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Enantioselective Synthesis of a Cyclopropane Derivative of Spliceostatin A and Evaluation of Bioactivity

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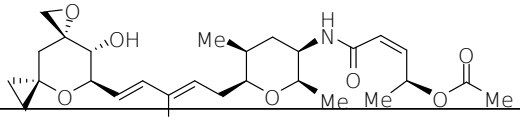
Supporting Information Available General experimental procedures, characterization data for all new products. This material is available free of charge via the Internet at http://pubs.acs.org.



cyclopropyl spliceostatin A

ABSTRACT: Spliceostatin A is a potent inhibitor of spliceosomes and exhibits excellent anticancer activity against multiple human cancer cell lines. We describe here the design and synthesis of a stable cyclopropane derivative of spliceostatin A. The synthesis involved a cross-metathesis or a Suzuki cross-coupling reaction as the key step. The functionalized epoxy alcohol ring was constructed from commercially available optically active tri-*O*-acetyl-D-glucal. The biological properties of the cyclopropyl derivative revealed that it is active in human cells and inhibits splicing *in vitro* comparable to spliceostatin A.

The majority of eukaryotic genes are expressed as precursor mRNAs (pre-mRNAs) which are then converted to mRNAs by splicing.[1,2] This event is carried out by the multi-megadalton ribonucleoprotein complex, spliceosome, which upon recognition of a splicing signal, catalyzes the removal of non-coding sequences (introns) and joins protein coding sequences (eons) to form messenger RNAs.[3,4,5] These get exported to cytoplasm for translation into proteins. While these transcription and translation processes are complex, recent studies have demonstrated that splicing is pathologically altered to promote the initiation and maintenance of cancer.[6,7] Splicing involves numerous protein-protein and protein-RNA interactions which offer opportunities to manipulate or inhibit the splicing cascade for therapeutic purposes, particularly in the area of anticancer drug development.[8,9] Presently, a number of natural products and their derivatives are known to potently inhibit spliceosome function by binding to the SF3B subunit of U2 SnRNP.[10,11,12] These include, FR901464 (**1**, Figure 1), spliceostatin A(**2**), pladienolide B (**3**), and a semisynthetic derivative E7O7, **4**.[13,14,15] The precise molecular interactions of these molecules with SF3B are being investigated using cryo-electron microscopy.[16,17,18] While these natural products display potent splicing activity their clinical use is limited due to chemical instability and inadequate physiochemical properties. To date, a semi synthetic derivative of pladienolide E7O7, (**4**), was developed with improved pharmacological properties for clinical development.[19] A number of spliceostatin derivatives

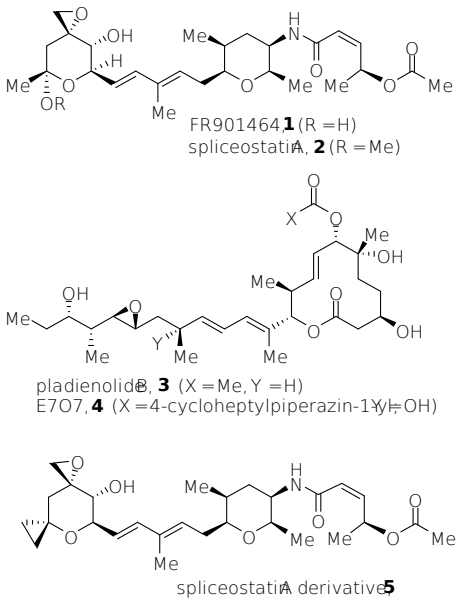
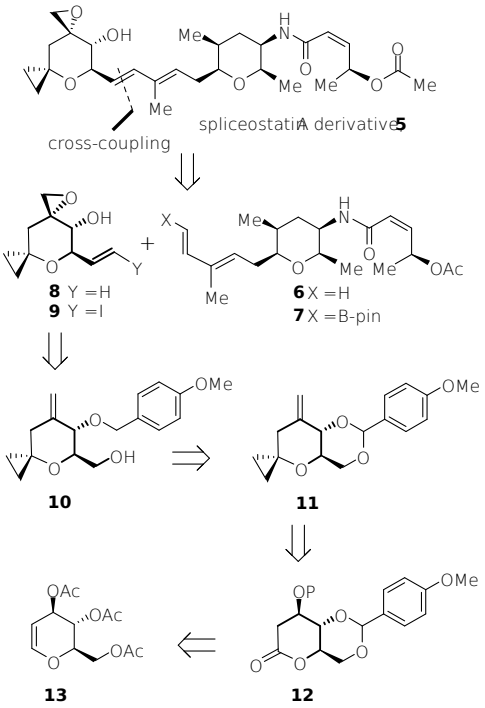


Figure 1. Structures of spliceosome inhibitors **1-5**

have seen specifically synthesized as payloads for antibody-drug conjugates.[20,21] Both FR901464 (**1**) and spliceostatin A(**2**) show very potent antitumor properties. FR901464 displayed IC_{50} values ranging from 0.6 to 3.4 nM against multiple human cancer cell lines. It also showed effectiveness against solid tumors implanted in mice at a dose range of 0.05 to 1 mg/kg.[14,22] Spliceostatin A showed similar activity. As a result, these compounds attracted much attention for synthesis and medicinal chemistry development. We recently reported the synthesis and structure-activity studies of both these compounds.[23,24] Nicolaou and co-workers also reported structural modifications of spliceostatin derivatives.[25] In our continued interest in developing molecular probes for splicing studies, we devised cyclopropyl derivative of FR901464 and spliceostatin A, where a cyclopropane is incorporated at the anomeric site of FR901464 and spliceostatin A to improve stability and potency. We have devised an enantioselective synthesis of the epoxide subunit using readily available tri-*O*-acetyl-D-glucal as the key starting material and investigated coupling using cross-metathesis and Suzuki reactions. The synthesis will provide ready access to stable spliceostatin derivatives for biological studies.

Our synthetic strategy for the construction of the cyclopropyl derivative of spliceostatin A is shown in Scheme 1. We initially planned cross metathesis of diene **6** and epoxy olefin **8** using Grubbs' 2nd generation catalyst. This cross metathesis reaction typically yields a mixture of olefin dimer, diene dimer, and the desired cross-coupling product. We also planned to investigate a Suzuki cross-coupling reaction between the known[26,27] pinacol borononate **7** and vinyl iodide derivative **9** to provide the desired cross-coupling product **5**. The synthesis of pinacol boronate **7** would be achieved in optically active form using our recently reported procedure.[27]

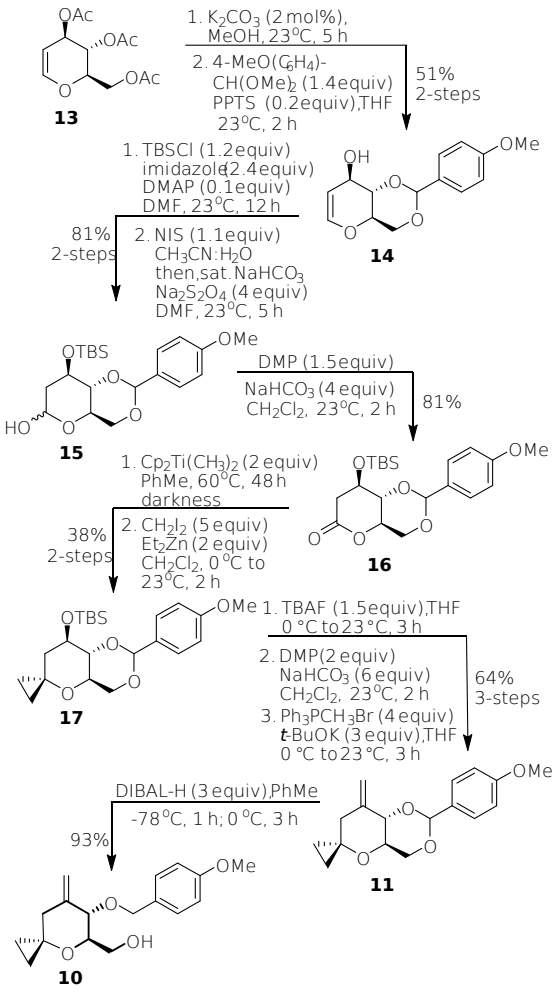
Scheme 1. Retrosynthesis of spliceostatin derivative



The synthesis of cyclopropyl epoxide segment **8** or **9** would be carried out from alcohol **10** which would be derived from 4-methoxybenzylidene acetal **11** by reduction with Dibal-H. Acetal **11** can be synthesized from δ-lactone **12** by synthetic manipulation from standard commercially available optically active tri-*O*-acetyl-*D*-glucal **13**.

Enantioselective synthesis of 7-methylene-4-oxaspiro[2.5]octane derivative **10** is shown in Scheme 2. The preparation of benzylidene acetal **14** was carried out in multi-gram scale using commercially available tri-*O*-acetyl-*D*-glucal **13** with minor modifications.[28] Treatment of **13** with K_2CO_3 in MeOH at 23 °C for 5 h provided the corresponding triol. Reaction of the resulting triol with 1-(dimethoxy)-4-methoxybenzene in the presence of a catalytic amount of PPTS at 23 °C for 2 h afforded benzylidene acetal **14**. It was protected as the TBS-ether using TBSCl and imidazole in the presence of

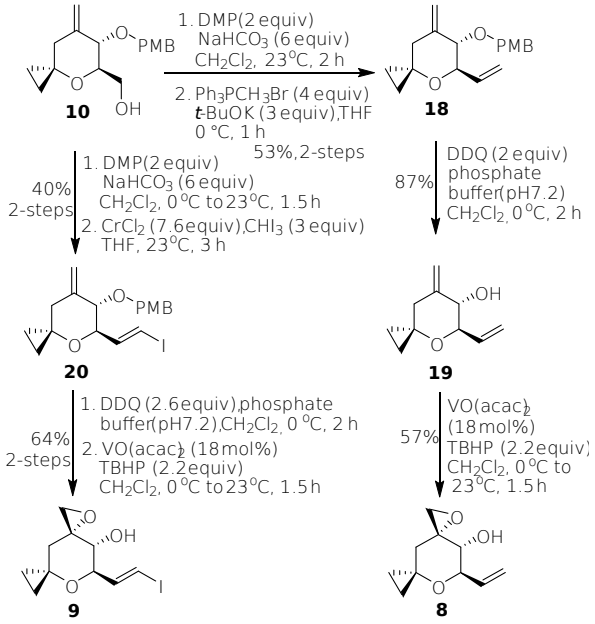
Scheme 2. Synthesis of dihydropyranone **18**



a catalytic amount of DMAP in DMF at 23 °C for 12 h. The resulting TBS-ether was treated with NIS in a mixture (95:5) of CH_3CN and water at 23 °C for 15 min and the mixture was concentrated. The residue was dissolved in DMF and saturated $NaHCO_3$ and $Na_2S_2O_4$ were added and the mixture was stirred at 23 °C for 5 h to provide lactol **15** in 81% yield over 2-steps.[29,30] Oxidation of lactol **15** with Dess-Martin periodinane (DMP) in the presence of $NaHCO_3$ in CH_2Cl_2 at 0 °C to 23 °C for 2 h furnished lactone derivative **16** in 81% yield. To install the cyclopropane ring, lactone **16** was treated with Petasis reagent[31] in toluene at 23 °C and the resulting mixture was heated at 60 °C for 48 h to provide the corresponding enol-ether in 90% yield. Simmons-Smith cyclopropanation[32] of the resulting enol ether with methylene diiodide and diethyl zinc in CH_2Cl_2 at 0 °C to 23 °C for 2 h afforded 4-oxaspiro[2,5]octane derivative **17** in 42% yield. Cyclopropane derivative **17** was converted to olefin **11** as follows. Treatment of TBS-ether **17** with TBAF in THF at 0 °C to 23 °C for 3 h provided the corresponding alcohol. Oxidation of the secondary alcohol to the corresponding ketone was achieved by treatment with DMP at 0 °C to 23 °C for 2 h. Wittig-olefination of the resulting ketone with methylenetriphenylphosphorane in THF at 0 °C to 23 °C for 3 h provided **11** in 64% yield over 3-steps. DIBAL-H reduction of benzylidene acetal **11** at -78 °C to 0 °C for 4 h furnished alcohol **10** in 93% yield.

Conversion of alcohol **10** to epoxy alcohol derivatives **8** and **9** is shown in Scheme 3. Oxidation of alcohol **10** with DMP in the presence of $NaHCO_3$ provided the corresponding aldehyde. Wittig-olefination of the resulting aldehyde with methylenetriphenylphosphorane in THF at 0 °C for 1 h afforded diene **18** in 53% yield over 2-steps. Removal of the PMB-group was carried out by exposure of **18** to DDQ in a mixture (10:1) of CH_2Cl_2 and phosphate buffer (pH 7.2) at 0 °C for 2 h

Scheme 3. Synthesis of epoxy alcohol derivatives

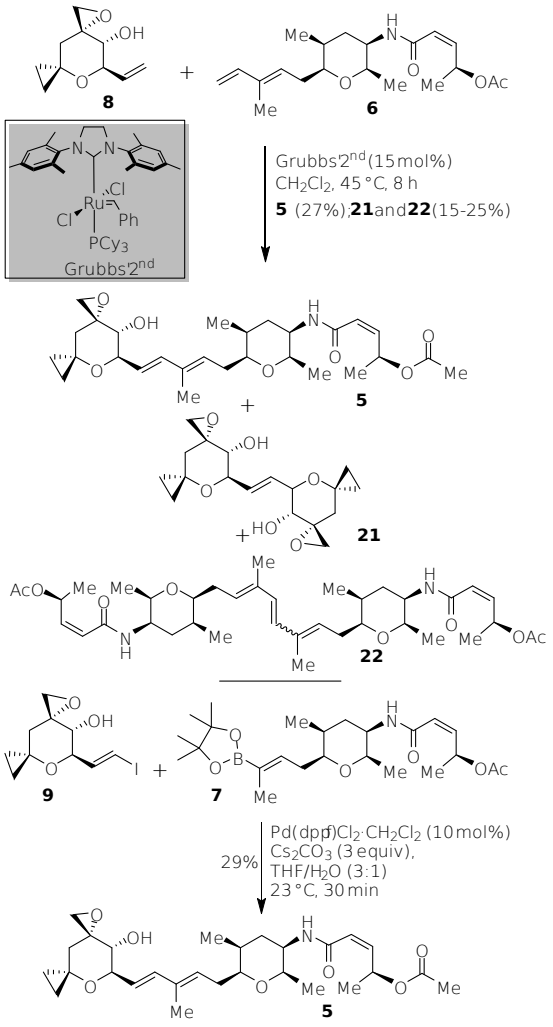


to furnish homoallylic alcohol **19**. Selective epoxidation of **19** with a catalytic amount (18 mol %) of $VO(acac)_2$ in the presence of *t*-butyl hydroperoxide in CH_2Cl_2 at 0 °C to 23 °C for 1.5 h provided epoxy alcohol derivative **8** as a single diastereomer (by ^{1}H-NMR analysis) in 57% yield (73% brsm). For the synthesis of vinyl iodide derivative **9**, alcohol **10** was oxidized with DMP in the presence of $NaHCO_3$ to provide the aldehyde in 48% yield. Takai olefination[33] was carried out by reaction of the resulting aldehyde with a mixture of $CrCl_2$ and CHI_3 in THF at 23 °C for 3 h to furnish vinyl iodide **20** in 85% yield. Exposure of PMB-derivative **20** to DDQ in a mixture of CH_2Cl_2 and phosphate buffer resulted in deprotection of the PMB-group. Directed epoxidation of the resulting allyic alcohol with *t*-butyl hydroperoxide in the presence of a catalytic amount (18 mol%) of $VO(acac)_2$ provided epoxy alcohol **9** in 64% yield over 2-steps.

For the synthesis of the cyclopropyl derivative of spliceostatin A, we first carried out a cross

metathesis[34] reaction of diene **6** and epoxy olefin **8** as shown in Scheme 4. A mixture of epoxy olefin **8** and Grubbs' II catalyst (15 mol%) in CH_2Cl_2 was prepared under argon atmostphere. To a stirred solution of

Scheme 4. The synthesis of spliceostatin derivative **5**



diene **6** in CH_2Cl_2, a one-third portion of epoxy olefin **8** and Grubbs catalyst was added and the resulting mixture was heated at reflux for 1.5 h. The other two portions of catalyst and olefin mixture were added successively in 1.5 h intervals. The resulting mixture was stirred at reflux for an additional 1.5 h (total 8 h). The reaction was cooled to 23 °C and the mixture was concentrated. The residue was purified by silica gel chromatography to furnish cyclopropane derivative **5** in 27% yield. The cross metathesis reaction also provided an inseparable mixture of epoxide dimer and diene dimer as the by-products (**21** and **22**, about 25-30%). We then investigated a Suzuki coupling[35] of known boronate **7** and vinyl iodide derivative **9** using $Pd(dppf)_2Cl_2$•DCM (10 mol%) catalyst in the presence of aqueous Cs_2CO_3 in THF at 23 °C for 30 min to provide the coupling product **5** in 29% yield. The yield for coupling product **5** via cross-metathesis or Suzuki coupling was comparable.

We evaluated the biological activity of this cyclopropane spliceostatin A (**5**) in an *in vitro* splicing system as previously described.[36] The compound inhibits splicing, but at a slightly reduced (~2-fold lower) potency relative to spliceostatin A (Figure 2). The compound is also active in HeLa cells. It induces the coalescence of nuclear speckles as observed by immuno-staining of the splicing factor SFRS2 at similar levels as spliceostatin A (Figure 3).

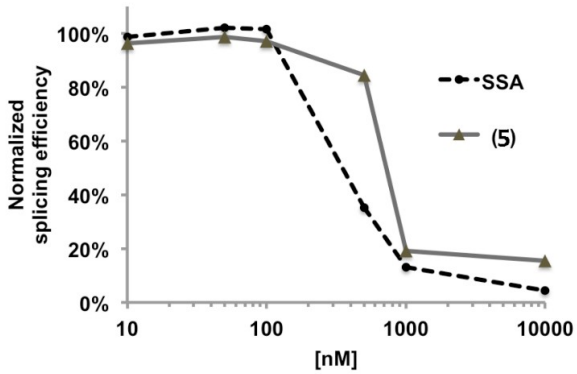


Figure 2. Impact of compound 5 on *in vitro* splicing. Average splicing efficiency relative to inhibitor concentration normalized to no-drug control. Compound **5**; SSA, spliceostatin A.

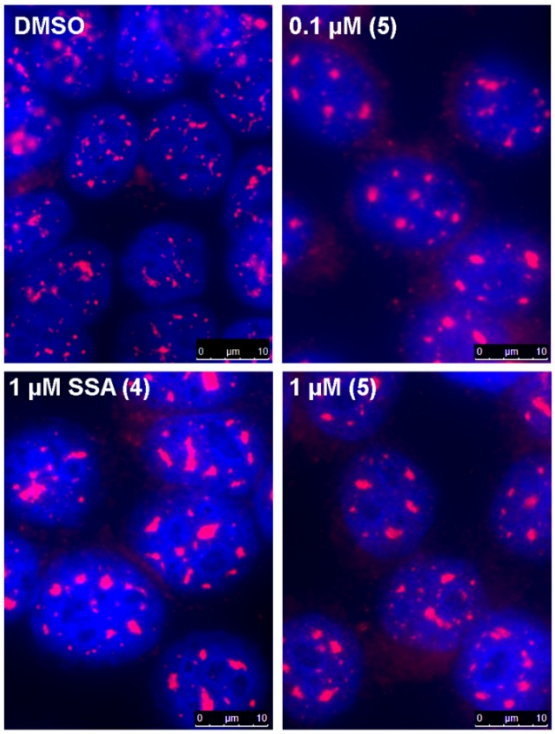


Figure 3. Changes in nuclear speckle morphology. Fluorescent images of in HeLa cells nuclei incubated four hours with the indicated compound, then fixed and stained with DAPI (blue) and anti-SRSF2 antibody (magenta).

In summary, we reported the design, synthesis, and biological evaluation of a cyclopropane derivative of spliceostatin A and FR901464. The synthesis of the cyclopropane derivatives **8** and **9** for coupling reactions was carried out enantioselectively from commercially available, optically active tri-*O*-acetyl-*D*-glucal. We have investigated both a cross-metathesis route as well as a Suzuki coupling and both coupling reactions provided the final derivative in comparable yield.

Our design of cyclopropane ring conceivably removed one chiral center and also improved chemical stability of the resulting cyclopropane derivative. We have evaluated spliceosome inhibitory activity of the cyclopropane derivative **5** and compared its activity with spliceostatin A. The compound is very active in HeLa cells. Also, compound **5** induces the coalescence of nuclear speckles at a similar level to spliceostatin A. As it turns out, the cyclopropane derivative exhibited comparable potency to spliceostatin A. Further design and synthesis of structural variants of spliceostatins are underway.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures in addition to ^{1}H- and ^{13}C-NMR spectra are available for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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