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### Authors

Ghosh, Arun K
Reddy, Guddeti Chandrashekar
MacRae, Andrew J
et al.



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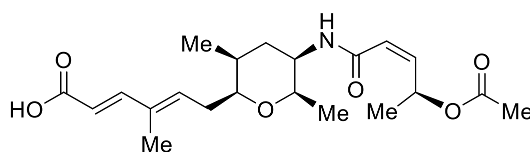
# Enantioselective Synthesis of Spliceostatin G and Evaluation of Bioactivity of Spliceostatin G and its Methyl ester

Arun K. Ghosh*,† Guddeti Chandrashekar Reddy,† Andrew J. MacRae,‡ and Melissa S. Jurica‡

†Department of Chemistry and Department of Medicinal Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907; ‡Department of Molecular Cell and Developmental Biology and Center for Molecular Biology of RNA, University of California, Santa Cruz, California 95064

akghosh@purdue.edu

*Supporting Information Available General experimental procedures, characterization data for all new products. This material is available free of charge via the Internet at http://pubs.acs.org.*




**Spliceostatin G**

**ABSTRACT:** An enantioselective total synthesis of spliceostatin G has been accomplished. The synthesis involved a Suzuki cross-coupling reaction as the key step to construct spliceostatin G. The functionalized tetrahydropyran ring was constructed from commercially available optically active tri-*O*-acetyl-D-glucal. Other key reactions include highly stereoselective Claisen rearrangement, 1,4 addition of MeLi to install C8 methyl group and reductive amination to incorporate the C10 amine functionality of spliceostatin G. Our 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 ribonuclear machinery, called spliceosome which upon recognition of splicing signal, catalyzes the removal of non-coding sequences (introns) and assembles protein coding sequences (exons) to form messenger mRNA prior to export and translation.[3,4] These transcription and translation steps 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 spliceosome may be an effective strategy for anticancer drug development. Among strategies modulation of spliceosome to target cancer therapeutically has become an area of significant interest. Pladienolides (pladienolide B, **1**, Figure 1) isolated from *Streptomyces*, were shown to be potently cytotoxic.[7,8] They inhibit spliceosome by binding to SF3B subunit of spliceosome.[9,10] While pladienolides are unsuitable for clinical use, a semisynthetic derivative E707, **2** 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 SF3B subunit of 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. Recently,[15,16,17] He and co-workers reported isolation and structural studies of a series of spliceostatin class of natural products from the fermentation broth FERM BP-3421 of *Burkholderia* sp.[18] Among these natural products, a less complex structure, spliceostatin G was isolated and full structure

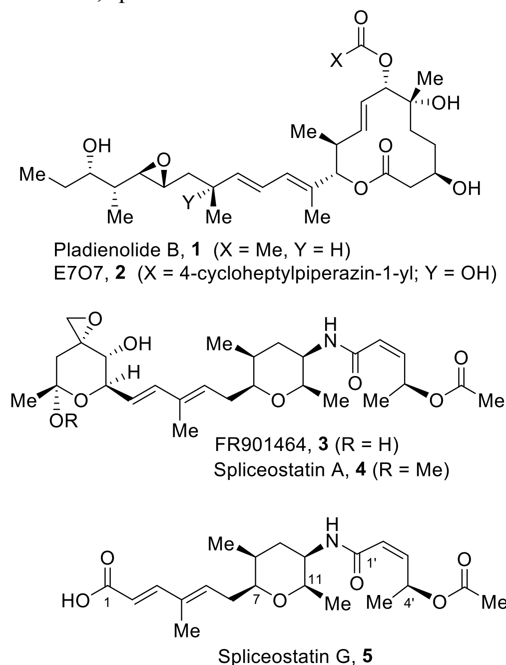
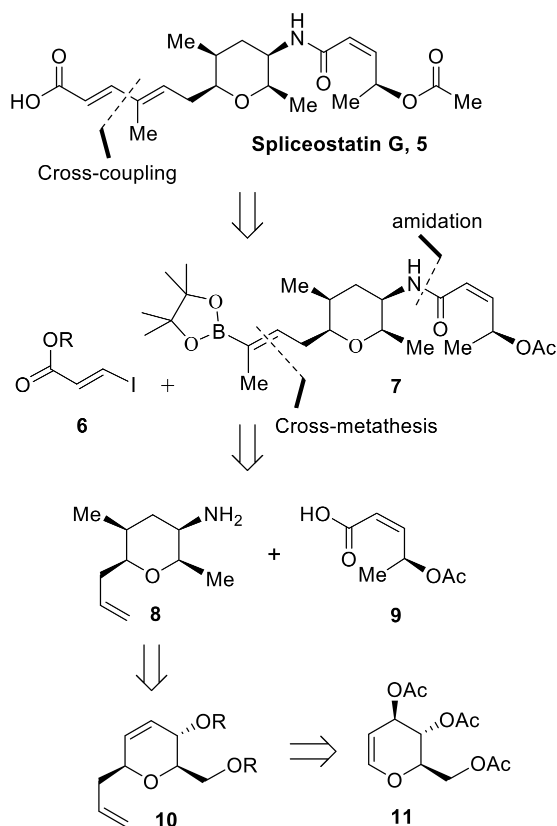



**Figure 1.** Structures of pladienolide B, E707, FR901464, spliceostatins A and G

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 epoxy alcohol on a tetrahydropyran framework or 5,6-dihydro-α-pyrone subunit present in more active natural products like spliceostatins A and E.[18] As part of continuing interests in the chemistry and biology of spliceostatins, we have devised an enantioselective synthesis of spliceostatin G using readily available tri-*O*-acetyl-D-glucal as the key starting material. Current synthesis will provide ready access to highly functionalized tetrahydropyran and the diene frameworks of spliceostatins.

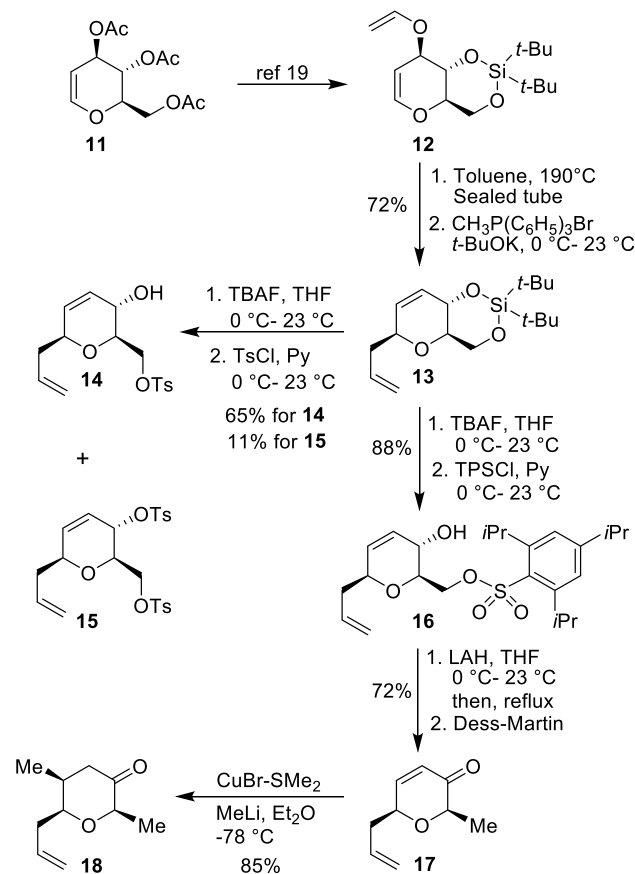
Our strategy for an enantioselective synthesis of spliceostatin G is shown in Scheme 1. To construct the diene component of spliceostatin G we planned a Suzuki cross-coupling reaction between the boronate segment **7** and iodoacrylate **6** at a late stage of the synthesis. The boronate derivative **7** would be obtained by a cross-metathesis reaction of amide derivatives of olefin **8** and commercially available pinacol boronate. Coupling of 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**. Optically active synthesis of this dihydropyran derivative would be carried out from commercially available tri-*O*-acetyl-D-glucal **11**.

**Scheme 1.** Retrosynthesis of Spliceostatin G




The synthesis of dihydropyran derivative **12** was carried out in 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 methylene-triphenylphosphorane at 0 °C afforded dihydropyran derivative **13** in 90% yield. The removal of the silyl group was

**Scheme 2.** Synthesis of dihydropyranone **18**

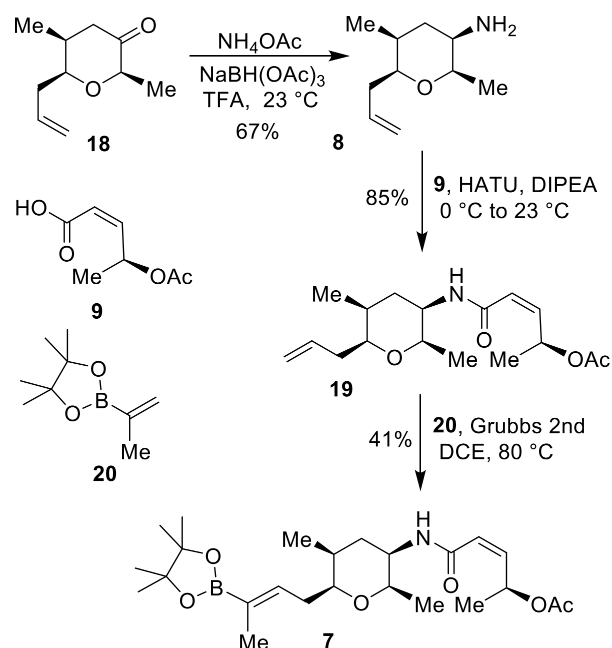



carried out by exposure to $n\mathrm{Bu_4N^+F^-}$ (TBAF) in THF at 0 °C to 23 °C for 12 h. The resulting alcohol was initially treated with *p*-toluenesulfonyl chloride in pyridine at 23 °C for 12 h to furnish a mixture (6 : 1) of tosylate derivatives **14** and **15**. For regioselective formation of primary sulfonate derivative, we chose a sterically bulkier 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCI). Reaction of diol with TPSCl in pyridine at 0 °C to 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 h provided the reduction product, the methyl derivative. Oxidation of the resulting allylic alcohol with Dess-Martin periodinane at 0 °C to 23 °C for 1.5 h furnished enone derivative **17** in 80% yield. For stereoselective installation of C5-methyl group, we carried out a 1,4-addition as developed by us previously.[17] Thus, treatment of **17** with MeLi in the presence of CuBr•$\mathrm{Me_2S}$ 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 boronate derivative **7** is shown in Scheme 3. A substrate control stereoselective reduction of ketone **18** with ammonium acetate and $\mathrm{NaBH(OAc)_3}$ 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 allyl derivative **19** with commercially available pinacol boronate **20** in the presence of Grubb's 2nd generation catalyst (10%) in

**Scheme 3.** Synthesis of boronate derivative **7**



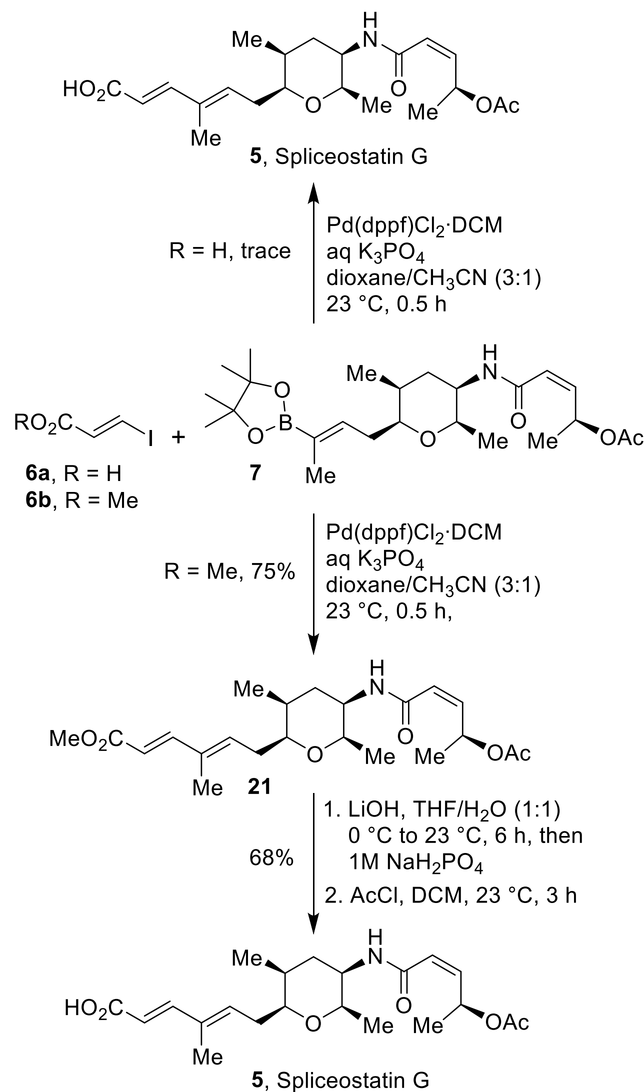

1,2-dichloroethane at 80 °C for 1 h afforded boronate derivative **7** in 41% yield.[21,22,23]

The synthesis of spliceostatin G is shown in Scheme 4. Initially, we conducted 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 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]

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

**Scheme 4.** The synthesis of spliceostatin G**.**



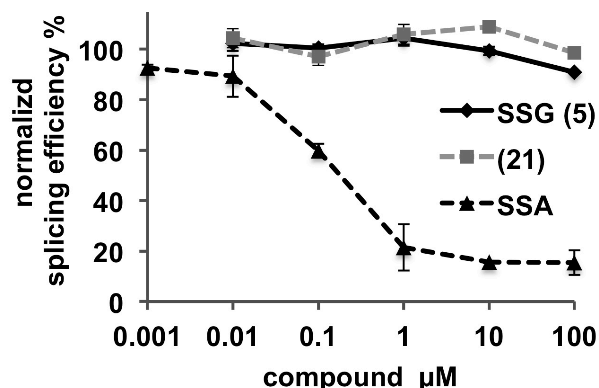




**Figure 2.** Impact of spliceostatin G on *in vitro* splicing. Average splicing efficiency relative to inhibitor concentration normalized to DMSO control. SSG, spliceostatin G; compound **21**; SSA, spliceostatin A.

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 derivative for the cross coupling reaction with iodoacrylic acid. The other stereoselective transformations include highly stereoselective 1,4 addition to construct 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 analogs will be important to clarify the link between splicing inhibition and changes in cellular function induced by these remarkable compounds.

**AUTHOR INFORMATION**

Corresponding Authors: *E-mail: akghosh@purdue.edu

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**Supporting Information**

Supporting Information Available General experimental procedures, characterization data for all new products. This material is available free of charge via the Internet at http://pubs.acs.org.

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