ORGANIC LETTERS

XXXX
Vol. XX, No. XX
000–000

First Synthesis of a Pentasaccharide Moiety of Ganglioside GAA-7 Containing Unusually Modified Sialic Acids through the Use of *N*-Troc-sialic Acid Derivative as a Key Unit†

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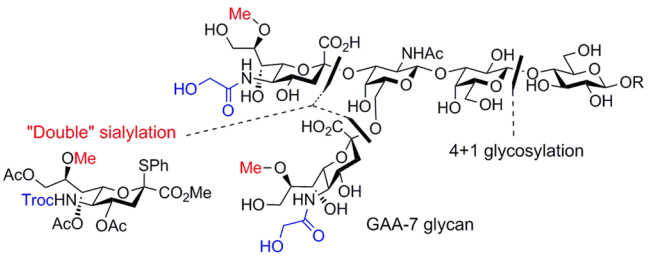
Received November 12, 2012

ABSTRACT



The pentasaccharide part of the potent neuritogenic ganglioside GAA-7 has been synthesized for the first time. The unique branched terminus constituting partially modified sialic acids and *N*-acetylgalactosamine was successfully established by stereoselective double-sialylation using 8-*O*-methyl-*N*-Troc-sialic acid as a donor. The final 4 + 1 coupling reaction provided a high yield of pentasaccharide, which was deprotected to deliver the target molecule.

It is well-documented that gangliosides produced in echinoderms (e.g., starfish and sea cucumber) show neuritogenic activity.[2] Since the activity of some echinodermatous gangliosides exceeds that of mammalian ganglioside GM1, which has been used in developing a drug for treating Alzheimer's disease,[3] echinoderms are considered an important source of bioactive gangliosides that are promising drug leads for combating neuronal disorders. According to results from the evaluation of neuritogenic activity toward PC-12 cells, four kinds of gangliosides (SJG-2,[4] LLG-5,[5a] LLG-3,[5b] and GAA-7[6]) showed comparable and highly potent activity.[2] However, the mechanism by which these gangliosides exert neuritogenic activity has not been elucidated, mainly due to the lack of available homogeneous gangliosides. The structures of echinodermatous gangliosides are substantially different from those of mammalian gangliosides in that they bear partially modified sialic acid residue(s) and/or repeated sequences of sialic acid not found in mammals. Therefore, such characteristic diversity in sialic acid structure is considered to be correlated with the potent neuritogenic activity of echinodermatous gangliosides. Our research group has

† Synthetic Studies on Sialoglycoconjugates. 158. For part 157, see ref 1.
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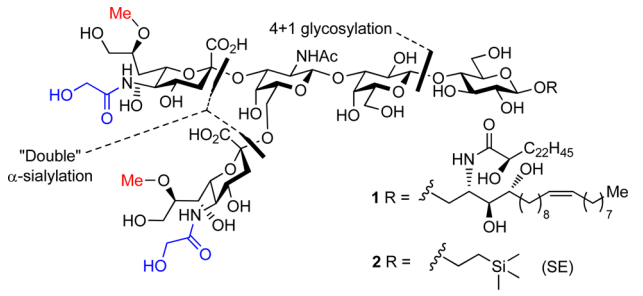
10.1021/ol303122w

previously reported the first total syntheses of HLG-2[7] and LLG-3,[8] which contain special tandem units consisting of partially modified sialic acid. With the ultimate aim of elucidating the neuritogenic activity of gangliosides, we herein report the synthesis of the glycan moiety of ganglioside GAA-7 (**1**), which was isolated from the starfish *Asterias amurensis versicolor* Sladen[6] (Scheme 1).

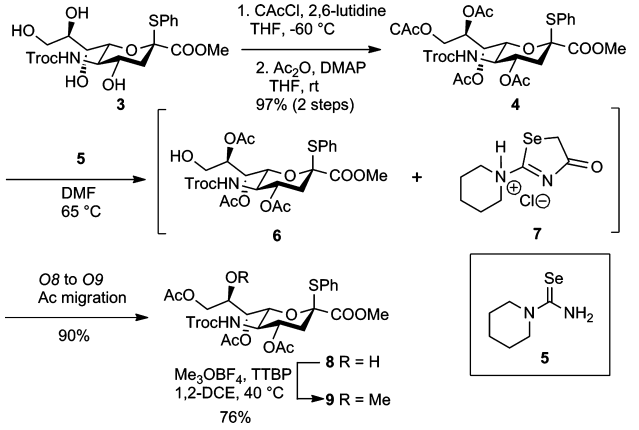
Scheme 1. Structure of Target Molecule **2** and Outline of Synthetic Strategy toward **2**



The glycan moiety of GAA-7 contains unusual sialic acid residues, which are methylated at the C8 hydroxyl group and glycolylated at the C5 amino group. Furthermore, the modified sialic acids are linked at the C3 and C6 hydroxyl groups of the galactosamine (GalN) residue through α-glycosidic bonds, forming a unique branched substructure at the terminus. This terminal branch is seen in only GAA-7 among all sialic acid-containing glycans identified. The terminal branch is a challenging class of molecule to synthesize because the reactivity of the C3 hydroxyl group is decreased by the adjacent acetamide group, making it difficult to establish an α-glycoside of sialic acid at the C3 position of GalN residue. Even if sialylation of the C3 and C6 hydroxyl groups were possible, the chromatographic separation of stereoisomers of sialyl oligosaccharides would generally be an arduous procedure due to the similar mobility of the isomers in silica gels, which impedes the purification of the desired product. Keeping this in mind, we envisaged the synthetic strategy depicted in Scheme 1. Target molecule **2** was fragmented into modified sialic acids, disaccharide (GalNAcβ(1,3)Gal), and glucose.

To introduce sialic acids at the two hydroxyl groups of GalNAc residue in an efficient and stereoselective manner, we chose a 2,2,2-trichloroethoxycarbonyl (Troc) group for orthogonal modification of the C5 amino group because it can be selectively removed and it endows high reactivity and stereoselectivity on the sialic acid donor.[9] According to the results reported in our previous paper,[9a] the use of a Troc group was also expected to facilitate the chromatographic separation of the anomers of sialylated products by widening the differences in their mobility on silica gel. As shown in Scheme 2, the 8-*O*-methyl-*N*-Troc-sialyl donor was designed as triacetate derivative **9** carrying a phenylthio group at the anomeric position based on the structure of the previously reported *N*-Troc sialyl donor.[9a] To access compound **9** in a short sequence, known compound **3**[9b] was first converted into 9-*O*-chloroacetyl (CAc) derivative **4** in very high yield (97%).[10] Next, the CAc group of compound **4** was selectively cleaved by the action of 1-selenocarbamoylpiperidine **5**[11] in DMF at 65 °C to afford **6**, which further underwent *O*8 to *O*9 acetyl migration promoted by an acidic tertiary ammonium salt with hydrogen chloride **7**, predominantly producing 8-OH derivative **8** in 90% yield. Finally, the hydroxyl group at C8 could be methylated most efficiently with Me_3OBF_4 in the presence of tri-*tert*-butylpyrimidine (TTBP) to give the 8-*O*-methyl sialyl donor **9** in 76% yield, whereas MeOTf gave a complex mixture of products.

Scheme 2. Synthesis of 8-Methyl-*N*-Troc Sialyl Donor **9**



To perform an efficient double-sialylation of GalN-Gal acceptor, it is crucial to boost the reactivity of the C3 hydroxyl group of the GalN residue since a hydroxyl group adjacent to an acetamide group is generally unreactive, likely due to hydrogen bonding. Because sialyl donor **9** is equipped with a Troc group at the C5 amino group, a TCA group, which is transformable into an acetyl group under conditions for reducing a Troc group (zinc in acetic acid),[12] was first selected as an orthogonal protecting group for the C2 amino group of the GalN residue. Furthermore, we aimed to deduce a suitable protection mode for the three hydroxyl groups of the GalN residue for double-sialylation by comparing a triol acceptor (**10**) and a diol acceptor (**11**) (Figure 1).[13]

It was difficult to obtain the disialylated product in the glycosylation of GalN-Gal acceptors **10** and **11** with sialyl

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(13) For the experimental procedure for the synthesis of compounds **10**, **11**, **12**, and **16**, see the Supporting Information.

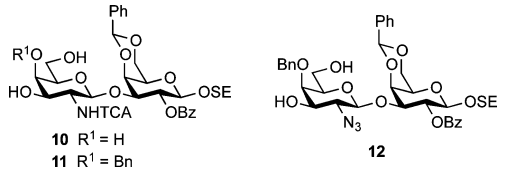
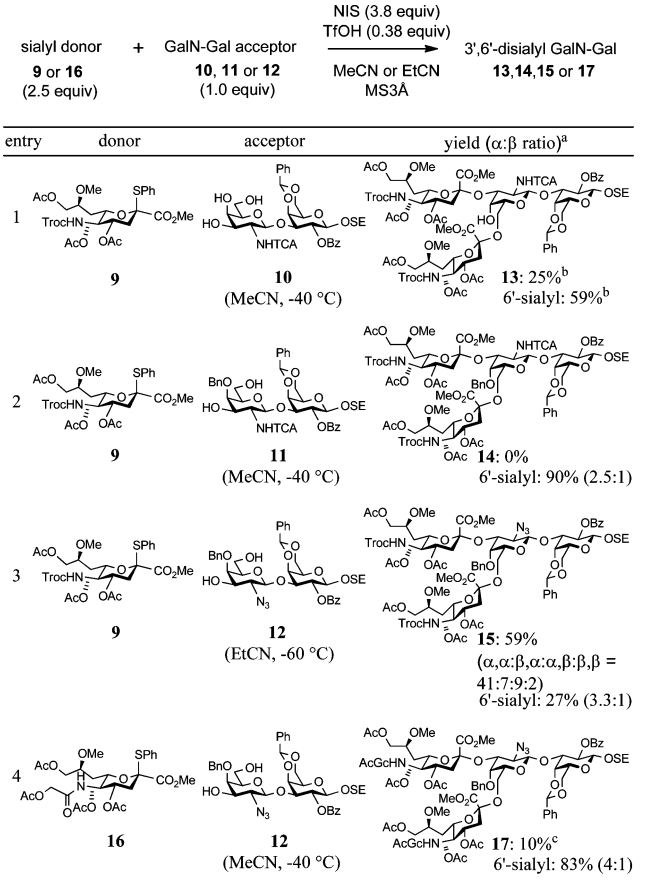


Figure 1. GalN-Gal acceptors used in this study.

donor **9**, which was promoted by *N*-iodosuccinimide (NIS) and trifluororomethanesulfonic acid (TfOH) in nitrile solvent.[14] The results of glycosylation are summarized in Table 1. In the case of triol acceptor **10**, doubly sialylated products were generated as a complex mixture of tetrasaccharides **13** including stereoisomers and their 1″, 4′-lactonated derivatives in about 25% yield, accompanied by 6′-sialyl GalN-Gal as an anomeric mixture (59%) (entry 1). Glycosylation of 4′-benzylated acceptor **11** with sialyl donor **9** did not produce tetrasaccharide **14** at all (entry 2), again giving an anomeric mixture of 6′-sialyl GalN-Gals as the main products in 90% yield (α/β = 2.5/1). In contrast, an alternative GalN-Gal acceptor **12** bearing an azido group at the C2 position of the GalN residue provided a good yield (59%) of disialylated product **15** as a mixture of stereoisomers (entry 3). The mixture consisted of the desired α(2,3)/α(2,6)-disialyl product (41%) and other stereoisomers (18%), namely, β(2,3)/α(2,6)-disialyl (7%), α(2,3)/β(2,6)-disialyl (9%), and β(2,3)/β(2,6)-disialyl isomers (2%) (α(2,3)/β(2,3) = 5.5:1; α(2,6)/β(2,6) = 4.3:1). As expected, all isomers were clearly separated on TLC with different R_f values (ΔR_f = 0.1−0.4), which facilitated chromatographic separation of each isomer.[15] This result indicated an important technical advantage in the use of the *N*-Troc-sialyl donor for accomplishing the synthesis of highly sialylated glycans. The anomeric configuration of the newly formed glycosides was determined on the basis of previous reports[16] by measuring the long-range $^3J_{\text{C-1, H-3}ax}$ coupling constants.

For the main product (α(2,6)/α(2,3)-disialyl GalN-Gal) in entry 3, the coupling constants were 6.3 and 6.0 Hz,

Table 1. Examination of Double Sialylation of GalN-Gal Acceptor



[a] The ratio of α:β was calculated on the basis of the isolated yield unless otherwise specified. [b] Yield of the mixture of stereoisomers and their lactonated derivatives, which were inseparable. [c] Yield of the inseparable mixture of stereoisomers. The ratio of α:β could not be estimated from the ^{1}H NMR spectrum because of its complexity.

whereas at least one coupling constant of the other products was less than 1.0 Hz. Although we examined double-sialylation using the *N*-acetylglycolyl sialyl donor **16**,[13,17] the disialylated product was obtained in poor yield (10%) as a mixture of anomers, which were hardly separated by column chromatography, owing to the same mobility of each anomer on the silica gel[15] (entry 4).

Scheme 3 shows the final part of the synthesis of target molecule **2**. To combine the tetrasaccharide part with a glucose residue, tetrasaccharide **15** (α-isomer) was converted into a suitable glycosyl donor. First, we screened various reaction conditions for transforming the azido group into an acetamide group in the presence of the Troc group ((i) TMSCl, AcCl;[18] (ii) Lindler catalyst; (iii) modified Staudinger reactions[19]). For compound **15**, Staudinger reaction using Me_2PPh, HOOBt, and acetic anhydride

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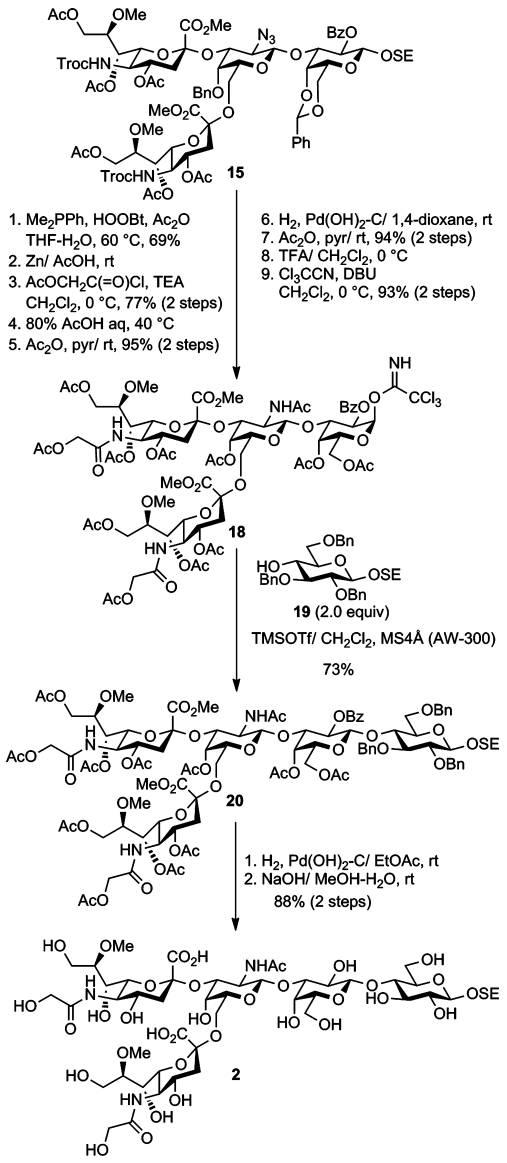
Scheme 3. Assembly of the Target Molecule **2**



in 1,4-dioxane–H_2O at 90 °C, which was reported by Ito et al.,[20] was the most effective, producing acetamide derivative in 69% yield together with a 1″,2′-lactam derivative (29%). Next, the two Troc groups were cleaved by the action of zinc in acetic acid[21] and the resulting amine group was subsequently acylated with acetoxyacetyl chloride (77% over two steps). Prior to the acidic cleavage of the 2-(trimethylsilyl)ethyl (SE) group at the anomeric position, the acid-labile benzylidene acetal was hydrolyzed with 80% aqueous acetic acid, followed by the acetylation of hydroxyl groups. Then, the benzyl group at the C4 hydroxyl group of the GalN residue was replaced with an acetyl group by a conventional reaction sequence. To provide a trichloroacetimidate group at the anomeric position as a leaving group,[22] the SE group was cleaved by exposure to TFA in CH_2Cl_2, and the resulting hydroxyl group was reacted with CCl_3CN and DBU[23] in CH_2Cl_2 at 0 °C. This reaction sequence produced the tetrasaccharyl donor **18** in 93% yield over two steps. To our delight, the final coupling of the tetrasaccharyl donor **18** (1.0 equiv) and glucosyl acceptor **19**[24] (2.0 equiv) in CH_2Cl_2, which was catalyzed with TMSOTf, provided pentasaccharide **20** in 73% yield. Finally, the synthesis of target molecule **2** was completed by full deprotection of compound **20** by hydrogenolysis on $Pd(OH)_2$ and subsequent removal of the ester groups.

In conclusion, the glycan moiety of ganglioside GAA-7 (**2**) has been synthesized for the first time. Taking advantage of the site-specific migration of an acetyl group in the glycerol moiety of sialic acid, key 8-*O*-Me-*N*-Troc sialic acid donor **9** was efficiently synthesized. The results of the double-sialylation of GalN-Gal acceptors demonstrated the efficacy of *N*-Troc type sialic acid donors in terms of glycosylation efficiency and facilitation of the chromatographic separation of anomeric isomers of sialylated products. The total synthesis of ganglioside GAA-7 using tetrasaccharide donor **18** and a glucosyl ceramide is underway and the results will be reported in due course.

Acknowledgment. The iCeMS is supported by World Premier International Research Center Initiative (WPI), MEXT, Japan. This work was financially supported in part by MEXT of Japan (a Grant-in-Aid for Scientific Research (B) No. 22380067 to M.K. and a Grant-in-Aid for Young Scientists (A) No. 23688014 and Grant-in-Aid on Innovative Areas No. 24110505, Deciphering sugar chain-based signals regulating integrative neuronal functions, to H.A.). We thank Ms. Kiyoko Ito (Gifu University) for providing technical assistance.

Supporting Information Available. All experimental procedures and ^1H and ^{13}C spectra of all new compounds.This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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