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COMMUNICATION

The total synthesis of a ganglioside Hp-s1 analogue possessing neuritogenic activity by chemoselective activation glycosylation†

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The total synthesis of ganglioside 2, an analogue of the ganglioside Hp-s1 (1) which displays neuritogenic activity toward the rat pheochromocytoma cell line PC-12 cell in the presence of nerve growth factor (NGF) with an effect (34.0%) greater than that of the mammalian ganglioside GM 1 (25.4%), was accomplished by applying a chemoselective-activation glycosylation strategy. Moreover, we also demonstrate that the synthesized ganglioside 2 exhibited neuritogenic activity toward the human neuroblastoma cell line SH-SY5Y without the presence of NGF.

Since around the early 1990s, many studies have demonstrated that activation of astrocytes,[1] differentiation of ependymal cells,[2] mesenchyme–epithelium interaction, expression of extracellular molecules, reformation of myelin, and migration of Schwann cells can make the injured neuron regenerate and remodel the tissue.[3] Many current data indicate that neutral stem cell can produce new neuron in another part of the human brain and epidermal growth factor (EGF) can promote differentiation and growth of the neural stem cell.[4–9] Therefore, it is important to search and develop efficient methods and efficacious drugs to enhance nerve regeneration.

Gangliosides, sialic acid-containing glycosphingolipids, are found ubiquitously in vertebrate cell plasma membranes and are especially abundant in the brain and nervous system.[10,11] Moreover, gangliosides, necessary for neuronal growth and development,[12–14] play a multifunctional role in neuronal regeneration and repair[12,13,15,16] and also possess a broad spectrum of biological functions, such as cell-cell recognition,[17] receptors for viral and bacterial toxins,[18] signal transduction,[19] oncogenesis,[20] and cell differentiation.[21] In addition, there have been numerous reports indicating that gangliosides extracted from marine invertebrates show neuritogenic activity toward the rat pheochromocytoma cell line PC-12 in the presence of NGF and that the activity of some gangliosides is superior to that of the mammalian ganglioside GM 1.[22] Hence, these echinodermatous gangliosides can be considered as lead compounds for the development of carbohydrate-based drugs used in the treatment of nerve degeneration diseases. However, for the total synthesis of these echinodermatous gangliosides, up to the present time, just only gangliosides M5, HLG-2, and LLG-3 have been synthesized by Shiba's, and Ando and Kiso's groups, respectively.[23]

Ganglioside Hp-s1 (**1**) (Fig. 1),[24] isolated from the ovary of the sea urchin *Diadema setosum* or the sperm of the sea urchin *Hemicentrotus pulcherrimus* and possessing the structure of Neu5Ac α2→6Glcβ1→1Cer, exhibits neuritogenic activity, and the effect of **1** (34.0%) is greater than that of the mammalian ganglioside GM 1 (25.4%). Owing to the difficulty of acquiring enough quantity and high purity of Hp-s1 from natural resources for further examination, and the lack of related syntheses in the literature, we are keenly interested in developing an efficient methodology for the synthesis of ganglioside Hp-s1. In order to understand the effect of the length of the carbon chain of the phytosphingosine moiety on biological activity, we report herein the total synthesis of the analogue ganglioside **2** (Fig. 1) which has one carbon less than Hp-s1 in the carbon chain of the phytosphingosine segment.

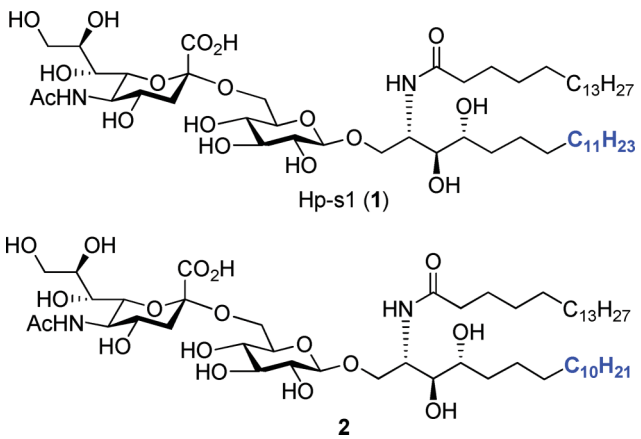

Fig. 1 Structures of gangliosides Hp-s1 (**1**) and **2**.

Our retrosynthetic analysis of **2** is shown in Scheme 1. The preparation of **2** was achieved *via* the amidization of disaccharide **24αβ** containing phytosphingosine with commercially available

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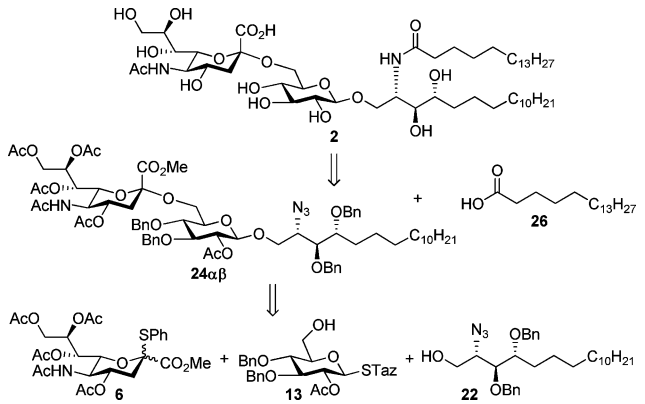
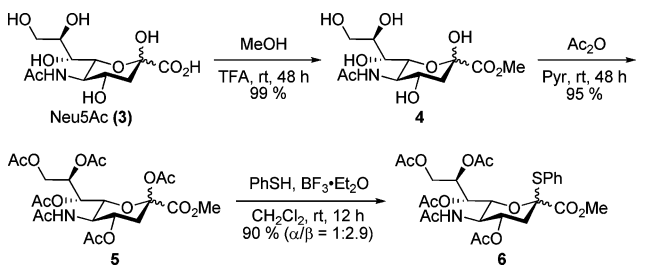
† Electronic Supplementary Information (ESI) available: General experimental procedures and ^{1}H-NMR, 13C, DEPT-90, and 135 NMR spectra for all key compounds and biological studies data. See DOI: 10.1039/b000000x/



Scheme 1 Retrosynthesis of ganglioside **2**.

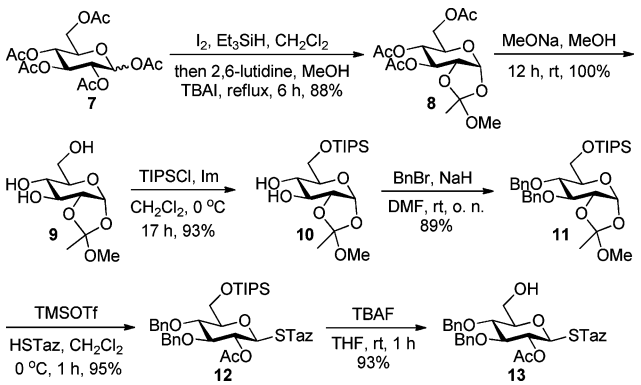
stearic acid. Furthermore, to develop the one-pot two-step glycosylations to conform to the essence of "Green Chemistry" [25] for synthetic methods in the future, we adopted the synthetic strategy of chemoselective-activation glycosylation for preparing disaccharide **24αβ** in this communication. The three building blocks required for chemoselective-activation glycosylation are Neu5Ac derivative **6**, glucose derivative **13**, and phytosphingosine derivative **22**. The leaving groups PhS- and TazS- were introduced at the anomeric positions of **6** and **13**, respectively.[26] As to the β-stereoselectivity of the glycosylation of **13** with **22**, a group resulting in neighboring-group participation will be installed at C2-OH of glucose. In order to enhance the reactivity of glycosylation, benzyloxy groups were assembled on C3 and C4 of glucose.

Scheme 2 describes the synthesis of the sialic acid derivative **6**. *N*-acetylneuraminic acid (**3**), the starting material, was transformed into the corresponding methyl ester **4** in 99% yield (α : β = 1 : 12) in dry CH_3OH by using CF_3CO_2H as a catalyst.[27] To assemble the leaving group PhS- at the anomeric carbon of **6** and to avoid any possible complication during glycosylation, per-*O*-acetylation can achieve the purpose of protecting the hydroxyls as OAc groups and rendering the OAc on the anomeric carbon of **4** a good leaving group for the ensuing nucleophilic substitution reaction. Per-*O*-acetylation of **4** was carried out with acetic anhydride in dry pyridine to afford the peracetylated compound **5** with excellent yield (95%). Then the peracetylated compound **5** was treated with thiophenol in the presence of boron trifluoride etherate to give the desired phenylthioglycoside **6** in 90% yield (α : β = 1 : 2.9).[28]



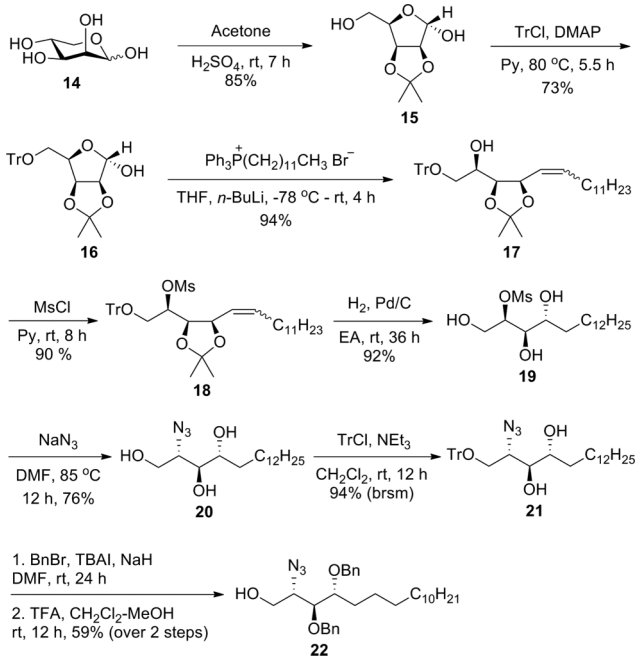
Scheme 2 Synthesis of *N*-acetylneuraminic acid derivative **6**.

Next, we turned our attention to the preparation of a suitable building block **13** that could function as both glycosyl acceptor and donor (Scheme 3). To carry out the chemoselective glycosylation, we chose 2-thiazoline-2-thiol (HSTaz) as an anomeric leaving-group for compound **13**. Under Ac_2O–imidazole–DMAP conditions, D-(+)-glucose was initially converted into peracetyl compound **7** in quantitative yield. In order to install an acetyl group at C2–OH of glucose to generate neighboring-group participation and shorten the reaction steps, the preparation of orthoester **8** and triol **9** were achieved by following Iadonisi's methodology,[29] through one-pot iodination and cyclization, and deacetylation under Zemplén conditions,[30] respectively. In order to get a glucose derivative with a free 6-hydroxyl group, an orthogonal protecting-group strategy was adopted. The regioselective silylation of C6–OH of the resulting triol **9** with TIPSCl in CH_2Cl_2 in the presence of imidazole at 0 °C provided the silyl compound **10** in 93% yield (over two steps). Subsequent benzylation of **10** with benzyl bromide yielded the dibenzyl compound **11** (89% yield). Thioglycoside **12** was obtained as a single β-stereoisomer in 95% yield by treatment of **11** with HSTaz in the presence of TMSOTf at 0 °C.[31] Upon exposure to TBAF in THF at room temperature, the TIPS group in **12** was selectively removed to give the C6-hydroxyl compound **13** in 93% yield ($[\alpha]_D^{30}$ + 41.0 (*c* 0.11, $CHCl_3$)).



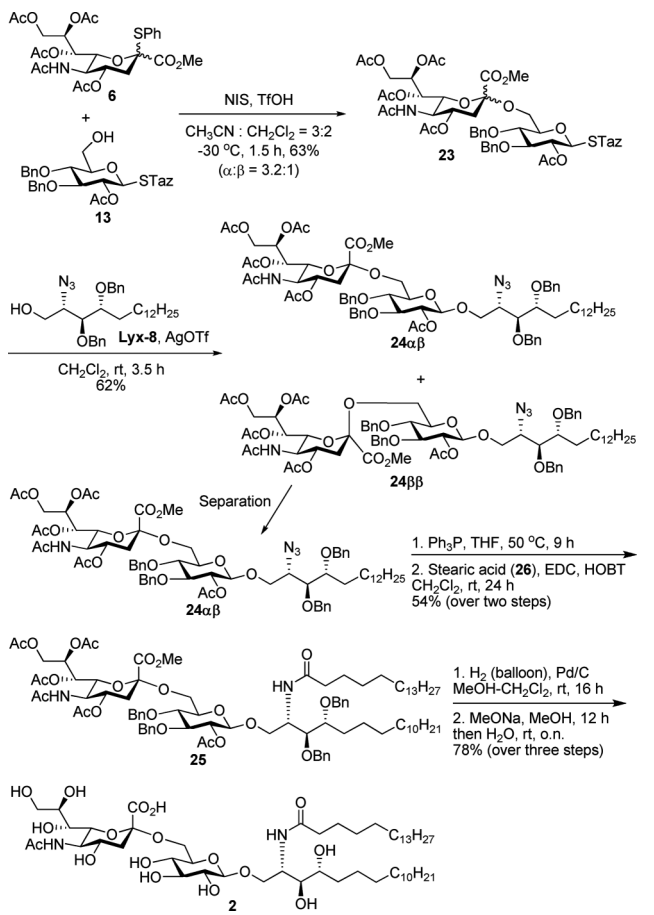
Scheme 3 Preparation of glucose building block **13**.

Herein, the required phytosphingosine **22** was constructed by following the Lin, and Akimoto methodology (Scheme 4)[32] with



Scheme 4 Synthesis of phytosphingosine **22**.

some modification. The suitably protected lyxofuranose **16** was generated (62%, two steps) from D-(−)-lyxose (**14**) *via* the sequence of regioselective isopropylidenation (dry acetone–H_2SO_4) and tritylation (TrCl–DMAP–pyridine). Wittig olefination of **16** with dodecyl(triphenyl)phosphonium bromide–*n*-butyl lithium followed by mesylation of the resulting enol **17** provided a mixture of *Z*- and *E*-stereoisomers (*ca.* 2.3 : 1) of **18** (85% overall yield), which was treated with H_2/10% Pd on charcoal in order to remove the trityl group. However, the triol **19** was actually produced, as a white solid in 92% yield ($[\alpha]_D^{25}$ +14.5 (*c* 0.32, $CHCl_3$: MeOH = 1 : 1 (v/v)), presumably due to the presence of trace acid and water to hydrolyze the acetonide. Subsequently, the mesylate group of **19** was transformed into azide (**20**) followed by regioselective tritylation of the primary hydroxyl group to afford the compound **21**. At this juncture, benzylation of **21** was carried out to give the corresponding protected azide, which on exposure to CF_3CO_2H in CH_2Cl_2–MeOH gave the desired phytosphingosine **22** ($[\alpha]_D^{25}$ −4.2 (*c* 0.68, $CHCl_3$)) in 42% overall yield from **19**.



Scheme 5 Assembly of all of the building blocks to produce the ganglioside **2**.

As illustrated in Scheme 5, the total synthesis of ganglioside **2** was accomplished by a sequence of assembly of the three building blocks. The sialylation of the glucosyl acceptor **13** with sialyl donor **6** was performed with NIS–TfOH as a promoter in CH_3CN–CH_2Cl_2 in the presence of 3 Å powdered molecular sieves at −30 °C to afford the disaccharide **23** as a mixture of α- and β-stereoisomers (3.2 : 1) in 63% yield.[26,33] Owing to the difficulty of separating the stereoisomers, **23** was directly utilized in the next step of the reaction. Compound **22** was glycosylated with **23** in the presence of a promoter (AgOTf–MS-3 Å) in anhydrous CH_2Cl_2 at room temperature to furnish a mixture of **24αβ** and **24ββ** in 62% yield and the stereochemistry of the glucose moiety was a single β-configuration as indicated by its ^{1}H NMR spectrum ($\delta_{(\text{anomeric H})}$ 4.27, J = 7.9 Hz).[26] After isolation of the anomers, reduction of the resultant **24αβ** under Staudinger's conditions followed by amidization of the yielded amine with commercially available stearic acid (**26**) in the presence of EDC–HOBt in dry CH_2Cl_2 produced the protected **25** in 54% yield over two steps. Finally, the protected ganglioside **25** went through a sequence of debenzylation and deacetylation to successfully provide the target ganglioside **2** ($[\alpha]_D^{26}$ −6.7 (*c* 0.23, MeOH)) as a white solid compound in 78% total yield from **25**.

After finishing the synthesis of the ganglioside **2**, the neuritogenic activity was evaluated by incubation of human neuroblastoma cell line SH-SY5Y with **2** for 72 h at a concentration of 1 μM, and 10 μM, respectively. As shown in Fig. S1 (see the ESI†), they showed dose-dependent results; that is, the neurite length of SH-SY5Y cells at 10 μM (Fig. S1C, see the ESI†) was longer than that at 1 μM (Fig. S1B, see the ESI†). Furthermore, 1 μM and 10 μM of **2** not only increased the portion of

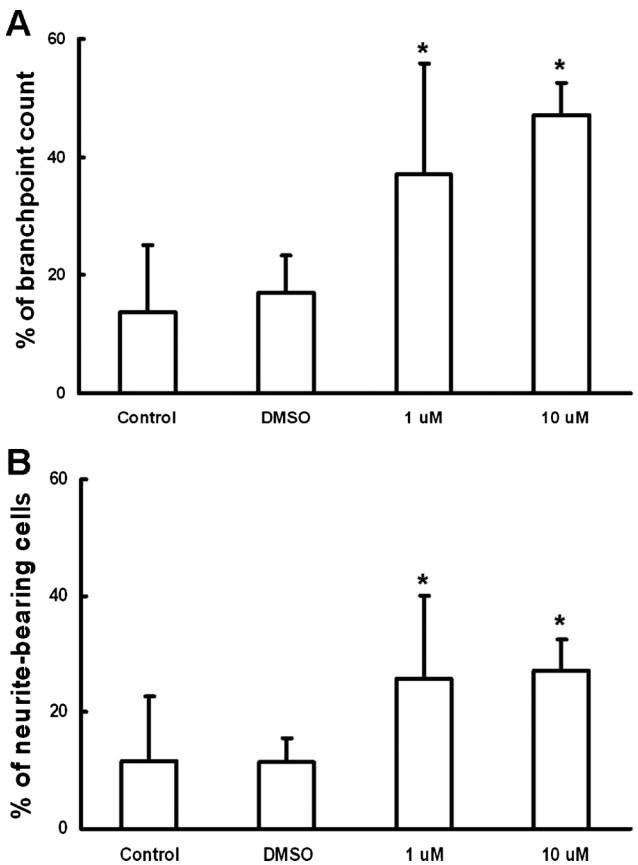


Fig. 2 Effect of neurite outgrowth in SH-SY5Y cells at different concentrations of **2**. Neurite outgrowth is represented by different parameters. A: branchpoint count. B: neurite-bearing cells. Significant difference from control group: *, p <0.05; **, p <0.01. Data were expressed as the mean from triplicate experiments; *bars*, +SD.

branchpoint count to 37% and 47% but also increased the neurite-bearing cells to 25% and 26%, respectively (Fig. 2). These results indicate that the synthesized ganglioside **2** possessed neuritogenic activity.

In conclusion, the total synthesis of ganglioside Hp-s1's analogue **2** has been achieved by a chemoselective-activation glycosylation methodology. The glycosylation of the glucose moiety in glycosyl donor **23** with phytosphingosine derivative **22** displays excellent stereoselectivity, providing a single β-isomer. The preparation of phytosphingosine **22** followed the Akimoto and Lin methodology with some modification, that is, hydrogenation of the double bond, deisopropylidenation, and detritylation of compound **18** proceeding simultaneously under H_2–Pd/C conditions. To the best of our knowledge, this is the first reported neuritogenic activity for the synthesized ganglioside **2** toward the human neuroblastoma cell line SH-SY5Y without the presence of NGF.

Acknowledgements

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