were carried out as depicted in Scheme 1. Compound **6a** was prepared from known 2-(trimethylsilyl)ethyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**4**)[15] via successive mono-alkylation of the diol, acylation and reductive ring opening of the 4,6-acetal. Unfortunately, alkylation of **4** via a stanylene intermediate did not proceed in a regiospecific manner. Although the formation of dialkylated product was avoided, the two regioisomeric monoalkylated products could not be separated, nor could their benzoylated derivatives **5a** and **5b** be separated. The structures of **5a** and **5b** were confirmed after reductive ring opening of the benzylidene acetal in **5**, which yielded a 1.5:1 ratio of **6a** and **6b** in a combined yield of 72%.

Glucopyranosyl donor **8** was obtained from phenyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**7**),[16] via a two-step procedure. First, reaction of the stannylidene derivative of **7** with benzyl bromide was found to be highly regiospecific in this case, which may be associated with the electron donating nature of the sulfur aglycon. Second, subsequent chloroacetylation of **7** afforded **8** (81% over two steps).

2.2. Synthesis of the target glycosphingolipid

Glycosylation of acceptor **6a** with **8** in the presence of *N*-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TfOH),[17] and 4 Å molecular sieves in dichloromethane gave the desired disaccharide (**9**) in 57% yield after purification. The stereochemistry of the newly formed glycosidic linkage could be determined by ^{1}H NMR spectroscopy (H-1′, 4.61 ppm, $J = 7.9$ Hz). Selective



Scheme 1. Reagents: (a) (i) *n*-Bu_2SnO, benzene; (ii) *n*-Bu_4NBr, *p*-MBnCl, toluene; (b) BzCl, pyridine, 71% two steps; (c) $NaBH_3CN$, HCl/Et_2O, THF, 72%; (d) (i) *n*-Bu_2SnO, benzene; (ii) *n*-Bu_4NBr, BnBr, toluene; (e) ClAcCl, CH_2Cl_2/pyridine, 81% two steps.