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Synthesis of the starfish ganglioside AG2 pentasaccharide

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ABSTRACT

This Letter reports the first synthesis of the AG2 pentasaccharide, using silylene-oxazolidinone double-locked sialic acid building blocks. The di-DTBS-protected sialic acid building block was easily prepared and readily activated with NIS and TfOH to provide the sialylated lactose unit in good yield with moderate selectivity. After obtaining the trisaccharide unit, the oxazolidinone-protected C4–OH on the sialic acid residue was readily deprotected by treatment with NaOMe. Coupling with the galactofuranosylβ(1-3)galactopyranosyl fluoride building block produced the desired AG2 pentasaccharide in a highly stereoselective manner. Finally, the desired AG2 pentasaccharide was obtained in good yield following global deprotection.

The structural diversity of sialyl-glycans on glycoproteins and glycolipids is important because of their several functions in biological systems.[1,2] Mammalian gangliosides mainly contain *N*-acetylneuraminic acid or *N*-glycolylneuraminic acid with α(2-3/6) binding modes on a penultimate galactose, glucosamine, or galactosamine residue. Such gangliosides are involved in cell adhesion and recognition, and in signal transduction through lipid rafts and caveolae.[3,4] The biosynthetic pathways of mammalian gangliosides are currently being elucidated;[5] the oligosaccharide chains of these gangliosides are terminated with sialic acid residue(s).

Concurrent with these developments, unique echinoderm gangliosides with potent mammalian nerve cell stimulating activity have been identified from starfish and sea cucumbers.[6,7] One of these gangliosides, AG2 (isolated from the starfish *Acanthaster planci*), has an unusual glycan sequence involving an inner sialic acid residue with a galactofuranose capping moiety (Scheme 1).[6a] In addition to this unique structure, AG2 exhibits potent nerve cell stimulating activity toward the mouse PC-12 cell line that is comparable in magnitude to that of the mammalian GM1 ganglioside.[7b] The GM1 receptor for nerve cell stimulation/suppression signals is the myelin-associated glycoprotein, MAG/Siglec-4.[8]

The galactofuranose residue on AG2's non-reducing terminus is widespread throughout bacteria, protozoa, and marine organisms.[9] However, galactofuranosylated glycans are not found in humans. Recently, it has been reported that such galactofuranosylated glycans on infectious organisms are recognized by human intelectin, which is involved in immune defense and inflammation.[10]

Recently, several echinoderm gangliosides involved in HLG-2 (isolated from sea cucumber *Holothuria leucospilota*; α-Neu5Ac-(2-4)-α-Neu5Ac-β-(2-6)-Glc-R) and LLG-3 (isolated from starfish *Linckia laevigata*; 8-*O*-Me-α-Neu5Ac-(2-11)-α-Neu5Gc-(2-3)-β-Gal(1-4)-β-Glc-R) have been synthesized.[11] These specific gangliosides are of particular interest due to their unique sialyl-oligosaccharide structures and potent nerve cell stimulating activity.[11,12] In order to elucidate the nerve cell stimulation mechanism and to investigate other biological functions of the unique AG2 structure, we synthesized the pentasaccharide part of AG2 **1**. The synthesis of the AG2 oligosaccharide presented two problems, namely, the introduction of a sialic acid residue onto the lactose unit, and the further glycosylation of the sialic acid C4–OH with the galactofuranosylβ(1-3)galactopyranoside unit. Based on the synthesis of HLG-2 and HPG-7 (isolated from sea cucumber *Holothuria pervicax*; α-Fuc-(1-4)-α-Neu5Ac-(2-11)-α-Neu5Gc(2-4)-α-Neu5Ac-(2-6)-β-Glc-R) gangliosides, the observed low efficiency of glycosylation of the sialic acid C4–OH resulted from low reactivity and the flexible side chain.[11a,12] To overcome these issues, 1,5-lactamized sialic acid acceptors have been developed for exposing the C4–OH.[12]

Previously, we developed a novel sialic acid building block **5** for efficient sialylation reactions. Compound **5** carries an oxazolidinone and di-*tert*-butylsilylene (DTBS) double-lock on the central pyranose ring.[13,14] We employed this building block for the synthesis of AG2 because the 5,7-*N*,*O*-DTBS ring would expose the C4–OH of the sialic acid, enhancing the reactivity of the hydroxy group. Scheme 1 shows the plan for the synthesis of AG2 pentasaccharide **1**. The galactofuranosylβ(1-3)galactopyranose unit **2** on the non-reducing terminus and sialylα(2-3)lactose trisaccharide **3** were

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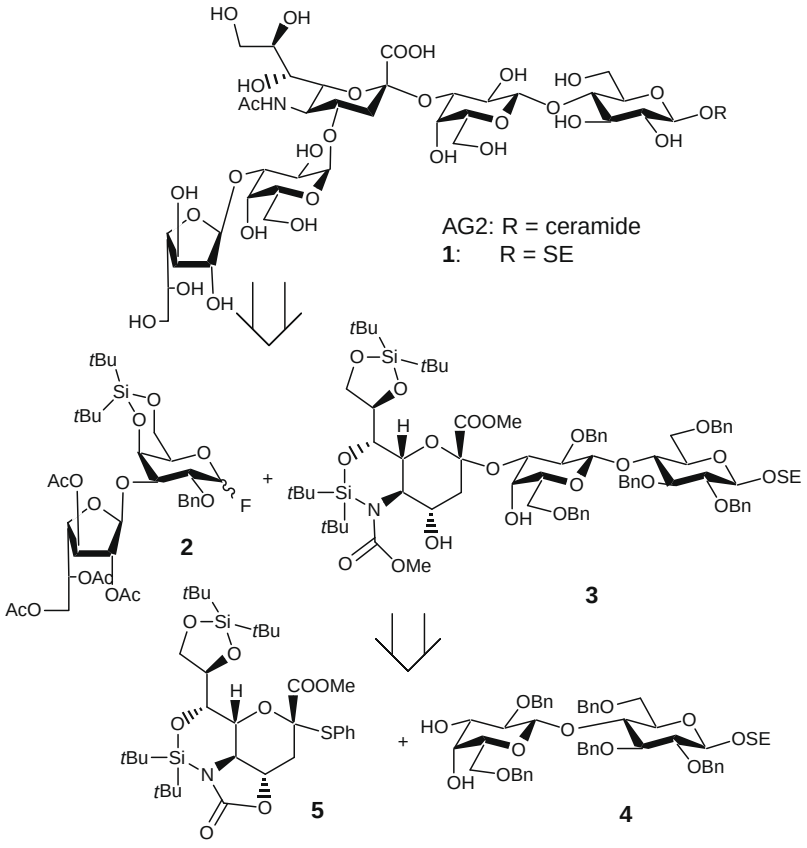
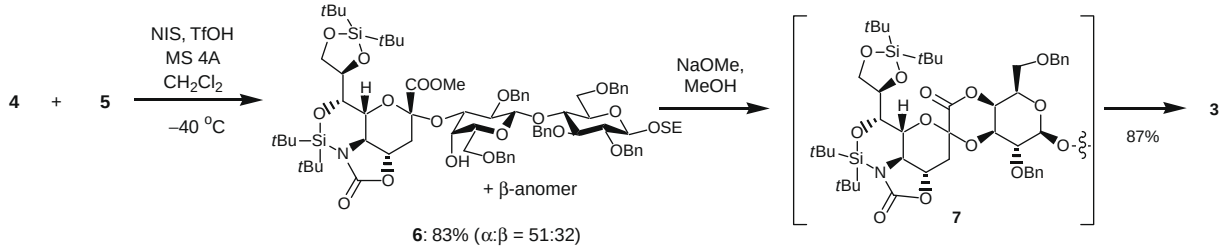


Scheme 1. Structure and synthetic scheme for starfish ganglioside AG2 pentasaccharide **1**. SE = 2-(trimethylsilyl)ethyl.

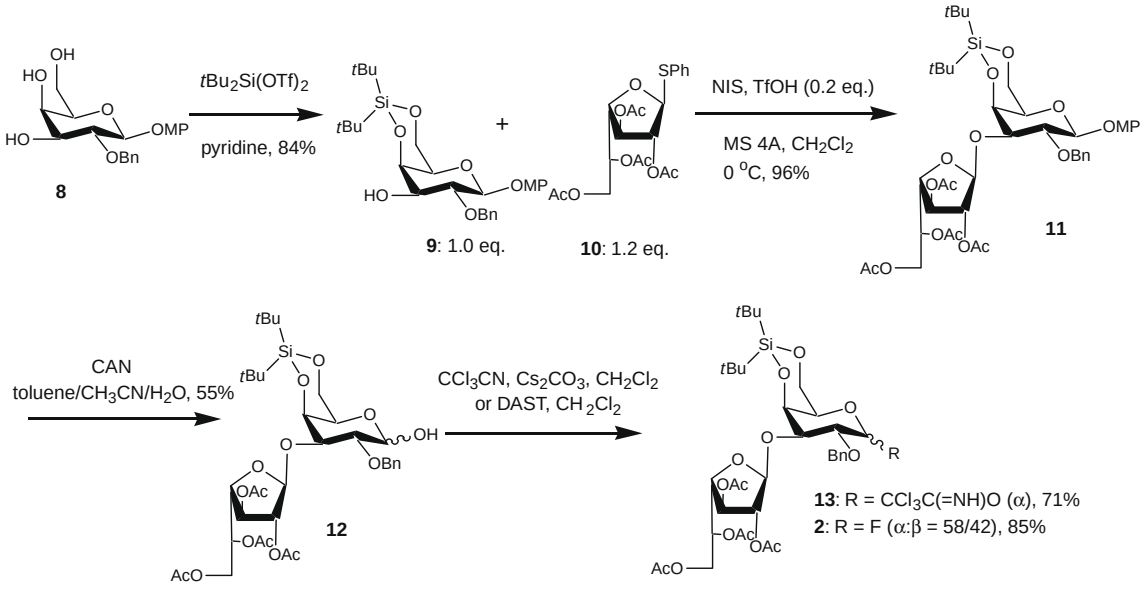
coupled at the final glycosylation stage. Glycosylation of the relatively exposed hydroxy group on **3** proceeded in good yield in a highly α-selective manner due to 4,6-*O*-DTBS protection of the galactose moiety on **2**.[15] The bulky *t*-butyl groups on the DTBS moiety of **2** inhibited glycosylation from the β-face. The sialic acid building block **5** was employed to introduce the sialic acid unit to the disaccharide **4**.[16] The sialylation reaction resulted in regio-selective opening of the oxazolidinone-ring, which liberated the C4–OH on the sialic acid unit to yield **3**. The acceptor hydroxy group of **3** was then sufficiently exposed to undergo further coupling with disaccharide **2,** since the DTBS-protecting group still fixes the side chain.

The sialylα(2-3)lactose trisaccharide unit **3**, which represents half the AG2 molecule, was efficiently synthesized as depicted in Scheme 2. First, the sialyllactose unit **3** was formed using the highly reactive silylene-oxazolidinone double-locked sialic acid building block **5**.[13] The sialylation reaction of lactose acceptor **4** with **5**, activated with *N*-iodosuccinimide(NIS) and TfOH at −40 °C,[17] produced trisaccharide **6** in 83% yield with moderate α-selectivity (α:β = 51:32). The generated **6α** and **6β** were readily separated by silica gel column chromatography (**6α**: $^{3}J_{C1,H3}$ = 5.1 Hz). A single-step transformation with NaOMe provided the desired trisaccharide acceptor **3**. Specifically, treatment of **6α** with NaOMe smoothly provided lactone intermediate **7**, then the oxazolidinone moiety as well as the lactone moiety on **7** were opened in a regio-selective manner to give **3** in 87% yield.[14]

Then, we synthesized galactofuranosylβ(1-3)galactopyranose unit **2**, which represents the other half of the AG2 pentasaccharide (Scheme 3). First, galactose triol **8**[18] was treated with $t\mathrm{Bu}_2\mathrm{Si(OTf)}_2$ in pyridine to produce 4,6-*O*-DTBS-protected galactose acceptor **9** in 84% yield. The subsequent glycosylation reaction between compound **9** (1.0 equiv) and phenylthiogalactofuranoside **10**[19] (1.2 equiv) in the presence of NIS and TfOH at 0 °C produced disaccharide **11** in 96% yield as a single isomer. Next, oxidative removal of the 4-methoxyphenyl functionality on the galactose residue of **11** using cerium ammonium nitrate (CAN) produced hemiacetal **12** in 55% yield. For further glycosylation reactions, disaccharide **12** was transformed into highly reactive trichloroacetimidate **13**



Scheme 2. Synthesis of the trisaccharide acceptor **3**.

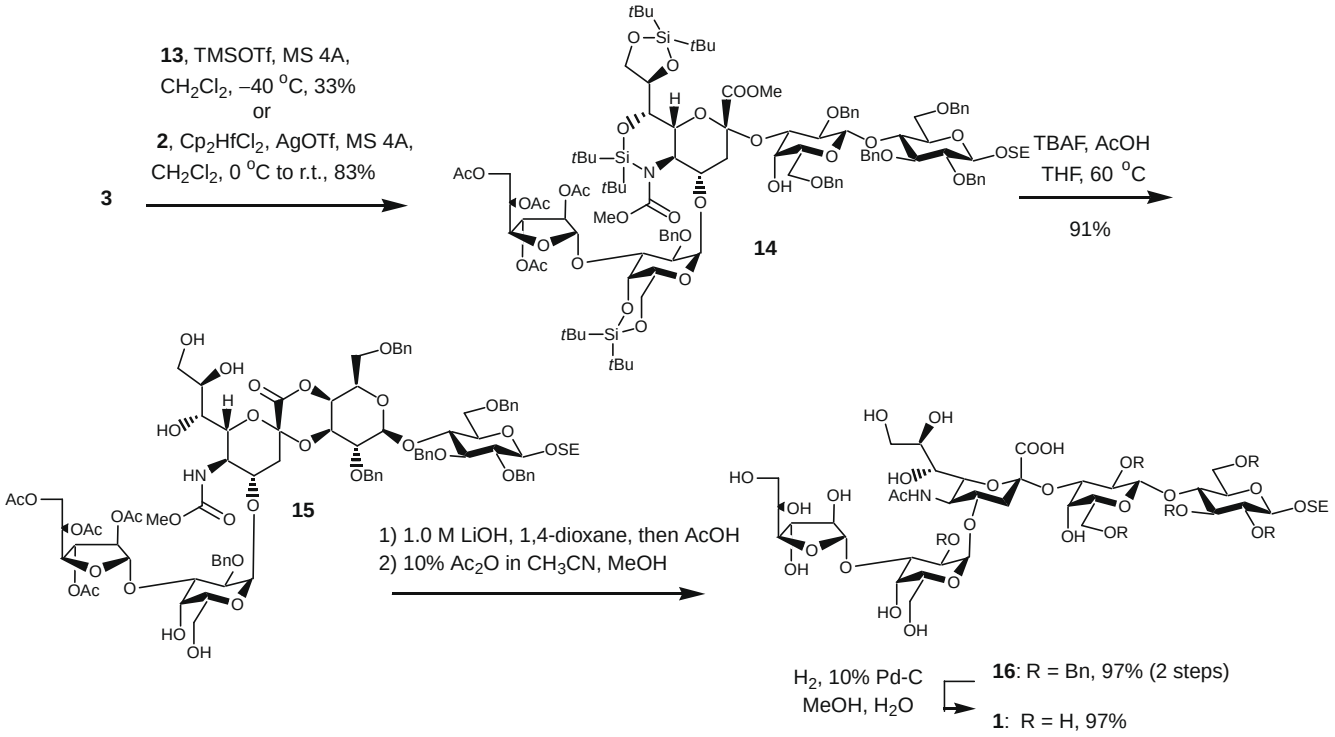


Scheme 3. Synthesis of galactofuranosyl galactose donors **2** and **13**. MP = 4-methoxyphenyl.

with CCl_3CN and Cs_2CO_3 (71% yield), and into fluoride **2** with *N,N*-diethylaminosulfur trifluoride (DAST, 85% yield;α:β = 58:42). We expected that the bulky 4,6-*O*-DTBS protection on the galactose moiety would favor α-selectivity due to the 'DTBS effect' in subsequent assembly with the sialyllactose trisaccharide.[15a]

To obtain fully protected AG2 pentasaccharide **14**, coupling reactions between trisaccharide acceptor **3** and disaccharide building blocks **2** and **13** were performed as shown in Scheme 4. Initially, we employed the highly reactive Schmidt-type trichloroacetimidate **13**.[20] Although imidate **13** (2 equiv) was readily activated with a catalytic amount of TMSOTf at −40 °C, the desired pentasaccharide **14** was obtained in an unsatisfactory 33% yield. On the other hand, the best yield was obtained with the less reactive glycosylfluoride **2**. Upon activation of fluoride **2** with Cp_2HfCl_2 and AgOTf at 0 °C,[21] the desired AG2 pentasaccharide **14** was readily obtained in 83% yield with predominant α-selectivity.

To obtain fully deprotected AG2 pentasaccharide **1**, a global deprotection process was conducted. First, three silylene-protecting groups on pentasaccharide **14** were simultaneously removed by treatment with TBAF and AcOH in THF at 60 °C to give lactone **15** in 91% yield. Next, the *N*-methoxycarbonyl group on **15** was hydrolyzed by 1.0 M aqueous LiOH with 1,4-dioxane at room temperature, then N-acetylation with Ac_2O in MeOH produced compound **16** in 97% yield (2 steps). During the treatment with base, the lactone moiety of **15** was simultaneously hydrolyzed to



Scheme 4. 2 + 3 coupling and deprotection for the synthesis of the AG2 pentasaccharide **1**.

transform the carboxyl function of **16**. The final removal of all benzyl-protecting groups by hydrogenation with 10% Pd-C in aqueous media produced fully deprotected AG2 pentasaccharide **1** in 97% yield. The structure of AG2 pentasaccharide **1** was confirmed by ^{1}H and ^{13}C NMR, and ESI-TOF-MS.

The pentasaccharide part of AG2 **1** was efficiently synthesized using novel sialic acid building blocks. The galactofuranosylβ(1-3)galactopyranose fluoride **2** was readily prepared by coupling with galactofuranosyl thioglycoside **10** and galactopyranose acceptor **9**. On the other hand, the side chain-fixed trisaccharide acceptor **3** was afforded by single-step transformation of trisaccharide **6**. In 2 + 3 coupling reactions with trisaccharide **3**, fluoride **2** performed the best for producing the desired fully protected pentasaccharide **14**. Finally, the synthesis of AG2 pentasaccharide **1** was achieved after global deprotection involving de-silylation, introduction of an acetamide group, and total removal of acyl- and benzyl-protecting groups.

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Supplementary data

Synthetic procedures, spectroscopic data, and ^{1}H and ^{13}C NMR spectra of compounds **1**–**3**, **9**, **11**–**16**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.08.071.

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