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Synthesis of glycosphingolipids from the fungus *Hirsutella rhossiliensis*

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ABSTRACT

The total synthesis of two neutral glycosphingolipids (GSLs) from the fungus *Hirsutella rhossiliensis* has been achieved. The GSLs possess a common neogala-core (Galβ1-6Gal) and have the following sequence: α-D-Man*p*(1 → 3)-β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1↔1)Cer (**1**) and α-D-Man*p*(1 → 3)-β-D-Gal*p*(1 → 6)[α-D-Glc*p*(1 → 4)]-β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1↔1)Cer (**2**). Our efficient synthetic strategy uses the different reactivity of the hydroxyl groups of galactose and the α-orienting solvent effect of dioxane-toluene to generate GSLs (**1**) and (**2**).

1. Introduction

Many glycosphingolipids (GSLs) sequences isolated from vertebrates terminate in sialic acid residues. In recent years, the synthesis of these GSLs and the understanding of the biological functions has attracted a lot of attention by various research groups.[1] In contrast, research on GSLs isolated from invertebrates has been neglected. There are only limited reports which describe naturally occurring sequences of GSLs in invertebrates.[2–21] These structures are significantly different from GSLs isolated from vertebrates. Moreover, the biological function of GSLs in invertebrates and fungi are unknown. For this reason we have been interested in the synthesis of glycolipids derived from various invertebrate sources in order to clarify their biological functions.[22–37]

Isolation and purification of glycolipids from invertebrate species are very difficult and the amount available is extremely small. As a result, controlled regio- and stereoselective synthetic approaches are required to produce sufficient amounts of homogenous material to explore biological structure/function relationships. Once the synthetic methodology has been developed this approach can also be applied to produce non-natural glycolipid analogs to exploit or manipulate their biological functions.

Tani et al. isolated and characterized three kinds of new GSLs, α-D-Man*p*(1 → 3)-β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1↔1)Cer (**1**), α-D-Man*p*(1 → 3)-β-D-Gal*p*(1 → 6)[α-D-Glc*p*(1 → 4)]-β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1↔1)Cer (**2**), and α-D-Glc*p*(1 → 2)-β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1↔1)Cer (**3**), which have neogala-series, β-D-Gal*p*(1–6)-β-D-Gal*p*(1–6)-β-D-Gal*p*, as core structure from the nematophagous fungus *Hirsutella rhossiliensis*.[38] Compound **3** was also isolated from mold, *Neurospora crassa* and the synthesis of compound **3** has been completed by Otsuka et al.[22] In this paper we describe our efforts to prepare GSLs (**1**) and (**2**). We were particularly interested to develop a synthetic scheme which minimizes the number of protection and deprotection steps by exploiting the different reactivities of hydroxyl groups in galactose. This approach is expected to reduce the number of synthetic steps while at the same time improving the overall yield of the desired products.

2. Result and discussion

2.1. Total synthesis of glycosphingolipid 1

The tetrasaccharide glycosphingolipid **1** contains the neogala-core sequence [β-D-Gal*p*(1 → 6)-β-D-Gal*p*(1 → 6)-β-D-Gal*p*] was prepared by stepwise synthesis of galactosyl donors and acceptors

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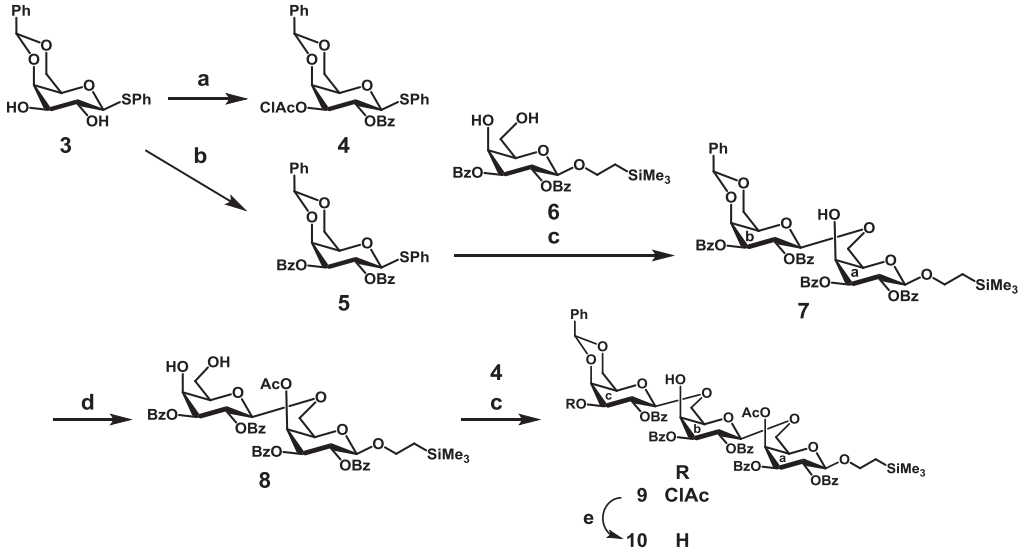
(Scheme 1). Galactopyranosyl donor **4** and **5** were obtained from phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**3**).[39] Regioselective chloroacetylation and subsequently benzoylation of **3** provided donor **4**.[40] Benzoylation of the two free hydroxyl groups of **3** using standard condition provided **5**.[41] We envisaged to achieve regioselective 6-O-glycosylation of galactoside acceptor **6** unprotected at both C-4 and C-6 by taking advantage of the greater steric hindrance between primary and secondary (axia) hydroxyl groups as well as using deactivating 2,3-*O*-benzoyl protecting groups. Disaccharide **7** was synthesized by selective glycosylation of diol-based glycosyl acceptor **6**[42] with thiogalactosyl donor **5** using *N*-iodosuccinimde (NIS)/trifluoromethanesulfonic acid (TfOH) as promoter.[43,44] The β-linkage in **7** was confirmed by ^{1}H-NMR spectroscopy. The nature of the new glycosidic linkage was determined by coupling constant of anomeric proton (H-1′, δ = 4.94, J = 8.1 Hz). This reaction was achieved by using small amount of donor **5** (1.1 equv.) which reacted regioselectively with the 6-OH of acceptor **6**. In addition, the glycosylation with the 6-OH was evidenced by HMBC correlation between the signal of C-6 at δ = 66.9 and H-1′ at δ = 4.94. The 4-OH group in **7** was acetylated and the benzylidene was cleaved by treatment with 80% AcOH to produce diol **8** in 72% yield. Comparing the ^{1}H-NMR data of **7** with those of **8** showed that H-4 signal of Gal a residue was shifted downfield by 1.49 ppm. This also indicates that the Gal b was bound at the 6-position of Gal a. The same previously described regioselective glycosylation strategy was used to generate trisaccharide **9** from diol **8**. NIS/TfOH promoted glycosylation of thiogalactoside donor **4** with acceptor **8** provided trisaccharide **9** in 78% yield as the only product. The anomeric proton of newly established anomeric center appeared as a doublet at δ = 7.83 (J = 8.2 Hz) consistent with the expected β-linkage. Trisaccharide acceptor **10** was synthesized by hydrolysis of the chloroacetyl group in **9** with aqueous pyridine in 92% yield.

Regioselective glycosylation of thiomannosyl donor **11**[45] with the trisaccharide diol-acceptor **10** in the presence of NIS and TfOH gave desired disaccharide **12**. As expected, the more reactive equatorial hydroxyl group at 3-position of acceptor **10** was successfully glycosylated. However, we were unable to isolate any other glycosylation product derived from glycosylation of the axial hydroxyl group indicating the highly regioselective nature of this glycosylation. The α-configuration of the new glycosidic linkage in **12** was indicated by the $J_{C\text{-}1',H\text{-}1'}$ value of 170 Hz. Furthermore, the binding to the 3-position was indicated by HMBC between the signal of C-3 of Gal c at δ = 74.8 and H-1 of Man at δ = 5.10. The successful selective introduction of mannose residue to reactive 3-OH makes this strategy very attractive and avoids time-consuming protection and deprotection steps. Removal of the benzylidene of **12** with 80% AcOH and acetylation gave protected tetrasaccharide **13** in 92% yield over two steps. Comparing the ^{1}H-NMR data of **12** with those of **13** showed that H-4 signal of Gal b residue was shifted downfield by 1.30 ppm, so it was confirmed that the mannose was bound to 3-position of the Gal c. Selective removal of 2-(trimethylsilyl)ethyl (TMS-ethyl) group in **13** with trifluoroacetic acid (TFA) in CH_2Cl_2, followed by treatment with CCl_3CN in the presence of 1,8-diazabicyclo[5,4,0]-undeca-7-ene (DBU)[46] afforded corresponding α-trichloroacetimidate **14** in 90% yield over two steps. Glycosylation of phytoceramide acceptor (2*S*,3*R*,4*R*)-3,4-di-*O*-benzoyl-2-hexadecanamido-octadecane-1,3,4-triol **15**[47] with glycosyl donor **14** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf)[48] afford desired protected glycosphingolipid **16** in 56% yield. Finally, Zemplén-based deacylation of **16** and purification by column chromatography on Sephadex LH-20 produced glycosphingolipid **1** (Scheme 2) in 84% yield. The structure and purity of **1** were demonstrated by its ^{1}H NMR and HR-FABMS data.

2.2. Total synthesis of glycosphingolipid *2*

The pentasaccharide glycosphingolipid **2** contains differs from **1** by addition of a glucose residue to the unreactive axial hydroxyl group in tetrasaccharide **12**. The pentasaccharide portion of glycolipid **2** was synthesized by glycosylation of reactive perbenzylated thioglucoside donor **17**[49] with tetrasaccharide acceptor **12**. We studied various reaction conditions varying solvent and temperature to optimize the desired α-selectivity in this reaction. Using a toluene/1,4-dioxane mixture[50] as solvent at −20 °C resulted in complete α-selectivity in this reaction to produce **18α** in 77% yield. (Scheme 3. Table 1). The α-linkage was assigned on the homonuclear coupling constant (br. d) of newly anomeric proton signal at δ = 5.10 and carbon signal at δ = 99.4. The β-linkage in **18β** was assigned on proton signal δ = 4.36 (d, J = 7.6 Hz) and carbon signal at δ = 103.8.



Scheme 1. Reagents and conditions: (a) see ref.[40]; (b) benzoyl chloride, pyridine, 87%; (c) NIS, TfOH, 4 Å MS, CH_2Cl_2, **7** 86%, **9** 78%; (d) 1) Ac_2O, pyridine, 2) 80% AcOH, 72% (two steps); (e) pyridine, H_2O, 92%.