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Total Synthesis of Agelagalastatin

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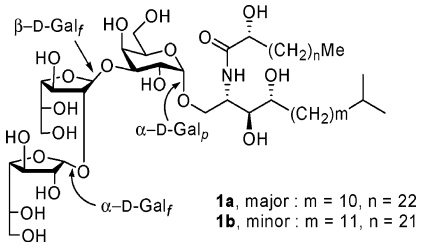
ABSTRACT



The total synthesis of agelagalastatin, an antineoplastic glycosphingolipid, has been achieved. The synthesis involved an α-selective glycosylation of the ceramide moiety with the trisaccharide fluoride. The trisaccharide component was constructed employing the CB glycoside method which permitted a completely α-stereoselective galactofuranosylation.

Agelagalastatin (**1**) was isolated from the Western Pacific marine sponge *Agelas* sp., and its structure was elucidated in 1991.[1] This compound is a member of a family of glycospingolipids found in *Agelas* sp. which include agelasphin-9b,[2] longiside,[3] triglycosylceramide,[4] and KRN7000.[5] These glycosphingolipids, which commonly contain α-*O*-galactopyranosyl ceramide moieties as their integral parts, have shown immunomodulating activity.[5,6] Among them, agelasphin-9b and KRN7000 exhibited immunostimulatory activity, which was suggested to be related to the interesting in vivo antitumoral properties of these compounds through an activation of the immune system,[2,7] and thus, KRN7000 is in clinical trials as a novel anticancer agent.[8] Agelagalastatin (**1**) displayed significant in vitro inhibitory activities against human cancer cell growth, its GI_{50} values ranging from 0.77 μg/mL for lung NCI-H460 to 2.8 μg/mL for the ovarian OVCAR-3.[1] Besides its biological activity, the unique structure of **1** has attracted our attention as it is composed of two galactofuranosides having α- and β-glycosyl linkages and a galactopyranoside having an α-glycosyl linkage with ceramide, as shown in structure **1**. The galactofuranoside moiety with α-configuration is quite rare in Nature and has been found only in a few microorganisms such as *Penicillium varians*,[9] *Leishmania major*,[10] and *Talaromyces flavus*.[11] In addition, agelagalastatin (**1**) was isolated as a mixture of two structural isomers (**1a**/**1b** = 4:1) in very low yield (7.42×10^{-6}%) because of the difficulty in separation.[1] Herein, we describe the first total synthesis of pure **1a** and **1b**.

Retrosynthesis of the target compound **1** leads to three galactoside building blocks **5**−**7** and a ceramide moiety **3** (Scheme 1). For the successful synthesis of **1**, it is essential to choose efficient glycosylation methods. Particularly challenging is its α-galactofuranosyl moiety, which is often a

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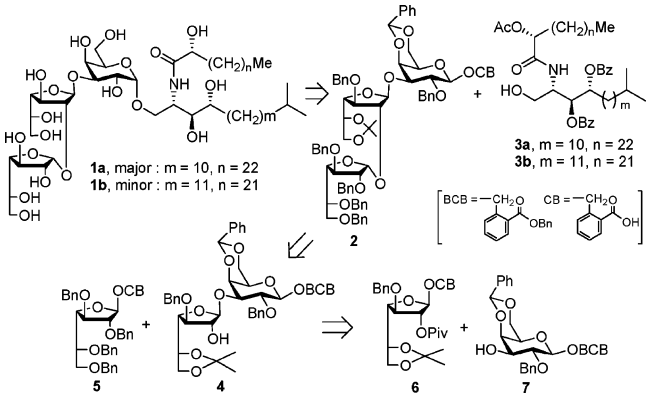
Scheme 1. Retrosynthesis of Agelagalastatin **1**

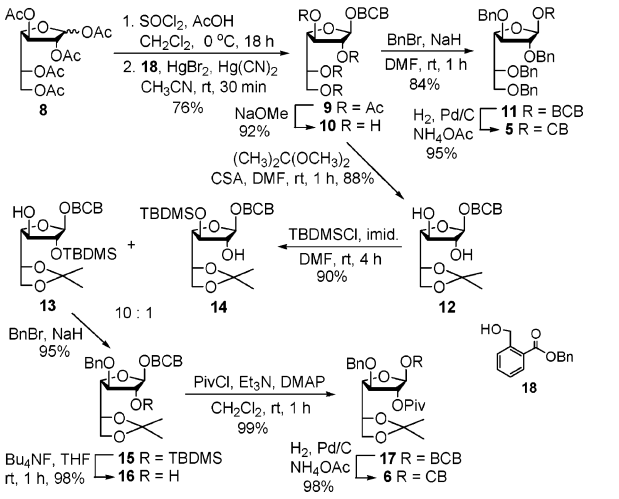


problematic subunit to incorporate stereoselectively. Attention needs to be paid to coupling the four building blocks, **3** and **5**−**7**, in the proper and correct order. The stereospecific construction of 1,2-*cis* α-galactofuranosyl and α-galactopyranosyl linkages has been one of the great challenges of D-glycoside synthesis.[12] In particular, there are no reliable methods available for the stereospecific synthesis of α-galactofuranosides, although a few attempts have been made employing galactofuranosyl trichloroacetimidates[13] and thiogalactofuranosides[14] as glycosyl donors. Our original plan was to employ 2′-carboxybenzyl (CB) glycosides as glycosyl donors[15] for the coupling of all four components of **1**. We envisioned that the construction of the α-galactopyranosyl linkage of **1** could be carried out by coupling between CB trisaccharide **2** and the ceramide moiety **3** in the later stages. We also believed that the crucial α-galactofuranosylation might be possible in the reaction between CB galactofuranoside **5** and 2′-(benzyloxycarbonyl)benzyl (BCB) disaccharide **4**, which would be readily obtainable from **6** and **7**.

Our synthesis commenced with the preparation of three building blocks **5**−**7** starting from D-galactose: compounds **5** and **6** were prepared via peracetylgalactofuranose **8**[16] as shown in Scheme 2 and compound **7** via 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (see the Supporting Information).

Anomeric chlorination of **8** followed by coupling of the resulting crude galactosyl chloride and benzyl 2-hydroxymethylbenzoate (**18**) afforded BCB tetraacetylgalactoside **9**. After conversion of **9** to **10** by deacetylation, the tetrol **10** was subjected to *O*-benzylation. Subsequent selective hydrogenolysis of the benzyl ester functionality[15] in the resultant

Scheme 2. Preparation of Monosaccharide Building Blocks **5** and **6**



BCB benzylgalactoside **11** thereafter provided the building block **5**. Protection of **10** with dimethoxypropane, on the other hand, gave the 5,6-*O*-isopropylidene derivative **12** and the subsequent protection of the diol **12** with TBDMSCl afforded the desired 2-*O*-silyl ether **13** along with a small amount of 3-*O*-silyl ether **14** (**13**/**14** = 10:1) in 90% yield. *O*-Benzylation of **13** followed by *O*-desilylation of the resulting **15** with Bu_4NF gave alcohol **16**. After *O*-pivaloylation of **16**, the resultant BCB galactoside **17** was converted into the building block **6**. Attempts to directly convert **12** into **17** by selective *O*-pivaloylation of the diol **12** by using PivCl in the presence of pyridine at 0 °C resulted in the formation of a mixture of 2-*O*-, 3-*O*- and 2,3-di-*O*-pivaloyl compounds.

To evaluate the exact reaction conditions needed and appropriate structures for glycosyl donors and acceptors in the crucial α-galactofuranosylation, we carried out a model study on the reaction of the donor **5** with the simpler acceptor **16** in the place of the disaccharide acceptor **4**. The model study began with dropwise addition of a diluted solution of Tf_2O in CH_2Cl_2 to a solution of **5**, **16**, and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in CH_2Cl_2 at −78 °C to afford the desired α-disaccharide **19** exclusively in 68% yield along with self-condensed ester **20**, which probably resulted from coupling between the carboxylate anion and the oxocarbenium ion generated from **5**, in 24% yield (method A in Scheme 3). To suppress formation of the ester **20**, the glycosylation was carried out with reversal of the order of the addition of reactants such that the concentration of **5** could be kept to a minimum during the glycosylation. Thus, slow addition of the donor **5** to a solution of acceptor **16**, DTBMP, and Tf_2O in CH_2Cl_2 at −78 °C afforded only the α-galactosyl disaccharide **19** in 82% yield (method B). The stereochemistry at the newly generated anomeric center of the disaccharide **19** was determined on the basis of its ^1H and ^{13}C NMR spectral data, especially the H1′−H2′ coupling constant ($J_{H1'-H2'}$ = 4.5 Hz) and the C1′ chemical shift ($\delta_{C1'}$ 99.0).[14]

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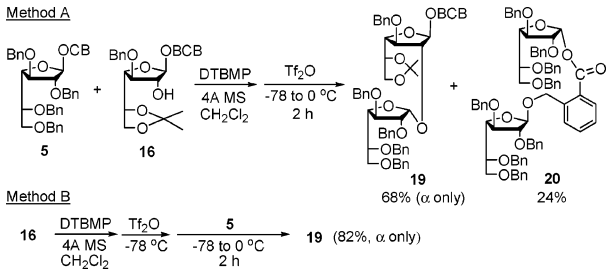
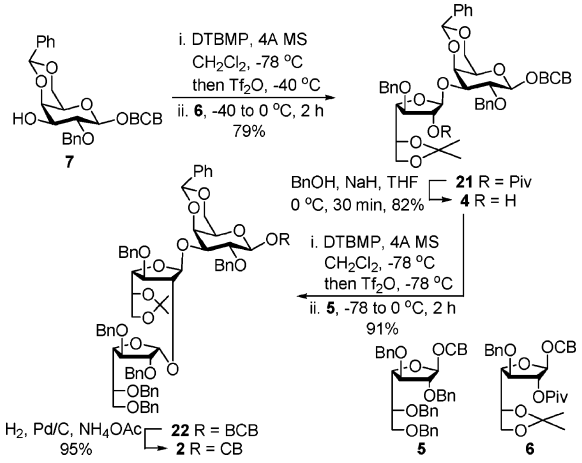
Scheme 3. Glycosylation of **16** with **5** for α-Galactofuranosylation



With this promising result for the α-galactofuranosylation with **5**, the stage was set for the assembly of building blocks **5**−**7** to make the trisaccharide **2** (Scheme 4). Glycosylation

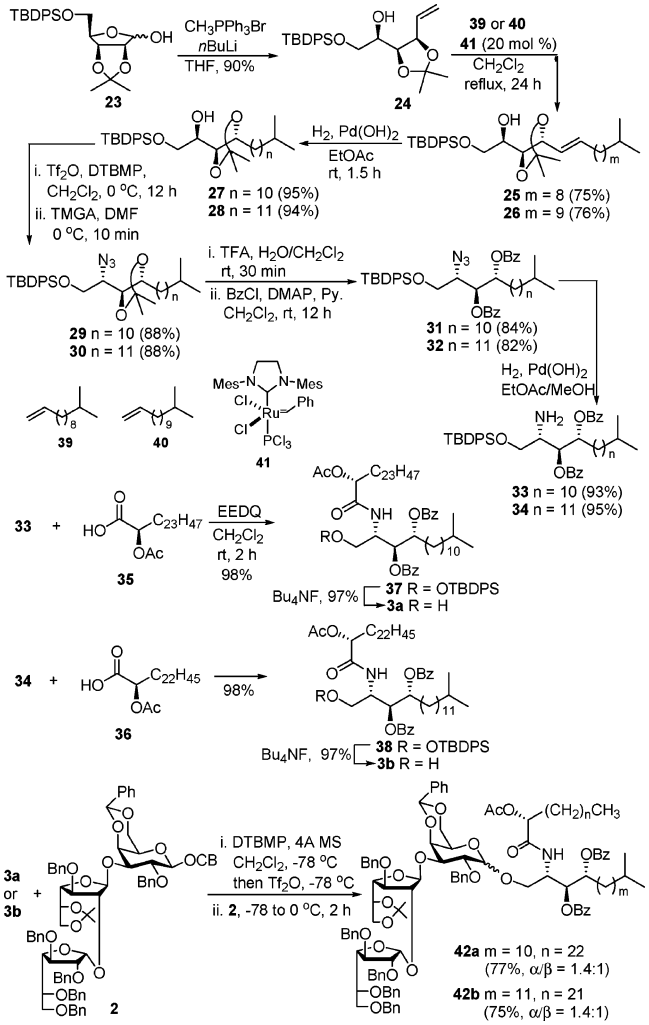
Scheme 4. Preparation of Trisaccharide Glycosyl Donor **2**



of **7** with **6** was carried out under the conditions of method B to give the *β*-disaccharide **21** exclusively in 79% yield. Removal of the *O*-pivaloyl group from **21** with NaOBn gave the alcohol **4**. Then, the crucial α-galactofuranosylation of the acceptor **4** with the donor **5** was successfully executed under the conditions of method B to afford the desired α-galactofuranosyl trisaccharide **22** ($J_{H1'-H2'}$ = 4.6 Hz and $\delta_{C1'}$ 98.5) exclusively in 91% yield. No *β*-trisaccharide was detected at all in the reaction mixture. The BCB trisaccharide **22** was converted into the CB trisaccharide **2** by selective hydrogenolysis. It is noteworthy that the reaction of the CB galactofuranoside **5** with the disaccharide **4** provided only the α-trisaccharide **22** in excellent yield, which is the first example of a completely stereoselective α-galactofuranosylation.

Our approach to the synthesis of ceramides **3a** and **3b** made use of the olefin cross-metathesis reaction to install the main carbon chain. Recently, the cross-metathesis olefination for the synthesis of sphingosines has become attractive due to the high *E*-selectivity and good yield of the process and its wide functional group tolerance.[17] Wittig reaction of known D-lyxose derivative **23**[18] with ylide CH_2=PPh_3 gave olefin **24**. The olefin cross-metathesis of **24** with 11-methyldodec-1-ene (**39**) and with 12-methyltridec-1-ene (**40**) in the presence of Grubbs' catalyst **41**[19] in refluxing CH_2Cl_2 provided the desired *trans*-olefins **25** and **26** in 75% and 76% yields, respectively (Scheme 5). After hydrogena-

Scheme 5. Preparation of Ceramides **3a** and **3b** and Their Glycosylations with CB Trisaccharide **2**



tion of **25**, the hydroxy group in **27** was activated as an *O*-triflate, and this subjected to an S_N2 reaction with tetramethylguanidinium azide (TMGA)[20] to give azido compound **29** with inverted configuration. Removal of the *O*-isopropylidene group of **29** by trifluoroacetic acid (TFA) followed by benzoylation of the resulting diol afforded

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compound **31** which, after hydrogenolysis, gave amine **33**. The homologue of **33**, compound **34**, was readily obtained in a like fashion from **26**. Coupling reaction of **33** with (*R*)-2-acetoxypentacosanoic acid (**35**) using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)[21] afforded amide **37**, and same reaction of **34** with (*R*)-2-acetoxytetracosanoic acid (**36**) provided amide **38**. Finally, a desilylation of **37** and **38** with Bu_4NF gave alcohols **3a** and **3b**, respectively.

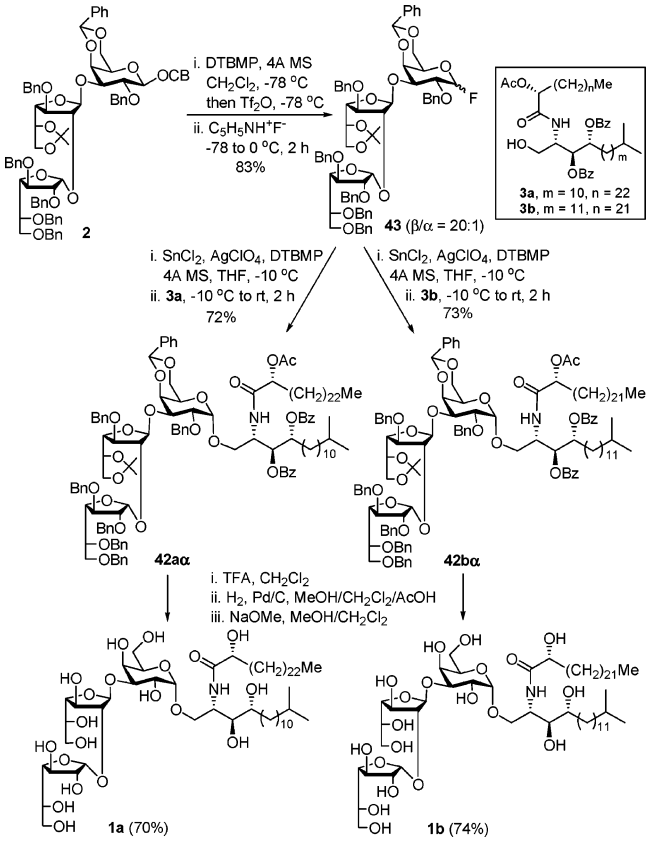
Glycosylation of the ceramide **3a** with the CB trisaccharide **2** afforded the desired protected agelagalastatin **42a**α (δ_{C1} 100.6) in 45% yield, along with undesired β-anomer **42a**β (δ_{C1} 104.5) in 32% yield, and the reaction of ceramide **3b** with **2** also gave a mixture of α- and β-anomers **42b** with the same ratio (α/β = 1.4:1) in 75% yield. Poor stereoselectivity in the glycosylation of **3** with the CB glycoside **2** led us to examine other glycosyl donors that could be readily prepared from **2**. We envisaged that sequential treatment of the CB glycoside **2** with Tf_2O and then with a fluoride reagent would give the glycosyl fluroride. Reaction of **2** with Tf_2O and then with (diethylamino)sulfur trifluoride (DAST) as a fluoride source afforded a mixture of α- and β-glycosyl fluoride **43** in 48% yield, whereas the same reaction with hydrogen fluoride pyridine as a fluoride source was quite efficient giving **43** in 83% (β/α = 20:1) yield (Scheme 6).

Glycosylations of **3a** and **3b** with **43** as the glycosyl donor were carried out by dropwise addition of a solution of **43** in THF to a solution of acceptor **3a** or **3b**, $SnCl_2$, $AgClO_4$,[22] and DTBMP in THF at −10 °C to afford desired α-glycosides **42a**α and **42b**α exclusively in 72% and 73% yield, respectively. The deprotection of **42a**α started with removal of the *O*-benzylidene and *O*-isopropylidene groups using TFA and was followed by hydrogenolysis to remove the benzyl groups. Finally, the *O*-acetyl and *O*-benzoyl protecting groups were removed using sodium methoxide in MeOH and CH_2Cl_2 to give the target compound **1a**. The other target compound **1b** was obtained from **42b**α by the same deprotection sequence under the same reaction conditions.

The ^1H NMR and ^{13}C NMR spectra of the authentic agelagalastatin isolated by Pettit and of our synthetic agelagalstatin (**1**) were identical in all respects.[23] Also, the optical rotations of synthetic compounds **1a** and **1b** were measured to be $[\alpha]_D$ +58.8 (*c* 0.65, CH_3OH) and $[\alpha]_D$ +59.8 (*c* 0.45, CH_3OH), respectively, which is exactly matched with the reported value of $[\alpha]_D$ +59 (*c* 0.65, CH_3OH) for the authentic agelagalastatin, which is a mixture of **1a** and **1b** with a ratio of 4:1.

We have thus achieved the first total synthesis of both the major component **1a** and the minor component **1b** of agelagalastatin (**1**). For the construction of the trisaccharide moiety **2**, we made use of the CB glycoside method.

Scheme 6. Synthesis of **1a** and **1b**



Particularly, the glycosylation of **4** with the CB galactofuranoside **5** showed complete α-stereoselectivity, giving only the α-galactofuranosyl trisaccharide **22**. On the other hand, for the coupling of the trisaccharide moiety and the ceramide part, trisaccharyl fluoride **43** was utilized as the glycosyl donor to afford α-glycosides **42a**α and **42b**α exclusively. For the synthesis of ceramides **3a** and **3b**, we employed olefin cross metathesis to install the main carbon chain efficiently. The biological activity of each component of agelagalastatin, **1a** and **1b**, will be reported in a separate communication.

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Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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