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Study on systematizing the synthesis of the a-series ganglioside glycans GT1a, GD1a, and GM1 using the newly developed *N*-Troc-protected GM3 and GalN intermediates ☆

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Dedicated to Professor Dr. Hans Kamerling on the occasion of his 65th birthday

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ABSTRACT

A first systematic synthesis of the glycan parts of the a-series gangliosides (GT1a, GD1a, and GM1) utilizing the newly developed *N*-Troc-protected GM3 and galactosaminyl building blocks is described. The key processes, including the assembly of the GM2 sequence and its conversion into the 3-hydroxy acceptor, were facilitated mainly by the high degree of participation and chemoselective cleavability of the Troc group in the galactosaminyl unit. Furthermore, the novel GM2 acceptor served as a good coupling partner during glycosylation with galactosyl, sialyl galactosyl, and disialyl galactosyl donors, successfully producing the GM1, GD1a, and GT1a glycans.

1. Introduction

There has been explosive growth in the field of glycobiology in the last two decades. The surfaces of animal cells are covered with glycolipids whose oligosaccharide chains are positioned outermost from the cell surface and often function as recognition molecules. Among the glycolipids, glycosphingolipids (GSLs) are the most abundant and intriguing molecules. GSLs contribute to the glycocalyx of the cell and provide binding sites for toxins, viruses, and bacteria, as well as mediate cell adhesion processes and other intercellular communication events. Gangliosides are distinguished from other GSLs by containing one or more sialic acid residues, which are considered as crucial structural and/or electronic elements for interplay with biological molecules or receptors on cells. Since gangliosides are extremely minor constituents in living organisms and are a huge family composed of diverse congeners at the functionality level, large quantities of homogeneous gangliosides are not available from natural sources. Therefore, the chemical reconstruction of gangliosides from monosaccharides that are abundant in nature has been needed. The a-series (GM2,[1] GM1,[1a,2] GD1a,[2a] and GT1a[3]) and b-series (GD2, GT1b, and GQ1b)[4] have been synthesized by several approaches, including those developed by our group, and very recently, the synthesis of the GP1c glycan has also been achieved.[5] However, the synthetic methods cannot always deliver a large amount of ganglioside for molecular biology or medicinal studies.

Also, in our earlier reports on the synthesis of GM1, GD1a, and GT1a,[2a,3] the low degree of accessibility of the GM2 tetrasaccharide acceptor (GM2 acceptor) impeded the efficient assembly of the glycan parts, decreasing the overall yields. The critical considerations in the synthesis of the GM2 acceptor are as follows: (1) α-selective sialylation of the C-3′ hydroxyl group of lactose and chromatographic separation of the α-sialylated product (GM3 glycan); (2) β-selective and efficient incorporation of the galactosamine moiety into the C-4′ hydroxyl group of the GM3 glycan, which is hampered by the adjacent sialyl moiety; and (3) the manipulation of protecting groups to convert GM2 glycan into the corresponding acceptor. After completion of the first total syntheses of gangliosides GD1a and GT1a, our efforts have turned to systematizing the synthesis of the a-series gangliosides with solutions for the above-mentioned synthetic problems aimed at obtaining a sufficient supply for cross-disciplinary studies. Meanwhile, we have devised a highly reactive *N*-Troc-sialyl donor,[6] with which we recently demon-

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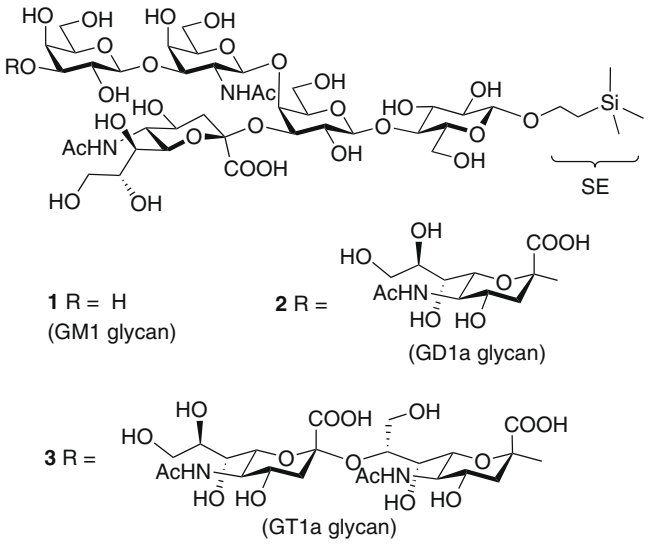


Figure 1. Structure of target glycans **1** (GM1), **2** (GD1a), and **3** (GT1a).

strated an efficient assembly of GM3 glycan.[7] Here, we report on the systematic syntheses of the a-series ganglioside glycans GM1 (**1**), GD1a (**2**), and GT1a (**3**), all of which feature the effective construction of the GM2 glycan and its conversion into the corresponding key acceptor (Fig. 1).

2. Results and discussion

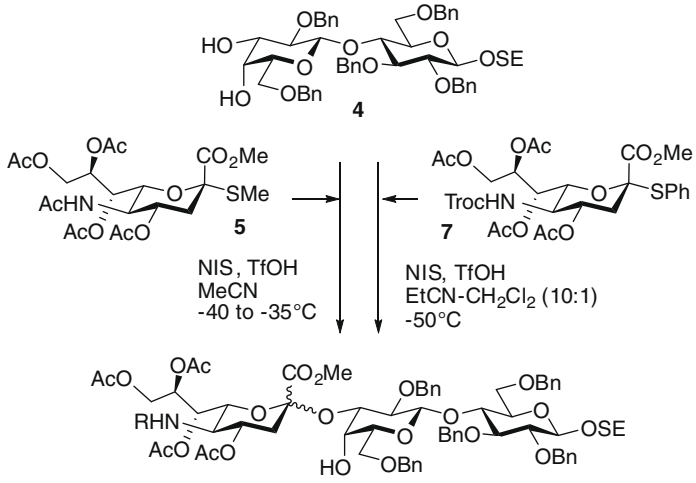
2.1. Lessons from our earlier results, and new synthetic strategy

Our revised strategy includes using an *N*-Troc sialyl lactose acceptor (GM3 acceptor) and novel galactosamine unit that is able to be glycosidated with a less reactive C-4′ hydroxyl group within the GM3 acceptor to fashion a GM2 glycan and then accept mono-, di-, and trisaccharyl glycosyl units at the C-3 hydroxyl group in the next elongation step toward the GM1, GD1a, and GT1a glycans. As we reported recently, the highly reactive *N*-Troc sialyl donor **7** improved the efficiency of the production of GM3 glycan.[7] In our original method of GM2 synthesis, GM3 acceptor **6** was produced in moderate yields (69%, α:β = 59:10) by the coupling of the *N*-acetyl sialyl donor **5** and 3′,4′-diol lactose acceptor **4**, which was promoted by NIS–TfOH in MeCN (Scheme 1).[1b] However, silica gel column chromatography was repeatedly conducted to separate the desired α-isomer from an anomeric mixture that also contained a 2,3-en sialic acid derivative, thereby making the GM3 assembly demanding and insufficient. In contrast, the *N*-Troc sialyl donor **7** improved, not only the yield of GM3 **8** (83%, α:β = 69:14) trisaccharide production, but also its chromatographic purification.[7]

Given the high degree of accessibility of GM3 acceptor **8**, we next examined the design of a linchpin unit, the GM2 acceptor, which is expected to be readily accessible through the minimum number of reaction steps and to possess a reactive hydroxyl group at the C-3 position. Therefore, the structure of the galactosaminyl donor (GalN donor) was considered. In the earlier report,[1b,2a,3] a phthaloyl group was employed as a β-directing functionality with the combination of a methyl sulfide group as an anomeric leaving group to establish the GM2 sequence (Scheme 2). That is, *N*-phthaloylgalactosaminyl donor **9**, which was derived from methylsulfenyl *N*-phthaloylgalactosamine in a 61% yield over two reactions, was reacted with GM3 acceptor **6** in the presence of NIS–TfOH to afford GM2 **10** in 68% yield. Then, the GM2 glycan was converted into the corresponding 3,4-diol acceptor **11** through protecting group manipulations, including demethylation, dephthaloylation, N-acetylation, remethylation, and acid hydrolysis of the isopropylidene group (57% over six reactions from **6**). Next, the coupling reaction with galactosyl donor **12** afforded GM1 **13**.[2a] Although the C-3 hydroxyl group in the *cis*-diol system was reactive to fashion the GM1 glycan sequence, the adjacent axial C-4 hydroxyl was also unexpectedly glycosylated, thereby producing 4-*O*-galactosyl and 3,4-di-*O*-galactosyl derivatives, **14** and **15**, as competitive products.

2.2. Assembly of a key GM2 unit

In this study, keeping these lessons in mind, a Troc group was chosen as a stereo-directing element in a novel GalN donor, which was first utilized in GM2 synthesis by Schmidt and co-workers,[1c] due to its compatible deprotection with ester groups present in the sialic acid residue at the C-3′ position after glycosylation. Furthermore, the C-4 hydroxyl group was designed to be protected as a benzylidene with a C-6 hydroxyl group in order to prevent the hydroxyl group from over-glycosylation at the next elongation



Scheme 1. Sialylations of the C′-3 hydroxyl group of lactose with *N*-Ac (**5**) or *N*-Troc-sialyl donor (**7**).