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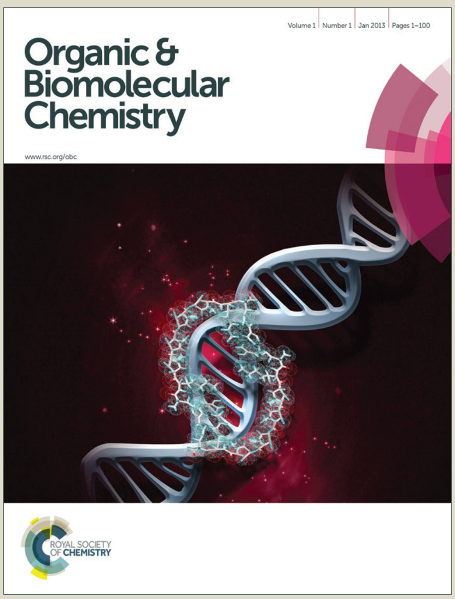

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COMMUNICATION

First total synthesis of ganglioside DSG-A possessing neuritogenic activity

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The first total synthesis of ganglioside DSG-A (1) has been achieved via chemoselective glycosylation and a [1+1+2] synthetic strategy. We have also developed an efficient method that can be handled in large scale (50 g) for the synthesis of the phytosphingosine.

Gangliosides, sialic acid-containing glycosphingolipids, are most abundant in the brain and nervous system where they comprise up to 6% of the total lipids. However, they are also ubiquitous in other tissues.[1] Many studies have indicated that gangliosides play a pivotal role not only in cell-cell and cell-matrix interactions,[2] but also in the development and the functions of the nervous system.[3] For example, mice lacking complex gangliosides, such as GD1a and GT1b, display progressive symptoms similar to those of axon degeneration and gross dysmyelination seen in neurodegenerative diseases.[4] Furthermore, numerous studies have demonstrated that administration of ganglioside GM1 to patients with Parkinson's disease or a number of different types of central nervous system lesion results in significant symptom reduction, as well as biochemical and behavioral recovery.[5] These results suggest that gangliosides may serve as potential candidates for treating neurodegenerative diseases.

Many gangliosides extracted from marine invertebrates show neuritogenic activity against the rat pheochromocytoma cell line PC-12 in the presence of NGF.[6] Among these gangliosides, SJG-2, LLG-3, GAA-7, LLG-5 and DSG-A (1) were found to be more effective than the mammalian ganglioside GM1, suggesting that these echinodermatous gangliosides can be considered as lead compounds for the development of chemotherapeutic agents for the treatment of neurodegenerative diseases. DSG-A, isolated from the ovary of the sea urchin *Diadema setosum*,[7] has a simpler structure, (9-*O*-Me-Neu5Acα(2→6)Glcβ(1→1)Cer possessing a 2-hydroxy octadecanoyl moiety), than the other gangliosides. However, to-date, DSG-A has not yet been synthesized and its mechanism of stimulating neurogenesis remains unclear because of the difficulty in obtaining a sufficient amount and purity of it from natural resources. Thus it is important to develop a powerful synthetic strategy for getting sufficient DSG-A, not only for efficacious validation but also for further structural modifications to examine the structure-activity relationships (SAR). Herein, we wish to report a total synthesis of DSG-A with an efficient methodology for a gram-scale (50 g) synthesis of the phytosphingosine moiety.

The retrosynthetic analysis of DSG-A (**1**) is outlined in Scheme 1. We intend adoptting a [1+1+2] strategy to assemble the four building blocks, including the sialyl donor **4** or **5**, the glucose derivative **6**, the succinimidyl acetoxyester **7**, and the phytosphingosine derivative **8**. The glucosyl acceptor **6** was first sialylated with sialyl donor **4** or **5** to give the disaccharide **2α** which was then glycosylated with the phytoceramide **3**, readily prepared by the amidization of amine **8** with the succinimidyl ester **7**, to generate the target molecule DSG-A. It is noteworthy that in order to achieve highly chemoselective glycosylations, the BoxS and EtS groups were introduced at the anomeric carbons of **4** (or **5**) and **6**, respectively. For the β-stereoselective glycosylation of disaccharide **2**□ with phytoceramide **3**, a group displaying a neighboring group effect to control the stereoselectivity was added to the C2-OH of glucose **6**. Moreover, to enhance the reactivity of glycosylation electron-donating groups were added to the C3- and C4-OH in **6**.

The preparative methods used to obtain **4** and **5** are shown in Scheme 2. Peracetyl phenylthioglycoside **9**[8] was first deacetylated with MeONa/MeOH to give tetraol **10**, which was used without further purification for the chemoselective methylation on C9-OH with Meerwein reagent[9] in MeCN in the presence of DTBMP at -10 ^{o}C to generate monomethylated **11** in 82% yield over two steps. To avoid affecting the subsequent conversion of the PhS group in compound **11** to BoxS, the free hydroxyl group of triol **11** was protected with Ac_2O/pyridine at room temperature to produce the acetate **12** in 95% yield. The PhS group was then converted into the BoxS group by chlorination of **12** with iodine chloride, followed by

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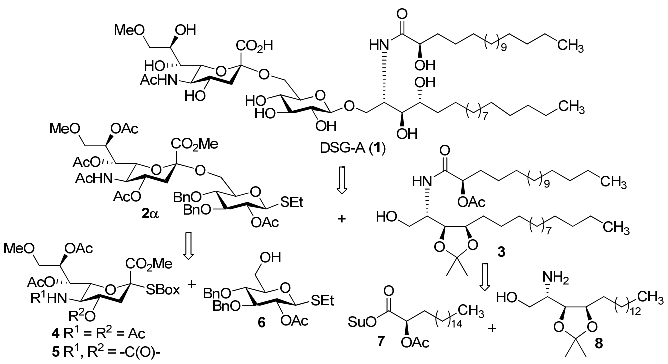
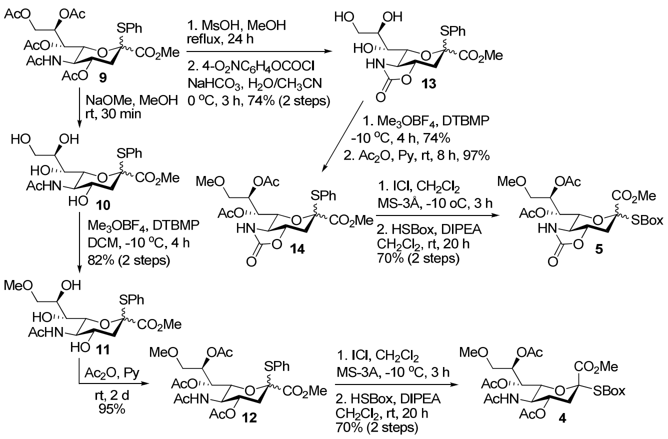
substitution of the resulting chloride with 2-mercaptobenzoxazole (HSBox) in the presence of Hunig's base.[10] The desired sialyl donor



Scheme 1. Retrosynthesis of the Ganglioside DSG-A (**1**).

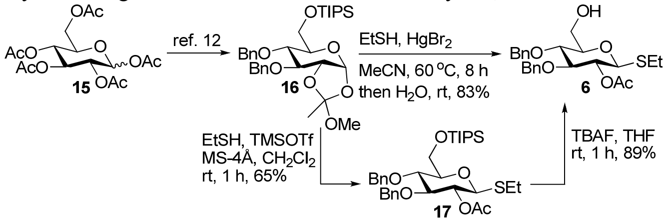


Scheme 2. Synthesis of the Sialyl Donor **4** and **5**.

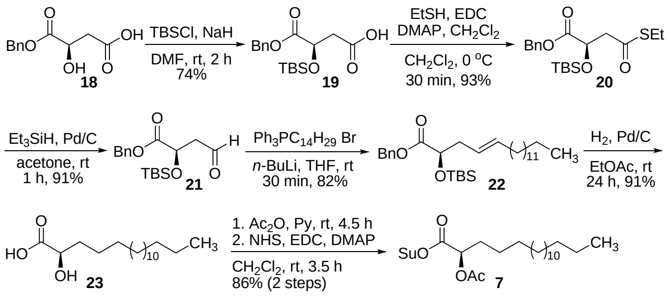
4 was obtained in 70% overall yield in two steps. For the synthesis of the sialyl donor **5**, the oxazolidinone **13** was synthesized using a documented protocol.[11] The subsequent procedure for the methylation of C9-OH in **13** and conversion of the PhS group in compound **14** to BoxS was the same as that of the conversion of compound **11** to compound **4**.

After preparing the desired sialyl donors **4** and **5**, we then focused on the synthesis of a suitable glucose derivative such as **6** that could function as both glycosyl acceptor and donor (Scheme 3). The commercially available pentaacetylated glucose **15**, which was employed as a starting material, was transformed into the orthoester **16** by a known method.[12] To simplify the reaction procedures, we developed a one-pot thioethylation and desilylation of orthoester **16** by treatment with EtSH in the presence of $HgBr_2$ at 60 °C followed by addition of H_2O at room temperature to observe the thioethyl glycoside **6** as a single β-stereoisomer with a free C6-OH in 83% yield.[13] Alternatively, a stepwise strategy was also used to prepare **6**, wherein **16** was thioethylated with EtSH in the presence of TMSOTf to generate the ethylthioglycoside **17** in 65% yield,[14] which was then treated with TBAF to generate the desilylated **6** in 89% yield.[15] However, the resulted overall yield (58%) through stepwise strategy was not better than that (83%) gained by one-pot two-step reactions.

Optically pure (*R*)-2-hydroxyoctadecanoic acid derivative **7** was synthesized (Scheme 4) from benzyl (+)-malate **18**, prepared with the known mehod.[16] In order to convert the carboxylic group to the aldehyde group via the thioester and to obtain a high yield of thioesterification, the hydroxyl group of **18** was first converted to **19** with the silyloxyl group by treatment with TBSCl in 74% yield. Thioesterification of **19** with EtSH in the presence of EDC/DMAP in dry CH_2Cl_2 generated the thioester **20** in 93% yield, which was then



Scheme 3. Preparation of the Glucose Derivative **6**.



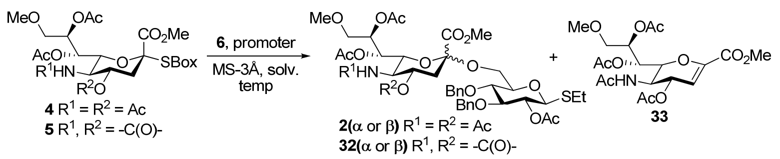
Scheme 4. Synthesis of the Optically Pure (*R*)-2-Hydroxyoctadecanoic Acid Derivative **7**.

transformed into aldehyde **21** with Fukuyama's method in 91% yield.[17] To establish the long-carbon side chain, Wittig olefination was carried out using aldehyde **21** as a substrate to generate the protected hydroxyalkenoate **22** in 82% yield as a single (*E*)-stereoisomer, fully characterized by its ^1H-^1H homodecoupling in the ^1H NMR spectrum ($\delta_{(\text{double bond H})}$ 5.47 (d, J = 15.6 Hz)). Treatment of **22** with H_2/10% Pd/C produced hydroxyalkanoic acid **23** as a white solid in 91% yield ($[\alpha]^{25}_D$ +16.6 (c 0.10, MeOH)) by debenzylation, saturation of the double bond and removal of the TBS group; the desilylation occurred presumably due to the presence of trace acid and water. The hydroxyl group in **23** was protected with Ac_2O/pyridine to form the acetate followed by esterification with *N*-hydroxysuccinimide to provide the desired succinimidyl acetoxyester **7** in 86% yield in two steps.

The preparations of the phytosphingosine derivative **8** and phytoceramide **3** are illustrated in Scheme 5. The primary hydroxyl group of substrate **24**[12] was first chemoselectively silylated with TIPSCl, and the resulted silyl ether **25** gave enol **26** by Wittig olefination in 73% overall yield in two steps. Enol **26** was converted into mesylate **27** (97% yield), which was then desilylated with TBAF to afford the hydroxymesylate **28** in 96% yield. To introduce the required amino group at C-2 in phytosphingosine, tandem addition and intramolecular cyclization of hydroxymesylate **28** with benzylisocyanate in the presence of NaH was employed to generate the 2-oxazolidone **29** in 81% yield,[18] which was then subjected to a cross-metathesis reaction with 1-tetradecene in the presence of Grubbs' catalyst to form phytosphingosine derivative **30** as a ca. 16:1 mixture of *E*- and *Z*-stereoisomers in 94% yield.[19] Upon exposure to NaOH in MeOH-H_2O, oxazolidone **30** was converted to the benzylaminoenol **31** in 94% yield. Hydrogenation of **31** followed by amidization of the corresponding aminoalcohol **8** (99%) with succinimidyl acetoxyester **7** generated the required phytoceramide **3** in 94% yield.

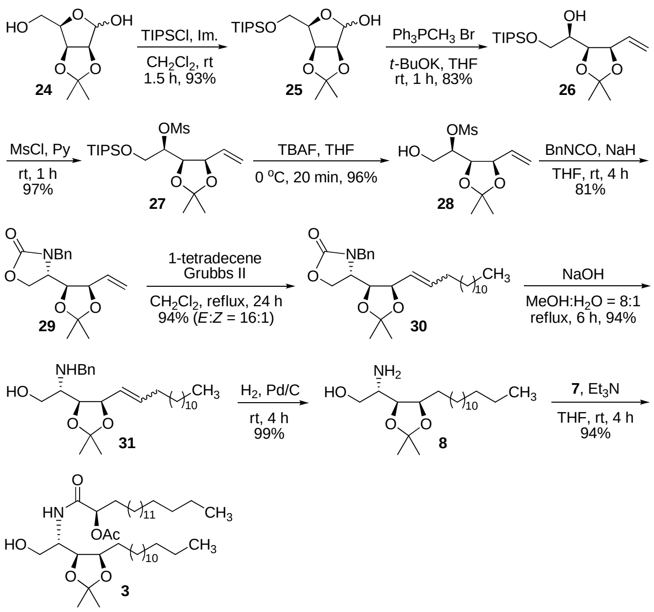
Before completing the synthesis of ganglioside DSG-A, glycosylation of the sialyl donors with a glucosyl acceptor was first evaluated (Table 1). The best result was obtained by glycosylating donor **5** with acceptor **6**, which was carried out in the presence of activator AgOTf in CH_2Cl_2 to give disaccharide **32α** as a single α-stereoisomer in excellent yield (93%) (entry 8).[20] To glycosylate the

Table 1. Glycosylation of sialyl donors **4** and **5** with glucosyl acceptor **6**.

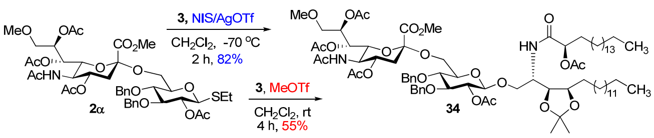


Entry	Donor	Promoter	Solvent	Temp (°C)	Time (h)	Yield of **2** or **32** (α/β ratio)[a]	Yield of **33**[a]
1	**4**	AgOTf	Et_2O	-40 to rt	11	54 (1.0:1.4)	43
2	**4**	AgOTf	CH_3CN	-40 to rt	7	-[c]	-
3	**4**	AgOTf	CPME	-40	7	46 (1.0:1.5)	52
4	**4**	AgOTf	THF/CH_2Cl_2[b]	-40	7	60 (1.5:1.0)	30
5	**4**	AgOTf	THF	-40	7	61 (3.0:1.0)	30
6	**4**	$Bi(OTf)_3$	THF	-40	7	37 (4.0:1.0)	52
7	**4**	$Cu(OTf)_2$	THF	-40 to rt	9	33 (8.0:1.0	52
8	**5**	AgOTf	CH_2Cl_2	-40	18	93 (α only)	0

[a] Isolated yield. [b] 3:1 (v/v). [c] No reaction.



Scheme 5. Construction of the protected Phytoceramide **3**.



Scheme 6. Examination of the Glycosylation of Disaccharide **2α** with Phytoceramide **3**.

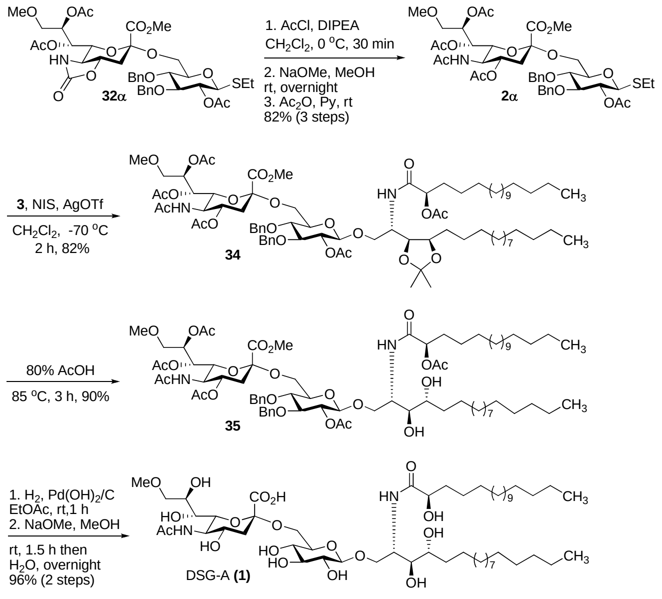
donor **4** with the acceptor **6**, eight sets of reaction conditions (promoter, solvent, and temperature) were used to generate glycal **33** and the disaccharide **2** as a mixture of the α- and β-stereoisomers; among of the tested conditions, only that in entry 5 resulted in a good yield and stereoselectivity for disaccharide **2**. For example, in Et_2O and in CPME (promoter AgOTf), the yields of the disaccharide were 54% or 46%, respectively, and the β-stereoisomer was the major product (entries 1 and 3). Although activation by $Cu(OTf)_2$ in THF resulted in a greater stereoselectivity (α:β = 8:1), the yield of the desired disaccharide was very poor (entry 7, 29%).

Next, we turned our attention to the coupling of the disaccharide **2α** with the protected phytoceramide **3**. As shown in Scheme 6, excellent stereoselectivity (β only) and a higher yield (82%) for the synthesis of the protected DSG-A analogue **34** were achieved by the use of NIS/AgOTf in CH_2Cl_2 at -70 °C. In contrast, activator MeOTf reduced the yield dramatically (55%) though excellent stereoselectivity (β only) was maintained.

Finally, the target molecule DSG-A was accomplished as shown in Scheme 7. The disaccharide **32α** was first converted into **2α**in 82% yield by a three-step sequence, involving amidization, deacetylation, decarbonylation and acetylation. The protected phytoceramide **3** was glycosylated with **2α** in the presence of the promoter NIS/AgOTf in anhydrous CH_2Cl_2 at -70 °C to generate the protected DSG-A analogue **34** in 82% yield with the glucose moiety in β-configuration, as demonstrated with its ^1H NMR spectrum ($\delta_{(anomeric\ H)}$ 4.31 (d, J = 8.0 Hz)). Deisopropylidenenation was then performed by treatment of **34** with 80% AcOH at 85 °C to give the diol **35** in 90% yield. Debenzylation of **35** using H_2/20% $Pd(OH)_2$ followed by deacetylation generated DSG-A (1) as a white solid compound in 96% yield in two steps.

Conclusions

In summary, we have achieved the first total synthesis of ganglioside DSG-A by employing a [1+1+2] synthetic strategy and chemoselective glycosylation. The glycosylation of sialyl donor **5** with glucosyl acceptor **6** and the conjugation of the synthesized disaccharide **2α** with the protected phytoceramide **3** both resulted in excellent yield and stereoselectivity. In addition, we have also developed an efficient method that can be applied to a large scale synthesis of phytosphingosine (50 g) in an efficient manner. Currently,



Scheme 7. Completion of the Total Synthesis of the Ganglioside DSG-A (**1**).

the application of one-pot two-step glycosylations to prepare a variety of DSG-A analogues for various biological tests, including neuritogenic activity, is under active investigation.

Acknowledgements

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