Oligosaccharides

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The Total Synthesis of the Neurogenic Ganglioside LLG-3 Isolated from the Starfish *Linckia laevigata***

Hideki Tamai, Hiromune Ando, Hide-Nori Tanaka, Ritsuko Hosoda-Yabe, Tomio Yabe, Hideharu Ishida, and Makoto Kiso**

Recently, echinodermatous gangliosides have attracted much attention because of their characteristic structure and their potent neurogenic activity towards neuron-like rat adrenal pheochromocytoma (PC-12) cells in the presence of the nerve growth factor (NGF); this activity can greatly exceed that of the mammalian ganglioside GM1.[1] Therefore, it is of great importance to cultivate their potential as drug leads for combating neurological disorders at the molecular level. However, the extensive structural diversity in echinodermatous gangliosides has impeded studies into their structure–activity relationships and the mechanism by which they promote neurogenesis. Their structure characteristically contains a range of modified sialic acid units connected by a variety of linker groups, and such units are never seen in mammalian gangliosides.[2] Previously, we carried out the first synthesis of two of these structures, namely, Neu5Gcα-(2,4)Neu5Ac and 8-*O*-SO_3H-Neu5Acα(2,8)Neu5Ac, by developing the *N*-Troc sialyl (Neu5Troc) donor as a key intermediate to modified sialic acid residues.[3] Recently, we were the first to synthesize the neurogenic ganglioside HLG-2, which is found in the sea cucumber.[4] Herein we report the first total synthesis of ganglioside LLG-3 (**1**; Scheme 1),[5] which contains the 8-*O*-Me-Neu5Acα(2,11)Neu5Ac structure, by using Neu5Troc chemistry combined with the glucosyl ceramide (Glc-Cer) cassette approach.

Ganglioside LLG-3 (**1**) was identified in the starfish *Linckia laevigata* by Higuchi and co-workers (Scheme 1).[6] They revealed that at a concentration of 10 μM LLG-3 caused neurogenesis of (63.1 ± 6.3)% of PC-12 cells in the presence of NGF (5 ng mL^{-1}), whereas in the control experiment (only NGF) (20.6 ± 2.2)% of PC-12 cells were affected. Ganglioside **1** is the second most potent enchinoderm ganglioside amongst the fifteen that have been examined. The most potent activity [(64.8 ± 7.6)%] was exhibited by the ganglioside SJG-2,[1,7] which is a heptasaccharide and contains a trisialyl tetrasaccharide unit. Therefore, even though **1** is a tetrasaccharide, it is considered to be more effective than SJG-2 and has attracted much attention in the field of medicinal chemistry.

The characteristic structure of modified sialic acids (8-*O*-Me-Neu5Acα(2,11)Neu5Gc) that is presumably responsible for the neurogenic activity, was expected to be efficiently synthesized from the common Neu5Troc donor **4** (Scheme 1).[8] Thus, it was envisioned that the synthesis of the 5-NH_2-Neu intermediate and the 8-*O*-methyl-Neu glycolate derivative could be achieved from **4** through the cleavage of the Troc group with subsequent migration of the acetyl group from O8 to N5. To avoid the loss of the glycan portion as a result of inefficient coupling with the lipid portion we anticipated using the glycosyl ceramide cassette approach, which was recently established in our laboratory.[9] Therefore, **1** was disconnected into the trisaccharide segment **2** and the Glc-Cer cassette **3**. Then, **3** was fragmented into the glucosyl donor **5** and the phytoceramide **6** as an aglycon.

As depicted in Scheme 2, the synthesis of the 8-*O*-methyl sialyl unit from **4** commenced with the stereoselective incorporation of benzylglycolate at the anomeric position to give **8**[5b] in a 91% yield ($\alpha/\beta = 7.5:1$). Next, **8** was treated with zinc under acidic conditions to produce the 8-hydroxy-*N*-acetyl sialyl derivative **9**, through the migration of the acetyl group from O8 to N5, in a high yield. The 8-*O*-methylation of **9** was attempted using two different methylating reagents (Me_3OBF_4 and MeOTf) but each attempt was unsuccessful, and only generated a complex mixture of 5-methylimidate derivatives and *N*-acetyl-*N*-methyl derivatives. Therefore, we masked the C8 hydroxy group with a chloroacetyl group [$(ClCH_2CO)_2O$, cat. DMAP, THF],[10] and then protected the C5 acetamide with an acetyl group (isopropenyl acetate, TsOH),[11] thereby obtaining **11** in a high yield (93% over two steps). Compound **11** was then subjected to a two-step sequence for 8-*O*-methylation. 1-Selenocarbamoylpiperidine (**12**), which was recently developed by our research group,[12] was found to be the best reagent for the selective dechloroacetylation at C8. The chloroacetyl group could also be removed by using the widely used DABCO method[13] (DABCO, EtOH), however this strategy promoted the migration of the acetyl group from N5 to O8, giving the fully acetylated derivative as the major product. Removal of the chloroacetyl group using **12** and 2,6-lutidine as an acid

[*] H. Tamai, Dr. H. Ando, Dr. H.-N. Tanaka, R. Hosoda-Yabe, Dr. T. Yabe, Dr. H. Ishida, Dr. M. Kiso
Department of Applied Bioorganic Chemistry, Gifu University
1-1 Yanagido, Gifu-shi, Gifu 501-1193 (Japan)
Fax: (+81) 58-293-3452
hando@ gifu-u.ac.jp
H. Tamai, Dr. H. Ando, Dr. H.-N. Tanaka, R. Hosoda-Yabe, Dr. M. Kiso
Department of Applied Bioorganic Chemistry
Institute for Integrated Cell-Material Sciences (WPI program)
Kyoto University
Yoshida Ushinomiya-cho, Sakyo-ku, Kyoto 606-8501 (Japan)

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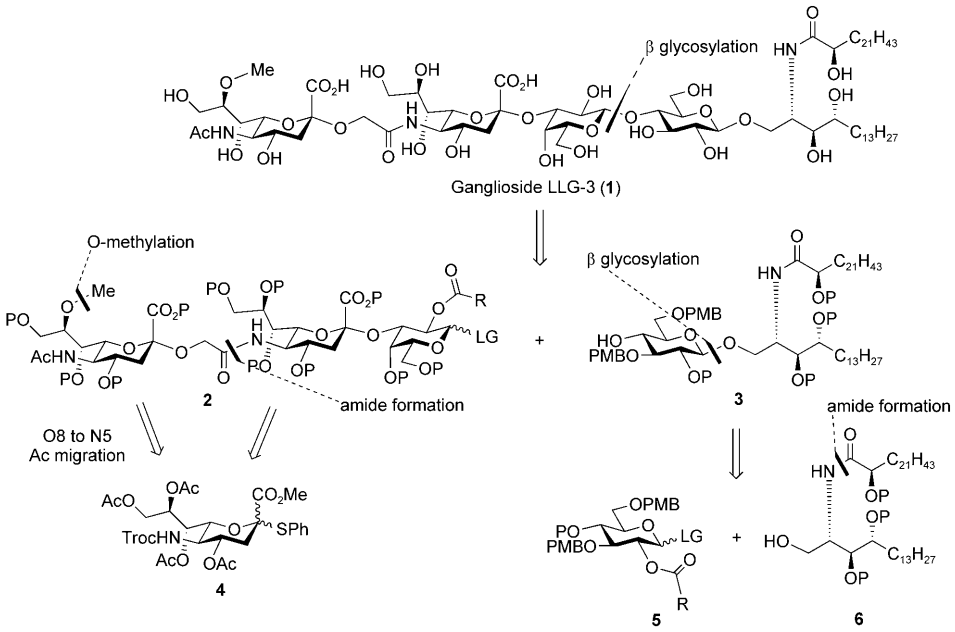
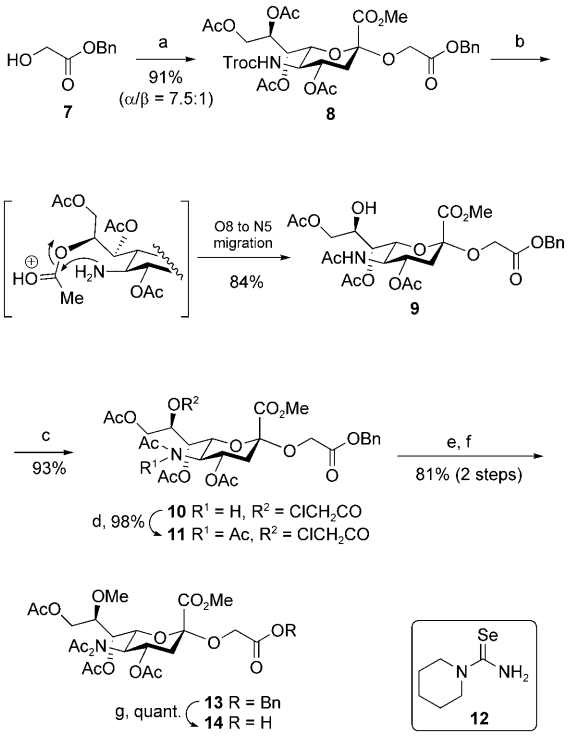


Scheme 1. Retrosynthetic scheme for target molecule **1**. Ac = acetyl, LG = leaving group, P = protecting group, PMB = *p*-methoxybenzyl, Troc = 2,2,2-trichloroethoxycarbonyl.

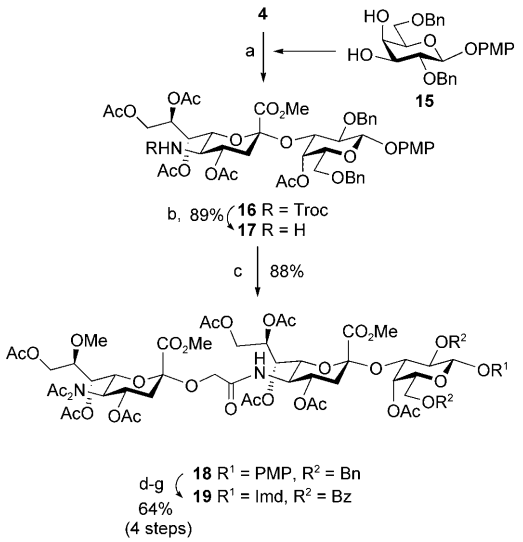
scavenger with subsequent methylation using Meerwein's reagent,[14] successfully introduced a methyl group at the C8 hydroxy group, thus generating **13** in an 81% yield (two steps). Upon hydrogenolysis, **13** was converted into the terminal sialyl glycolic unit **14** in a quantitative yield.

To synthesize the 5-NH_2-sialyl unit **17**, **4** was reacted with the galactosyl acceptor **15** according to the procedure reported in our previous paper[8a,c] to give disaccharide **16** (Scheme 3). MeCN suppressed the migration of the acetyl group from O8 to N5 during the Troc removal, which was achieved using zinc under acidic conditions. This reaction produced the 5-amino sialyl unit **17** in a satisfactory yield. The carboxylic unit **14** and the amine unit **17** were successfully coupled to deliver the key terminal trisaccharide **18** in an 88% yield; **18** was then manipulated in a straightforward manner to furnish the imidate **19**.[15]

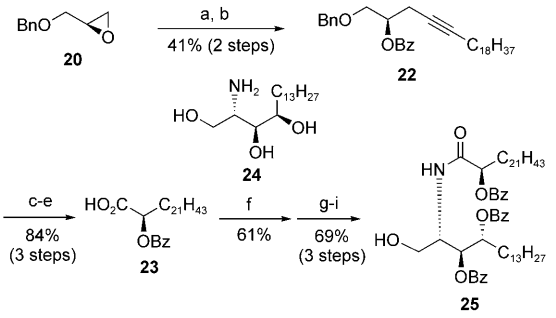
The synthesis of the Glc-Cer portion (**30**) was conducted in accordance with our earlier report.[4,9] The (*R*)-2-hydroxytricosanoic acid derivative **23** was successfully assembled



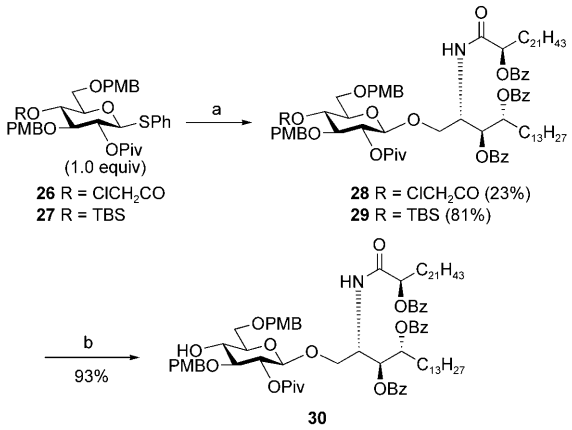
Scheme 2. Synthesis of 8-*O*-Me-sialyl glycolic acid **14**. a) **4**, NIS, TfOH, EtCN, molecular sieves (4 Å), −80°C, 91% (α/β = 7.5:1); b) Zn, DMF/AcOH (4:1), 84%; c) $(ClCH_2CO)_2O$, DMAP, THF, 93%; d) $TsOH\cdot H_2O$, isopropenyl acetate, 85°C, 98%; e) **12**, 2,6-lutidine, DMF, 65°C; f) Me_3OBF_4, DTBMP, CH_2Cl_2, reflux, 81% (2 steps); g) H_2, 20% $Pd(OH)_2/C$, EtOAc, quant. Bn = benzyl, DMAP = 4-(dimethylamino)pyridine, DMF = *N*,*N*′-dimethylformamide, DTBMP = 2,6-di-*tert*-butyl-4-methylpyridine, NIS = *N*-iodosuccinimide, Tf = trifluoromethanesulfonyl, THF = tetrahydrofuran, Ts = *p*-toluenesulfonyl.



Scheme 3. Synthesis of trisaccharide unit **19**. a) See Ref 8a and 8c for reaction conditions; b) Zn, MeCN/AcOH (4:1), 89%; c) **14**, EDC·HCl, HOBt, $NaHCO_3$, MeCN, 88%; d) H_2, 20% $Pd(OH)_2/C$, EtOAc; e) Bz_2O, DMAP, Py, 40°C, 96% (2 steps); f) CAN, toluene/MeCN/H_2O (5:6:3), 0°C, 80%; g) CCl_3CN, DBU, CH_2Cl_2, 0°C, 84%. Bz = benzoyl, CAN = cerium(IV) ammonium nitrate, DBU = 1,8-diazabicyclo-[5.4.0]undec-7-ene, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt = 1-hydroxybenzotriazole, Imd = trichloroacetimidoyl, PMP = *p*-methoxyphenyl, Py = pyridine.



Scheme 4. Synthesis of the ceramide portion **25**. a) **21**, *n*BuLi, HMPA; b) BzCl, DMAP, Py, 41% (2 steps); c) H_2, 10% $Pd(OH)_2$/C, EtOAc, RT; d) DMP, $NaHCO_3$, CH_2Cl_2, RT; e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, THF/*t*BuOH/H_2O (4:5:1), RT, 84% (3 steps); f) **24**, EDC·HCl, HOBt, CH_2Cl_2, 61%; g) TrCl, Py, 50 °C; h) BzCl, DMAP, Py, 50 °C, 78% (2 steps); i) H_2, 10% $Pd(OH)_2$/C, 88%. DMP = Dess–Martin periodinane, HMPA = hexamethylphosphoric acid triamide, Tr = triphenylmethyl.



Scheme 5. Synthesis of the Glc-Cer cassette **30**. a) **25** (1 equiv), NIS, TfOH, molecular sieves (4 Å), CH_2Cl_2, 0 °C; b) TBAF, THF, 30 °C, 93%. Piv = pivaloyl, TBAF = tetra-*n*-butylammonium fluoride, TBS = *tert*-butyldimethylsilyl.

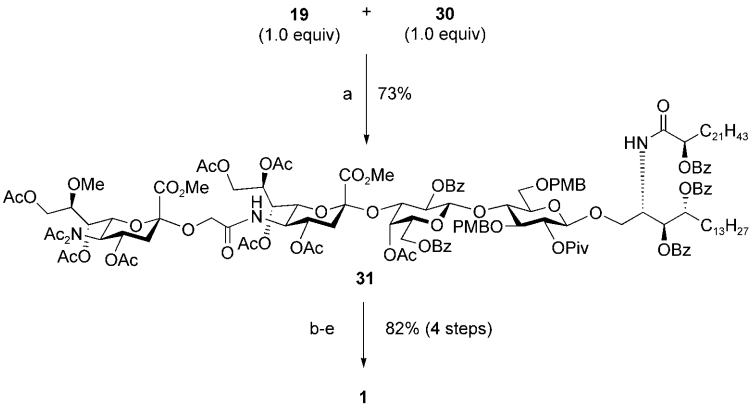
from oxirane **20** and icos-1-yne (**21**) using a five-step reaction sequence (Scheme 4). Phytosphingosine (**24**), which was synthesized from 1,2:4,6-diacetone-D-mannose using procedures reported by Tzou and Lin et al.,[16,17] was coupled with **23** using a carbodiimide (EDC·HCl, HOBt) to yield the phytoceramide framework. In preparation for the subsequent coupling with the Glc section, the hydroxy groups were protected with benzoyl groups and the resulting product was additionally manipulated to provide the 1-hydroxy derivative **25**.

The protecting group at C4 of the Glc donor was shown to strongly influence the yield of the coupling reaction with the ceramide acceptor **25** (Scheme 5). As previously reported,[9] the 4-chloroacetyl Glc donor **26**[17] provided Glc-Cer **28** in poor yield (23%). In contrast, the use of the TBS derivative **27**[17] increased the yield to 81%. Upon exposure to TBAF, the TBS group of **29** was removed to afford the Glc-Cer acceptor **30**, which was now ready for the assembly of the glycolipid framework of LLG-3.

Pleasingly, the final glycosidation of the trisaccharide donor **19** (1.0 equiv) with the Glc-Cer acceptor **30** (1.0 equiv), promoted by TMSOTf in CH_2Cl_2 at 0 °C, successfully produced the protected LLG-3 **31** in good yield (73%; Scheme 6). Treatment of **31** with TFA resulted in removal of the PMB groups, and subsequent treatment with LiCl liberated the carboxylic acid, and then removal of the acyl groups[5e] successfully delivered ganglioside LLG-3 (**1**, 25 mg).

Examination of the neurite growth of PC-12 cells revealed that the synthesized ganglioside LLG-3 (**1**) potentiated the neurite outgrowth in the presence of NGF (5 ng mL^{-1}).[18] LLG-3 (1 μM), NGF (5 ng mL^{-1}, control), and NGF at high concentration (50 ng mL^{-1}, positive control) all gave a similar increase in the number of neurites that had a length between one and two cell-body diameters. In contrast, the number of neurites that were more than twice the cell-body diameter varied depending on the exogenous factors; for example, NGF at 5 ng mL^{-1} (0.57/cell), NGF at high concentration (0.85/cell), and LLG-3 at 1 μM (0.73/cell). These results suggest that in the presence of NGF LLG-3 enhances the elongation of the neurites rather than their formation.

In summary, we have presented the first synthesis of ganglioside LLG-3 (**1**). The efficient assembly of the unusual structure comprising of an 8-*O*-Me-sialic acid and an *N*-glycolyl-sialic acid reinforces the utility of the *N*-Troc sialyl donor **4** for the synthesis of different sialyl glycosides. The Glc-Cer cassette approach provides an efficient route to gangliosides that contain a phytoceramide moiety. It was also revealed that silyl protection of the C4 hydroxy group of the glucosyl donor remarkably improved the yield of the coupling reaction with the phytoceramide acceptor. Finally, we could demonstrate the neurogenic activity of the synthesized LLG-3 (**1**) towards the PC-12 cells. We are also investigating the



Scheme 6. Final coupling and global deprotection to deliver LLG-3 **1**. a) TMSOTf, CH_2Cl_2, acid washed molecular sieves (4 Å), 0 °C, 73%; b) TFA, CH_2Cl_2, 0 °C; c) hydrazine acetate, THF, 0 °C; d) LiCl, Py, reflux; e) 0.1 M NaOH (aq.), 0 °C to 45 °C, 82% (4 steps). TFA = trifluoroacetic acid, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

neurogenic activity towards PC-12 cells by using other synthesized gangliosides such as GM1,[19] GM2,[20] GQ1b,[5] X1,[21] and HLG-2.[4] The detailed results of their activities will be reported in due course.

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