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Enantioselective Synthesis of Spliceostatin G and Evaluation of Bioactivity of Spliceostatin G and Its Methyl Ester

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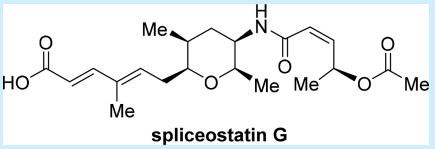
Supporting Information

ABSTRACT: An enantioselective total synthesis of spliceostatin G has been accomplished. The synthesis involved a Suzuki cross-coupling reaction as a key step. The functionalized tetrahydropyran ring was constructed from commercially available optically active tri-*O*-acetyl-D-glucal. Other key reactions include a highly stereoselective Claisen rearrangement, a Cu(I)-mediated 1,4 addition of MeLi to install the C8 methyl group, and a reductive amination to incorporate the C10 amine functionality of spliceostatin G. Biological evaluation of synthetic spliceostatin G and its methyl ester revealed that it does not inhibit splicing in vitro.



In mammalian cells, the splicing of pre-mRNAs is a fundamental process for gene expression.[1,2] Splicing is carried out by a complex ribonucleoprotein machinery, called the spliceosome, which upon recognition of splicing signals catalyzes the removal of intervening sequences (introns) and assembles protein coding sequences (exons) to form messenger RNA (mRNA) prior to export and translation.[3,4] Spliceosome assembly and catalysis are generally very complicated. Recent studies have revealed that splicing is pathologically altered in many different ways in cancer cells.[5,6] Therefore, manipulation or inhibition of splicing events by targeting the spliceosome may be an effective strategy for anticancer drug development. Pladienolides (pladienolide B, **1**, Figure 1) isolated from *Streptomyces* have been shown to be potently cytotoxic.[7,8] They inhibit the spliceosome by binding to the SF3B subunit of the spliceosome.[9,10] While pladienolides are unsuitable for clinical use due to inadequate physicochemical properties, a semi-synthetic derivative E707, **2**, with improved properties underwent clinical trials.[11,12] Subsequently, other natural products, such as FR901464, **3**, and its methylated derivative spliceostatin A, **4**, were shown to potently inhibit spliceosome through binding to the SF3B subunit of the spliceosome.[13,14] Total synthesis and further design of structural isomers were pursued for these natural products to improve stability and reduce structural complexities of these agents.[15−17] Recently, He and co-workers reported a series of spliceostatin classes of natural products isolated from the fermentation broth FERM BP-3421 of *Burkholderia* sp.[18] Among these natural products, a less complex structure, spliceostatin G, was isolated, and the full structure of spliceostatin G was confirmed by detailed ^{1}H and ^{13}C NMR studies.[18] Spliceostatin G did not exhibit potent cytotoxicity inherent to other spliceostatins. Spliceostatin G does not contain an epoxy alcohol on a tetrahydropyran framework nor a 5,6-dihydro-α-pyrone subunit present in the more active natural products like spliceostatins A and E.[18] As part of our continuing interest in the chemistry and biology of spliceostatins, we have devised an enantioselective synthesis of

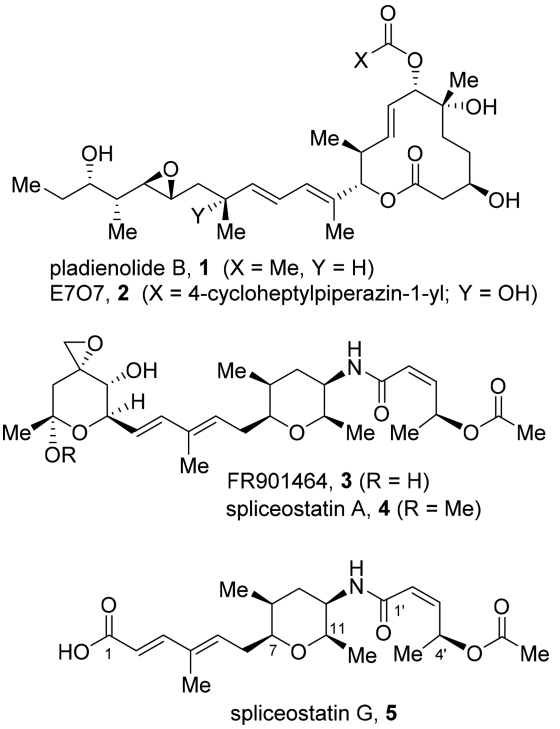


Figure 1. Structures of pladienolide B, E707, FR901464, and spliceostatins A and G.

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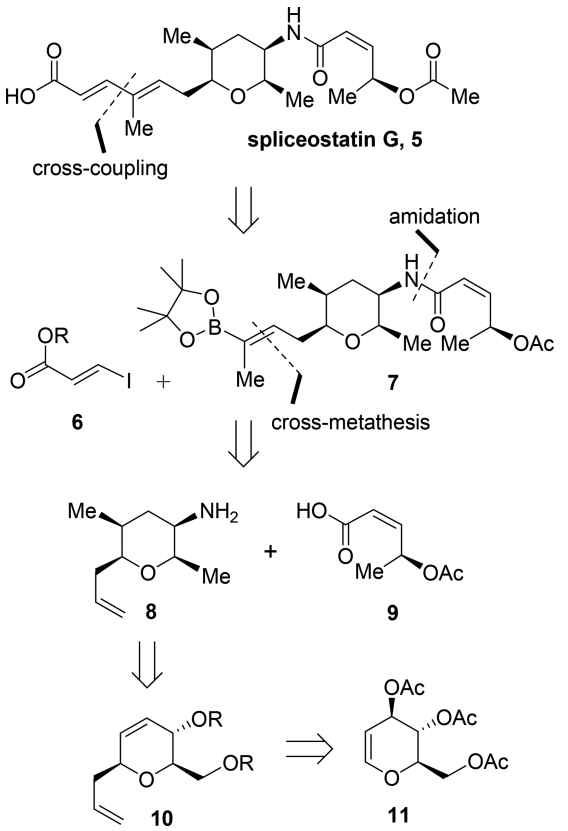
spliceostatin G using readily available tri-*O*-acetyl-D-glucal as the key starting material. The current synthesis will provide ready access to highly functionalized tetrahydropyrans and the diene frameworks of the spliceostatins.

Our strategy for an enantioselective synthesis of spliceostatin G is shown in Scheme 1. To construct the diene component of

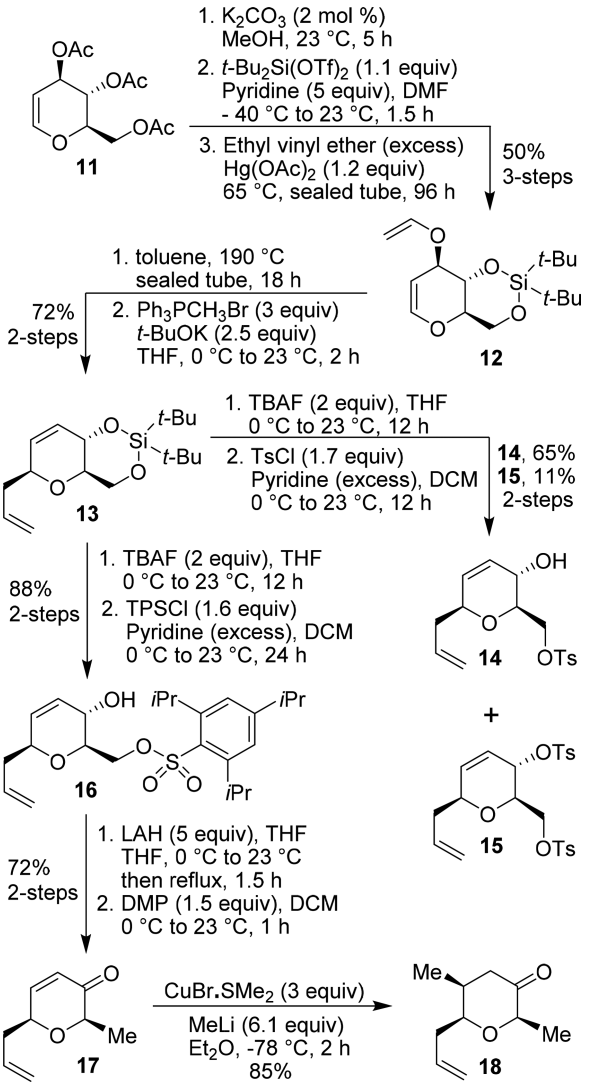
Scheme 1. Retrosynthesis of Spliceostatin G



spliceostatin G, we planned a Suzuki cross-coupling reaction between the boronate segment **7** and iodoacrylate **6** at a late stage of the synthesis. The boronate derivative **7** would be obtained by a cross-metathesis of the amide derivative of olefin **8** and commercially available pinacol boronate. Coupling of the amine functionality of **8** with acid **9** would provide the requisite amide for cross-metathesis. The functionalized tetrahydropyran ring **8** could be constructed from dihydropyran derivative **10**. Asymmetric synthesis of this dihydropyran derivative would be carried out from commercially available tri-*O*-acetyl-D-glucal **11**.

The preparation of dihydropyranone **18** is shown in Scheme 2. The synthesis of dihydropyran derivative **12** was carried out on a multigram scale from commercially available tri-*O*-acetyl-D-glucal **11** as reported in the literature.[19] This was subjected to heating in a sealed tube in toluene at 190 °C for 18 h to provide the Claisen rearrangement product, the corresponding aldehyde. Wittig olefination of the aldehyde with methylenetriphenylphosphorane at 0 °C afforded dihydropyran derivative **13** in 90% yield. The removal of the silyl group was carried out by exposure to tetrabutylammonium fluoride (TBAF) in THF at 0−23 °C for 12 h. The resulting diol was initially treated with *p*-toluenesulfonyl chloride in pyridine at 0 °C to 23 °C for 12 h to furnish a mixture (6:1) of tosylate derivatives **14** and **15**. For regioselective formation of the primary sulfonate derivative, we chose the sterically bulkier 2,4,6-triisopropylbenzenesulfonyl

Scheme 2. Synthesis of Dihydropyranone 18



chloride (TPSCl). Reaction of the diol with TPSCl in pyridine at 0−23 °C for 24 h afforded primary sulfonate derivative **16** in 92% yield. Reduction of sulfonate **16** by LAH in THF at 0 °C to 65 °C for 1.5 h provided the reduced product, the methyl derivative. Oxidation of the resulting allylic alcohol with Dess−Martin periodinane at 0−23 °C for 1 h furnished enone derivative **17** in 80% yield. For stereoselective installation of the C8-methyl group, we carried out a Cu(I)-mediated 1,4-addition as developed by us previously.[17] Thus, treatment of **17** with MeLi in the presence of CuBr·SMe_2 complex at −78 °C for 2 h provided dihydro-2*H*-pyranone **18** in excellent yield and excellent diastereoselectivity (25:1 by ^{1}H NMR and ^{13}C NMR analysis).

Elaboration of dihydropyranone **18** to the boronate derivative **7** is shown in Scheme 3. A substrate-controlled stereoselective reduction of ketone **18** with ammonium acetate and $NaBH(OAc)_3$ in the presence of trifluoroacetic acid (TFA) at 23 °C for 12 h afforded primary amine **8** with high diastereoselectivity (96:4 by ^{1}H NMR analysis).[17,20] Coupling of optically active acid **9** and amine **8** using HATU in the presence of diisopropylethylamine (DIPEA) resulted in amide derivative **19** in 85% yield. Cross-metathesis of the allyl derivative **19** with commercially available pinacol boronate **20**

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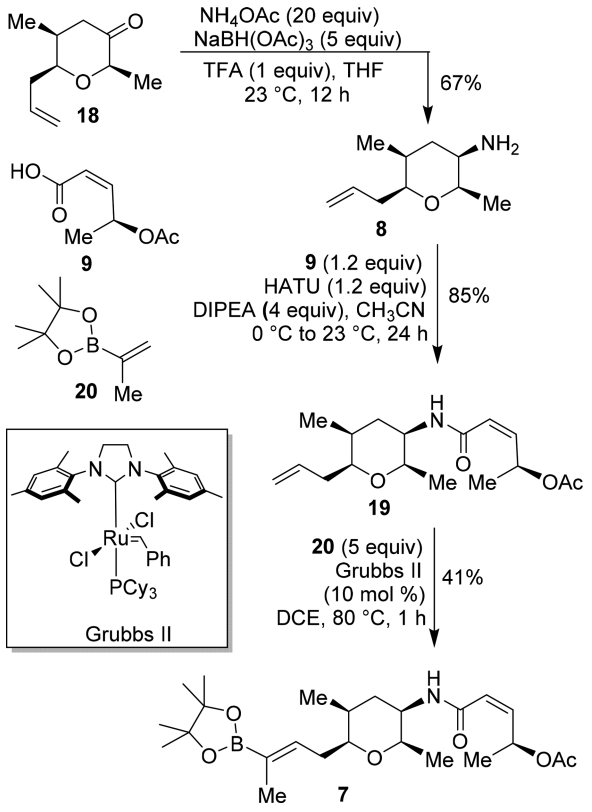
Scheme 3. Synthesis of Boronate Derivative 7

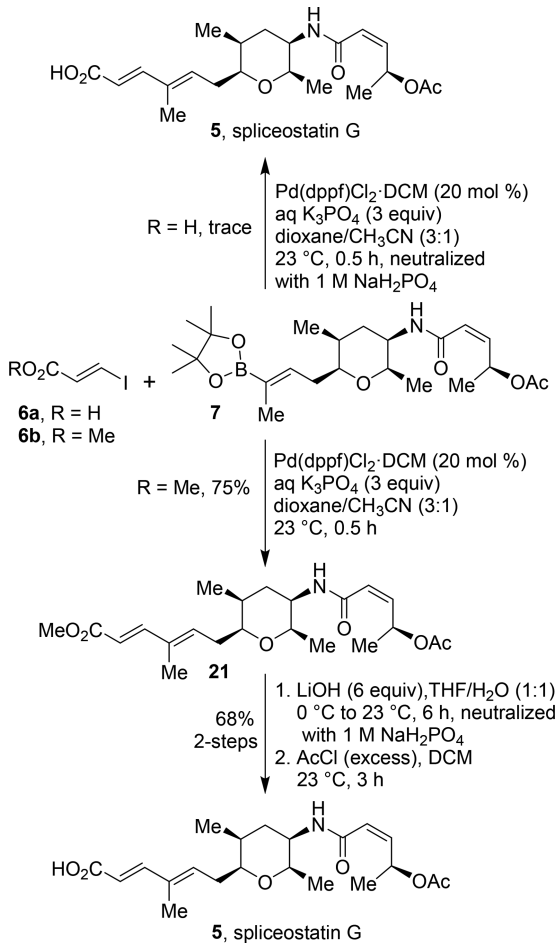


in the presence of Grubb's second-generation catalyst (10 mol %) in 1,2-dichloroethane at 80 °C for 1 h afforded boronate derivative **7** in 41% yield.[21−23]

The synthesis of spliceostatin G is shown in Scheme 4. Initially, we conducted a Suzuki coupling of boronate **7** and iodocarboxylic acid **6a** using Pd(dppf)Cl_2·DCM catalyst (20 mol %) in the presence of aqueous K_3PO_4 in a mixture of dioxane and acetonitrile at 23 °C for 30 min. However, this condition only provided trace amount of coupling product spliceostatin G. We have also carried out this coupling using $Pd(Ph_3P)_4$ catalyst (10 mol %) in the presence of Cs_2CO_3 at 55 °C. This condition only provided a trace amount of desired product.[24] We then carried out this Suzuki reaction[25] with methyl iodoacrylate **6b** using Pd(dppf)$_2Cl_2$·DCM (20 mol %) catalyst in the presence of aqueous K_3PO_4 at 23 °C for 30 min to furnish coupling product **21** in 75% yield after silica gel chromatography. The methyl ester **21** was converted to spliceostatin G by saponification with aqueous LiOH in THF at 23 °C for 6 h, followed by reaction of the resulting hydroxyl acid with acetyl chloride in CH_2Cl_2 at 23 °C for 3 h. Spliceostatin G was obtained in 68% yield over two steps. The ^{1}H NMR and ^{13}C NMR of our synthetic spliceostatin G [$[\alpha]_D^{23}$ −71.7 (*c* 0.53, $CHCl_3$)[26]] are in full agreement with the reported spectra of natural spliceostatin G.[18]

The biological properties of synthetic spliceostatin G (**5**) and the precursor **21** were evaluated in an in vitro splicing system as previously described[27] (Figure 2). Neither compound showed inhibition of splicing in this system, even at 100 μM concentration. In contrast, spliceostatin A in the same assay shows strong splicing inhibition. This result is consistent with previous reports showing that spliceostatin G (**5**) does not affect the growth of several cancer cell lines.[18]

Scheme 4. Synthesis of Spliceostatin G



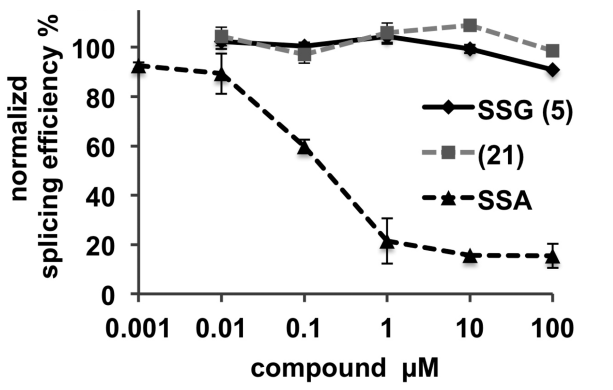


Figure 2. Impact of spliceostatin G on in vitro splicing. Average splicing efficiency relative to inhibitor concentration normalized to DMSO control. Key: SSG, spliceostatin G; compound **21**; SSA, spliceostatin A.

In summary, we have achieved an enantioselective synthesis of spliceostatin G and confirmed the assignment of relative and absolute stereochemistry of spliceostatin G. The synthesis involved a Suzuki cross-coupling reaction as the key step. Enantioselective synthesis of the functionalized tetrahydropyran ring was achieved from commercially available optically active tri-*O*-acetyl-D-glucal using a highly stereoselective Claisen rearrangement.

A cross-metathesis of commercially available pinacol boronate using Grubbs' catalyst provided the vinyl boronate

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derivative for the cross-coupling reaction with iodoacrylic acid. The other stereoselective transformations include a highly stereoselective 1,4 addition to construct the C8 methyl group and reductive amination to incorporate the C10 amine functionality of spliceostatin G. The synthesis is convergent and amenable to the synthesis of structural variants. We have also evaluated spliceosome inhibitory activity of spliceostatin G and compared its activity with spliceostatin A. Spliceostatin G does not inhibit in vitro splicing assembly or chemistry. The design and synthesis of structural variants of spliceostatins are in progress. These analogues will be important to clarify the link between splicing inhibition and changes in cellular function induced by these remarkable compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03456.

Experimental procedures in addition to ^{1}H and ^{13}C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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