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Synthesis of a new glycosphingolipid, neurosporaside, from *Neurospora crassa*

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ABSTRACT

The glycosphingolipid neurosporaside (α-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→)-Cer) occurs in *Neurospora crassa*. We attempted to synthesize neurosporaside by block synthesis (route A) and linear synthesis (route B). Oligosaccharide derivatives were synthesized using trimethylsilyltrifluoromethanesulfonate and *N*-iodosuccinimide/trifluoromethane sulfonic acid as promoters. The target tetrasaccharide could not be attained via route A, but route B showed potential: glycosidic bonds (β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*) were formed stereoselectively, leading to the synthesis of glycosphingolipid **2**.

1. Introduction

Glycosphingolipids have attracted attention recently due to their biological activities. Sialic acid-containing glycosphingolipids such as gangliosides are especially well known for their involvement in extracellular recognition, cell–cell interactions, differentiation, oncogenesis, and the immune system.[1,2] Many carbohydrate researchers have focused on glycosphingolipids containing sialic acid from higher animals. Systematic studies of their structure and function at the molecular level were undertaken via synthesis of analogs. The biological functions in invertebrates of glycolipids lacking sialic acid, however, are unknown.[5] Thus, it is hoped that synthesis and investigation of the functions of such glycolipids may help to elucidate disease mechanisms and facilitate the development of new drugs.[6,7] Organic synthesis is a powerful method for exploring structure–activity relationships, providing access to large numbers of homogeneous and structurally defined oligosaccharides, including both natural and unnatural compounds. We are interested in the synthesis of novel naturally occurring glycosphingolipids.

Neurospora crassa is prominent in the long history of genetics, biochemistry, and molecular biology, having been employed as a convenient model for studies of fungal growth and development. In 2011, Costantina et al. isolated and characterized a novel glycosphingolipid, neurosporaside α-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→)-Cer (**1**, Fig. 1), from *N. crassa*.[8] A previous study of *N. crassa* reported only one neutral glycosphingolipid, β-glucopyranosylceramide. The structure of neurosporaside is unique, however, in that the sugar moiety linked to the ceramide is formed by a chain of three β-galactopyranoside residues, with a glucopyranoside α-linked to the outer galactopyranoside. The biological activities of these compounds cannot be compared with those of glycosphingolipids from other animal species because those molecules contain different ceramide structures. Therefore, ceramide structures must also be considered in functional studies of the carbohydrate moieties in glycosphingolipids. The ceramides of the major natural glycosphingolipids comprise a fatty acid and a saturated or unsaturated sphingosine.[4,9] In this paper, we have attempted to synthesize structures containing general ceramides (**2**, Fig. 1).

2. Results and discussion

The aim of this study was an efficient and stereoselective synthesis of compound **2**. We considered two retrosynthetic plans: block glycosylation with two types of disaccharide derivatives (route A), and linear glycosylation with monosaccharide derivatives coupled one by one from the reducing end of sugar residues (route B; Fig. 2).[10] Route A is very efficient from the perspective of total yield, but this route faces a critical obstacle: glycosylation by Gal (II) and Gal (III) is α-/β-non-selective. In contrast, route B

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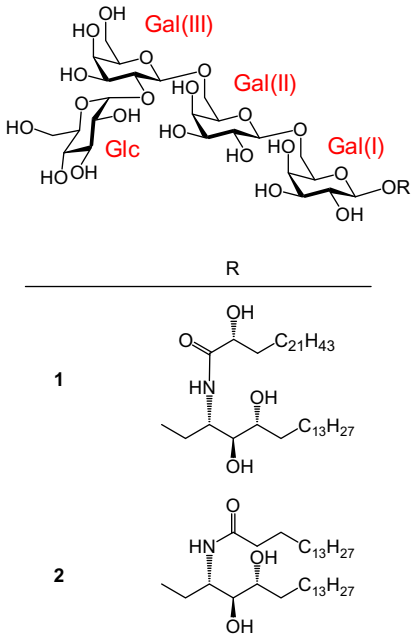

Figure 1. Structure of glycosphingolipids neurosporaside (**1**) from *Neurospora crassa* and its motif **2**.

relies on glycosylation, which is expected to proceed with high stereoselectivity; however, we expect a lower total yield than route A given the number of additional reaction steps. We attempted to synthesize compound **2** via both routes A and B.

2.1. Monosaccharide derivatives 7, 8 and disaccharide derivative 12

Compound **3** was protected at the 3,4-di-OH groups with an isopropylidene group, and at 6-OH with a *t*-butyl dimethylsilyl group (TBS), resulting in 2-OH derivative **5**.[11] These protecting groups in **5** were removed with TsOH after protection at 2-OH with a levulinoyl group (Lev), followed by benzoylation at 3-, 4-, and 6-OH, giving monosaccharide derivative **6** (81% over three steps). Compound **7** was obtained when the Lev group of compound **6** was selectively deprotected by treatment with hydrazine acetate (NH_2NH_2–AcOH) (92%).[12] Trichloroacetimidate derivative **8** was synthesized by selective removal of the *p*-methoxyphenyl group with ceric ammonium nitrate (CAN) in CH_3CN/H_2O (6:1)[13] and treatment with trichloroacetonitrile and 1,8-diazabicyclo[5,4,0]-7-undecane (DBU; 90% over two steps; Scheme 1).

The synthesis of disaccharide derivative **11** using acceptor **9**[14] and donor **10**[15] was achieved with *N*-iodosuccinimide (NIS) and trifluoromethane sulfonic acid (TfOH)[16] in 72% yield. The structure of **11** was confirmed by ^1H and ^{13}C NMR spectroscopy. Selective removal of the TBS group of **11** was achieved with TsOH; we thus obtained glycosyl acceptor **12** for synthesis of the tetrasaccharide via route A and for synthesis of the trisaccharide via route B (Scheme 2).

2.2. Tetrasaccharide derivative 19 via routes A and B

We expected that tetrasaccharide derivative **19** could be obtained by block synthesis using disaccharide donor **15** and acceptor **12** (route A). Synthesis of disaccharide derivative **14** by coupling of monosaccharide acceptor **7** and donor **13** was achieved

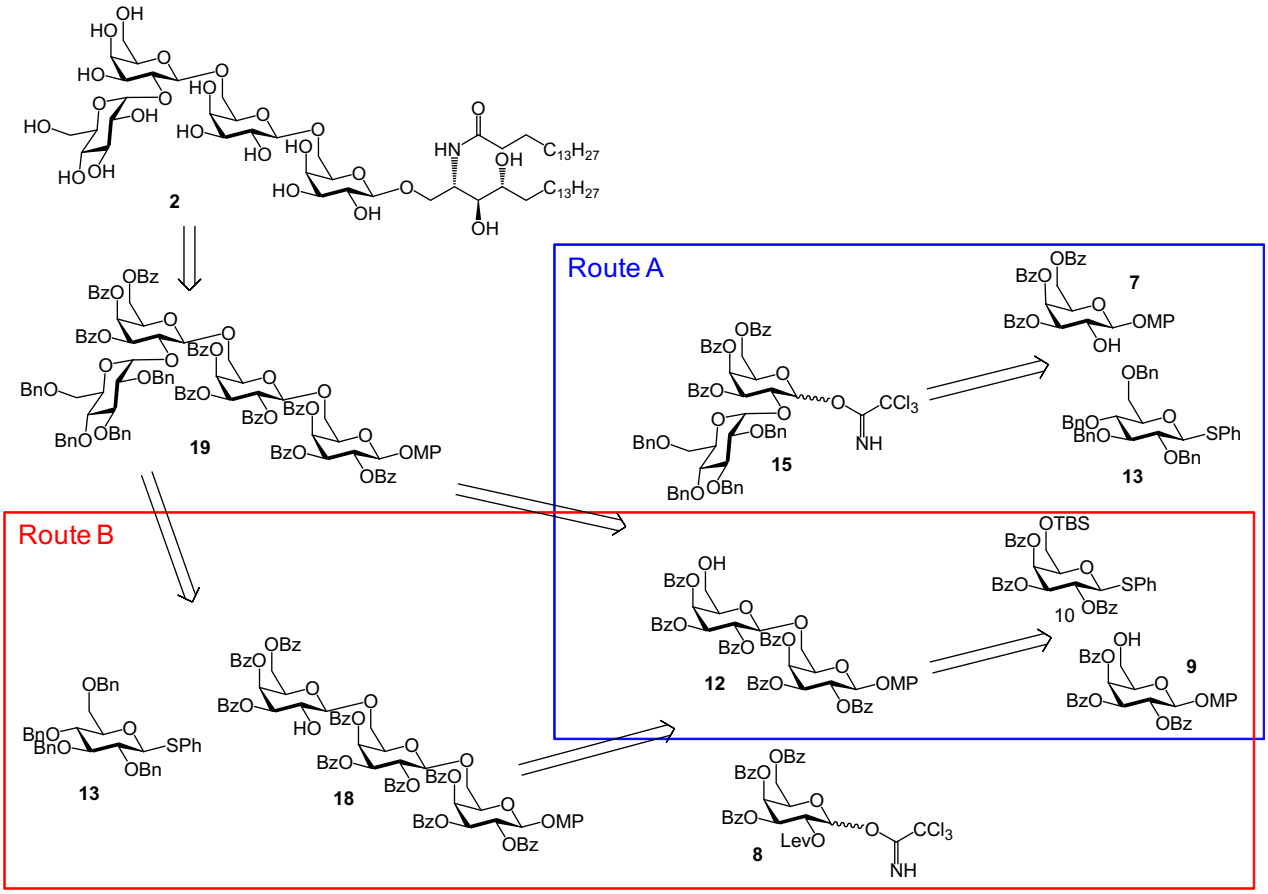

Figure 2. Retrosynthesis of **2** by route A and route B.