Stereoselective Synthesis of the Trisaccharide Moiety of Ganglioside HLG-2

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Supporting Information

ABSTRACT: The glycan portion of ganglioside HLG-2, which was identified in the extracts of the sea cucumber Holothuria leucospilota, was synthesized in a highly efficient and stereoselective manner. The unusual sequence of the trisaccharide moiety, α-N-glycolylsialyl-(2,4)-α-N-acetylsialyl-(2,6)-glucoside, was assembled by stereoselective coupling of a 5-N,4-O-carbonyl-protected sialyl phosphate donor, a N-2,2,2-trichloroethoxycarbonyl (Troc)-protected sialyl acceptor, and a (trimethylsilyl)ethyl-β-glucosyl acceptor in high yield. The synthesis featured the high-yielding construction of two α-sialyl linkages.

Echinodermatous gangliosides, one kind of sialic acid-containing glycosphingolipids, have recently attracted much attention because of their specific structures and their neuritogenic activity, which is similar to that of mammalian gangliosides. However, their structure−activity relationships have not been investigated yet, mainly because of the lack of homogeneous gangliosides.

Ganglioside HLG-2, which was first isolated from the sea cucumber Holothuria leucospilota by Higuchi and co-workers, showed neuritogenic activity toward the rat pheochromocytoma cell line PC-12 in the presence of nerve growth factor. What attracts us more is the fact that the glycan portion of HLG-2 contains two unique structural features shown in Figure 1. An α(2,4) linkage between sialic acids is only found in rare natural products such as the HLG series gangliosides and the ganglioside HPG series. It is known that a sialylation reaction in high yield and with complete α stereoselectivity is a great challenge because of the presence of a destabilizing electron-withdrawing carboxylic group together with a tertiary anomic center and the lack of a participating group. In addition, the low nucleophilic activity of 4-OH of the sialic acid as an acceptor makes it harder to build the α(2,4) linkage between sialic acids. As a result, there are few methods to date to synthesize such a linkage efficiently. The other remarkable characteristic of the glycan moiety of HLG-2 is the tandem of N-glycolylsialic acid (NeuGc) and N-acetylsialic acid (NeuAc). All of these features lead to difficulties in synthesizing the glycan portion of HLG-2.

Previously, Kiso and co-workers reported the synthesis of the glycan moiety of HLG-2 in 4.7% overall yield in 14 steps using a novel 1,5-lactamized sialyl acceptor with the fixed boat form, which could result in the increased activity of the C4-hydroxyl group. In that process, the α/β ratio was 66/18 for the formation of the (2,4) linkage between sialic acids. By the same strategy, they completed the total synthesis of the ganglioside HLG-2.

Our group has been interested in the development of new methodologies for chemical synthesis of sialosides and their synthetic applications. In fact, in the past decades, various sialylation methods have been developed. Among all of these sialyl donors, an N-2,2,2-trichloroethoxycarbonyl (Troc)-protected thiophenyl sialyl donor showed high α stereoselectivity and good yield toward glycosylation promoted by NIS/TfOH, especially when the primary alcohol of glucose was used as the acceptor. Recently, Wong and co-workers identified 5-N,4-O-carbonyl-protected sialyl phosphates to be more efficient donors than the corresponding 5-N,4-O-carbonyl-protected thiophenol donors developed earlier. In this work, we tried to develop a more efficient strategy to assemble the ganglioside HLG-2 glycan moiety (1).

Figure 1. Structure of the novel disialyl ganglioside HLG-2.
For this purpose, S-N,4-O-carbonyl-protected sialyl phosphate donor 2, Troc-protected sialyl building block 3, and (trimethylsilyl)ethyl (SE)-protected β-glucosyl acceptor 4 were chosen as building blocks (Scheme 1). The reason why we chose the S-N,4-O-carbonyl-protected sialyl phosphate donor and the N-Troc-protected thiophenylsialyl block is that they both have proved to be good α-sialylation donors, allowing us to construct the glycan structure more efficiently. In addition, the Troc and oxazolidinone protecting groups could be orthogonally removed under certain conditions to afford the amino functionality, which could be further transformed into the corresponding N-glycolylsialic acid and N-acetylsialic acid, respectively.

We tried to couple sialyl phosphate donor 2 with N-Troc-protected acceptor 3 to produce disaccharide 5. However, the glycosylation reaction showed a poor stereoselective ratio (α/β = 1:1.26) even though it gave a high yield (96%) (Table 1, entry 1). In this process, we did not obtain the disaccharide with the (2,7) linkage because of the low reactivity of the 7-OH group. The configuration of the disaccharide was examined by NMR spectroscopy, and the newly formed α-glycosidic bond was confirmed by a $3^J_{C1-H3a}$ coupling constant of 6.0 Hz. Other solvents and additives were tried, but unfortunately, the results were not improved (Table 1). We hypothesized that the poor stereoselectivity may come from the low nucleophile reactivity of the C4-hydroxyl group in the sialyl acceptor. On the other hand, the trimethylsilyloxy moiety is very attractive in the chemistry of protecting groups because of its selective introduction and selective removal under mild conditions. Actually, trimethylsilyl (TMS) ethers were previously used as acceptors in some glycosylation reactions. In our case, we presumed that trimethylsilylation of the C4-hydroxyl group could improve its nucleophilic reactivity as an acceptor, which might lead to good stereoselectivity. Therefore, the sialyl block 6 was prepared quantitatively from acceptor 3 by treatment with 0.55 equiv of hexamethyldisilazane (HMDS) and a catalytic amount (up to 2 mol %) of iodine under solvent-free conditions at room temperature. Although compound 6 seems not to be a typical acceptor, it may be used as a glycosyl acceptor because the TMS protecting group is acid-sensitive and may be easily removed in situ under glycosylation conditions such as promoted by trimethylsilyl trifluoromethanesulfonate (TMSOTf). As expected, the glycosylation reaction of sialyl phosphate donor 2 and sialyl acceptor 6 yielded the corresponding sialyl-α(2,4)sialyl sequence 5 in a highly stereoselective manner. However, when the reaction was quenched with triethylamine, the C7-hydroxyl group in disaccharide 5 was trimethylsilylated (up to 50% yield) because the promoter TMSOTf can be used as the reagent for silylation in an organic alkali environment. To avoid this problem, saturated NaHCO$_3$ aqueous solution was used to quench the reaction; finally, disaccharide 5 was successfully isolated in 95% yield as the pure α isomer (Table 1, entry 8).

With the success of the efficient construction of disaccharide 5, we tried to directly conjugate 5 with the glucosyl acceptor 4. However, the result did not meet our expectation, as the trisaccharide 7 was collected in only 36% isolated yield (Scheme 2), and all of our attempts to improve the yield failed. We supposed that the low yield may be related to the 8,9-O-isopropylidene group. Therefore, the 8,9-O-isopropylidene...
dene functionality in 5 was manipulated to give 7,8,9-tri-O-acetyl-protected disaccharide 8 in the hope that the glycosylation efficiency would be improved. Fortunately, the glycosyl coupling of disaccharide 8 with monosaccharide 4 afforded trisaccharide 9 in 96% isolated yield with only the α isomer. Finally, as shown in Scheme 2, after replacement of the Troc group in trisaccharide 9 by an acetyl group, the oxazolidinone ring was removed to expose the amino group, which was then functionalized with a glycolyl group. Full deprotection of the benzyl groups then yielded the target molecule ganglioside HLG-2 glycan structure 1 smoothly. The structure of compound 1 was confirmed by NMR spectral analyses.

In conclusion, we have completed the efficient synthesis of the glycan moiety of ganglioside HLG-2 in 44.5% overall yield in nine steps by using a 5-N,N'-O-carbonyl-protected sialyl phosphate donor, an N-Troc-protected sialyl block, and a (trimethylsilyl)ethyl-β-glucosyl acceptor. The two key glycosylation reactions were performed in high yields with excellent α-stereoselectivity. It is noteworthy that in this process, the oxidation efficiency would be improved. Fortunately, the oxazolidinone functionality in 5 was manipulated to give 7,8,9-tri-O-acetyl-protected disaccharide 8 in the hope that the glycosylation efficiency would be improved. Fortunately, the glycosyl coupling of disaccharide 8 with monosaccharide 4 afforded trisaccharide 9 in 96% isolated yield with only the α isomer. Finally, as shown in Scheme 2, after replacement of the Troc group in trisaccharide 9 by an acetyl group, the oxazolidinone ring was removed to expose the amino group, which was then functionalized with a glycolyl group. Full deprotection of the benzyl groups then yielded the target molecule ganglioside HLG-2 glycan structure 1 smoothly. The structure of compound 1 was confirmed by NMR spectral analyses.

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Methyl (p-Tolyl 8,9-O-isopropylidene-3,5-dideoxy-4-(methyl 7,8,9-Tri-O-acyl-5-amino-5,4-O-carbonyl-3,5-dideoxy-o-glycéro-a-o-galactonon-2-ulopyranosyl)onate)-(2,2,2-trichloroethoxy-2-carbonylamino)-o-glycéro-a-o-galactonon-2-ulopyranoside-2-ol (4). A mixture of the 95% solution of NaHCO₃ (150 mg, 2.24 mmol) and camphorsulfonic acid (CSA, 26.7 mg, 0.25 mmol) were added. The reaction mixture was stirred for 3 h and concentrated under reduced pressure. The residue was dissolved in EtOAc, and the solution was washed with water. The aqueous layer was washed successively with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was redisolved in CH₂CN (10 mL), and 2,2-dimethoxypropane (DMP, 0.3 mL, 5.06 mmol) and camphorsulfonic acid (CSA, 26.7 mg, 0.25 mmol) were added. The reaction mixture was stirred for 2 h, and then the reaction was quenched with triethylamine (TEA). The mixture was concentrated in vacuo, and the residue was purified by column chromatography on silica gel eluting with ethyl acetate/petroleum ether (2:3) to yield 3 as a white foam (549.4 mg, 76%). [α]D = [160.8° (c 3.14, CHCl₃)]. H NMR (400 MHz, CDCl₃) δ 7.40 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.66 (d, J = 10.8 Hz, 1H), 4.58 (d, J = 12.0 Hz, 1H), 4.63 (d, J = 12.0 Hz, 1H), 4.39 (d, J = 10.4 Hz, 1H), 4.19–4.10 (m, 2H), 3.98 (d, J = 5.2 Hz, 2H), 3.75 (q, J = 10.0 Hz, 1H), 3.67 (d, J = 7.6 Hz, 1H), 3.51 (s, 3H), 2.78 (dd, J = 13.6, 4.8 Hz, 1H), 2.33 (s, 3H), 2.09 (d, J = 13.2, 2.0 Hz, 1H), 1.41 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 168.8, 153.9, 140.1, 136.3, 129.7, 126.0, 109.2, 95.5, 90.1, 74.9, 74.7, 72.5, 70.6, 67.7, 67.3, 55.0, 52.6, 41.3, 27.0, 25.6, 21.5; HMRS (ESI) calcld for C₂₃H₃₄Cl₃N₂O₉S⁺ [M + Na]⁺ 619.1045, found 619.1039.

2-Trimethylsilyl-2,3,4-Tri-O-benzyl-β-D-glucopyranoside (4). This compound was prepared according to the known procedure.¹¹ H NMR (400 MHz, CDCl₃) δ 7.38–7.26 (m, 15H), 4.95 (d, J = 11.2 Hz, 1H), 4.93 (d, J = 10.8 Hz, 1H), 4.86 (d, J = 10.8 Hz, 1H), 4.81 (d, J = 10.8 Hz, 1H), 4.73 (d, J = 10.8 Hz, 1H), 4.64 (d, J = 10.8 Hz, 1H), 4.45 (d, J = 7.8 Hz, 1H), 4.02–3.95 (m, 1H), 3.87 (dd, J = 11.8, 5.6 Hz, 1H), 3.73–3.54 (m, 4H), 3.43–3.35 (m, 2H), 1.92 (dd, J = 7.6, 6.0 Hz, 1H), 1.08–0.99 (m, 2H), 0.30 (s, 3H), 1H). The yield was 119.8 mg, 24%.

Methyl (p-Tolyl 8,9-O-isopropylidene-4,7-Di-O-methoxymethyl-3,5-dideoxy-2-thio-5-(2,2,2-trichloroethoxy-carbonylamino)-o-glycéro-a-o-galactonon-2-ulopyranosyl)onate-(2,2,2-trichloroethoxy-2-carbonylamino)-o-glycéro-a-o-galactonon-2-ulopyranoside-2-ol (6). To compound 3 (602.9 kg, 1.0 mmol) was added iodine (5.1 mg, 0.02 mmol) followed by hexamethyldisilazane (HMDS) (0.089 g, 0.55 mmol). The mixture was stirred at room temperature until the starting material was completely consumed. The crude reaction mixture was dissolved in tert-butyl methyl ether (TBME) (4 mL) and stirred with the addition of 10% Na₂CO₃ solution until the iodine color was achieved. The solids were filtered off, and the solution was evaporated under the reduced pressure, yielding 6 as a light-yellow foam quantitatively. [α]D = +123.4° (c 3.40, CHCl₃). H NMR (400 MHz, CDCl₃) δ 7.35 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 4.96 (d, J = 8.0 Hz, 1H), 4.78 (d, J = 12.0 Hz, 1H), 4.66 (t, J = 12.4 Hz, 2H), 4.44 (td, J = 10.4, 8.8 Hz, 1H), 4.23 (s, 3H), 3.87–3.85 (m, 1H), 3.77 (t, J = 7.8 Hz, 1H), 3.61 (s, 3H), 3.52 (t, J = 7.6 Hz, 1H), 3.28 (q, J = 10.0 Hz, 1H), 2.54 (dd, J = 14.6, 4.4 Hz, 1H), 2.32 (s, 3H), 1.95 (dd, J = 14.0, 11.2 Hz, 1H), 1.41 (s, 3H), 1.25 (s, 3H), 0.14 (m, 18H). ¹³C NMR (100 MHz, CDCl₃) δ 169.1, 153.8, 140.1, 136.3, 129.7, 126.2, 107.5, 95.4, 89.3, 74.8, 73.0, 70.9, 67.4, 64.9, 55.6, 52.6, 41.3, 26.4, 24.7, 21.4, 11.0, 3H; HMRS (ESI) calcld for C₃₃H₄₄Cl₃N₂O₉S⁺ [M + Na]⁺ 693.1638, found 693.1623.

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reaction was quenched with MeOH. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether = 1:3 → 1:2) to give 10 (159.0 mg, 73%) as a colorless syrup. 

**ASSOCIATED CONTENT**

**Supporting Information**

NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.
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