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# Enantioselective Syntheses of FR901464 and Spliceostatin A: Potent Inhibitors of Spliceosome

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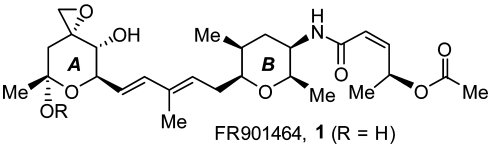
## ABSTRACT




FR901464, **1** (R = H)
Spliceostatin A, **2** (R = Me)

**Enantioselective syntheses of FR901464 and spliceostatin A, potent spliceosome inhibitors, are described. The synthesis of FR901464 has been accomplished in a convergent manner in 10 linear steps (20 total steps). The *A*-tetrahydropyran ring was constructed from (*R*)-isopropylidene glyceraldehyde. The functionalized tetrahydropyran *B*-ring was synthesized utilizing a Corey–Bakshi–Shibata reduction, an Achmatowicz reaction, and a stereoselective Michael addition as the key steps. Coupling of *A*- and *B*-ring fragments was accomplished via cross-metathesis.**

In 1996, Nakajima and co-workers from the Fujisawa Pharmaceutical Co. isolated FR901464 (**1**) (Scheme 1) from the fermentation broth, *Pseudomonas* sp. No. 2663.[1] FR901464 exhibited enhanced transcriptional activity of promoter SV40 at a very low concentration (10 nM). It exhibited remarkable antitumor activity, displaying $IC_{50}$ values ranging from 0.6 to 3.4 nM against multiple human cancer cell lines. Furthermore, it showed significant effectiveness against human solid tumors implanted in mice at a dose range of 0.056–1 mg/kg.[1b] Subsequently, Yoshida and co-workers reported that a more stable methylated derivative of FR901464, named spliceostatin A (**2**), retained similar potent antitumor activity as FR901464.[2] More significantly, both FR901464 and spliceostatin A potently inhibited in vitro splicing and promoted pre-mRNA accumulation by binding to SF3b, a ribonuclear protein in the spliceosome.[2b] Thus, structural analogues of FR901464 may have potential clinical applications with a novel mechanism of action. The biology and chemistry of FR901464 attracted much interest among the synthetic community. The first total synthesis of FR901464 was accomplished by Jacobsen and co-workers.[3] Two other syntheses were reported later by Kitahara and co-workers and Koide and co-workers.[4,5] The reported syntheses were carried out in a total of 29–41 chemical steps. In our continuing interest in natural products[6] that inhibit splicing activity, we sought to develop a convergent synthesis of FR901464 and spliceostatin A in an effort to facilitate the synthesis of structural variants. Herein, we report a concise,

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(2) (a) Motoyoshi, H.; Horigome, M.; Ishigami, K.; Yoshida, T.; Horinouchi, S.; Yoshida, M.; Watanabe, H.; Kitahara, T. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2178–2182. (b) Kaida, D.; Motoyoshi, H.; Tashiro, E.; Nojima, T.; Hagiwara, M.; Ishigami, K.; Watanabe, H.; Kitahara, T.; Yoshida, T.; Nakajima, H.; Tani, T.; Horinouchi, S.; Yoshida, M. *Nature Chem. Biol.* **2007**, *3*, 576–583. (c) Zhang, F.; He, H.-Y.; Tang, M.-C.; Tang, Y.-M.; Zhou, Q.; Tang, G.-L. *J. Am. Chem. Soc.* **2011**, *133*, 2452–2462. (d) Fan, L.; Lagisetti, C.; Edwards, C. C.; Webb, T. R.; Potter, P. M. *ACS Chem. Biol.* **2011**, *6*, 582–589.

(3) (a) Thompson, C. F; Jamison, T. F.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2000**, *122*, 10482–10483. (b) Thompson, C. F.; Jamison, T. F.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2001**, *123*, 9974–9983.

(4) (a) Horigome, M.; Motoyoshi, H.; Watanabe, H.; Kitahara, T. *Tetrahedron Lett.* **2001**, *42*, 8207–8210. (b) Motoyoshi, H.; Horigome, M.; Watanabe, H.; Kitahara, T. *Tetrahedron* **2006**, *62*, 1378–1389.

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(6) (a) Ghosh, A. K.; Anderson, D. D. *Org. Lett.* **2012**, *14*, 4730–4733. (b) Ghosh, A. K.; Li, J. *Org. Lett.* **2011**, *13*, 66–69.

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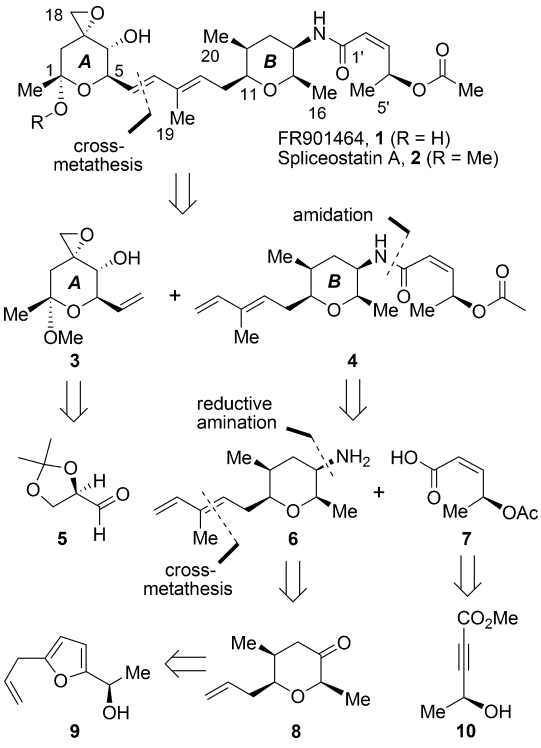
enantioselective synthesis of FR901464 which has been accomplished in 10 linear steps.

As shown in Scheme 1, we planned to utilize cross-metathesis[7] to couple the epoxy alcohol segment **3** and the amide segment **4** at a late stage of the synthesis. A related strategy was utilized by Koide and co-workers.[5b]

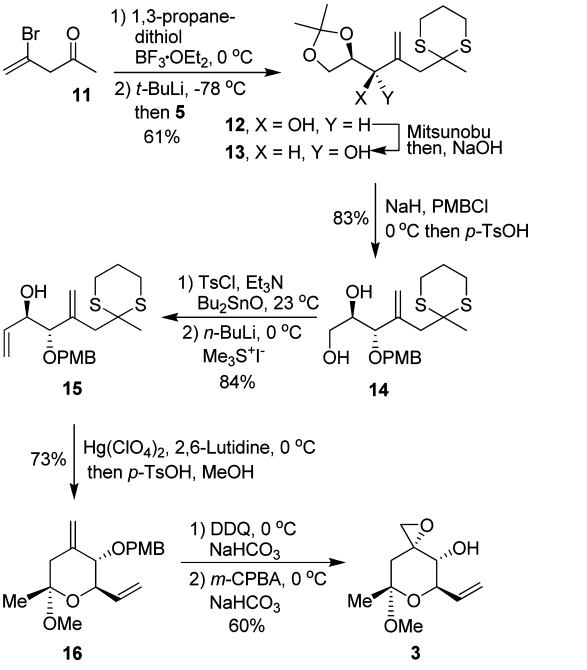
**Scheme 1.** Retrosynthesis of FR901464




The highly functionalized tetrahydropyran ring (**A**) could be constructed by utilizing a versatile chiral pool (*R*)-isopropylidene glyceraldehyde **5**.[8] The amide fragment **4** could be formed via coupling of amine **6** and acid **7**. Amine **6** could be obtained by a reductive amination of pyranone derivative **8**. The functionalized pyranone ring (**B**) would be derived from furan derivative **9** via an Achmatowicz rearrangement[9] as the key step. This optically active furan derivative could be obtained by CBS-reduction of a commercially available ketone. Acid **7** would be derived from the known optically active alcohol **10**.[10]

The synthesis of epoxy alcohol segment **3** is shown in Scheme 2. Commercially available bromo ketone **11** was protected as its dithiane derivative. Lithiation of the

**Scheme 2.** Synthesis of Epoxy Alcohol Segment **3**




resulting dithiane with *t*-BuLi at −78 °C for 1 h followed by reaction with (*R*)-isopropylidene glyceraldehyde provided a mixture (1:1) of diastereomers **12** and **13** in 61% yield in two steps.[11] This lack of stereoselectivity was somewhat unexpected, especially given the presence of chelating atoms at both α and *β* positions of (*R*)-isopropylidene glyceraldehyde. In an attempt to improve *anti*-diastereoselectivity, we investigated this addition reaction in the presence of a number of Lewis acids such as $CeCl_3$,[12] $ZnCl_2$,[13] and $MgBr_2$[14] in THF and ether. However, there was no further improvement in the diastereomeric ratio. The isomers were separated by silica gel chromatography. The *syn*-isomer **12** was converted to desired *anti*-isomer **13** by a Mitsunobu reaction in the presence of *p*-nitrobenzoic acid followed by NaOH-mediated hydrolysis of the benzoate ester.[15,16] The hydroxyl group of **13** was protected as a PMB ether, and subsequent removal of the isopropylidene group was carried out by the addition of *p*-TsOH in a one-pot operation to provide diol **14**. The primary alcohol was selectively monotosylated using TsCl and $Et_3N$ in the presence of dibutyltin oxide. Reaction of the resulting monotosylate with an excess of Corey−Chaykovsky dimethylsulfonium methylide,[17] prepared by treatment of trimethylsulfonium iodide with *n*-BuLi, furnished allylic

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(8) (*R*)-Isopropylidene glyceraldehyde is commercially available and also can be conveniently prepared from D-mannitol in gram scale; see: *Organic Synthesis*; Wiley: New York, 1998; Collect. Vol. IX, p 6; *Org. Synth.* 1995, *72*, 6.

(9) (a) Achmatowicz, O.; Bukowski, P.; Szechner, B.; Zwierzchowska, Z.; Zamojski, A. *Tetrahedron* **1971**, *27*, 1973–1996. (b) Georgiadis, M. P.; Albizati, K. F.; Georgiadis, T. M. *Org. Prep. Proc. Int.* **1992**, *24*, 95–118.

(10) Trost, B. M.; Quintard, A. *Org. Lett.* **2012**, *14*, 4698–4700.

(11) The absolute stereochemistry of **12** and **13** were ultimately identified by comparing the 2D-NMR spectroscopy of advanced intermediate **16** and its C-4 epimer.

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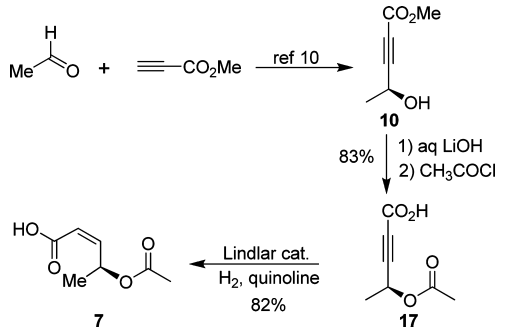
(15) Prasad, K. R.; Gholap, S. L. *J. Org. Chem.* **2008**, *73*, 2–11.

(16) For details, see the Supporting Information.

alcohol **15** in 84% yield. A similar functional group transformation was previously reported by Carreira and co-workers.[18] The dithiane group of **15** was then removed by using an excess of $Hg(ClO_4)_2$ in methanol in the presence of dry 2,6-lutidine. This condition resulted in the formation of the corresponding methyl ketal as a mixture of anomers, which upon treatment with a catalytic amount of p-TsOH in methanol at 0 °C provided a single diastereomer **16**.[19] Removal of the PMB group in **16** with DDQ[20] followed by alcohol directed epoxidation with *m*-CPBA afforded the desired epoxy alcohol segment **3** stereoselectively as a white solid in 19% overall yield from **11** (8 steps). The methyl ketal **3** is quite stable and easy to handle for subsequent reactions.

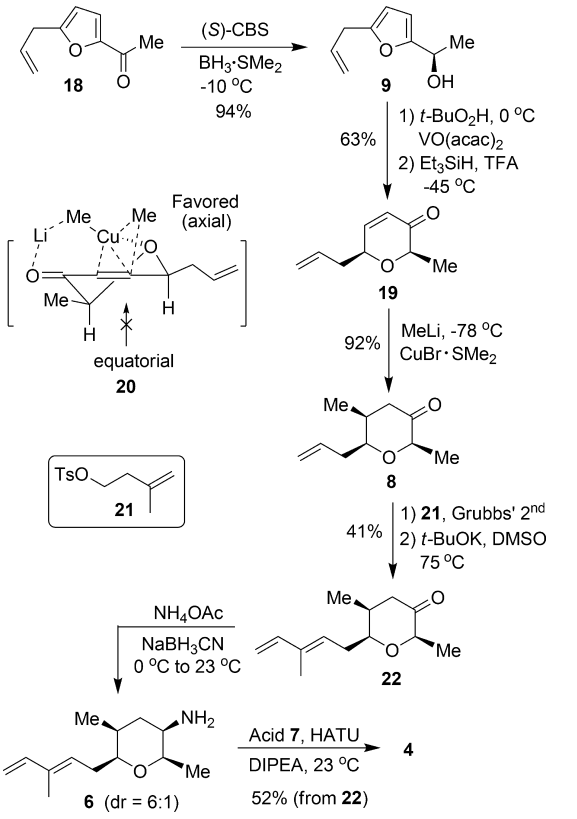
The preparation of the *Z*-allylic acetate side chain **7** is shown in Scheme 3. Optically active alcohol **10** was efficiently prepared by utilizing a catalytic asymmetric addition protocol reported by Trost and co-workers to provide **10** in 98% ee.[10] Saponification of methyl ester **10** with aqueous LiOH followed by acetylation with acetyl chloride provided acetate **17** in excellent yield. Hydrogenation over Lindlar's catalyst afforded the desired *cis*-alkene **7**.

**Scheme 3.** Synthesis of Acid **7**




The synthesis of amide segment **4** is shown in Scheme 4. Enantioselective reduction of commercially available acetyl furan **18** with (*S*)-2-Me-CBS and $BH_3 \cdot Me_2S$ afforded chiral alcohol **9** in 94% yield (93% ee).[21] An Achmatowicz rearrangement was then carried out by treatment of alcohol **9** with $^tBuO_2H$ in the presence of a catalytic amount of $VO(acac)_2$ to furnish a hemiketal,[9] which was directly reduced to enone **19** as a single diastereomer by employing

**Scheme 4.** Synthesis of Amide **4**




the protocol described by Kishi and co-workers.[22] Our subsequent synthetic plan required installation of the C20 (*S*)-methyl-bearing stereocenter. We elected to carry out a 1,4-addition to enone **19**. Accordingly, treatment of **19** with MeLi/CuBr·$Me_2S$ at −78 °C for 2 h provided the desired pyranone **8** in excellent yield (92%) and diastereoselectivity (25:1 dr, by $^1H$ and $^{13}C$ NMR analysis). The observed diastereoselectivity can be explained based upon the conformational analysis of enone **19**. The stereochemical outcome of Michael addition can be rationalized by assuming stereoelectronically favorable axial attack of the cuprate as shown in the transition-state model **20**.[23] Further studies regarding the origin of high diastereoselectivity is under investigation.

Pyranone **8** and known alkene **21**[24] were then subjected to cross-metathesis conditions using Grubbs' second generation catalyst[25] to provide the corresponding terminal tosylate. Treatment of the resulting tosylate with *t*-BuOK in DMSO at 75 °C for 12 h resulted in diene **22** via base-promoted elimination in 41% yield over two steps.[26] For the introduction of the amine group at C-14, we anticipated that a reductive amination could proceed stereoselectively via substrate control. Indeed, reductive amination of **22**

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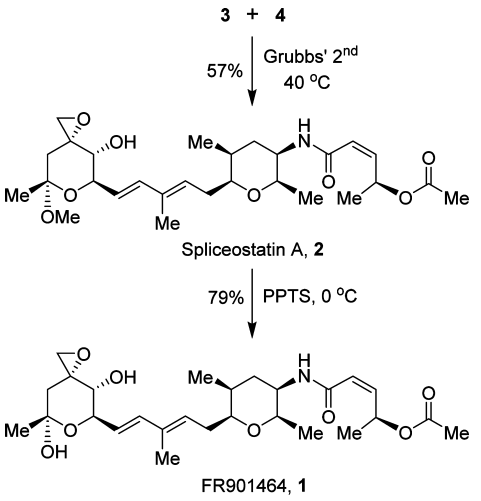
**Scheme 5.** Synthesis of FR901464 and Spliceostatin A




with ammonium acetate and $NaBH_3CN$ afforded the corresponding primary amine **6** as a major product (6:1 dr, by $^1H$- and $^{13}C$ NMR analysis).[27] The crude amine **6** and its epimer were directly treated with acid **7** using standard amidation conditions to give the amide **4** along with minor C-14 epimer, which were separated by column chromatography.

With the stereoselective syntheses of segments epoxy alcohol **3** and amide **4**, we then turned our attention to construct the C6–C7 double bond of the target molecules. As shown in Scheme 5, cross-metathesis of the two fragments proceeded smoothly in the presence of Grubbs' second-generation catalyst to afford spliceostatin A (**2**) as a white solid in 57% isolated yield based upon one recycle of unreacted **3** and **4** under the same conditions. The removal of the methyl ketal in **2** was achieved by exposure of **2** to PPTS in wet THF[28] at 0 °C which provided FR901464 (**1**) as a white powder in good yield. The $^1H$ and $^{13}C$ NMR of our synthetic FR901464 $[[\alpha]^{23}_D$ −13.0 (*c* 0.45, $CH_2Cl_2$)] is identical to the reported spectra of natural $[[\alpha]^{23}_D$ −12.0 (*c* 0.5, $CH_2Cl_2$)][1a] and synthetic[3−5] FR901464.

In summary, we have accomplished a concise and enantioselective strategy for the syntheses of FR901464 and spliceostatin A in 20 and 19 total steps with the longest linear sequence of 10 and 9 steps, respectively. This represents a significant improvement over previously reported routes. The key features in our syntheses include the use of readily availalable chiral pool (*R*)-isopropylidene glyceraldyhyde **5** to form an ***A***-ring fragment, a CBS reduction, an Achmatowicz rearrangement, and a stereoselective Michael addition for the construction of a ***B***-ring fragment, and a cross-metathesis reaction for coupling the two fragments. The synthesis is short, convergent and amenable to the synthesis of structural variants. Further syntheses of structural variants and biological studies are in progress.

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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.

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The authors declare no competing financial interest.