UNIVERSITY OF CALIFORNIA SAN DIEGO

# Development of Splice Modulators as Potent Anti-Tumor Therapeutics

A dissertation submitted in partial satisfaction of the
requirement for the degree Doctor of Philosophy

in

Chemistry

by

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The dissertation of Warren C. Chan is approved, and it is acceptable in quality and form for publication on microfilm or electronically.

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DEDICATION

This dissertation would not have been possible without the following people, in no particular order: My parents, for their continual support; Michael D. Burkart, for his mentorship; Jim La Clair, for his mentorship; Katia Charov, for her friendship; Tony D. Davis, for his support and mentorship; John and Jen Michaud-Lee, for being my home away from home; Rebecca Re, for being my shrink; the Dümpers crew, for letting me be my true self; Miriam Zawadzki and Marie Kuzemchak, for their emotional support; Abigail Dommer, for our fulfilling life experiences and everlasting friendship; Taryn Lucas, for her unmatched kindness; Kevin Sweeney, for being there; Jon Salamon, for reaching out no matter the distance between us; Sauntee Braddock, for your genuine friendship; and to all those who I've laughed and cried with, I am freer because of you.

EPIGRAPH

"And this I believe: that the free, exploring mind of the individual human is the most valuable thing in the world. And this I would fight for: the freedom of the mind to take any direction it wishes, undirected. And this I must fight against: any idea, religion, or government which limits or destroys the individual. This is what I am and what I am about."

John Steinbeck, *East of Eden*

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FIELDS of STUDY

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ABSTRACT OF THE DISSERTATION

Development of Splice Modulators as Potent Anti-Tumor Therapeutics

by

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Professor Michael D. Burkart, Chair

Since its discovery in 1977, the study of alternative RNA splicing has led to its implication as a major factor in the onset of disease. The recent identification of natural product and synthetic modulators of RNA splicing has opened access for a chemical-based interrogation of RNA splicing processes and has provided a new platform for molecular discovery and therapy. While splice modulators have entered clinical trials, limited clinical efficacy in splicing factor mutation driven malignancies, such as acute myeloid leukemia, has remained a challenge.

There is a pressing unmet medical need for developing potent small molecule splice modulators (SPLMs) for the treatment of a broad array of malignancies characterized by splicing deregulation. However, the inability to practically access gram scale lead molecules with viable pharmacological properties continues to hinder their application. The dissertation herein details two projects that addresses these issues: 1. A scalable approach to prepare 17*S*-FD-895, a potent *in vivo* active splice modulator, that not only provided material to enable clinical translation but also furthered lead validation by expanding the structure-activity relationships that guide splice modulation. 2. Utilizing established synthetic methods and RNA isolation strategies to develop and profile a suite of splice modulator analogues to expand the structure-activity relationships of SPLMs and to gain a clearer understanding of the mechanisms underlying inherent gene selectivity in splicing.

## Chapter 1: Introduction and Background

Life operates through the orchestrated translation of the four-digit genetic code into a 20 amino acid code to give proteins. During this process, DNA, the storage oligonucleotide, passes its information on through conversion into RNA, which in turn serves as an intermediary for the translation of a gene into a protein. To match the needs of complex eukaryotes, most genes are transcribed as pre-mRNAs that contain intervening sequences (introns), as well as expressed sequences (exons). Introns are now known to be removed during the process of pre-mRNA splicing, a reorganization process that joins exons together to produce mature mRNAs (*1-2*) that allows the cell and organism to rapidly alter the gene product in response to temporal or environmental challenges (Figure 1.1).

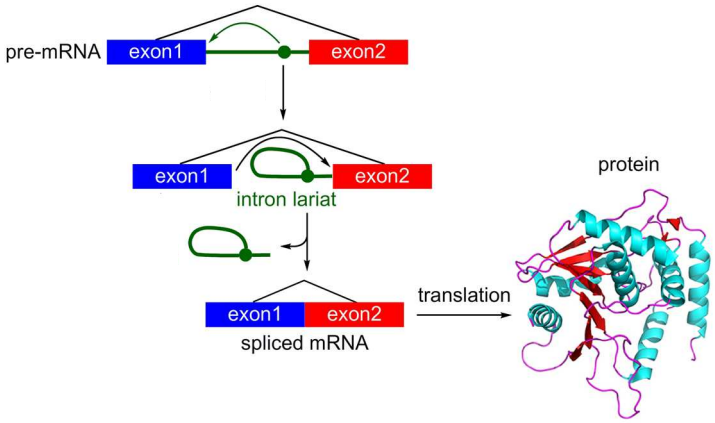



**Figure 1.1** Overview of the splicing process depicting the conversion of pre-mRNA to spliced mRNA for translation to protein.

Since most human genes contain multiple introns, splicing is a crucial step in gene expression. Splicing is catalyzed in two distinct transesterification steps by a dynamic ribonucleoprotein (RNP) machine called the spliceosome, remarkable for its ability to maintain fidelity amongst tens of thousands of *cis* and *trans* gene sequences. The spliceosome is composed of five different small nuclear RNP subunits (U1-U5 snRNPs) (*3-4*), along with many associated protein cofactors (Figure 1.2). In addition to being controlled by a complex array of splicing signals

located at exon-intron boundaries spliceosomes are stepwise assembled on their targets by a multi-step process in which *cis* and *trans*-acting elements are recruited to control higher order assembly and gene selectivity (*5*).

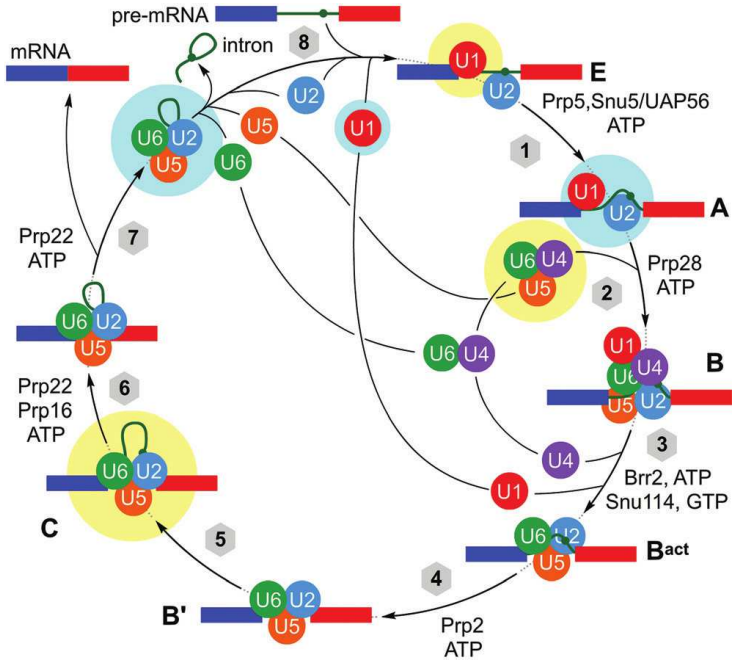
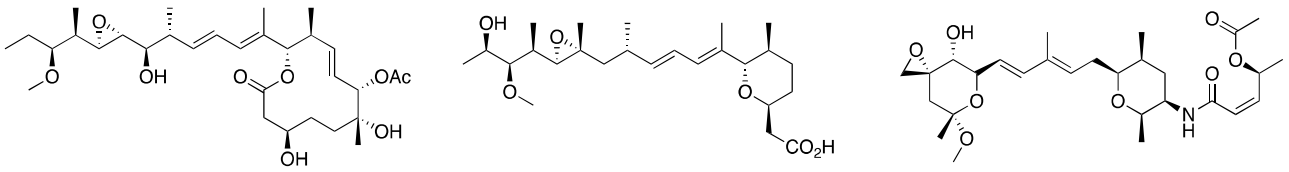



**Figure 1.2** The mechanism of splicing, depicting complexes, RNA binding proteins, and the energetic costs associated with each complex formation.

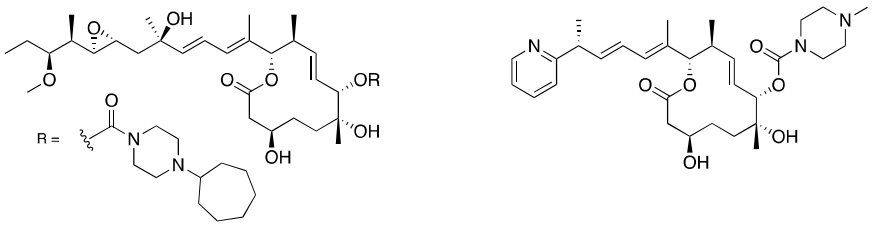
An early crucial step in the process involves recognition of the branch point adenosine (BPA), directly involved in the first transesterification step, and its adjacent sequences on the pre-mRNA. Recent crystallographic data has suggested that the interface of the subunits SF3B1 and PHF5A within the U2 snRNP are responsible for interacting with the BPA and enabling its bulged nature to pre-organize its nucleophilic attack to the 3'ss of the gene sequence of focus (*6-7*). In 2007 Kaida et al. and Kotake et al., (*8-9*) through a combination of affinity enrichment, UV cross-linking and protein analysis by western blotting and mass spectroscopy, demonstrated that the spliceostatins and pladienolides (Figure 1.3), antitumor compounds isolated from *Pseudomonas* sp. 2663 and *Streptomyces platensis,* respectively, inhibit *in vitro* splicing and promote pre-mRNA accumulation by binding to SF3B. This impairment of the spliceosome was directly correlated to



FD-895 Herboxidiene Spliceostatin A

**Figure 1.3** Representative molecules from the three main classes of SPLMs. These families share structural similarities characterized by a cyclic moiety joined by a central diene to highly oxidated functionality.

inhibition of cell growth as well as the increased number of snRNP-enriched nuclear speckles in *in vitro* studies, an observation often noted when established splicing factors are inactivated by RNAi, antisense oligonucleotides or antibodies. The observed splicing modulation was further validated by co-crystal structures obtained by the Pena and Burkart laboratories (*10-11*) which demonstrated that the SPLMs inhabit the same chemical space as the BPA and thus preventing the SF3B1 subcomplex from adopting the necessary confirmation to form a stable duplex with the RNA as required for high fidelity splicing.



E7107 H3B-8800

**Figure 1.4** SPLMs that have entered the clinic. E7107 progressed to Phase II clinical trials before removal due to ocular toxicity. H3B-8800 is currently in Phase I trials.

The lack of approved drugs targeting splicing factor mutation driven malignancies and the implications of alternatively spliced products in various diseases and cancers fueled the entry of E7107 in 2013 (*12-13*), a synthetic pladienolide analogue, into clinical trials (Figure 1.4) to treat solid tumors and leukemia. Although the trials were suspended early during Phase II due to ocular toxicity, it was clear that the spliceosome presented itself as a promising new chemotherapeutic

target as evidenced by the recent entry of H3B-8800 (*14*) by Eisai Co. Ltd. Since its description in 1994 (*15*), FD-895, a natural product derived from *Streptomyces hygroscopicus,* and the first member isolated from the pladienolide family, has since shown significant promise as a viable anti-tumor pharmaceutical against hematologic malignancies, especially chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML). Efforts by Burkart and Castro demonstrated that FD-895 not only possessed single nanomolar toxicity against leukemia-lymphoma cell lines, but did so preferentially in cancer cells as compared to normal lymphocytes (Figure 1.5A) (*16*). Additionally, RNA-seq

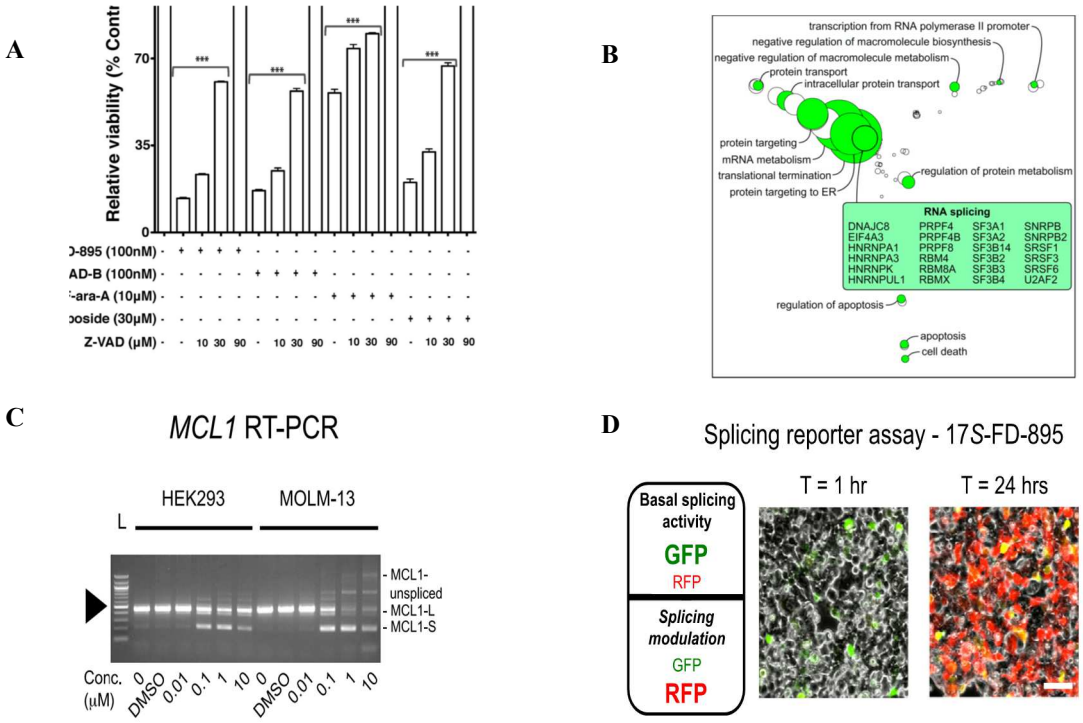



**Figure 1.5** In vitro and qPCR analysis of SPLMs. (**A)** Caspase activity of FD-895 and pladienolide B in normal B cells and patient CLL cells. **(B)** RNA-seq analysis shows that 15% of the population of splice modulated genes code for RNA splicing machinery itself. **(C)** RT-PCR and gel electrophoresis analyses of HEK293 and MOLM-13 treated with 17*S*-FD-895 for 4 hr indicate that splice modulation can reverse splicing of anti-apoptotic (MCL1-L) genes often upregulated in tumor cells in favor of pro-apoptotic genes (MCL1-S). **(D)** Summary of the predicted fluorescence readout using a dual fluorescence (RFP/GFP) alternative splicing reporter assay in HEK293 cells. Right: confocal microscopy images showing C increased RFP expression in 17S- FD-895-treated (10 μM) HEK293 cells.

transcriptome analysis showed that FD-895 induced a generalized process of intron retention (IR) in a majority of genes (82%) with the genes exhibiting the highest levels of IR belonging to the gene regulation/RNA splicing pathway (Figure 1.5B) as well as genes implicated for anti-apoptotic characteristics associated with tumor cells (Figure 1.5C). This strongly suggested that the IR effect mediated by FD-895, which could be cleverly visualized using differential fluorescence of GFP and RFP modified intron/exon junctions (Figure 1.5D), involved a broad process that is not stochastic in nature but that certain pathways, mainly RNA processing/editing, are highly sensitive to the effect of these compounds. This was compared with other chemotherapy agents such as bendamustine and F-ara-A which, although showed toxicity, did not exhibit IR.

A synthetic analogue of FD-895, 17*S*-FD-895, exhibited similar effectiveness against leukemic stem cells (LSCs) in secondary acute myeloid leukemia (*17*). In splicing reporter assays and pre-clinical patient-derived AML murine models, treatment with 17*S*-FD-895 reversed anti-apoptotic, cancer related splice isoform switching of genes such as *MCL-1* in the *BCL* family of genes responsible for apoptosis. RT-PCR analysis indicated that pharmacologic splicing modulation triggered *MCL1* exon 2 skipping, producing the pro-apoptotic *MCL-1* short isoform, as well as reducing expression of the secondary AML-associated transcript *PTK2B-202*, indicating splicing modulation could suppress sAML splice isoform expression patterns or favor survival of cells with less-perturbed spliceosome function. 17*S*-FD-895 also demonstrated *in vitro* and *in vivo efficacy* on par with FD-895, which was advantageous due to the greater stability and synthetic feasibility of 17*S*-FD-895.

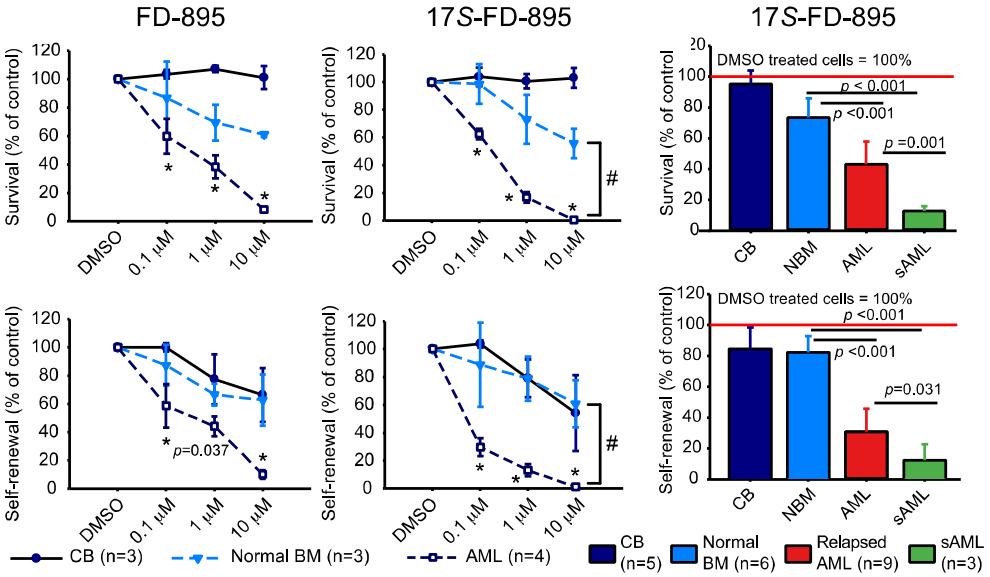



**Figure 1.6** Splicing modulation with 17S-FD-895 (1 μM) impairs LSC maintenance in patients resistant to 5-azacytidine (Vidaza). (A, B) In Vitro Efficacy #1: CD34+ AML cells, normal bone marrow or cord blood (CB) were co-cultured with SL/M2 stroma for two with increasing doses of FD-895 (A) or 17S-FD-895 (B) or vehicle control (DMSO) and assessed for survival (upper panels) and self-renewal (lower panels) (*p<0.05, ANOVA). (C) In Vitro Efficacy #2: CD34+ LSC from relapsed de novo AML or sAML patient samples, normal bone marrow or CB in co-culture were treated with 1 μM 17*S*-FD-895 or vehicle control and assessed for survival (upper panels) and self-renewal (lower panels) in methylcellulose (p<0.05, ANOVA).

Underlying these efforts to develop the pladienolide family of splice modulators into potential drug candidates are the synthetic challenges in supplying material for preclinical work and subsequent clinical trials. While access to SPLMs has evolved from the early stages of natural product isolation to the current state of high-fidelity total synthesis (*18*), playing a key role in structural determination and medicinal chemistry optimization, gram scale quantities required for preclinical efforts could only be accessed *via* fermentation (*19*). Even so, these natural product pladienolides were also unstable in aqueous media (Figure 1.7), hydrolyzing into its *seco*-acids which have been implicated in the observed toxicity seen in the E7107 Phase II trials.

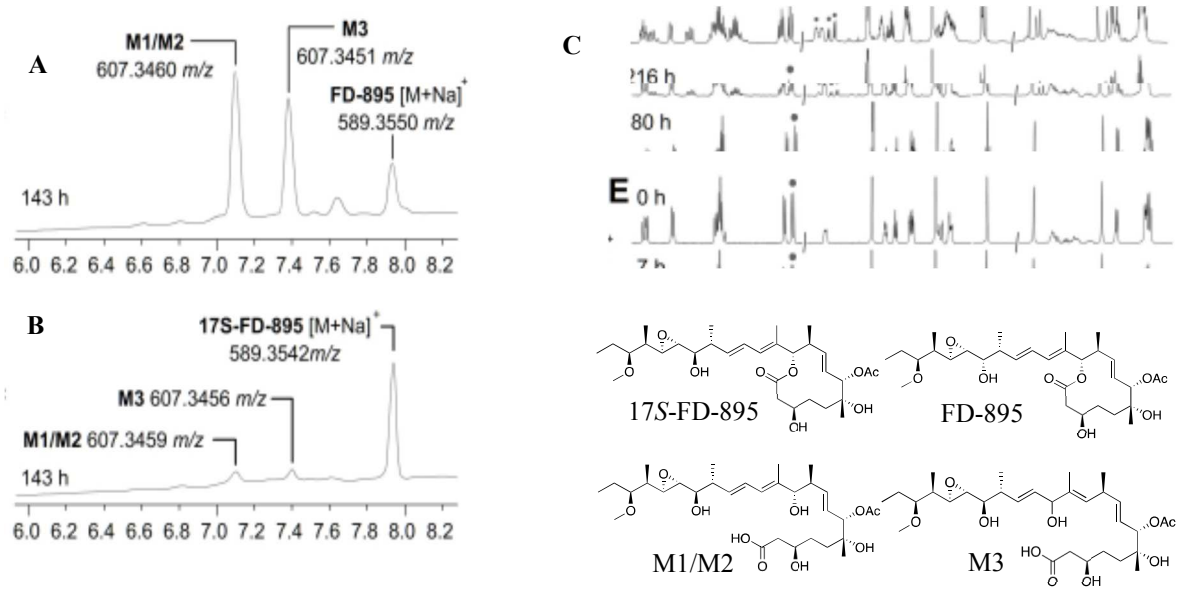



**Figure 1.7** Schematic representation of the hydrolytic products obtained from degradation of FD-895. Acids M1-M3 are readily observed in in vitro and in vivo conditions. LC−HRMS traces depicting the products from incubating samples of (A) 10 mM FD-895 in D2O/DMSO-d6 (10:1); (B) 10 mM 17S-FD-895 in D2O/DMSO-d6 (10:1); (C) NMR monitor of the hydrolytic decomposition of 10 mM FD-895 in D2O/DMSO-d6 (10:1) at 37 °C.

While these efforts had established a solid synthetic foundation, the methods previously published were ultimately not amenable for translation to the gram-scale production required to facilitate preclinical evaluation. This thesis is a detailed description of the culmination of efforts across multiple institutions to not only surpass these synthetic challenges but also to utilize these optimized methods to develop a panel of analogues for interrogation of stereochemical SAR of the SF3B binding pocket. These efforts not only involved overcoming synthetic walls but required substantial crystallographic and computational expertise as well as in depth analysis of the specific interactions of these splice modulators with RNA.

Chapter 1 discusses the identification of 17*S*-FD-895, a synthetic analogue of FD-895 as a potential anti-tumor drug candidate and the successful efforts in developing a multi-gram scale synthesis. The feasibility and robustness of the route was validated in the amounts of the target molecule that was produced. Its viability for Good Manufacturing Practices production was also

demonstrated via production of a gram scale lot of $^{13}C$ labeled isotopic material on two positions in the molecule. These improved methods also allowed further SAR exploration by enabling synthesis of a panel of analogues by utilizing stockpiles of intermediates of diastereomers and enantiomers produced along the route, its design which also facilitated generation of new stereocenters with only changes in chiral reagents.

Chapter 2 discusses the specific interactions splice modulators engage in the pocket of SF3B1. A detailed analysis of the co-crystal structures of SF3B1 and SPLMs obtained suggest that SPLMs are highly organized in their 3D structure within the binding pocket, possessing both rigid and flexible characteristics that allow the necessary contacts to induce splice modulation.

Chapter 3 summarizes the expansion of the currently known SAR of SPLMs in the SF3B1 pocket *via* analogue development and qPCR analyses. A suite of analogues, developed utilizing the synthetic methods optimized from the scale-up route discussed in Chapter 1, were assessed not only for their *in vivo* potency, but also for their potential to induce selective splice modulation.

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## Chapter 2: Developing a Scalable Approach to 17*S*-FD-895

The pladienolides share a common motif comprised of two functional moieties united through a central diene. The routes developed by Kotake and co-workers from Eisai Co, Ghosh, and partial routes by Maier, Skaanderup, Chandrasekhar groups, derive the macrolide core using ring-closing metathesis (*1*-*5*). The core is then attached to the respective side chains using either a Julia–Kocienski olefination or Stille coupling. In the Eisai Co. synthesis of pladienolide B (**1**) (Figure 2.1a), core **19** was assembled by using a Sm(II)-type Reformatsky reaction to create the C(3) carbinol and a Paterson aldol reaction to install the C(10)-C(11) stereodiad. The C(6)-C(7) centers were created early on through Sharpless dihydroxylation. The synthesis of side chain **9** was conducted using an Evans aldol to install the C(20)-C(21) stereodiad followed by Shi epoxidation at C(18)-C(19).

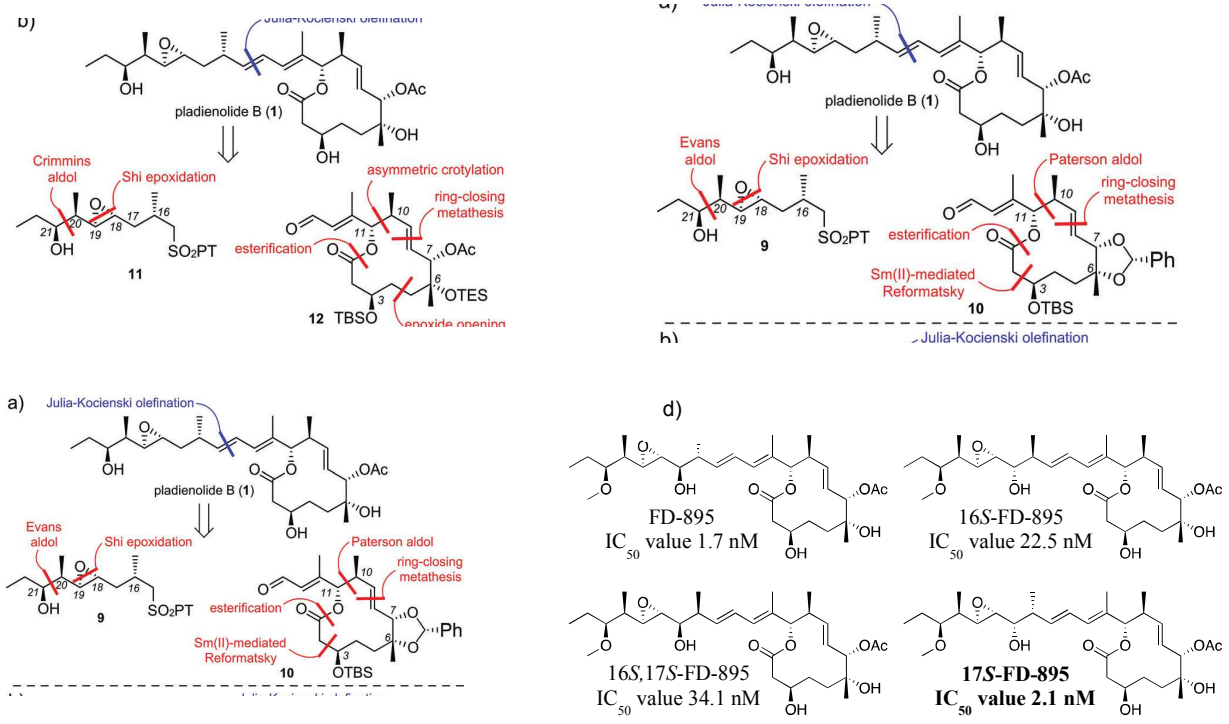



**Figure 2.1** Synthetic disconnections implemented in the total syntheses of a–b) pladienolide B (1) or c) FD-895 (3). Bond disconnections for component coupling steps (blue) and key steps in component syntheses (red) are shown.

In the Ghosh route to **1** (Figure 2.1b), C(20)-C(21) in component **11** was set using a Crimmins aldol. This was followed by the implementation of a Shi epoxidation to set C(18)-C(19). Core **12** was assembled by an epoxide ring opening to afford the C(4)-C(5) diad and a subsequent asymmetric crotylation to install the C(10)-C(11) stereocenters. Esterification, followed by ring-closing metathesis, completed the synthesis of **12**.

In our previous studies on FD-895 (Figure 2.1c) (*6*-*7*), the C(3) center was installed in core **14** using a Sammakia aldol on material that contained the C(6)-C(7) stereodiad. This diad was prepared by a Brown allylboration followed by 2-methoxyethoxymethyl (MEM) ether directed installation of the methyl group at C(6). The C(10)-C(11) centers were prepared using a Brown crotylboration. In this route, component **13** was constructed using a Crimmins aldol to install the C(20)-C(21) stereodiad, a Sharpless epoxidation to install C(18)-C(19) and a Marshall allenyl addition to create the C(16)-C(17) diad. The use of Marshall allenylstannane chemistry was advantageous, since it allowed access to all four of the C(16)-C(17) diastereomers which completed the stereochemical assignment of the FD-895 natural product.

Through synthetic efforts to assign the prior ambiguous C(16)-C(17) stereocenter in FD-895 (*6*), the Burkart laboratory had identified 17*S*-FD-895 not only as an analogue that had comparable activity in leukemic stem cells but demonstrated a significant increase in stability (Figure 2.1d) in aqueous phosphate buffer as well as presenting itself as a more synthetically feasible target for gram scale production. Its promising pharmacological profile in patient cells and murine models and potential entry into human clinical trials (*8*) called for a retooling of the synthesis of 17*S*-FD-895 to supply the gram amounts needed to acquire sufficient preclinical data. These synthetic efforts led to the successful production of 17+ grams of 17*S*-FD-895 as well as the

development of a route amenable for isotope labeling, analogue synthesis, and exploration of SAR as described below.

# Scalable Synthesis of 17*S*-FD-895 Expands the Structural Understanding of Splice Modulatory Activity


## Summary

While splice modulators have entered clinical trials, limited clinical efficacy in splicing factor mutation driven malignancies, such as acute myeloid leukemia, has remained a challenge. There is a pressing unmet medical need for developing potent small molecule splice modulators for the treatment of a broad array of malignancies characterized by splicing deregulation. However, the inability to practically access gram scale lead molecules with viable pharmacological properties continues to hinder their application. Here we report a scalable approach to prepare 17*S*-FD-895, a potent *in vivo* active splice modulator. The strategy described herein not only provides material to enable clinical translation but also furthers lead validation by expanding the structure-activity relationships that guide splice modulation.


## Introduction

Since their first discovery in the mid-1990s, families of polyketide natural products, including FD-895, the pladienolides, the spliceostatins, herboxidiene, and the thailanstatins, have garnered interest due to selective antitumor activities (*9-13*). In recent years, two lead candidates, E7107 (*14*) and H3B-8800 (*15*), have advanced to Phase I clinical trials for solid tumors and leukemia. Mode of action studies (*16-17*) indicate that they share similar abilities to modulate splicing through interactions within the SF3B component of the spliceosome. First suggested as a consensus motif (*18-20*) and later validated by structural analyses, these small molecules uniquely position themselves at an interface between SF3B1, PHF5A, and SF3B3, a hinge region involved in regulating the branch site adenosine-binding pocket (*21-24*). These splice modulators all possess

a similar structural backbone containing a macrolactone ring linked by a diene to a side chain. Here, the importance and positioning of the stereochemical centers within these molecules clearly indicates a unique geometrical requirement for activity (*25-26*).

While many of these splice modulators display the necessary functional spatiality to enable facile binding to the SF3B pocket *in vitro*, the high density of their functional groups results in a low stability in biological media resulting in short half-lives ($t_{1/2} \leq 30$ min) (*27*). Recent studies now indicate that synthetic modifications along the side chain are not only tolerated, but allow for access to a three-dimensional arrangement that reduces the rate of degradation. These studies also indicate that synthetic analogs meet the requirements for active binding to the spliceosome pocket *in vivo*. This ultimately led to our identification of 17*S*-FD-895 (**1**) as a therapeutic lead.

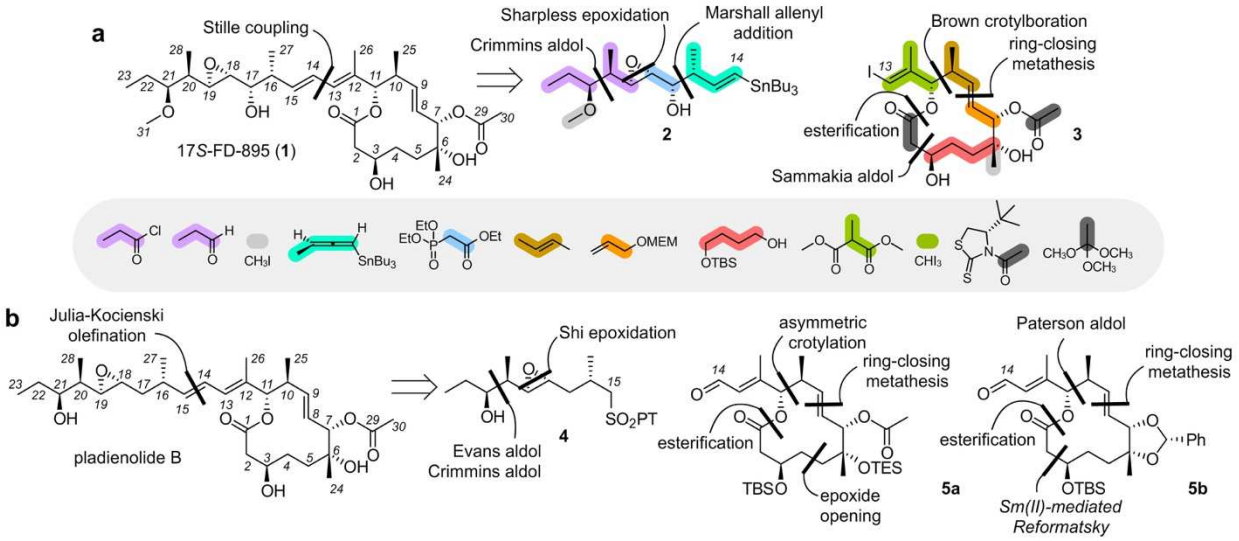
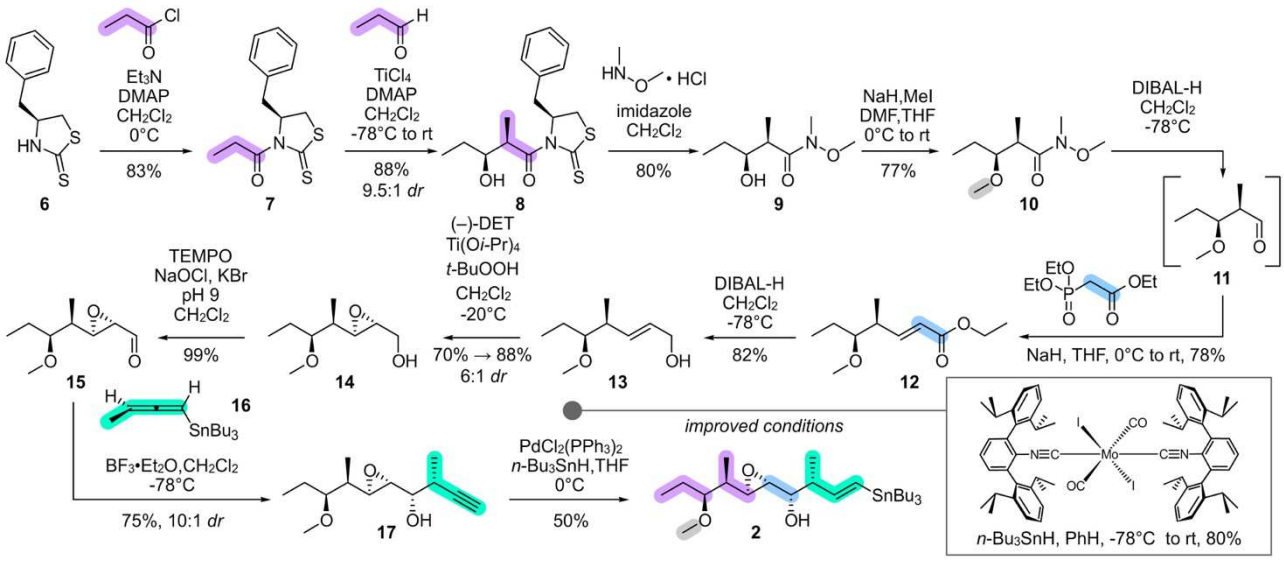



**Figure 2.2.** Synthetic design. **(a)** The synthesis of 17*S*-FD-895 (**1**) arises through the coupling of side chain **2** and core **3**. The 11 $sp^3$ stereocenters and stereochemistry of the 3 olefins of **1** arose from 12 precursors (inset) that are available on the kg scale. The key steps used to prepare each component are noted. **(b)** The retro-analysis of the related macrolide, pladienolide B, as developed by Ghosh (*25*) from core **5a** and Kotake (*27*) from core **5b**. Colored highlights denote the sourced components as shown in grey inset.

While efforts have been developed to access gram scale quantities of pladienolides *via* fermentation (*28*), these approaches have been limited to the production of natural materials. To

access the non-natural C17 stereocenter in 17*S*-FD-895 (*29*), we focused on a synthetic approach. To date, reported gram scale synthesis has enabled access only to the less-complex herboxidiene (*30*). The synthetic challenges in facing gram scale preparation of 17*S*-FD-895 (**1**, Figure 2.2), include: 11 total stereocenters (6 contiguous), a substituted diene, remote functionality, a quaternary carbon and a 12-membered lactone. Our approach (Figure 2.2a) expanded on prior milligram-scaled campaigns (*1*-*5*)(Fig. 1b) that identified the importance of component assembly. As **1** possesses potent biological activity, with a human maximum tolerated dose (MTD) estimated at 4 mg/m$^2$ based on E7107, we opted for a route that avoided production of active materials until the final step. In general, we targeted a process that would be amenable for large-scale synthesis by reducing operations and chromatographic requirements.




**Scheme 2.1.** Side chain **2** was synthesized in 11 steps beginning from Crimmins auxilary **6**. The yields and stereoselectivities indicated reflect the improvements made that enabled gram scale production. Compounds **6** and **7** were purified by recrystallization. Colored highlighting denotes carbons from sourced precursors (Fig. 1). Abbreviations: DMAP, dimethylaminopyridine; DIBAL-H, diisobutylaluminium hydride; DET, diethyltartrate; *i*-Pr, *iso*-propyl; *t*-Bu, *tert*-butyl; TEMPO, 2,2,6,6-tetramethylpiperidine-*N*-oxyl; *n*-Bu, butyl.

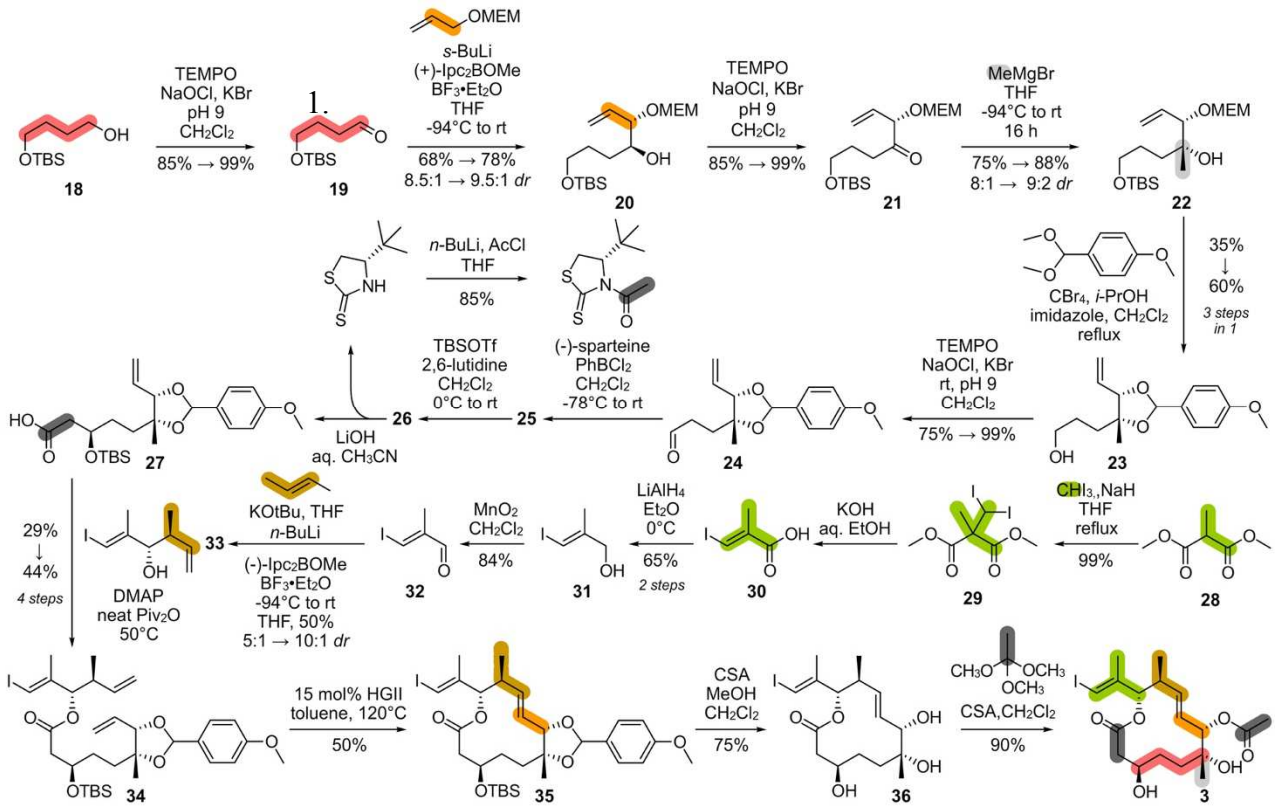
**Results**

We began by developing methods to prepare 20 g (0.039 mol) of side chain **2** (Scheme 2.1) to secure over 15 g (0.027 mol) of **1**. This started with optimization and preparation of Crimmins' auxiliary **7** (*31*) on a kilogram scale. Diastereoselective aldol addition, followed by aminolysis and subsequent methylation, enabled the successful transition to 155 g (0.82 mol) of Weinreb amide **10** per batch from 235 g (0.94 mol) of **7**. Fortunately, we were able to recover 65 ± 5% of **6**. At this point, we encountered our first challenge: the high volatility of aldehyde **11**. This was circumvented by a solvent change to 2-methyltetrahydrofuran, enabling reduction of **10** and homologation to **12** without isolation of **11**. Next, DIBAL-H reduction afforded alcohol **13**, which could be stored at 4 °C for over 2 years. Sharpless epoxidation of **13** provided **14** with a 6:1 *dr* (diastereomeric ratio), which was oxidized to **15** by use of TEMPO. Subsequent condensation of aldehyde **15** with Marshall allenylstannane **16** (*32*) provided alkyne **17**.

The next issue arose in the hydrostannylation of **17**, where the use of a palladium catalyst generated only a 1:5 α:β regioselectivity. This led to contamination by traces of the undesired α-vinylstannane, which was reduced by use of Figueroa's molybdenum catalyst (*33*) (insert, Scheme 2.1) to a 1:10 *dr* favoring the desired β-stannane. Ultimately, effective chromatographic conditions assisted access to **2** with 95+% purity that was established by LC/MS analysis. To date, we have stocked over 200 g (1.3 mol) of **13**. Over multiple repetitions, we were able to synthesize 6.5 ± 0.5 g (0.013 mol) of **2** from 25 g (0.16 mol) of **13** in a week.

Parallel efforts were also launched to produce 20 g (0.043 mol) of **3** (Scheme 2.2). We developed scalable methods to prepare intermediate **22** in 300 g batches from *mono*-protected **18**. To achieve this, TEMPO oxidations enabled scalable conversion of **18** to **19** and **20** to **21** without chromatography. Reducing the reaction temperature (-78 °C to -94 °C) improved the *dr* (85% to

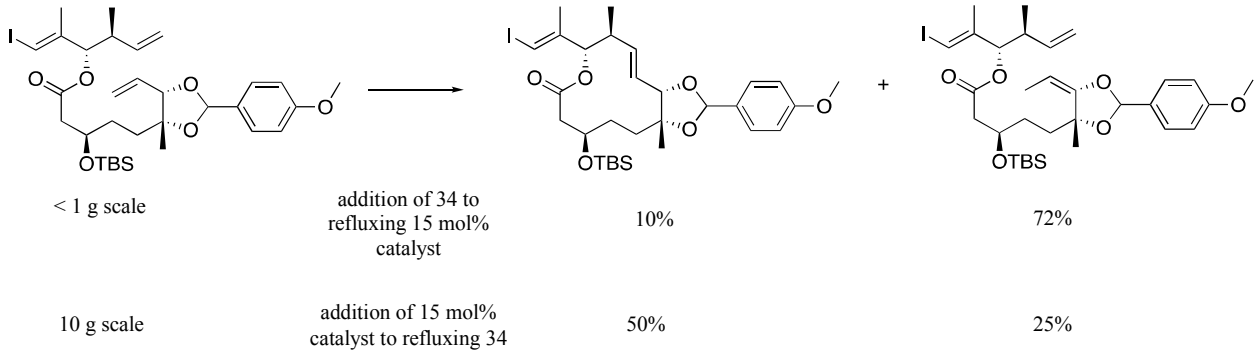
95%) of the allylboration of aldehyde **19** to **20**. Solvent change (THF to $Et_2O$) and reaction temperature optimization (-78 °C to -94 °C) improved the selectivity of the Grignard addition (85% to 90% *dr*) to **21** affording **22**. This process currently requires a single chromatographic step (**20**, Scheme 2.2). With a stability of over 4 years at -20 °C, compound **22** provides an ideal storage point for batch preparation of core **3**.




**Scheme 2.2.** Synthesis of core **3** from mono-protected 1,4-butanediol **18.** The yields and stereoselectivities indicated reflect the improvements made that enabled gram scale production. Colored highlighting denotes carbons from sourced precursors (Fig. 1). Abbreviations: Ipc, isopinocampheyl; Ph, phenyl; TBSOTf; *tert*-butyldimethylsilyl trifluoromethylsulfonyl; HGII, 2nd generation Hoveyda-Grubbs catalyst; CSA, (1*S*)-(+)-10-camphorsulfonic acid.

The conversion of **22** to **3** provided the most significant challenge. Previously established methods to convert **22** to **23** relied on extremely pure $ZnBr_2$, which caused issues due to its hygroscopicity. After reaction screening, we observed that the *in situ* decomposition of $CBr_4$ in *i*-PrOH (*34*) reproducibly returned 65 ± 5% of **23**, enabling three transformations in one step. The

next challenge arose in the installation of the C1-C3 fragment. Upon oxidation to **24**, we installed the remote C3 stereocenter in 9:1 *dr* using a chiral *tert*-leucine derived thiazolidinethione auxiliary (*35*). Subsequent protection and saponification afforded acid **27**, which was esterified with alcohol **33** in neat pivalic anhydride to afford **34** (*36*). These conditions operated without solvent and were high yielding and reproducible. This 6-step sequence could be conducted in 3 days, accessing 10 g (0.015 mol) batches of **34** from 25 g (0.069 mol) of **22**. At this point, we had installed the remaining 5 stereocenters required for **1** with 95+% purity in **34**.




**Scheme 2.3.** Simple procedural changes lead to drastic improvement in yields and reactivity selectivity.

Next, we turned our attention to the challenging ring closing metathesis (Scheme 2.3). Previously, the reaction had been performed at a maximum of 1 g and suffered from allylic isomerization despite the use of additives (*37*). After screening catalysts and reaction conditions, we discovered that inverting the order of addition (a solution of 2$^{nd}$ Hoveyda-Grubbs catalyst in toluene to **34** in refluxing toluene) provided acceptable yields of **35** on the 5-10 g scale. Subsequent global deprotection of **35** with mild acid, followed by selective acetylation of C7 in **36** *via* orthoester formation, yielded core **3**. After optimization, we are now able to convert 30 g (0.083 mol) of **22** to 1.8 ± 0.2 g (0.0039 mol) of **3** (95+% purity *via* LC/MS) in less than 2 weeks.

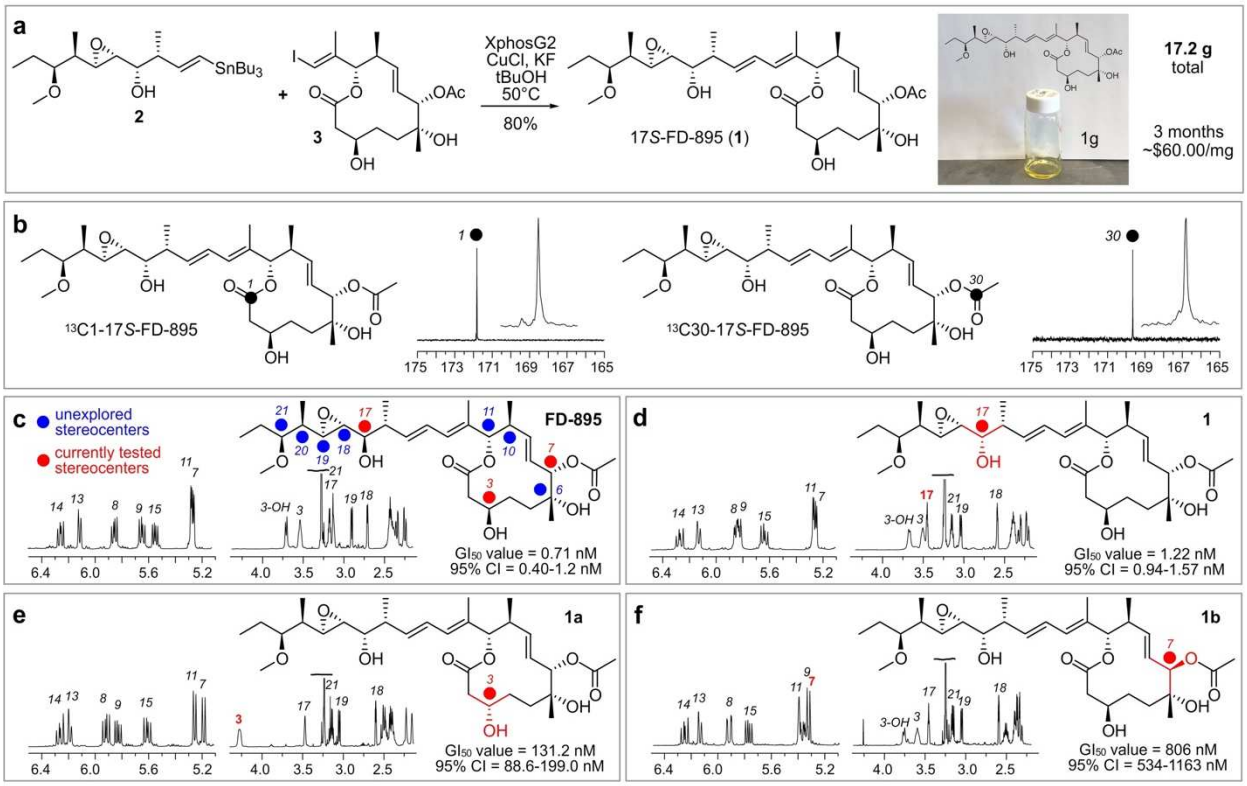



**Figure 2.3.** Synthesis of 17*S*-FD-895 (**1**), single-carbon isotopically-labeled materials and stereoisomeric analogs. **(A)** Stille coupling of side chain **2** and core **3** yield **1** with an effective mass balance. The 17 g batch was prepared by 5 full time employees (FTEs) over the 3 months period, only the effort of 3 FTEs was required the entire period. **(B)** Synthesis of $^{13}$C1-17*S*-FD-895 (Scheme S1) and $^{13}$C30-17*S*-FD-895 (Scheme S2) were prepared by installing $^{13}$C-containing precursors into the routes in Schemes 1-2. $^{13}$C-NMR returned a single peak, suggestive that a single isomeric material was present within these batches. **(C)** SARs in FD-895 were identified through analog development. Red and blue spheres denote stereochemical centers evaluated in this study and those yet to be explored, respectively. **(D-F)** Analogs **1**, **1a** and **1b** were synthesized and $GI_{50}$ values were evaluated in HCT-116 cells. Select regions of $^{1}$H NMR spectra are provided to illustrate chemical shift modifications. **(D)** 17*S*-FD-895 (**1**). **(E)** Replacing dichlorophenylborane and (-)-sparteine in the Sammakia aldol addition with $TiCl_4$ and diisopropylamine afforded the inverted C3 stereocenter in **1a** (Scheme S3). **(F)** C7 core isomer **1b** was synthesized from **34** in 6 steps (Scheme S4). While inversion at C17 retained activity, inversion within the macrolactone core at C3 or C7 resulted in marked loss in activity.

At this stage, we were set for the final step (Figure 2.3a). We opted for a Stille cross-coupling at C13-C14 (*38*), as alternate installation of the C14-C15 olefin by cross-metathesis or Julia-Kocienski olefination (*39*) can be complicated by the formation of undesired *cis*-olefins. After parallelized-reaction screening, we settled on an olefin coupling using Buchwald's XPhos Pd G2 catalyst with CuCl and KF in anhydrous *t*-BuOH. Under Class III safety conditions, we prepared **1** in 80 ± 2% yield, with a worker exposure of less than 3 h per 5 g batch (a key step in

deducing risk from this potent agent, MTD at 4 mg/kg). Fortunately, we were able to recover 16 ± 3% of **3**, which could be recycled, providing an effective mass balance in the conversion of **3** to **1**. Side chain **2** was not recoverable.

To provide materials to assist purity analyses, we introduced $^{13}C$ labels in **1** independently at C1 and C30 (Figure 2.3b). The $^{13}C$ isotopic tag at C1 was installed by preparing the Sammakia auxiliary with 1-$^{13}C$ acetyl chloride (Scheme S1), relaying it to the corresponding $^{13}C1$-labeled core **3**, and coupling it with side chain **2** to afford 1 g of $^{13}C1$-17*S*-FD-895. The $^{13}C$ tag at C30 was introduced by selective acetylation of **36** with 1-$^{13}C$ acetic anhydride (Scheme S2). The resulting $^{13}C30$-labeled **3** was coupled to **2** to prepare 100 mg of $^{13}C30$-17*S*-FD-895. $^{13}C$-NMR spectroscopy confirmed that batches of $^{13}C1$-17*S*-FD-895 and $^{13}C30$-17*S*-FD-895 were a single compound with 99% purity. Overall, this improved route has produced over 17 g of 17*S*-FD-895 (**1**), with all 11 stereocenters installed in high selectivity and reproducibility. Furthermore, the ability to produce gram scale lots of stable, isotopically labeled material is especially advantageous for *in vivo* pharmacological assessments.

Next, we wanted to expand the stereochemical structure activity relationship (s-SAR) (*40*) profile of FD-895 (*41*) (Fig. 2.3c-f) by utilizing our route to access non-natural analogs from late stage intermediates. The C3-isomer **1a** (Figure 2.3e) and the C7-isomer **1b** (Figure 2.3f) were synthesized by changes in chiral reagents (**1a**, Scheme 2.3e and **1b**, Scheme 2.3f) and along with **1** (Figure 2.3d) were confirmed by NMR peak shift comparisons (Figure 2.3e). Comparing the activity of **1, 1a** and **1b** to the natural product, FD-895, in human colorectal tumor HCT-116 cells indicated that inverting the C3 and C7 stereocenters in **1a** and **1b**, respectively, compromised activity, while the C17 isomer in **1** retained potency.

These results were consistent with established X-ray crystal structure (Figure 2.S1) of the SF3B core complexed with pladienolide B. In this and related structures, inverting the C3 hydroxyl-group in **1a** ablates its interaction with K1071 of the SF3B1 subunit (Figure 2.S1a-f). The lack in activity of the C7 isomer followed a similar reasoning, as inversion of the C7 acetate in **1b** disrupts its interaction with R38 in PHF5A. These findings support a strict SAR within the 12-membered core, as it bridges the interface between SF3B1 and PHF5A. Tolerance for inversion of C17 in **1**, was also supported structurally. Rotational freedom within the side chain (Figure 2.S1g-h) permitted pladienolide B and associated analogs to adopt distinct conformations to access the same binding pocket. Overall, this synthesis has facilitated material access to complete preclinical evaluation (setting the stage for development of improved GMP manufacturing protocols), delivered isotopic materials, filled gaps in the SAR data, and contributed to an understanding of structural features required to engage small molecule splice modulation.

Chapter 2, in full, is a reprint of the material as it appears in Scalable Synthesis of 17*S*-FD-895 Expands the Structural Understanding of Splice Modulatory Activity, *Cell Rep. Phys. Sci.* **1**, https://doi.org/10.1016/j.xcrp.2020.100277, (2020) Co-authors James J. La Clair, Brian León, Kelsey A. Trieger, Martijn Q. Slagt, Mark T. Verhaar, Dominika U. Bachera, Minze T. Rispens, Remco M. Hofman, Vincent L. de Boer, Rory van der Hulst, Rutger Bus, Pieter Hiemstra, Michael L. Neville, Kyle A. Mandla, Joshua S. Figueroa, Catriona Jamieson, Michael D. Burkart express their consent for inclusion of this published material in Chapter 2 of this dissertation. The dissertation author was an investigator and author on this paper.

## SUPPLEMENTARY MATERIALS

## TABLE OF CONTENTS

(*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one

Adduct **25**

Adduct **26**

Acid **27**

Alcohol **33**

Ester **34**

Macrolide **35**

Triol **36**

Core **3**

D. Procedure for the Stille coupling of stannane **2** to core **3** to deliver 17*S*-FD-895 (**1**)

E. Procedures for the synthesis of $^{13}$C1-17*S*-FD-895

F. Procedures for the synthesis of $^{13}$C30-17*S*-FD-895

G. Procedures for the synthesis of 3*S*-17*S*-FD-895 (**1a**)

H. Procedures for the synthesis of 7*R*-17*S*-FD-895 (**1b)**

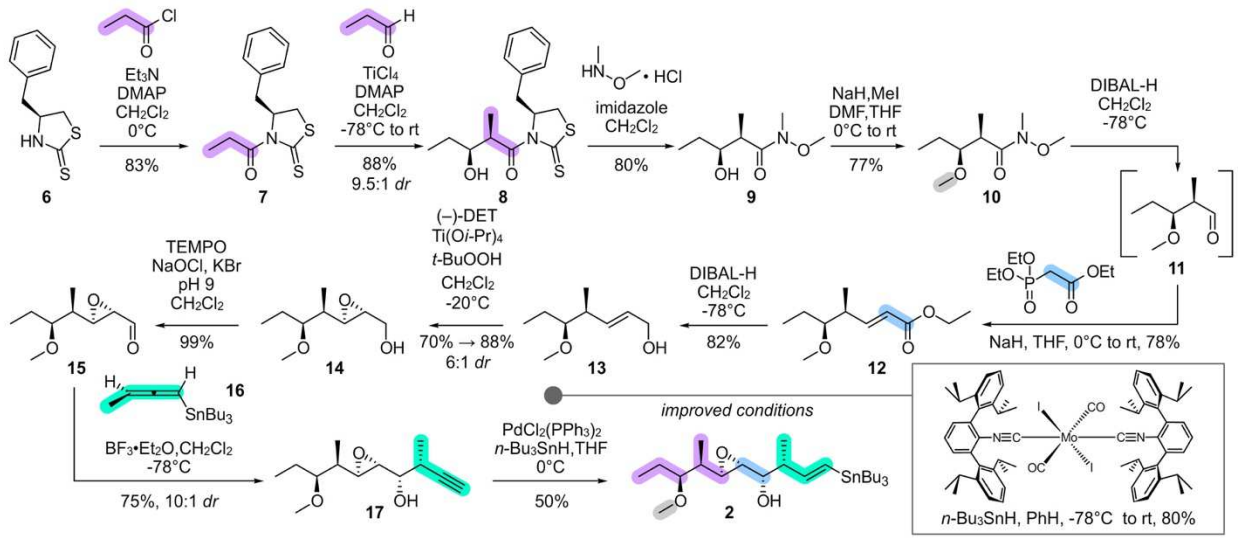
I. Cell viability assays

J. Additional references

**A. General experimental methods**: Chemical reagents were obtained from Acros Organics, Alfa Aesar, Chem-Impex Int., CreoSalus, Fischer Scientific, Fluka, Oakwood Chemical, Sigma-Aldrich, Spectrum Chemical Mfg. Corp., or TCI Chemicals. Deuterated NMR solvents were obtained from Cambridge Isotope Laboratories. All reactions were conducted with rigorously dried anhydrous solvents that were obtained by passing through a column composed of activated A1 alumina or purchased as anhydrous. Anhydrous *N*,*N*-dimethylformamide was obtained by passage over activated 3Å molecular sieves and a subsequent NaOCN column to remove traces of dimethylamine. Triethylamine ($Et_3N$) was dried over Na and freshly distilled. Ethyl-*N,N*-diisopropylamine (Et*N*-$Pr_2$) was distilled from ninhydrin, then from KOH. Anhydrous $CH_3CN$ was obtained by distillation from $CaH_2$. All reactions were performed under positive pressure of Ar in oven-dried glassware sealed with septa, with stirring from a Teflon coated stir bars using an IKAMAG RCT-basic stirrer (IKA GmbH). Solutions were heated on adapters for IKAMAG RCT-basic stirrers. Analytical Thin Layer Chromatography (TLC) was performed on Silica Gel 60 F254 precoated glass plates (EM Sciences). Preparative TLC (pTLC) was conducted on Silica Gel 60 plates (EM Sciences). Visualization was achieved with UV light and/or an appropriate stain ($I_2$ on $SiO_2$, $KMnO_4$, bromocresol green, dinitrophenylhydrazine, ninhydrin, and ceric ammonium molybdate). Flash chromatography was carried out on Fischer Scientific Silica Gel, 230-400 mesh, grade 60 or SiliaFlash Irregular Silica Gel P60, 40-63 μm mesh, grade 60. Yields correspond to isolated, chromatographically and spectroscopically homogeneous materials. $^1H$ NMR and $^{13}C$ NMR spectra were recorded on a Varian VX500 spectrometer equipped with an Xsens Cold probe. Chemical shift δ values for $^1H$ and $^{13}C$ spectra are reported in parts per million (ppm) and multiplicities are abbreviated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. All $^{13}C$ NMR spectra were recorded with complete proton decoupling. FID files were

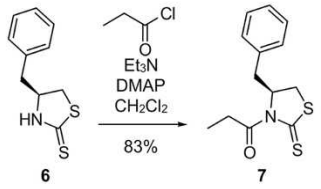
processed using MestraNova 12.0.3. (MestreLab Research). Electrospray (ESI) mass spectrometric analyses were performed using a ThermoFinnigan LCQ Deca spectrometer, and high-resolution analyses were conducted using a ThermoFinnigan MAT900XL mass spectrometer with electron impact (EI) ionization. A Thermo Scientific LTQ Orbitrap XL mass spectrometer was used for high-resolution electrospray ionization mass spectrometry analysis (HR-ESI-MS). FTIR spectra were obtained on a Nicolet magna 550 series II spectrometer as thin films on either KBr or NaCl discs, and peaks are reported in wavenumbers ($cm^{-1}$). Optical rotations $[\alpha]_D$ were measured using a Perkin-Elmer Model 241 polarimeter with the specified solvent and concentration and are quoted in units of deg $cm^2$ $g^{-1}$. Spectral data and procedures are provided for all new compounds and copies of select spectra have been provided.

**B. Procedures for the synthesis of side chain 2.** An eleven step sequence was developed to prepare 20 g of component **2** beginning with auxiliary **6**.




This procedure was optimized, in part, from published methods (*19*). Although the known compound **9** had been previously synthesized in decagram quantities (*33*), large amounts of toxic $AlMe_3$ were required to hydrolyze the oxazolidinone auxiliary. Switching to the more labile thiazolidinethione auxiliary allowed for mild hydrolysis and facilitated decagram production of alcohol **13** and subsequent gram scale production of vinylstannane **2**. Each 25 g batch of **13** provided 6.5 g of **2** at 95% purity with a total of 20 g of **2** produced to date.

**Synthesis of auxiliary 7**




**Reagents:**

$Et_3N$, 98% (Fischer Scientific): redistilled before use

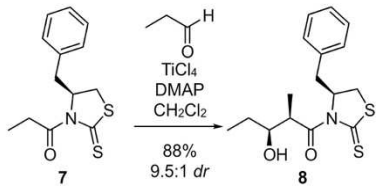
DMAP, 98% (CreoSalus): used without further purification

Propionyl chloride, 98% (Sigma-Aldrich): freshly distilled before use

**(*R*)-1-(4-Benzyl-2-thioxothiazolidin-3-yl)propan-1-one (7).** $Et_3N$ (700 mL, 5.20 mol) and DMAP (105 g, 862 mol) were added at rt to a 20 L reaction vessel containing a solution of **6** (892 g, 4.26 mol) in anhydrous $CH_2Cl_2$ (9 L). The mixture was cooled to 0 °C, and propionyl chloride (490 mL, 5.61 mol) dissolved in $CH_2Cl_2$ (2.3 L) was added dropwise over 1.5 h while maintaining the temperature at 0 °C. The mixture was then stirred at rt. After 18 h, the mixture was cooled to 0 °C, and satd. $NH_4Cl$ (5.8 L) was added dropwise while keeping the temperature below 0 °C. The mixture was extracted with $CH_2Cl_2$ (3 × 2 L). The combined organic phases were washed with satd. $NaHCO_3$ (4 L) and brine (4 L), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure auxiliary **7** (950 g, 83%) was obtained by crystallization from $CH_3CN$. Characterization data matched literature values (*43*).

Auxiliary **7**: $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.30 (m, 3H), 7.24 (m, 2H), 5.34 (ddd, $J$ = 10.9, 7.2, 3.8 Hz, 1H), 3.36 (m, 2H), 3.17 (dd, $J$ = 13.2, 3.8 Hz, 1H), 3.05 (m, 2H), 2.84 (d, $J$ = 11.6, 1H), 1.15 (t, $J$ = 7.2 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 201.2, 175.0, 136.7, 129.6, 129.0, 127.3, 68.8, 36.8, 32.5, 32.0, 8.9; LCMS (ES-API) *m/z* calcd. for $C_{12}H_{13}NOS_2$ $[M+1]^+$: 266.40.

**Synthesis of adduct 8**




**Reagents:**

Propionaldehyde, 98% (Alfa Aesar): redistilled before use

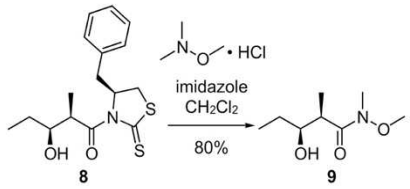
EtN(*i*-Pr)$_2$, 97% (Fisher Scientific): redistilled before use

$TiCl_4$, 98% (Alfa Aesar): used without further purification

**(2*R*,3*S*)-1-((*S*)-4-Benzyl-2-thioxothiazolidin-3-yl)-3-hydroxy-2-methylpentan-1-one (8).** (*S*)-1-(4-Benzyl-2-thioxothiazolidin-3-yl)propan-1-one (**7**) (235 g, 887 mmol) was added to a 20 L reaction flask and dissolved in $CH_2Cl_2$ (7 L) with mechanical stirring. The mixture was cooled below 0 °C. $TiCl_4$ (1 M solution in $CH_2Cl_2$, 922 mL, 922 mmol) was added dropwise over 1 h, while maintaining the temperature below 0 °C, at which point the mixture turned orange. EtN(*i*-Pr)$_2$ (168 mL, 966 mmol) was added dropwise over 30 min, at which point the resulting black mixture was stirred at 0 °C for 15 min. After cooling the reaction to -94 °C, a solution of propionaldehyde (71.0 mL, 984 mmol) in anhydrous $CH_2Cl_2$ (350 mL) was added dropwise over 6 h. The mixture was stirred at -94 °C for 30 min before being slowly warmed to rt overnight. The mixture was cooled to 0 °C and satd. $NaHCO_3$ (1.7 L) was slowly added. **CAUTION RAPID HEATING**. The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 1 L). The combined organic phases were washed with brine (2 L), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure adduct **8** (250 g, 88%) was obtained in a 9.5:1 *dr* by flash chromatography, eluting with a gradient of heptane to 1:3 EtOAc/heptane.

Adduct **8**: TLC (1:3 EtOAc/heptane): $R_f$ = 0.63 (CAM stain); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.34 (m, 2H), 7.29 (m, 3H), 5.37 (ddd, *J* = 11.2, 7.1, 4.4 Hz, 1H), 4.73 (qd, *J* = 7.1, 2.3 Hz, 1H), 3.97 (ddd, *J* = 8.1, 5.3, 2.2 Hz, 1H), 3.38 (ddd, *J* = 11.5, 7.2, 1.1 Hz, 1H), 3.25 (dd, *J* = 13.2, 4.1 Hz, 1H), 3.05 (dd, *J* = 13.2, 10.5 Hz, 1H), 2.89 (dd, *J* = 11.6, 0.8 Hz, 1H), 2.77 (bs, 1H), 1.61 (m, 1H), 1.45 (m, 1H) 1.18 (d, *J* = 7.1 Hz, 3H), 0.98 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 201.7, 178.7, 136.5, 129.6, 129.1, 129.1, 127.4, 72.6, 69.1, 42.3, 37.1, 31.9, 26.7, 10.6, 10.5; FTIR (film) $\nu_{max}$ 3444, 3027, 2964, 2937, 2876, 1689, 1455, 1352, 1258, 1191, 1164, 1041, 1029, 960 $cm^{-1}$; LCMS (ES-API) *m/z* calcd. for $C_{15}H_{19}NO_2S_2$ $[M+1]^+$: 324.40; $[\alpha]^{25}_D$ = 199.5 ° (c = 1.0 $CH_2Cl_2$).

**Conversion of alcohol 8 to Weinreb amide 9**




**Reagents:**

*N*,*O*-Dimethylhydroxylamine hydrochloride, 99% (Alfa Aesar): used without further purification

Imidazole, 99% (Sigma-Aldrich): used without further purification

**(2*R*,3*S*)-3-Hydroxy-*N*-methoxy-*N*,2-dimethylpentanamide (9).** *N*,*O*-Dimethylhydroxylamine hydrochloride (174 g, 1.78 mol) and imidazole (182 g, 2.68 mol) were added in succession to a solution of **8** (288 g, 892 mmol) in $CH_2Cl_2$ (13 L) in a 20 L reaction vessel at rt. The mixture was stirred at rt for an additional 16 h. $H_2O$ (3 L) was added, and the mixture was separated followed by extraction of the aqueous phase with $CH_2Cl_2$ (3 × 2.5 L). The combined organic phases were washed with brine (5 L), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator to afford a yellow oil. Pure amide **9** (131 g, 80%) was obtained by flash chromatography, eluting with a gradient of heptane to 3:1 EtOAc/heptane.

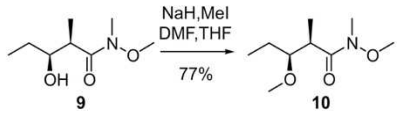
*Note 1: 65±5% of auxiliary **6** was recovered after chromatography*

*Note 2: Rotational isomers were observed by NMR*

Amide **9**: TLC (3:1 EtOAc/heptane): $R_f$ = 0.17 ($KMnO_4$); $^1$H NMR (500 MHz, $CDCl_3$) δ 3.79 (bs, 1H), 3.76 (td, *J* = 5.4, 2.6 Hz, 1H), 3.69 (s, 3H), 3.17 (s, 3H), 2.90 (bs, 1H), 1.77 (bs, 1H), 1.57 (m, 1H), 1.39 (m, 1H), 1.15 (d, *J* = 7.1 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 178.5, 73.1, 61.7, 38.1, 32.0, 26.8, 10.5, 10.1; FTIR (film) $\nu_{max}$ 2969, 2917, 2855, 1719,

1449, 1265, 1178, 1108, 1020, 715 $cm^{-1}$; LCMS (ES-API) *m/z* calcd. for $C_8H_{17}NO_3$ $[M+1]^+$: 176.40; $[\alpha]^{25}_D$ = -11.3 ° (c = 1.0, $CH_2Cl_2$).

**Methylation of amide 9 to 10**




**Reagents:**

NaH, 60% in mineral oil (Alfa Aesar): used without further purification

MeI, 98% (Sigma-Aldrich): used without further purification

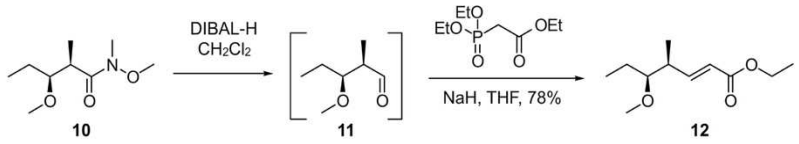
**(2*R*,3*S*)-*N*,3-Dimethoxy-*N*,2-dimethylpentanamide (10).** MeI (1.12 L, 18.0 mol) was added at rt to a solution of amide **9** (155 g, 886 mmol) in a mixture of anhydrous THF (6 L) and anhydrous DMF (1.5 L) in a 20 L reaction vessel. The mixture was cooled to 0 °C and NaH (60% in mineral oil, 88.5 g, 2.21 mol) was added in portions ensuring the mixture remained at 0 °C. The mixture was slowly warmed to rt and stirred for 16 h. After cooling the mixture to 0 °C, a solution of phosphate buffered saline pH 7 (1.5 L) was added dropwise. The volatiles were concentrated on a rotary evaporator. $H_2O$ (4.5 L) was added to the residue, and the obtained mixture was extracted with *t*-butyl methyl ether (3 × 3 L). The combined organic phases were washed with brine (3 L), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure amide **10** (129 g, 77%) was obtained as a colorless oil by flash chromatography, eluting with a gradient of heptane to 1:1 EtOAc/heptane.

*Note 1: Rotational isomers are observed by NMR*

Amide **10**: TLC (3:1 EtOAc/heptane): $R_f$ = 0.27 ($KMnO_4$); $^1$H NMR (500 MHz, $CDCl_3$) δ 3.68 (s, 3H), 3.41 (s, 3H), 3.30 (tdd, *J* = 7.0, 4.0, 1.0 Hz, 1H), 3.18 (s, 3H), 3.03 (bs, 1H), 1.58 (dqd, *J* = 14.9, 7.5, 3.9 Hz, 1H), 1.42 (dt, *J* = 14.4, 7.2 Hz, 1H), 1.21 (d, *J* = 6.9 Hz, 3H), 0.93 (t, *J* = 7.4 Hz,

3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 176.5, 83.9, 61.6, 58.7, 39.6, 32.2, 25.3, 14.5, 9.6; FTIR (film) $\nu_{max}$ 3581, 3502, 2969, 2934, 2882, 2820, 1658, 1457, 1379 $cm^{-1}$; LCMS (ES-API) *m/z* calcd. for $C_9H_{19}NO_3$ $[M+1]^+$: 190.40; $[\alpha]^{25}_D$ = -13.0 ° (c = 1.0 $CHCl_3$)

**Conversion of 10 to ester 12**




**Reagents:**

DIBAL-H, 1.0 M in hexanes (Sigma-Aldrich): used without further purification

NaH, 60% in mineral oil, (Alfa Aesar): used without further purification

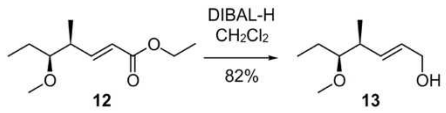
Triethyl phosphonoacetate, 99% (Oakwood Chemical): used without further purification

**Ethyl (4*S*,5*S*,*E*)-5-methoxy-4-methylhept-2-enoate (12)** Amide **10** (107 g, 565 mmol) was dissolved in anhydrous $CH_2Cl_2$ (2 L) in a 5 L flask. The mixture was cooled to -78 °C. DIBAL-H (1.0 M, 880 mL, 886 mol) was added dropwise over 45 min at -78 °C and stirred for 15 min. Acetone (100 mL) was added dropwise over 10 min, and the mixture was warmed to 0 °C. Satd. Rochelle's salt (2 L) was added over 30 min, and the mixture was stirred at rt for 1.5 h. The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 500 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. The residue was then dried *via* azeotropic removal of toluene to deliver aldehyde **11**, which was used immediately after preparation. A solution of triethyl phosphonoacetate (572 mL, 2.88 mol) in anhydrous 2-methyltetrahydrofuran (400 mL) was added dropwise over 30 min to a 5 L reaction flask containing a suspension of NaH (60% in mineral oil, 97.4 g, 2.44 mol) in anhydrous 2-methyltetrahydrofuran (1 L) cooled to 0 °C. **CAUTION RAPID EVOLUTION OF $H_2$**. The mixture was stirred at 0 °C for 15 min and a solution of **11** in anhydrous 2-methyltetrahydrofuran (1 L) was added dropwise over 30 min. The mixture was stirred at rt for 16 h, cooled to 0 °C and quenched with satd. $NH_4Cl$ (1.6 L). The organics were concentrated on a rotary evaporator.

The mixture was extracted with EtOAc (2 × 1 L), and the combined organic phases were dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure ester **12** (88.3 g, 78% over two steps) was obtained as a colorless oil by flash chromatography, eluting with a gradient of $CH_2Cl_2$ to 1:10 EtOAc/$CH_2Cl_2$.

Ester **12**: TLC ($CH_2Cl_2$): $R_f$ = 0.14 (CAM stain); $^1$H NMR (500 MHz, $CDCl_3$) δ 6.95 (dd, *J* = 15.8, 7.7 Hz, 1H), 5.82 (dd, *J* = 15.8, 1.3 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.36 (s, 3H), 3.00 (ddd, *J* = 7.4, 5.6, 4.4 Hz, 1H), 2.57 (m, 1H), 1.51 (m, 1H), 1.41 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 166.8, 151.3, 121.1, 85.6, 60.4, 58.0, 39.3, 20.0, 14.9, 14.4, 10.0; FTIR (film) $\nu_{max}$ 2978, 2934, 2882, 2820, 1719, 1650, 1466 cm$^{-1}$; LCMS (ES-API) *m/z* calcd. for $C_{11}H_{20}O_3$ $[M+NH_4]^+$: 218.6; $[\alpha]^{25}_D$ = -45.4 ° (c = 1.0, $CH_2Cl_2$).

**Reduction of 12 to alcohol 13**



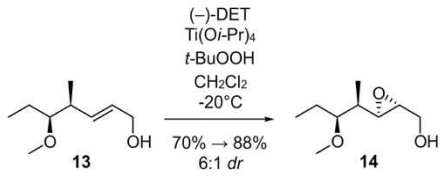

**Reagents:**

DIBAL-H, 1.0 M in hexanes (Sigma-Aldrich): used without further purification

**(4*S*,5*S*,*E*)-5-Methoxy-4-methylhept-2-en-1-ol (13).** DIBAL-H (1.0 M, 700 mL, 0.85 mol) was added dropwise over 60 min to a 5 L reaction flask containing a solution of ester **12** (56.5 g, 282 mmol) in anhydrous $CH_2Cl_2$ (1.5 L) cooled to -78 °C. The mixture was stirred for 1 h at -78 °C. Acetone (100 mL) was then added dropwise over 25 min. The mixture was warmed to 0 °C, satd. Rochelle's salt (1 L) was added, and the mixture was stirred at rt for 2 h. The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 500 mL). The combined organic phases were washed with brine (250 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure alcohol **13** (36.5 g, 82%) was obtained by flash chromatography, eluting with a gradient of heptane to 1:1 EtOAc/heptane.

Alcohol **13**: TLC (1:3 EtOAc/heptane): $R_f$ = 0.26 (CAM stain); $^1$H NMR (500 MHz, $CDCl_3$) δ 5.65 (m, 2H), 4.10 (bs, 2H), 3.36 (s, 3H), 2.92 (ddd, *J* = 7.5, 5.7, 4.2 Hz, 1H), 2.44 (m, 1H), 1.52 (m, 1H), 1.40 (m, 1H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 135.2, 129.0, 86.4, 64.0, 57.7, 38.9, 23.5, 16.0, 10.0; FTIR (film) $\nu_{max}$ 3388, 2968, 2932, 2876, 2826, 1460, 1375 cm$^{-1}$; LCMS (ES-API) *m/z* calcd. for $C_9H_{18}O_2$ $[M+1]^+$: 158.20; $[\alpha]^{25}_D$ = -34.5 ° (c = 0.2, $CHCl_3$).

**Epoxidation of alcohol 13 to epoxide 14**




**Reagents:**

Ti(O*i*-Pr)$_4$ , 97% (Sigma-Aldrich): vacuum distilled at 90 °C, 5 mbar

(-)-Diethyltartrate, 99% (Alfa Aesar): used without further purification

*t*-Butylhydroperoxide, 3.3 M in toluene: dried from a 70% solution in water according to methods developed by the Sharpless laboratory (*44*)

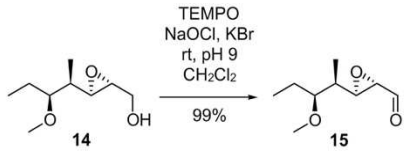
**((2R,3R)-3-((2R,3S)-3-Methoxypentan-2-yl)oxiran-2-yl)methanol (14)**. *t*-Butylhydroperoxide (3.3 M, 76.6 mL, 253 mmol) was added to a 1 L flask containing a stirring solution of Ti(O*i*-Pr)$_4$ (2.73 mL, 12.6 mmol), (-)-diethyl tartrate (2.21 mL, 12.6 mmol) and powdered 4Å molecular sieves (2 g) in anhydrous $CH_2Cl_2$ (400 mL). The mixture was cooled to -20 °C and stirred for 30 min. A solution of alcohol **13** (20.0 g, 127 mmol) in $CH_2Cl_2$ (50 mL) was added dropwise. The reaction was stirred at -20 °C for 4 h. The reaction was quenched *via* addition of 10% NaOH (25 mL). The mixture was then extracted into $CH_2Cl_2$ and concentrated on a rotary evaporator. Pure epoxyalcohol **14** (22.1 g, 88%) was obtained as a 6:1 mixture of diastereomers by flash chromatography, eluting with a gradient of hexanes to 1:1 EtOAc/hexanes.

*Note 1: Diastereomers were not separable and carried on directly to the next step.*

Epoxyalcohol **14**: TLC (1:2 EtOAc/hexanes): $R_f$ = 0.10 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 3.55 (m, 1H), 3.33 (m, 1H), 3.20 (s, 3H), 3.08 (td, *J* = 6.3, 4.5 Hz, 1H), 2.89 (dd, *J* = 7.6, 2.3 Hz, 1H), 2.63 (dt, *J* = 4.9, 2.6 Hz, 1H), 1.59 (tt, *J* = 13.9, 7.4 Hz, 1H), 1.41 (m, 1H), 1.35 (m, 1H), 1.02

(d, $J$ = 6.9 Hz, 1H), 0.85 (t, $J$ = 7.4 Hz, 3H), 0.84 (d, $J$ = 7.4 Hz, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 83.8, 62.2, 58.0, 57.9, 57.7, 38.8, 24.0, 10.4, 10.1; FTIR (film) $\nu_{max}$ 3422, 2972, 2930, 2879, 1468, 1103 $cm^{-1}$; HR-ESI-MS *m/z* calcd. for $C_9H_{18}O_3$ $[M]^+$: 174.1250, found 174.1249; $[\alpha]^{25}_D$ = +182.4 ° (c = 1.0, $CHCl_3$).

**Oxidation of epoxyalcohol 14 to epoxyaldehyde 15**




**Reagents**

TEMPO, 99% (Oakwood Chemical): used without further purification

KBr, (Spectrum Chemical Mfg. Corp.): used without further purification

NaOCl, 2 M, 10-15% active chlorine (Spectrum Chemical Mfg. Corp.): used without further purification

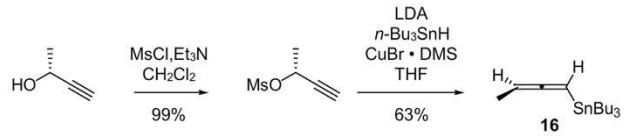
**(2*S*,3*R*)-3-((2*R*,3*S*)-3-Methoxypentan-2-yl)oxirane-2-carbaldehyde (15).** A solution of KBr (1.21 g, 10.2 mmol) in $H_2O$ (50 mL), satd. $NaHCO_3$ (100 mL) and TEMPO (1.33 g, 8.50 mmol) were added sequentially to a 2 L flask containing a solution of epoxyalcohol **14** (22.1 g, 127 mmol) in $CH_2Cl_2$ (600 mL). The mixture was cooled to 0 °C and a solution of NaOCl (2 M, 85 mL, 170 mmol) and satd. $NaHCO_3$ (100 mL) were added dropwise *via* an addition funnel. The mixture was allowed to warm to rt and stirred for 2 h. The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 300 mL). The combined organic phases were washed with brine (500 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Aldehyde **15** (21.8 g, 99%) was obtained without further purification and was carried on directly to the next step.

*Note 1: Diastereomers obtained from epoxidation were not separable at this step and thus carried forward.*

Aldehyde **15**: TLC (1:2 EtOAc/hexanes): $R_f$ = 0.55 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 8.67 (d, *J* = 6.4 Hz, 1H), 3.10 (s, 3H), 2.90 (td, *J* = 6.4, 4.0 Hz, 1H), 2.84 (dd, *J* = 7.5, 2.0 Hz, 1H),

2.79 (dd, $J$ = 6.4, 2.0 Hz, 1H), 1.44 (m, 1H), 1.21 (m, 1H), 0.82 (m, 1H), 0.74 (t, $J$ = 7.4 Hz, 3H), 0.63 (d, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 197.7, 83.4, 58.6, 58.5, 57.7, 38.3, 23.7, 10.0, 9.8; FTIR (film) $\nu_{max}$ 2972, 2930, 2879, 2828, 1732, 1468, 1103 cm$^{-1}$; HR-ESI-MS *m/z* calcd. for $C_9H_{16}O_3$ $[M+H]^+$: 173.1172, found 173.1174; $[\alpha]^{25}_D$ = -89.0 ° (c = 1.0, $CH_2Cl_2$).

**Synthesis of allenylstannane 16.** A two step sequence to prepare grams of allenylstannane **16** beginning with commercially available (*R*)-but-3-yn-2-ol (*29*).




**Reagents:**

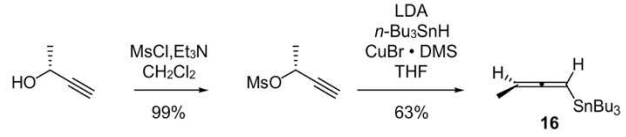
$Et_3N$, 98% (Fischer Scientific): redistilled over $CaH_2$ before use

MsCl, 98% (Alfa Aesar): used without further purification

**(*R*)-But-3-yn-2-yl methanesulfonate.** $Et_3N$ (198 mL, 1.43 mol) was added dropwise over 15 min to a 3 L three-necked flask containing a solution of (*R*)-but-3-yn-2-ol (50.0 g, 713 mmol) in $CH_2Cl_2$ (750 mL) cooled to -78 °C. After 10 min, MsCl (83.4 mL, 1.07 mol) was added dropwise over 2 h. The mixture was stirred at -78 °C for 1 h, at which point satd. $NaHCO_3$ (500 mL) was added slowly. The mixture was warmed to rt, and the phases were separated. The aqueous phase was extracted with $CH_2Cl_2$ (3 × 500 mL). The combined organic phases were washed with brine (250 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. The crude was passed through a plug of $SiO_2$, and the elutants were concentrated. (*R*)-But-3-yn-2-yl methanesulfonate (99%, 107.5 g) was obtained without further purification and was carried directly to the next step. Characterization data matched literature values.

**(*R*)-But-3-yn-2-yl methanesulfonate**: $^1$H NMR (500 MHz, $CDCl_3$) δ 5.27 (qd, $J$ = 6.7, 2.1 Hz, 1H), 3.11 (s, 3H), 2.71 (d, $J$ = 2.2 Hz, 1H), 1.65 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 80.2, 76.4, 67.6, 39.2, 22.5; LCMS (ES-API) *m/z* calcd. for $C_6H_8O_3S$ $[M+1]^+$: 148.08.

**Conversion of (*R*)-but-3-yn-2-yl methanesulfonate to allenylstannane 16**




**Reagents:**

*n*-BuLi, 2.5 M in hexanes (Acros Organics): used without further purification

*i*$Pr_2NH$, 98% (Alfa Aesar): distilled over $CaH_2$

*n*-$Bu_3SnH$, 97% contains 0.05% BHT as stabilizer (Acros Organics): used without further purification

CuBr•DMS, 99% (Acros Organics): used without further purification

**(*S*)-Buta-1,2-dien-1-yltributylstannane (16).** *n*-BuLi (2.5 M, 172 mL, 429 mmol) was added dropwise to a solution of *i*$Pr_2NH$ (60.7 mL, 429 mmol) in anhydrous THF (800 mL) in a 5 L flask at 0 °C over 10 min. After 15 min, *n*-$Bu_3SnH$ (135 mL, 501 mmol) was added dropwise over 10 min, and the mixture was stirred at 0 °C for 2.5 h. After cooling the mixture to -85 °C, CuBr•DMS (88.2 g, 429 mmol) was added in portions over 40 min. The mixture was stirred at for 30 min at -85 °C. (*R*)-But-3-yn-2-yl methanesulfonate (53.0 g, 358 mmol) was added dropwise, and the mixture was stirred for 10 min. The mixture was poured into a mixture of *t*-butyl methyl ether (2 L), 25% aqueous $NH_3$ (260 mL) and satd. $NH_4Cl$ (2 L) and stirred vigorously for 1 h. The phases were separated, and the organics were dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Allenylstannane **16** (77.2 g, 63%) was obtained in 96% *ee* by vacuum distillation (1 mbar, 150 °C). Characterization data matched literature values.

*Note 1: This procedure was repeated to deliver a total over 500 g of **16**.*

Allenylstannane **16:** $^{1}$H NMR (500 MHz, $CDCl_3$) δ 5.20 (dq, *J* = 6.9, 4.0 Hz, 1H), 4.68 (p, *J* = 6.9 Hz, 1H), 1.64 (dd, *J* = 6.9, 1.4 Hz, 3H), 1.60 (m, 12H), 1.37 (m, 6H), 0.93 (t, *J* = 7.4 Hz, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 210.0, 75.6, 74.9, 29.4, 27.6, 14.0, 10.6; LCMS (ES-API) *m/z* calcd. for $C_{13}H_{32}Sn$ $[M+1]^+$: 345.15.

**Derivatization of 16 for determination of enantiomeric excess.**

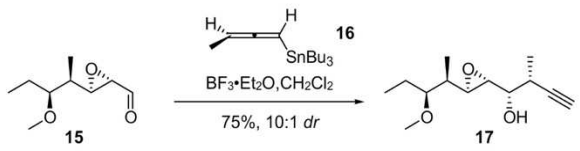
**Reagents:**

Isobutyraldehyde (Alfa Aesar): used without further purification

$BF_3$•$Et_2O$, 46.5% $BF_3$ (Alfa Aesar): used without further purification

Isobutyraldehyde (40 μL, 0.44 mmol) in $CH_2Cl_2$ (4 mL) was added dropwise to a solution of allenylstannane **16** (200 mg, 583 μmol) and $BF_3$•$OEt_2$ (210 μL, 1.66 mmol) cooled to -78 °C. After stirring at -78 °C for 1 h, the reaction was quenched with a satd. $NaHCO_3$ (4 mL). The mixture was allowed to warm to rt, and the phases were separated. The organic phase was stirred with KF on Celite (50 wt%, 100 mg) and $Na_2SO_4$ (100 mg). The solid was removed by filtration and an aliquot of the filtrate was used for chiral GC analysis indicating 96% *ee*.

**Marshall addition of allenylstannane 16 to aldehyde 15**




**Reagents:**

$BF_3$•$Et_2O$, 46.5% $BF_3$ (Alfa Aesar): used without further purification

**(1*S*,2*R*)-1-((2*R*,3*R*)-3-((2*R*,3*S*)-3-Methoxypentan-2-yl)oxiran-2-yl)-2-methylbut-3-yn-1-ol (5).** Aldehyde **15** (7.01 g, 40.8 mmol) and allenylstannane **16** (21.0 g, 61.0 mmol) in a 1 L flask were dissolved in anhydrous $CH_2Cl_2$ (400 mL) and purged with an Ar atmosphere. The mixture was cooled to -78 °C and $BF_3$•$Et_2O$ (7.53 mL, 61.0 mmol) was added dropwise over 5 min. The reaction was stirred for 1 h at -78 °C. A mixture of MeOH (50 mL) and satd. $NaHCO_3$ (10 mL) was added, and the solution was warmed to rt. The phases were separated, and the aqueous phases were extracted with $Et_2O$ (3 × 400 mL). The organic phases were combined, dried with $Na_2SO_4$ and concentrated on a rotary evaporator. Alkyne **17** (6.92 g, 75%) was obtained in a 10:1 *dr* as a colorless oil by flash chromatography, eluting with a gradient of hexanes to 1:3 $Et_2O$/hexanes.

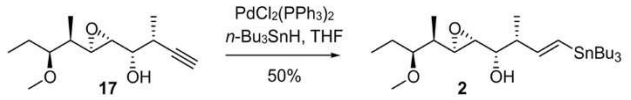
*Note 1: Minor C16-C17 Marshall diastereomers were removed chromatographically.*

*Note 2: The remaining C18-C19 epoxide diastereomer from the Sharpless epoxidation was resolved after purification of the next step.*

Alkyne **17**: TLC (1:2 EtOAc/hexanes); $R_f$ = 0.50 (CAM stain); $^1$H NMR (500 MHz, $CDCl_3$) δ 3.58 (dd, *J* = 4.4, 4.4 Hz, 1H), 3.41 (s, 3H), 3.20 (td, *J* = 6.5, 4.1 Hz, 1H), 3.06 (dd, *J* = 8.1, 2.3 Hz, 1H), 2.91 (dd, *J* = 4.5, 2.3 Hz, 1H), 2.81 (qdd, *J* = 7.0, 4.7, 2.4 Hz, 1H), 2.17 (d, *J* = 2.6 Hz, 1H), 2.05 (d, *J* = 4.8 Hz, 1H), 1.67 (ddd, *J* = 14.2, 7.6, 6.7 Hz, 1H), 1.48 (m, 2H), 1.31 (dd, *J* = 7.2, 0.7

Hz, 3H), 0.97 (d, $J$ = 7.1 Hz, 3H), 0.90 (t, $J$ = 7.4 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 84.4, 83.8, 72.3, 71.4, 58.9, 58.3, 38.9, 30.4, 23.9, 17.1, 10.6, 10.1; FTIR (film) $\nu_{max}$ 3438, 3310, 2973, 2937, 2879, 1457, 1090 $cm^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{13}H_{22}O_3$ $[M+H]^+$ 226.1642, found 226.1641; $[\alpha]^{25}_D$ = +45.4 ° (c = 1.0, $CH_2Cl_2$).

**Hydrostannylation of 17**



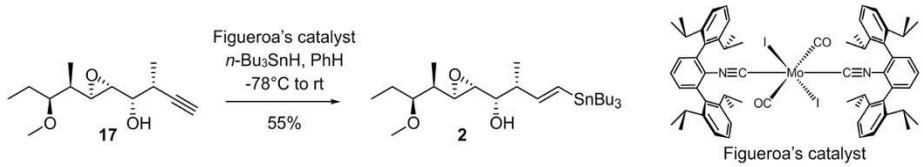

**Reagents:**

*n*-$Bu_3SnH$, 97% contains 0.05% BHT as stabilizer (Acros Organics): used without further purification

$PdCl_2(PPh_3)_2$ (Oakwood Chemical): dried *via* azeotropic distillation of benzene

**(1*S*,2*R*,*E*)-1-((2*R*,3*R*)-3-((2*R*,3*S*)-3-Methoxypentan-2-yl)oxiran-2-yl)-2-methyl-4-(tributylstannyl)but-3-en-1-ol (2).** $PdCl_2(PPh_3)_2$ (1.55 g, 2.21 mmol) was added to a solution of alkyne **17** (5.01 g, 22.1 mmol) in a 500 mL flask in anhydrous THF (200 mL). The mixture was cooled to 0 °C and *n*-$Bu_3SnH$ (17.9 mL, 66.3 mmol) was added dropwise. The mixture was stirred for 45 min at 0 °C, at which point the resulting mixture was concentrated to yield a black crude oil. The material was extracted into hexanes, filtered through a pad of Celite and was eluted with hexanes. The elutant was concentrated on a rotary evaporator, and this process was repeated twice until a clear black solution was achieved. Pure vinylstannane **2** (5.72 g, 50%) was obtained as a mixture of 1:5 α:β regioisomers by flash chromatography, eluting with a gradient of hexanes to $CH_2Cl_2$ to 1:20 $Et_2O/CH_2Cl_2$. The desired regioisomer can be obtained in 95+% purity by additional flash chromatography, eluting with a gradient of hexanes to $CH_2Cl_2$ to 1:20 $Et_2O/CH_2Cl_2$.

**Alternate Procedure using Figueroa's Catalyst.**

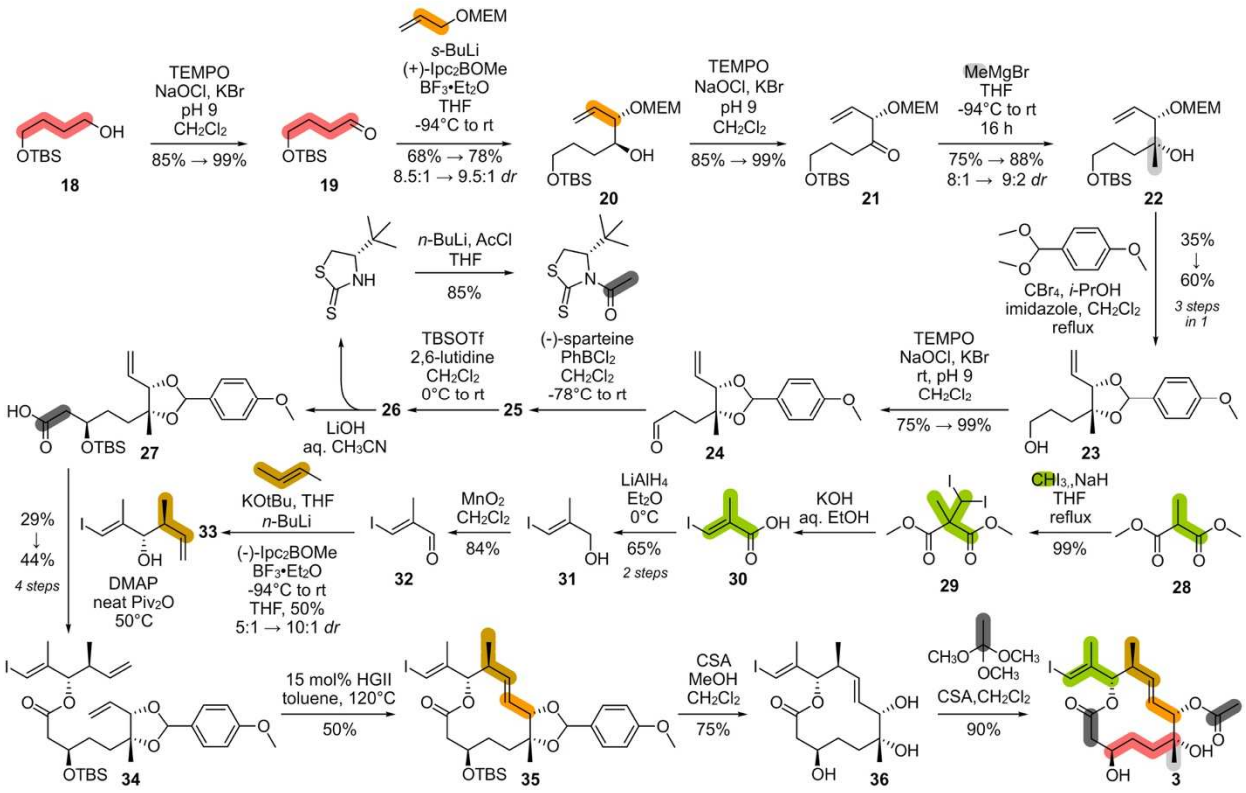



Alkyne **17** (5.01 g, 22.1 mmol) in a 500 mL flask was dissolved in benzene (200 mL) and cooled to -78 °C. *n*-$Bu_3SnH$ (17.9 mL, 66.3 mmol) was added dropwise. Figueroa's catalyst ($MoI_2(CO)_2(CNAr^{Dipp2})_2$) (*31*) was added as a solid. The resulting frozen red mixture was slowly thawed with stirring to rt over 4 h. The mixture was concentrated on a rotary evaporator. Pure vinylstannane **2** (11.3 g, 55%) was obtained as a 1:10 α:β regioisomers by flash chromatography, eluting with a gradient of hexanes to $CH_2Cl_2$ to 1:20 $Et_2O/CH_2Cl_2$.

*Note 1: The unwanted epoxide diastereomer byproduct is also removed by chromatography.*

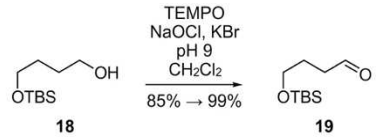
Vinylstannane **2**: TLC (1:10 $Et_2O$/hexanes): $R_f$ = 0.28 (CAM stain); $^1H$ NMR (500 MHz, $C_6D_6$) δ 6.27 (dd, *J* = 19.1, 6.8 Hz, 1H), 6.19 (d, *J* = 19.1 Hz, 1H), 3.45 (m, 1H), 3.23 (s, 3H), 3.16 (m, 1H), 3.07 (dd, *J* = 8.0, 2.3 Hz, 1H), 2.73 (dd, *J* = 4.4, 2.3 Hz, 1H), 2.51 (td, *J* = 6.9, 5.2 Hz, 1H), 1.61 (m, 8H), 1.39 (m, 8H), 1.19 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 7.1 Hz, 3H), 1.00 (d, *J* = 8.1 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 12H), 0.86 (t, *J* = 7.4 Hz, 3H); $^{13}C$ NMR (125 MHz, $C_6D_6$) δ 150.8, 129.0, 83.7, 73.1, 59.3, 57.8, 57.7, 46.1, 39.3, 29.6, 27.7, 23.9, 16.2, 14.0, 10.9, 10.0, 9.8; FTIR (film) $\nu_{max}$ 3454, 3310, 2973, 2937, 2890, 1459, 1101, 840 $cm^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{25}H_{50}O_3Sn$ $[M+H]^+$ 519.2843, found 519.2839; $[\alpha]^{25}_D$ = +12.3 ° (c = 1.0, $CH_2Cl_2$).

**C. Procedures for the synthesis of core 3.** A twelve step sequence optimized from published methods (*1*) was developed to prepare **3** at gram scale, beginning with commercially available **18** (Scheme 2) and shown below.




Alcohol **22** was prepared in hectogram quantities. Each 20 g batch of alcohol **22** produced 6 g of **27** with a total of 90 g of **27** synthesized to date. Each 6 g batch of acid **27** then yielded 1.1 g of core **3** with a total of 18 g of **3** synthesized to date.

**Oxidation of 18 to aldehyde 19**




**Reagents:**

TEMPO, 99% (Oakwood Chemical): used without further purification

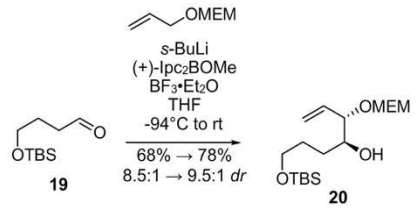
KBr (Spectrum Chemical Mfg. Corp.): used without further purification

NaOCl 2M, 10-15% active chlorine (Spectrum Chemical Mfg. Corp.): used without further purification

**4-((*tert*-Butyldimethylsilyl)oxy)butanal (19).** A solution of KBr (6.99 g, 58.7 mmol) in $H_2O$ (60 mL) was added to a 3 L flask containing a solution of **18** (100 g, 489 mmol) in $CH_2Cl_2$ (1 L) followed by satd. $NaHCO_3$ (100 mL) and TEMPO (2.29 g, 14.7 mmol). The mixture was cooled to 0 °C and a mixture of NaOCl (2 M, 318 mL, 636 mmol) and satd. $NaHCO_3$ (300 mL) was added in portions *via* a dropping funnel. The mixture was allowed to warm to rt and stirred for 3 h. The mixture was extracted with $CH_2Cl_2$ (3 × 250 mL). The combined organic phases were washed with $H_2O$ (500 mL), brine (500 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Aldehyde **19** (100 g, 99%) was obtained as a clear oil without further purification. Characterization data matched literature values.

Aldehyde **19**: TLC (1:10 EtOAc/hexanes): $R_f$ = 0.20 ($KMnO_4$); $^1$H NMR (500 MHz, $CDCl_3$) δ 9.79 (t, *J* = 1.7 Hz, 1H), 3.65 (t, *J* = 6.0 Hz, 2H), 2.50 (td, *J* = 7.1, 1.7 Hz, 2H), 1.86 (tt, *J* = 7.1, 5.9 Hz, 2H), 0.90 (m, 9H), 0.04 (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 202.5, 62.1, 40.8, 25.9, 25.5, 18.2, -5.4; LCMS (ES-API) *m/z* calcd. for $C_{10}H_{22}O_2Si$ $[M+1]^+$: 203.14.

**Brown addition to aldehyde 19**




**Reagents:**

*s*-BuLi, 1.4 M in cyclohexane (Sigma-Aldrich): used without further purification

(+)-B-Methoxydiisopinocampheylborane, 99% (Sigma-Aldrich): used without further purification.

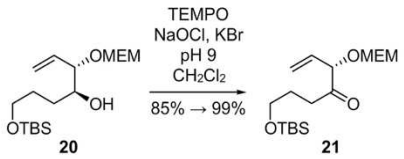
$BF_3$•$Et_2O$, 46.5% $BF_3$ (Alfa Aesar): used without further purification

**(8*S*,9*S*)-14,14,15,15-Tetramethyl-8-vinyl-2,5,7,13-tetraoxa-14-silahexadecan-9-ol (20).** A solution of *s*-BuLi (1.4 M, 353 mL, 494 mmol) was added dropwise over 30 min to a 3 L three-necked flask containing a solution of MEM-protected allyl alcohol (86.7 g, 593 mmol) in anhydrous THF (1 L) cooled to -78 °C. The resulting solution was stirred at -78 °C for 1 h followed by addition of a solution of (+)-B-methoxydiisopinocampheylborane (156 g, 494 mmol) in anhydrous THF (500 mL). The resulting clear mixture was stirred again at -78 °C for 1 h. $BF_3$•$Et_2O$ (79.3 mL, 642 mmol) was added followed by an addition of a solution of 4-((*t*-butyldimethylsilyl)oxy)butanal (**19**) (100 g, 494 mmol) in anhydrous THF (200 mL). The mixture was stirred at -78 °C for 3 h and then warmed to rt overnight. After cooling to 0 °C, satd. $NH_4Cl$ (500 mL) was added to the mixture, which was extracted with $CH_2Cl_2$ (3 × 250 mL). The combined organic phases were washed with $H_2O$ (500 mL), brine (500 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure alcohol **20** (134 g, 78%) was obtained in 90.5% *dr* as

determined by chiral HPLC by flash chromatography, eluting with a gradient of heptane to 1:1 EtOAc/heptane.

Alcohol **20**: TLC (1:5 EtOAc/hexanes): $R_f$ = 0.25 (CAM stain); $^1$H NMR (500 MHz, $CDCl_3$) δ 5.68 (ddd, *J* = 17.3, 10.5, 8.0 Hz, 1H), 5.32 (m, 2H), 4.79 (d, *J* = 7.0 Hz, 1H), 4.70 (d, *J* = 7.0 Hz, 1H), 3.91 (t, *J* = 7.9 Hz, 1H), 3.83 (ddd, *J* = 10.9, 5.3, 3.5 Hz, 1H), 3.64 (m, 3H), 3.55 (ddd, *J* = 5.3, 3.6, 1.9 Hz, 2H), 3.39 (s, 3H), 2.98 (bs, *J* = 3.5 Hz, 1H), 1.71 (m, 1H), 1.63 (m, 2H), 1.40 (m, 1H), 0.88 (s, 9H), 0.04 (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 134.9, 120.0, 93.1, 81.6, 73.3, 71.7, 67.5, 63.3, 59.2, 29.5, 29.0, 26.1, 18.5, -5.2; FTIR (film) $\nu_{max}$ 3347, 2927, 2856, 1616, 1250, 1021 $cm^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{17}H_{36}O_5SiNa$ $[M+Na]^+$: 371.2224, found 371.2223; $[\alpha]^{25}_D$ = +51.5 ° (c = 1.0, $CH_2Cl_2$).

**Oxidation of 20 to ketone 21**




**Reagents:**

TEMPO, 99% (Oakwood Chemical): used without further purification

KBr (Spectrum Chemical Mfg. Corp.): used without further purification

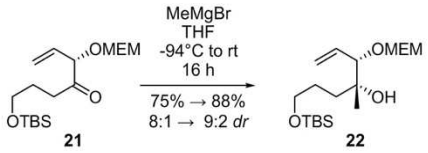
NaOCl 2 M, 10-15% active chlorine (Spectrum Chemical Mfg. Corp.): used without further purification

**(*S*)-14,14,15,15-Tetramethyl-8-vinyl-2,5,7,13-tetraoxa-14-silahexadecan-9-one (21)**. A solution of KBr (3.65 g, 30.6 mmol) in $H_2O$ (100 mL), satd. $NaHCO_3$ (250 mL) and TEMPO (3.99 g, 25.5 mmol) were added sequentially to a 2 L flask containing a solution of **20** (89.0 g, 255 mmol) in $CH_2Cl_2$ (400 mL). The mixture was cooled to 0 °C and a solution of NaOCl (2 M, 255 mL, 511 mmol) and satd. $NaHCO_3$ (300 mL) were added in portions (20 mL at a time) while maintaining the temperature below 0 °C. The mixture was warmed to rt and stirred for 2 h. The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (2 × 200 mL). The combined organic phases were washed with brine (500 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Ketone **21** (88.0 g, 99%) was obtained without further purification.

Ketone **21**: TLC (1:3 EtOAc/hexanes): $R_f$ = 0.40 (CAM stain); $^1$H NMR (500 MHz, $CDCl_3$) δ 5.77 (ddd, *J* = 17.2, 10.4, 6.8 Hz, 1H), 5.46 (dt, *J* = 17.2, 1.3 Hz, 1H), 5.36 (dt, *J* = 10.4, 1.0 Hz, 1H), 4.80 (d, *J* = 7.0 Hz, 1H), 4.74 (d, *J* = 7.0 Hz, 1H), 4.62 (dt, *J* = 6.7, 1.2 Hz, 1H), 3.76 (dt, *J* = 11.0,

4.4 Hz, 1H), 3.67 (m, 1H), 3.59 (t, $J$ = 6.1 Hz, 2H), 3.52 (t, $J$ = 4.6 Hz, 2H), 3.37 (s, 3H), 2.62 (m, 2H), 1.76 (m, 2H), 0.87 (s, 9H), 0.02 (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 208.2, 132.6, 120.2, 93.7, 82.7, 71.8, 67.5, 62.1, 59.2, 34.8, 26.4, 26.0, 18.4, -5.2; FTIR (film) $\nu_{max}$ 2954, 2929, 2857, 1720, 1472, 1256, 1101 $cm^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{17}H_{34}O_5SiNa$ $[M+Na]^+$: 369.2068, found 369.2067; $[\alpha]^{25}_D$ = +22.0 ° (c = 1.0, $CH_2Cl_2$).

**Stereoselective Grignard addition to ketone 21**




**Reagents:**

MeMgBr, 3 M solution in $Et_2O$ (Sigma-Aldrich): used without further purification

**(8*S*,9*R*)-9,14,14,15,15-Pentamethyl-8-vinyl-2,5,7,13-tetraoxa-14-silahexadecan-9-ol (22)**. MeMgBr (3 M, 462 mL, 1.39 mmol) was added dropwise to a 5 L reaction flask containing a solution of ketone **21** (160 g, 462 mmol) in anhydrous THF (1.5 L) at -94 °C. The mixture was stirred at -94 °C for 2 h, allowed to warm to rt and then stirred for an additional 16 h. After re-cooling to -78 °C, satd. $NH_4Cl$ (500 mL) was added to the mixture dropwise. The mixture was diluted with $H_2O$ (1 L) and extracted with *t*-butyl methyl ether (2 × 500 mL). The combined organic phases were washed with $H_2O$ (500 mL) and brine (500 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. The crude was filtered through a pad of Celite eluting with EtOAc, and the elutants were concentrated on a rotary evaporator. Alcohol **22** (155 g, 88%) was obtained in a 90% *dr* as determined by chiral HPLC without further purification.

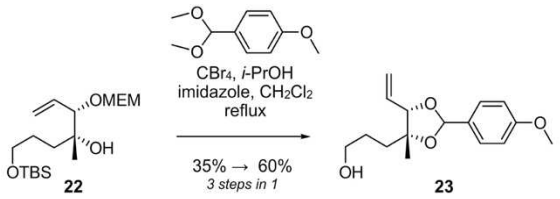
*Note 1: Average batches of crude **22** contained <5% of starting material **21**.*

*Note 2: Solutions of MeMgBr in $Et_2O$ gave better yields and selectivity as compared to that in THF (≤ 70% yield, ≤90% de).*

Alcohol **22**: TLC (1:5 EtOAc/hexanes): $R_f$ = 0.30 (CAM stain); $^1$H NMR (500 MHz, $CDCl_3$) δ 5.73 (ddd, *J* = 17.2, 10.5, 8.1 Hz, 1H), 5.29 (ddd, *J* = 14.7, 1.9, 0.8 Hz, 1H), 5.26 (ddd, *J* = 21.6, 1.9, 0.8 Hz, 1H), 4.75 (d, *J* = 7.0 Hz, 1H), 4.70 (d, *J* = 7.0 Hz, 1H), 3.86 (d, *J* = 8.0 Hz, 1H), 3.82

(dd $J$ = 5.2, 3.7 Hz, 1H), 3.80 (dd, $J$ = 5.5, 3.4 Hz, 1H), 3.61 (m, 3H), 3.53 (dd, $J$ = 3.3, 2.3 Hz 1H), 3.52 (dd, $J$= 3.3, 1.9 Hz, 1H), 3.36 (s, 3H), 2.69 (s, 1H), 1.64 (m, 1H), 1.59 (m, 2H), 1.42 (m, 1H), 1.14 (s, 3H), 0.87 (s, 9H), 0.02 (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 134.3, 120.3, 93.3, 87.5, 73.4, 71.8, 67.5, 63.9, 59.1, 33.9, 26.6, 26.1, 23.6, 18.5, -5.2; FTIR (film) $\nu_{max}$ 2954, 2929, 2857, 2359, 1472, 1255, 1097, 1037 $cm^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{18}H_{38}O_5SiNa$ $[M+Na]^+$: 385.2401, found 385.2403; $[\alpha]^{25}_D$ = +57.3 ° (c = 1.0, $CH_2Cl_2$).

**Conversion of 22 to alcohol 23**




**Reagents:**

$CBr_4$, 99% (TCI Chemicals): used without purification

Imidazole, 99% (Sigma-Aldrich): used without purification

*p*-Anisaldehyde dimethyl acetal, 98% (Acros Organics): used without further purification

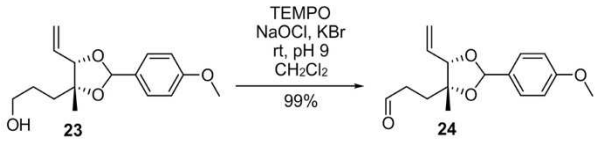
*i*-PrOH, 99% (Fischer Scientific): used as provided without further drying

**3-((4*R*,5*S*)-2-(4-Methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)propan-1-ol (23).** $CBr_4$ (27.7 g, 63.8 mmol) and imidazole (500 mg, 7.34 mmol) were added to a solution of alcohol **22** (20.0 g, 55.2 mmol) in *i*-PrOH (2 L). The mixture was heated to reflux and stirred overnight at 100 °C, at which point an orange color appeared, and NMR analyses indicated complete consumption of starting material. The mixture was cooled to rt and concentrated on a rotary evaporator. The resulting brown crude oil was immediately taken up in anhydrous $CH_2Cl_2$ (700 mL) and purged with Ar. Anisaldehyde dimethyl acetal (20.0 mL, 117 mmol) was added in one aliquot, and the mixture turned purple after 10 min of stirring at rt. The reaction was stirred overnight. Satd. $NaHCO_3$ (100 mL) was added, and the mixture was extracted with $CH_2Cl_2$ (2 × 500 mL). The organics were combined and concentrated on a rotary evaporator to yield a brown oil. Pure alcohols **23** (9.21 g, 60%) was obtained by flash chromatography, eluting with a gradient of hexanes to 1:3 EtOAc/hexanes.

*Note 1: Batches of **23** were obtained in an inconsequential mixture of acetal diastereomers, as noted in its structure.*

Alcohols **23**: TLC (1:1 EtOAc/hexanes): $R_f$ = 0.37 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 7.55 (d, *J* = 8.6 Hz, 2H), 7.50 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 8.6 Hz, 2H), 6.16 (s, 1H), 5.91 (s, 1H), 5.79 (m, 1H), 5.71 (m, 1H), 5.30 (dt, *J* = 3.5, 1.6 Hz, 1H), 5.27 (dt, *J* = 3.5, 1.6 Hz, 1H), 5.07 (dd, *J* = 1.7, 1.7 Hz, 1H), 5.05 (dd, *J* = 1.7, 1.7 Hz, 1H), 4.17 (dt, *J* = 6.7, 1.2 Hz, 1H), 4.09 (dt, *J* = 6.7, 1.2 Hz, 1H), 3.42 (m, 2H), 3.38 (m, 2H), 3.27 (s, 3H), 3.26 (s, 3H), 1.73 (m, 2H) 1.53 (m, 2H), 1.33 (m, 1H) 1.19 (s, 3H) 1.17 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 160.8, 160.6, 133.9, 133.8, 133.8, 133.7, 132.8, 131.1, 128.5, 128.3, 117.9, 117.9, 117.8, 117.6, 114.0, 113.9, 107.7, 102.5, 102.2, 96.3, 88.0, 86.5, 86.2, 86.2, 83.6, 82.5, 82.4, 81.8, 63.1, 63.0, 63.0, 58.4, 58.4, 33.8, 32.7, 31.3, 29.9, 28.6, 27.5, 27.3, 27.2, 27.1, 22.9, 22.5, 22.0, 21.8; FTIR (film) $\nu_{max}$ 3421, 3080, 2938, 1718, 1614, 1516, 1932, 1303, 1249, 1170, 1032 cm$^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{16}H_{22}O_4Na$ $[M+Na]^+$: 301.1410, found 301.1411; $[\alpha]^{25}_D$ = +14.8 ° (c = 0.4, $CH_2Cl_2$).

**Oxidation of 23 to aldehyde 24**




**Reagents:**

TEMPO, 99% (Oakwood Chemical): used without further purification

KBr (Spectrum Chemical Mfg. Corp.): used without further purification

NaOCl, 2 M, 10-15% active chlorine (Spectrum Chemical Mfg. Corp.): used without further purification

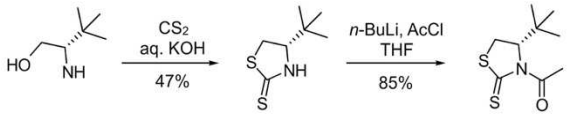
**3-((4*R*,5*S*)-2-(4-Methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)propanal (24).** A solution of KBr (0.699 g, 5.87 mmol) in $H_2O$ (60.0 mL) was added to a 2 L flask containing a solution of alcohol **23** (11.2 g, 40.2 mmol) in $CH_2Cl_2$ (750 mL) followed by satd. $NaHCO_3$ (75 mL) and TEMPO (229 mg, 1.47 mmol). The mixture was cooled to 0 °C, and a mixture of NaOCl (2 M, 32.0 mL, 63.6 mmol) and satd. $NaHCO_3$ (50 mL) was added in portions (20 mL). The mixture was allowed to warm to rt. After stirring at rt for 3 h, the mixture was extracted with $CH_2Cl_2$ (3 × 250 mL). The combined organic phases were washed with $H_2O$ (500 mL) and brine (500 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Aldehyde **24** (11 g, 99%) was used without further purification.

*Note 1*: *Aldehydes **24** are susceptible to rearrangement when purified over unbuffered silica gel.*

Aldehydes **24**: TLC (1:1 EtOAc/hexanes): $R_f$ = 0.70 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 9.39 (s, 1H), 9.29 (s, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 4.3 Hz, 2H), 6.79 (d, *J* = 4.3 Hz, 2H), 6.02 (s, 1H), 5.83 (s, 1H), 5.70 (m, 1H), 5.65 (m, 1H), 5.28 (dt, *J* =

13.0, 1.6 Hz, 1H), 5.24 (dt, *J* = 12.8, 1.8 Hz, 1H), 5.04 (dt, *J* = 4.7, 1.5 Hz, 1H), 5.02 (dt, *J* = 4.6, 1.4 Hz, 1H), 4.10 (dt, *J* = 6.6, 1.3 Hz, 1H), 4.02 (dt, *J* = 6.6, 1.2 Hz, 1H), 3.27 (s, 3H), 3.25 (s, 3H), 2.26 (m, 2H), 2.04 (m, 3H), 1.87 (ddd, *J* = 13.0, 9.8, 5.5 Hz, 1H), 1.41 (ddd *J* = 14.3, 9.7, 5.5 Hz, 1H), 1.00 (s, 3H), 0.99 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 200.5, 200.4, 160.8, 160.6, 133.2, 133.1, 132.7, 130.8, 128.4, 128.4, 118.2, 118.2, 114.0, 113.9, 102.5, 102.2, 87.6, 87.5, 82.7, 81.4, 54.8, 38.9, 38.4, 29.3, 25.7, 22.6, 21.9; FTIR (film) $\nu_{max}$ 2935, 2838, 2730, 1724, 1612, 1515, 1392, 1257, 1249, 1172, 1114, 1033, 1006 cm$^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{16}H_{20}O_4Na$ $[M+Na]^+$: 299.3181, found 299.3175; $[\alpha]^{25}_D$ = +35.7 ° (c = 1.0, $CH_2Cl_2$).

**Synthesis of auxiliary 39.** A two step sequence to prepare auxiliary **(***S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one beginning with commercially available (*S*)-4-(*tert*-Butyl)thiazolidine-2-thione, was optimized from developed methods (*32*).




**Reagents:**

KOH, 99% (Fischer Scientific): used without further purification

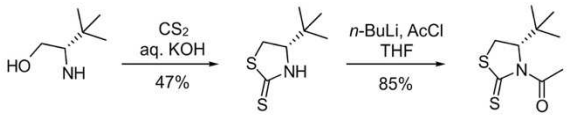
$CS_2$, 98% (Alfa Aesar): used without further purification

**Preparation of (*S*)-4-(*tert*-butyl)thiazolidine-2-thione**

**(*S*)-4-(*tert*-Butyl)thiazolidine-2-thione.** KOH (2.63 kg, 46.9 mol) was dissolved in $H_2O$ (9 L) and stirred in a 20 L reactor equipped with a mechanical stirrer and two reflux condensers. (*S*)-2-Amino-3,3-dimethylbutan-1-ol (250 g, 2.13 mol) was added followed by dropwise addition of $CS_2$ (1.03 L, 17.1 mol). The mixture was heated at 95 °C for 16 h. After cooling to 50 °C, an additional portion of $CS_2$ (1.03 L, 17.1 mol) was added dropwise, and the mixture was heated at 70 °C for 16 h. The mixture was cooled to 50 °C, and a third portion of $CS_2$ (500 mL) was added dropwise. The mixture was heated to 65 °C and stirred for 48 h. After cooling the mixture to rt, the solids were collected by filtration and washed with $H_2O$ (2 L). The white solids were dried at rt by airflow. Pure (*S*)-4-(*tert*-butyl)thiazolidine-2-thione (176 g, 47%) was obtained by flash chromatography, eluting with $CH_2Cl_2$.

**(*S*)-4-(*tert*-Butyl)thiazolidine-2-thione**: TLC ($CH_2Cl_2$): $R_f$ = 0.70, UV; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.58 (s, 1H), 4.01 (t, $J$ = 9.6, 8.5, 1.2 Hz, 1H), 3.41 (m, 2H), 1.01 (s, 9H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 73.3, 34.5, 34.4, 25.9; LCMS (ES-API) *m/z* calcd. for $C_7H_{13}NS_2$ $[M+1]^+$: 176.05.

**Acetylation of (*S*)-4-(*tert*-butyl)thiazolidine-2-thione**




**Reagents:**

*n*-BuLi, 2.5 M in hexane (Acros Organics): used without further purification

Acetyl chloride, 98% (Sigma-Aldrich): used without further purification

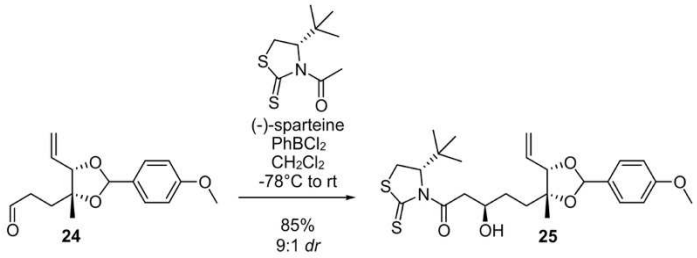
**(*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one.** *n*-BuLi (2.5 M, 460 mL, 1.15 mol) was added dropwise to a 5 L flask containing a solution of **(***S*)-4-(*tert*-butyl)thiazolidine-2-thione (182 g, 1.04 mol) in anhydrous THF (1.8 L) at -78 °C. The mixture was stirred at −78 °C for 30 min. Acetyl chloride (89.0 mL, 1.25 mol) was added dropwise, and the mixture was stirred at -78 °C for 1.5 h. The mixture was then warmed to rt, stirred for 1 h, recooled to 0 °C and quenched with satd. $NH_4Cl$ (800 mL). The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (2 × 200 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure (*S*)-1-(4-(*tert*-butyl)-2-thioxothiazolidin-3-yl)ethan-1-one (191 g, 85%) was obtained by flash chromatography, eluting with a gradient of heptane to $CH_2Cl_2$.

*Note 1: This procedure was repeated to deliver a total of 186 g of (S)-1-(4-(tert-butyl)-2-thioxothiazolidin-3-yl)ethan-1-one, which was routinely recycled throughout this program.*

**(*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one**: TLC (1:1 $CH_2Cl_2$/heptane): $R_f$ = 0.80, UV; $^1$H NMR (500 MHz, $CDCl_3$) δ 5.28 (dd, *J* = 8.4, 1.0 Hz, 1H), 3.51 (dd, *J* = 11.8, 8.5 Hz,

1H), 3.08 (d, $J$ = 11.0 Hz, 1H), 2.77 (s, 3H), 1.03 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 205.3, 170.3, 72.0, 38.0, 30.5, 26.9, 26.8; LCMS (ES-API) *m/z* calcd. for $C_9H_{15}NS_2$ $[M+1]^+$: 217.06.

**Stereoselective aldol addition of 24 to 25**




**Reagents:**

Dichlorophenylborane, 97% (Acros Organics): used without further purification

(-)-Sparteine, 98% (TCI Chemicals), S0461: used without further purification

(*S*)-1-(4-(*tert*-butyl)-2-thioxothiazolidin-3-yl)ethan-1-one: dried *via* azeotropic removal of toluene by rotary evaporation

**(3*R*)-1-((*R*)-5-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)-3-hydroxy-5-((4*R*,5*S*)-2-(4-methoxy-phenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentan-1-one (25).** (*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one (11.7 g, 53.7 mmol) was added to a 3 L flask and dissolved in anhydrous $CH_2Cl_2$ (800 mL). An Ar atmosphere was introduced, and dichlorophenylborane (6.20 mL, 47.8 mmol) was added at rt and stirred for 15 min. (-)-Sparteine (21.9 mL, 95.5 mmol) was added neat, at which point the mixture appeared cloudy but became homogeneous upon further stirring within 1 min. After stirring at rt for 30 min the mixture was cooled to -78 °C, and aldehyde **24** (11.0 g, 39.8 mmol) in a solution of anhydrous $CH_2Cl_2$ (80 mL) was added dropwise over 15 min. The mixture was stirred at -78 °C for 1 h and slowly warmed to 0 °C over 3 h, at which point NMR analyses indicated complete consumption of starting material. The mixture was quenched with satd. $NaHCO_3$ (200 mL), and the organic phase was separated. The aqueous phase was washed with $CH_2Cl_2$ (200 mL), and the organic phases were combined, dried over $Na_2SO_4$, filtered

and concentrated on a rotary evaporator. Alcohol **25** (16.7 g, 85%) was obtained in a 9:1 *dr* as a yellow oil by vacuum filtration over neutral silica gel eluting with $CH_2Cl_2$ (1.5 L, elution of unreacted auxiliary) and 1:1 EtOAc/hexanes (1.5 L, elution of product).

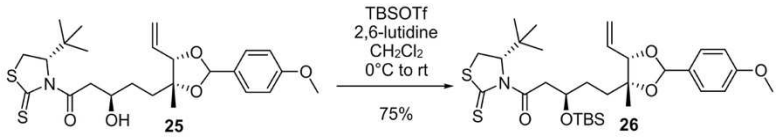
*Note 1: Aldol adduct **25** was susceptible to hydrolysis when purified on untreated silica gel. Flash chromatography on neutral silica gel eluting with a gradient of hexanes to 1:1 EtOAc/hexanes can be used to obtain **25** in 95%+ purity. In practice this material is sufficiently clean after passing it through a vacuum funnel plug of neutral silica.*

*Note 2: Minor unwanted C3 isomers were observable by NMR and carried forward.*

Alcohols **25**: TLC (1:3 EtOAc/hexanes): $R_f$ = 0.23 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 7.62 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.34 (m, minor), 6.86 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 8.7 Hz, 2H), 6.78 (d, *J* = 8.8 Hz, minor), 6.26 (s, 1H), 5.94 (s, 1H), 5.84 (m, 2H), 5.76 (m, minor), 5.33 (dt, *J* = 2.0, 1.0 Hz, 1H), 5.29 (dt, *J* = 2.0, 1.0 Hz, 1H), 5.27 (dd, *J* = 1.9, 1.3 Hz, 1H, minor), 5.23 (dd, *J* = 1.9, 1.3 Hz, minor), 5.10 (dt, *J* = 2.0, 1.2 Hz, 1H), 5.07 (dt, *J* = 2.1, 1.2 Hz, 1H), 5.06 (dd, *J* = 1.9, 1.2 Hz, minor), 5.05 (d, *J* = 0.8 Hz, 1H), 5.04 (dd, *J* = 1.9, 1.2 Hz, minor), 5.03 (d, *J* = 7.6 Hz, 1H), 4.98 (d, *J* = 7.6 Hz, 1H), 4.87 (d, *J* = 0.8 Hz, 1H), 4.21 (dt, *J* = 6.7, 1.3 Hz, 1H), 4.12 (d, *J* = 6.7, 1.2 Hz 1H), 4.09 (m, 2H), 4.02 (d, *J* = 9.4 Hz, minor), 3.99 (d, *J* = 9.4 Hz, minor), 3.87 (t, *J* = 1.2 Hz, minor), 3.85 (t, *J* = 1.2 Hz, minor), 3.61 (m, 2H), 3.30 (d, *J* = 2.9 Hz, minor) 3.29 (s, minor), 3.28 (s, 3H), 3.27 (s, 3H), 3.23 (d, *J* = 2.6 Hz, minor) 3.19 (d, *J* = 2.6 Hz, minor), 2.49 (m, 2H), 2.45 (m, minor), 2.27 (ddd, *J* = 13.9, 11.8, 4.4 Hz, 1H), 2.21 (ddd, *J* = 13.5, 11.8, 4.6 Hz, 1H), 2.01 (m, 2H), 1.93 (m, 1H), 1.89 (m, minor), 1.83 (m, minor), 1.63 (m, 2H), 1.44 (ddd, *J* = 13.5, 11.5, 4.8 Hz, 1H), 1.31 (m, 1H), 1.23 (s, 3H), 1.20 (s, 3H), 1.10 (s, minor) 0.74 (s, 3H), 0.73 (s, minor), 0.71 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 205.2, 205.2, 173.0, 173.0, 172.4, 172.0, 160.8, 160.6, 159.6, 135.8, 133.9, 133.8, 133.8, 133.0, 131.2, 128.7, 128.4, 128.2, 128.1,

127.6, 118.1, 118.0, 117.9, 114.2, 114.1, 113.9, 113.5, 102.8, 102.3, 93.7, 88.0, 86.5, 86.3, 83.4, 82.3, 81.8, 72.1 72.0, 72.0, 70.6, 68.8, 68.8, 68.8, 54.8, 54.8, 47.3, 45.8, 45.7, 45.5, 37.9, 37.9, 33.2, 31.3, 31.1, 30.9, 30.8, 29.8, 29.8, 29.4, 26.7, 22.8, 22.2, 22.0; FTIR (film) $\nu_{max}$ 3640, 3427, 2966, 2877, 1685, 1594, 1501, 1452, 1352, 1338, 1320, 1248, 1155, 1140, 1075, 1024 $cm^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{25}H_{35}NO_5S_2Na$ $[M+Na]^+$: 516.6689, found 516.6694; $[\alpha]^{25}_D$ = +245 ° (c = 1.0, $CH_2Cl_2$).

**TBS protection of 25 to adduct 26**




**Reagents:**

2,6-Lutidine, redistilled, 99% (Chem-Impex Int.): used without further purification

TBSOTf, 99% (Chem-Impex Int.): used without further purification

**(3*R*)-1-((*R*)-5-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)-3-((*tert*-butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentan-1-one (26)**. Alcohol **25** (15.0 g, 30.4 mmol) was dissolved in anhydrous $CH_2Cl_2$ (600 mL) in a 2 L flask followed by addition of 2,6-lutidine (18.54 mL, 159 mmol). The mixture was purged with Ar and cooled to 0 °C. TBSOTf (27.4 mL, 119 mmol) was added dropwise, and the mixture was warmed to rt and stirred overnight, at which point NMR analyses indicated complete consumption of starting material. The solution was quenched with addition of solid $NaHCO_3$ (5 g) and stirred for 15 min. The mixture was concentrated to 50 mL under rotary evaporation. Adduct **26** (13.9 g, 75%) was obtained as a yellow oil by vacuum filtration over neutral silica gel eluting with $CH_2Cl_2$.

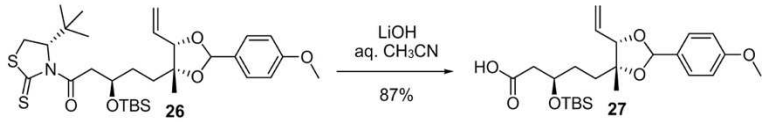
*Note 1: **26** can be further purified (95+%) via flash chromatography on neutral silica gel eluting with a gradient of hexanes to 1:10 EtOAc/hexanes. In practice the material is sufficiently clean to proceed to the next step without chromatography.*

*Note 2: Minor unwanted C3 isomers were carried forward.*

Adducts **26:** TLC ($CH_2Cl_2$): $R_f$ = 0.40 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 7.61 (d, *J* = 8.6 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.81 (d, *J* = 8.7 Hz, 2H), 6.31 (s, 1H),

5.94 (s, 1H), 5.87 (m, 2H), 5.36 (dt, *J* = 3.1, 1.6 Hz, 1H), 5.33 (dt, *J* = 2.9, 1.5 Hz, 1H), 5.14 (m, minor), 5.12 (t, *J* = 1.5 Hz, 1H), 5.11 (t, *J* = 1.5 Hz, 1H), 5.10 (t, *J* = 1.4 Hz, 1H), 5.09 (t, *J* = 1.5 Hz, 1H), 5.06 (d, *J* = 7.9 Hz, 1H), 5.03 (d, *J* = 7.6 Hz, 1H), 4.97 (d, *J* = 8.1 Hz, minor), 4.54 (m, 1H), 4.46 (m, 1H), 4.23 (dt, *J* = 6.4, 1.3 Hz, 1H), 4.14 (dt, *J* = 6.5, 1.3 Hz, 1H), 3.80 (dd, *J* = 17.2, 5.9 Hz, 1H), 3.76 (m, minor), 3.73 (m, 1H), 3.69 (m, 1H), 3.66 (m, minor), 3.61 (dd, *J* = 17.3, 5.3 Hz, 1H), 3.31 (s, 3H), 3.30 (s, minor), 3.26 (s, 3H), 2.56 (ddd, *J* = 11.8, 10.9, 8.3 Hz, 1H), 2.54 (m, minor), 2.17 (m, 1H), 2.03 (m, 1H), 1.93 (m, 2H), 1.90 (m, minor), 1.50 (m, 1H), 1.41 (ddd, *J* = 13.5, 11.4, 5.1 Hz, 1H), 1.28 (s, 3H), 1.26 (s, minor), 1.26 (s, 3H), 1.23 (s, minor), 1.03 (s, 3H), 1.03 (s, minor), 1.00 (s, 9H), 0.99 (s, minor), 0.78 (s, minor), 0.77 (s, 9H), 0.27 (s, minor), 0.22 (s, 3H), 0.21 (s, minor), 0.19 (s, 3H), 0.19 (s, 3H), 0.16 (s, minor), 0.14 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 205.1, 205.0, 170.9, 170.9, 170.8, 160.8, 160.6, 133.9, 133.7, 133.6, 133.0, 131.3, 128.7, 128.4, 128.2, 127.9, 127.7, 127.5, 118.0, 117.9, 114.1, 113.9, 102.7, 102.4, 88.0, 87.9, 86.1, 83.6, 83.5, 82.4, 82.2, 72.2, 72.1, 70.3, 69.5, 69.4, 54.8, 54.8, 53.3, 46.4, 46.1, 37.9, 37.8, 34.0, 32.8, 32.0, 31.5, 29.9, 29.8, 28.9, 26.8, 26.2, 26.2, 25.9, 22.7, 22.2, 18.4, 18.3, -3.4, -4.2, -4.2, -4.3, -4.3; FTIR (film) $\nu_{max}$ 2966, 2858, 1697, 1369, 1319, 1265, 1261, 1195, 1037, 1029 cm$^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{31}H_{49}NO_5S_2SiNa$ $[M+Na]^+$: 630.2689, found 630.2691; $[\alpha]^{25}_D$ = +210 ° (c = 1.0, $CH_2Cl_2$).

**Saponification of adduct 26 to acid 27**




**Reagents:**

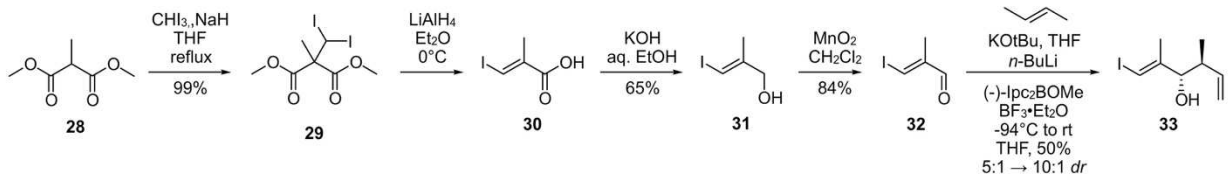
LiOH•$H_2O$, 98% (Alfa Aesar): used without further purification

**(3*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentanoic acid (27).** LiOH•$H_2O$ (5.01 g, 119 mmol) was added to a 3 L flask containing a solution of **26** (13.5 g, 22.2 mmol) in 20% aq $CH_3CN$ (500 mL). The mixture was stirred at rt overnight, at which point the deep yellow color dissipated into a light brown solution. The mixture was diluted with $H_2O$ (500 mL) and $Et_2O$ (600 mL). The aqueous phase was collected, and the organic phase was extracted with $H_2O$ (2 × 400 mL). The aqueous phases were combined, and the pH was adjusted to 6.5 with 1 M HCl. The mixture was extracted into EtOAc (3 × 700 mL), and the organics were combined, dried over $Na_2SO_4$, filtered and concentrated by rotary evaporation. Acid **27** (8.76 g, 87%) was obtained as a colorless oil by vacuum filtration over silica gel eluting with $CH_2Cl_2$ (elution of auxiliary) and 1:5 EtOAc/hexanes (elution of product).

Acids **27**: TLC (1:1 EtOAc/hexanes): $R_f$ = 0.54 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 7.55 (d, *J* = 8.8 Hz, 2H), 7.53 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.20 (s, 1H), 5.92 (s, 1H), 5.81 (ddd, *J* = 17.4, 10.7, 6.6 Hz, 1H), 5.78 (ddd, *J* = 17.4, 10.7, 6.6 Hz, 1H), 5.71 (m, minor), 5.35 (dd, *J* = 1.5, 1.5 Hz, 1H), 5.31 (dd, *J* = 1.5, 1.5 Hz, 1H), 5.29 (m, minor), 5.25 (m, minor), 5.11 (dt, *J* = 3.4, 1.5 Hz, 1H), 5.09 (dt, *J* = 3.3, 1.4 Hz, 1H), 5.08 (m, minor), 5.06 (m, minor), 4.19 (m, 1H), 4.11 (m, 1H) 3.31 (s, 3H), 3.27 (s, 3H), 2.47 (dd, *J* = 15.0, 7.2 Hz, 1H), 2.39 (dd, *J* = 15.0, 7.4 Hz, 1H), 2.31 (dd, *J* = 15.0, 5.0 Hz, 1H), 2.31 (dd, *J* = 15.0, 4.7 Hz, 1H),

1.89 (m, 2H), 1.66 (m, 2H), 1.22 (m, 1H), 1.17 (s, 3H), 1.06 (s, 9H), 0.97 (s, minor), 0.96 (s, 9H), 0.15 (s, 3H), 0.12 (s, minor), 0.11 (s, 3H), 0.08 (s, minor) 0.06 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 177.4, 177.4, 160.8, 160.6, 133.6, 133.5, 132.9, 131.2, 128.5, 128.4, 128.2, 128.0, 127.7, 127.7, 127.6, 118.0, 117.9, 114.0, 114.0, 93.7, 87.9, 86.3, 86.0, 83.3, 82.0, 81.6, 70.0, 69.9, 54.8, 54.8, 32.6, 31.8, 31.4, 30.2, 28.8, 26.6, 26.1, 26.0, 22.6, 22.1, 21.9, 18.3, 18.2, -4.3, -4.4, -4.4, -4.6, -4,6, -4.7; FTIR (film) $\nu_{max}$ 3683, 2958, 2931, 2858, 1731, 1612, 1265, 1250, 1072 cm$^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{24}H_{38}O_6SiNa$ $[M+Na]^+$: 473.2287, found 473.2290; $[\alpha]^{25}_D$ = +11.95 ° (c = 0.8, $CH_2Cl_2$).

**Synthesis of intermediate 33.** A four step sequence was optimized from developed methods (*45*) to prepare aldehyde **32** at multi-gram scale. Conversion of **32** to **33** produced a *dr* of 91%.




**Reagents:**

Dimethyl 2-methylmalonate, 97% (Sigma-Aldrich): used without further purification

NaH, 60% dispersion in mineral oil, (Alfa Aesar): used without further purification

$CHI_3$, 98% (Oakwood Chemicals): used without further purification

KOH, 99% (Fischer Scientific): used without further purification

$LiAlH_4$, 99%. (Sigma-Aldrich): used without further purification

**(*E*)-3-Iodo-2-methylprop-2-en-1-ol (31).** The conversion of **28** to alcohol **31** was completed without purification of **29** and **30**. A solution of dimethyl 2-methylmalonate (**28**) (310 mL, 2.33 mol) in anhydrous THF (800 mL) was added dropwise over 20 min to a suspension of NaH (60% in a mineral oil, 150 g, 3.75 mol) in anhydrous THF (800 mL) in a 10 L reaction vessel. The reaction was stirred at reflux for 1.5 h. A solution of $CHI_3$ (802 g, 2.04 mol) in anhydrous THF (2 L) was added dropwise over 40 min. The mixture was cooled to 50 °C and stirred for 16 h. After cooling to 0 °C, 2 M HCl (1.5 L) was slowly added to the mixture. **CAUTION RAPID HEATING AND BUBBLING**. The phases were separated, and the aqueous phase was extracted with EtOAc (2 × 300 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator to yield diester **29** (1.01 kg, 99%), which was then dissolved in 80% EtOH (2.5 L) in a 5 L flask. KOH (700 g, 12.5 mol) was added dropwise as a solution in $H_2O$ (1 L) over 1 h. The mixture was heated at reflux and stirred for 16 h. After cooling to rt, the mixture was

concentrated on a rotary evaporator. The resulting crude material was acidified to pH 1 with conc. HCl and extracted into $CH_2Cl_2$ (1 L). The organic phase was washed with $H_2O$ (1 L), and the aqueous phase was extracted with $CH_2Cl_2$ (3 × 600 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. The resulting crude acid **30** (289 g, 1.36 mol) was dissolved in anhydrous $Et_2O$ (400 mL) and added dropwise over 20 min to a 3 L three-necked flask containing a suspension of $LiAlH_4$ (76.4 g, 2.01 mol) in anhydrous $Et_2O$ (800 mL) cooled to -20 °C. The mixture was stirred at -20 °C for 1 h, warmed to rt and stirred for a further 2 h. After cooling the mixture to -78 °C, acetone (200 mL) was added dropwise over 30 min, followed by a dropwise addition of 2 M HCl (800 mL) over 1 h. The resulting mixture was filtered through a Büchner filter fitted with Whatman filter paper #1. The phases were separated, and the aqueous phase was extracted with *t*-butyl methyl ether (3 × 1 L). The combined organic phases were washed with brine (3 × 500 mL), dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure alcohol **31** (146 g, 65% over two steps) was obtained by flash chromatography, eluting with a gradient of heptane to $CH_2Cl_2$ in incremental increases of 1:5 $CH_2Cl_2$/heptane. Characterization data matched literature values.

Alcohol **31**: TLC (1:1 $CH_2Cl_2$/heptane): $R_f$ = 0.60 ($KMnO_4$); $^1$H NMR (500 MHz, $CDCl_3$) δ 6.27 (h, $J$ = 1.2 Hz, 1H), 4.11 (bs, 2H), 1.83 (bs, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 147.3, 67.2, 21.5; HR-ES-MS *m/z* calcd. for $C_4H_7IONa$ $[M+Na]^+$: 220.9498, found 220.9499.

**(3*S*,4*S*,*E*)-1-Iodo-2,4-dimethylhexa-1,5-dien-3-ol (33)**. The conversion of alcohol **31** to vinyl iodide **33** was completed without purification of aldehyde **32**. Activated $MnO_2$ (643 g, 7.39 mol) was added to a 2 L three-necked flask containing a solution of **31** (146 g, 739 mmol) in anhydrous $CH_2Cl_2$ (1 L). The mixture was stirred vigorously at rt for 16 h. The mixture was then passed through a pad of Celite, followed by concentration on a rotary evaporator, to yield crude aldehyde

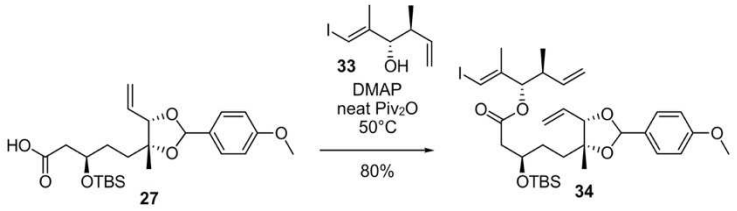
**32** (142.4 g, 84%). (*E*)-But-2-ene (200 mL, 2.00 mol) was condensed and added to a 10 L reaction flask containing anhydrous THF (1.5 L) at -78 °C. KO*t*-Bu (114 g, 1.01 mol) was added, and the mixture was stirred at -78 °C for 30 min. *n*-BuLi (2.5 M in hexane, 400 mL, 1.00 mol) was added dropwise over 15 min, and the resulting yellow mixture was stirred at -78 °C for an additional 30 min. A solution of (-)-B-methoxydiisopinocampheylborane (253 g, 800 mmol) in anhydrous THF (1 L) was added dropwise over 15 min, and the mixture turned clear. After stirring the mixture for 30 min, $BF_3$•$Et_2O$ (170 mL, 1.34 mol) was added dropwise over 10 min, and the mixture was stirred for an additional 10 min. After cooling the mixture to -94 °C, a solution of **32** (121 g, 617 mmol) in anhydrous THF (750 mL) was added dropwise over 45 min. The mixture was allowed to warm to rt and stirred for 16 h. $H_2O$ (2 L) was added, and the mixture was concentrated on a rotary evaporator. Vinyl iodide **33** (78.0 g, 50%) was obtained at a 10:1 *dr* by flash chromatography, eluting with $CH_2Cl_2$.

*Note 1: Efficacy of $MnO_2$ may vary depending on supplier. An alternative procedure involving stirring alcohol **31** with 2 eq. of IBX in DMSO at rt for 30 min will also produce comparable yields of aldehyde **32**.*

*Note 2: Aldehyde **32** is volatile and will evaporate upon exposure to high vacuum.*

Vinyl iodide **33:** TLC ($CH_2Cl_2$): $R_f$ = 0.40 ($KMnO_4$); $^1$H NMR (500 MHz, $CDCl_3$) δ 6.26 (s, 1H), 5.72 (m, 1H), 5.18 (d, *J* = 16.0 Hz, 1H), 5.18 (d, *J* = 11.3 Hz, 1H), 3.87 (dd, *J* = 8.1, 2.9 Hz, 1H), 2.36 (h, *J* = 7.4 Hz, 1H), 1.88 (d, *J* = 2.9 Hz, 1H), 1.82 (bs, 3H), 0.92 (d, *J* = 6.8 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 148.1, 140.0, 117.4, 80.2, 80.0, 42.4, 19.4, 16.6; HR-ES-MS *m/z* calcd. for $C_8H_{13}IONa$ $[M+Na]^+$: 274.9998, found 274.9997; $[\alpha]^{25}_D$ = -23.6 ° (c = 1.0, $CH_2Cl_2$).

**Esterification of acids 27 with alcohol 33 to afford 34**




**Reagents:**

DMAP, 98% (Sigma-Aldrich): used without further purification

Pivalic anhydride, 99% (Alfa Aesar): used without further purification

**(3*S*,4*S*,*E*)-1-Iodo-2,4-dimethylhexa-1,5-dien-3-yl-(3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentanoate (34)**. DMAP (150 mg, 1.22 mmol) and pivalic anhydride (3.71 mL, 18.3 mmol) were added sequentially to a 250 mL flask containing **27** (5.51 g, 12.2 mmol) and alcohol **33** (3.23 g, 12.8 mmol). The mixture was purged with Ar and stirred neat at 50 °C for 8 h. Pivalic anhydride was removed from the mixture under airflow. Crude material was then loaded directly onto silica gel in hexanes and eluted with a gradient of hexanes to 1:10 $Et_2O$/hexanes. Pure esters **34** (6.72 g, 80%) were obtained as a clear oil.

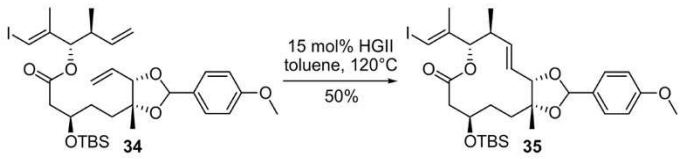
*Note 1: The removal of pivalic anhydride led to improved chromatographic conditions.*

*Note 2: C3 isomers were also removed after chromatography*

Esters **34**: TLC (1:4 $Et_2O$/hexanes): $R_f$ = 0.40 and 0.38 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 7.57 (d, *J* = 8.7 Hz, 2H), 7.55 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 6.24 (s, 1H), 6.22 (s, 1H), 6.19 (s, 1H), 5.93 (s, 1H), 5.83 (m, 1H), 5.80 (m, 2H), 5.65 (m, 1H), 5.63 (m, 1H), 5.33 (dt, *J* = 17.2, 1.6 Hz, 1H), 5.19 (d, *J* = 8.1 Hz, 1H), 5.16 (d, *J* = 8.1 Hz,

1H), 5.10 (dq, *J* = 10.4, 1.4 Hz, 1H), 4.96 (m, 2H), 4.21 (m, 1H), 4.16 (p, *J* = 5.8 Hz, 1H), 4.12 (dt, *J* = 6.6, 1.3 Hz, 1H), 3.30 (s, 3H), 3.26 (s, 3H), 2.50 (dd, *J* = 15.0, 6.3 Hz, 1H), 2.43 (dd, *J* = 15.0, 6.6 Hz, 1H), 2.30 (dd, *J* = 15.0, 5.6 Hz, 1H), 2.26 (m, 1H), 2.22 (dd, *J* = 15.0, 5.7 Hz, 1H), 1.99 (dt, *J* = 13.0, 4.0 Hz, 1H), 1.87 (m, 1H), 1.79 (m, 1H), 1.71 (d, *J* = 1.1 Hz, 3H), 1.69 (d, *J* = 1.1 Hz, 3H), 1.67 (m, 1H), 1.25 (s, 3H), 1.24 (m, 2H), 1.22 (s, 3H), 1.01 (s, 9H), 0.98 (s, 9H), 0.71 (d, *J* = 5.3 Hz, 3H), 0.69 (d, *J* = 5.3 Hz, 3H), 0.15 (s, 3H), 0.14 (s, 3H), 0.12 (s, 3H), 0.10 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 170.0, 170.0, 160.8, 160.6, 144.9, 144.9, 139.7, 137.7, 137.6, 132.9, 131.3, 128.6, 128.4, 128.2, 128.1, 127.6, 118.0, 117.9, 115.8, 115.8, 114.0, 114.0, 102.7, 102.3, 87.9, 86.0, 83.3, 82.1, 82.0, 81.9, 80.4, 80.4, 69.9, 69.7, 54.8, 54.8, 42.9, 42.7, 40.4, 40.4, 32.9, 31.8, 31.3, 29.0, 26.2, 26.1, 22.8, 22.2, 20.3, 18.3, 18.3, 16.4, 16.4, -4.4, -4.4, -4.4, -4.5; FTIR (film) $\nu_{max}$ 2956, 2929, 2856, 1739, 1616, 1517, 1378, 1249, 1170, 1070 cm$^{-1}$; HR-ES-MS *m/z* calcd. for $C_{32}H_{49}NO_5S_2SiNa$ $[M+Na]^+$: 707.2203, found 707.2199; $[\alpha]^{25}_D$ = -13.1 ° (c = 1.0, $CH_2Cl_2$).

**Ring-closing metathesis of 34 to lactone 35**




**Reagents:**

2nd Generation Hoyveda Grubbs catalyst, 97% (Sigma-Aldrich): used without further purification

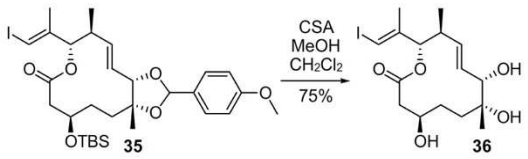
**(3a*S*,6*S*,7*S*,11*R*,13a*R*,*E*)-11-((*tert*-Butyldimethylsilyl)oxy)-7-((*E*)-1-iodoprop-1-en-2-yl)-2-(4-methoxyphenyl)-6,13a-dimethyl-3a,6,7,10,11,12,13,13a-octahydro-9*H*-[1,3]dioxolo[4,5-*f*][1]oxacyclododecin-9-one (35).** Esters **34** (5.15 g, 7.52 mmol) in a two-necked 3 L flask equipped with a 1 L addition funnel were dissolved into anhydrous, degassed toluene (700 mL). The mixture was purged with Ar and heated to reflux. 2nd Generation Hoyveda-Grubbs catalyst (706 mg, 1.13 mmol) in anhydrous, degassed toluene (700 mL) purged under Ar was dropwise added to the solution of **34** in boiling toluene. After stirring for 20 min the mixture turned from a clear green color into a black solution and was further stirred at reflux for 5 h. The mixture was then cooled to rt and concentrated by a rotary evaporator. The crude black semi-solid was then suspended in hexanes and filtered through a pad of Celite and eluted with hexanes. The elutants were concentrated on a rotary evaporator to yield a crude green oil. Pure lactones **35** (2.47 g, 50%) was obtained as a white solid by flash chromatography, eluting with a gradient of hexanes to 1:10 $Et_2O$/hexanes.

*Note 1: Allylic isomerization is the main byproduct of this reaction. Although literature suggests certain additives (i.e. hydroquinone) may inhibit such competing reactions, no improvements in yields were observed with **34** or similar analogues (i.e. other protecting groups) as the substrate.*

*Note 2: The acetal diastereomers were separable by chromatography, and their spectroscopic data are recorded individually below.*

Lactones **35**: TLC (1:2 $Et_2O$/hexanes): $R_f$ = 0.38, 0.35 (CAM stain); $^1H$ NMR (500 MHz, $C_6D_6$) Isomer A δ 7.61 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.29 (d, *J* = 1.2 Hz, 1H), 6.03 (s, 1H), 5.66 (dd, *J* = 15.2, 9.4 Hz, 1H), 5.00 (d, *J* = 10.6 Hz, 1H), 4.99 (dd, *J* = 15.2, 9.6 Hz, 1H), 4.07 (d, *J* = 9.6, 1H), 3.93 (ddt, *J* = 9.2, 7.4, 4.3 Hz, 1H), 3.24 (s, 3H), 2.36 (dd, *J* = 14.4, 4.5 Hz, 1H), 2.31 (dd, 14.4, 9.4 Hz, 1H), 2.18 (m, 2H), 1.80 (m, 1H), 1.66 (d, *J* = 1.1 Hz, 3H), 1.41 (m, 2H), 1.20 (s, 3H), 1.01 (m, 1H), 0.95 (s, 9H), 0.49 (d, *J* = 6.8 Hz, 3H), 0.05 (s, 3H), 0.00 (s, 3H); Isomer B δ 7.60 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.32 (s, 1H), 5.75 (dd, *J* = 15.1, 9.9 Hz, 1H), 5.00 (d, *J* = 10.6 Hz, 1H), 4.98 (dd, *J* = 15.1, 9.6 Hz, 1H), 4.20 (d, *J* = 9.9 Hz, 1H), 3.93 (ddt, *J* = 9.1, 7.7, 3.9 Hz, 1H), 3.26 (s, 3H), 2.36 (dd, *J* = 14.3, 4.3 Hz, 1H), 2.30 (dd, *J* = 14.3, 9.3 Hz, 1H), 2.23 (m, 1H), 2.16 (dt, *J* = 12.9, 7.0 Hz, 1H), 1.81 (m, 1H), 1.72 (d, *J* = 1.2 Hz, 3H), 1.42 (m, 1H), 1.34 (m, 1H), 1.27 (s, 3H), 1.03 (m, 1H), 0.97 (s, 9H), 0.55 (d, *J* = 6.8 Hz, 3H), 0.09 (s, 3H), 0.03 (s, 3H); $^{13}C$ NMR (125 MHz, $C_6D_6$) Isomer A δ 168.2, 160.6, 144.3, 137.2, 132.4, 128.4, 128.1, 128.0, 127.7, 127.6, 114.0, 102.7, 86.0, 84.0, 83.6, 80.0, 72.2, 54.8, 43.7, 40.6, 35.0, 32.5, 26.2, 26.0, 19.0, 18.2, 16.4, -4.5, -4.5; Isomer B δ 168.2, 160.8, 144.3, 136.4, 131.5, 131.2, 128.4, 128.4, 128.2, 128.0, 127.7, 127.5, 114.0, 101.6, 85.2, 84.0, 83.6, 80.0, 72.1, 54.8, 43.9, 40.4, 35.1, 31.9, 26.0, 22.8, 19.0, 18.2, 16.4, -4.5; FTIR (film) $\nu_{max}$ 2948, 2915, 2899, 1741, 1625, 1500, 1381, 1263, 1171, 1071 $cm^{-1}$; HR-ES-MS *m/z* calcd. for $C_{30}H_{45}IO_6SiNa$ $[M+Na]^+$: 679.1902, found 679.1899; $[\alpha]^{25}_D$ = -10.3 ° (c = 0.5, $CH_2Cl_2$).

**Deprotection of 35 to triol 36**



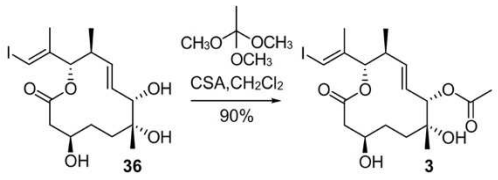

**Reagents:**

(1*S*)-(+)-10-Camphorsulfonic acid, 98% (TCI Chemicals): used without further purification

**(4*R*,7*R*,8*S*,11*S*,12*S*,*E*)-4,7,8-Trihydroxy-12-((*E*)-1-iodoprop-1-en-2-yl)-7,11-dimethyloxacyclododec-9-en-2-one (36).** Lactones **35** (2.47 g, 3.77 mmol) were dissolved in 1:3 MeOH/$CH_2Cl_2$ (300 mL) in a 1 L flask. (1*S*)-(+)-10-Camphorsulfonic acid (3.45 g, 14.9 mmol) was added as a solid in one portion. The mixture was stirred for 5 h, at which point TLC analyses indicated complete conversion of starting material. Satd. $NaHCO_3$ (50 mL) was added, and the mixture was extracted into $CH_2Cl_2$ (3 × 200 mL). The organics were collected and concentrated on a rotary evaporator to a crude oil. Pure triol **36** (1.19 g, 75%) was obtained as a white solid by flash chromatography, eluting with a gradient of $CH_2Cl_2$ to 1:2 acetone/$CH_2Cl_2$.

Triol **36**: TLC (1:2 acetone/$CH_2Cl_2$): $R_f$ = 0.25 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 6.18 (bs, 1H), 5.56 (dd, *J* = 15.2, 9.7 Hz, 1H), 5.16 (d, *J* = 10.7 Hz, 1H), 4.95 (dd, *J* = 15.2, 9.8 Hz, 1H), 3.54 (d, *J* = 11.0 Hz, 1H), 3.46 (ddq, *J* = 10.7, 7.1, 3.4 Hz, 1H), 3.41 (dd, *J* = 9.7, 4.4 Hz, 1H), 2.20 (dd, *J* = 14.9, 4.0 Hz, 1H), 2.13 (m, 1H), 2.08 (dd, *J* = 15.0, 2.8 Hz, 1H), 1.65 (d, *J* = 1.1 Hz, 3H), 1.55 (m, 1H), 1.30 (m, 2H), 1.14 (s, 3H), 1.10 (m, 1H), 0.56 (d, *J* = 6.8 Hz, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 171.6, 143.6, 135.4, 131.2, 127.2, 83.9, 79.7, 76.7, 72.9, 69.0, 40.6, 37.9, 35.7, 30.0, 24.3, 16.0; FTIR (film) $\nu_{max}$ 3683, 3602, 3552, 2977, 2958, 2935, 1708, 1616, 1365, 1284, 1172 cm$^{-1}$; HR-ES-MS *m/z* calcd. for $C_{16}H_{25}IO_5Na$ $[M+Na]^+$: 447.0601, found 447.0606; $[\alpha]^{25}_D$ = -57.0 ° (c = 1.0, $CH_2Cl_2$).

**Selective acetylation of triol 36 to core 3**




**Reagents:**

(1*S*)-(+)-10-Camphorsulfonic acid, 98% (TCI Chemicals): used without further purification

Trimethyl orthoformate, 99% (Sigma-Aldrich): used without further purification

**(2*S*,3*S*,6*S*,7*R*,10*R*,*E*)-7,10-Dihydroxy-2-((*E*)-1-iodoprop-1-en-2-yl)-3,7-dimethyl-12-oxooxacyclododec-4-en-6-yl acetate (3).** Triol **36** (1.10 g, 2.59 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (120 mg, 0.259 mmol) were dissolved in anhydrous $CH_2Cl_2$ (100 mL) in a 100 mL flask and cooled to 0 °C. Trimethyl orthoformate (400 µL, 3.13 mmol) was added dropwise as a solution of $CH_2Cl_2$ (20 mL), and the mixture was stirred at 0 °C for 1 h, at which point satd. $NH_4Cl$ (5 mL) was added. The mixture was stirred for 20 min and extracted into $CH_2Cl_2$ (150 mL). The organics were concentrated on a rotary evaporator. Pure core **3** (1.09 g, 90%) was obtained as a white semi-solid by flash chromatography, eluting with a gradient of $CH_2Cl_2$ to 1:3 acetone/$CH_2Cl_2$.

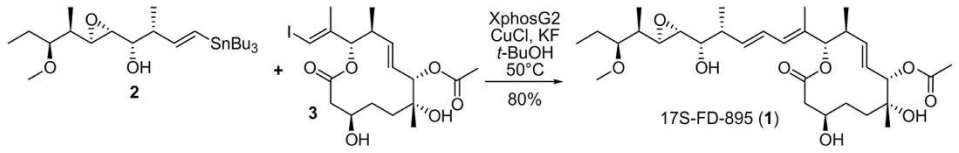
*Note 1: TLC analyses of the mixture taken prior to quench with aq. $NH_4Cl$ indicates two spots with $R_f$ values of 0.30 and 0.65. The higher $R_f$ spot corresponds to the unstable cyclic acetal that rearranges to the desired $C_7$ acetate upon exposure to aq. $NH_4Cl$.*

Core **3**: TLC (1:8 acetone/$CH_2Cl_2$): $R_f$ = 0.30 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 6.12 (s, 1H), 5.74 (dd, *J* = 15.3, 9.8 Hz, 1H), 5.47 (dd, *J* = 15.3, 10.1 Hz, 1H), 5.18 (d, *J* = 9.8 Hz, 1H), 5.13 (d, *J* = 10.6 Hz, 1H), 3.46 (bs, 1H), 2.20 (d, *J* = 14.9, 1H), 2.15 (m, 1H), 2.08 (d, *J* = 14.9 Hz,

1H), 1.78 (bs, 1H), 1.64 (m, 1H), 1.61 (s, 3H), 1.60 (d, *J* = 1.1 Hz, 3H), 1.55 (m, 1H), 1.44 (m, 1H), 1.16 (m, 2H), 0.98 (s, 3H), 0.51 (d, *J* = 6.7 Hz, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 171.7, 169.0, 143.8, 139.8, 126.9, 84.4, 80.0, 79.0, 73.2, 69.3, 41.1, 38.4, 35.8, 30.2, 24.7, 20.8, 19.1, 16.1; FTIR (film) $\nu_{max}$ 3502, 3058, 2959, 2873, 1733, 1616, 1368, 1243, 1168, 1021 cm$^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{18}H_{27}IO_6Na$ $[M+Na]^+$: 489.0745, found 489.0742; $[\alpha]^{25}_D$ = -67.5 ° (c = 1.0, $CH_2Cl_2$).

**D. Procedures for the Stille coupling of vinylstannane 2 to core 3 to deliver 17S-FD-895 (1).**

This procedure was optimized from El Marrouni and co-workers (*36*).




**Reagents:**

CuCl, anhydrous, beads, 99.99% (Sigma-Aldrich): beads were powdered prior to addition

KF, anhydrous, powder, 99.9% (Sigma-Aldrich): used without further purification

XPhos Pd G2 (Sigma-Aldrich): used without further purification

*t*-BuOH, anhydrous, 99.5% (Sigma-Aldrich): used without further purification

**CAUTION: 17*S*-FD-895 is a highly potent biological agent. Full body suits, facemasks, and respiratory masks are recommended for production of greater than 1 g of 17*S*-FD-895**

**17*S*-FD-895 (1).** Vinylstannane **2** (1.33 g, 2.57 mmol) and core **3** (1.00 g, 2.14 mmol) were combined in a 100 mL flask and dried *via* rotary evaporation of benzene. To the mixture was then sequentially added CuCl (0.425 g, 4.29 mmol), KF (0.249 g, 4.29 mmol) and XPhos Pd G2 (0.169 g, 0.214 mmol) and anhydrous *t*-BuOH (25 mL). The reaction vessel was purged under Ar, heated to 50 °C and stirred overnight, at which point solution turns into a gray cloudy mixture. The mixture was then filtered through a plug of Celite and eluted with acetone (200 mL). The elutants were concentrated on a rotary evaporator to yield a crude brown semi-solid. Pure 17*S*-FD-895 (**1**) (1.21 g, 80%) was obtained as a white semi-solid by flash chromatography over neutral silica gel, eluting with a gradient of hexanes to 1:3 acetone/hexanes.

*Note 1: An additional chromatographic step on mixed fractions may be needed to maximize yield.*

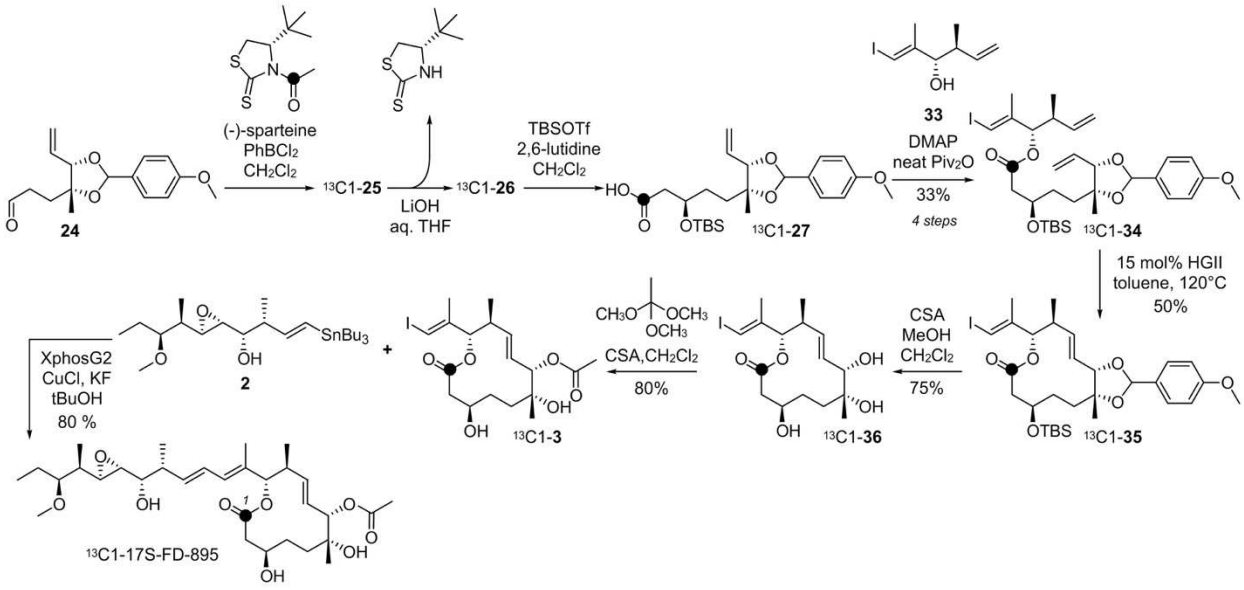
*Note 2: This reaction was performed on a **MAXIMUM** of 1 g of core **3** due to toxicity.*

17S-FD-895 (**1**): TLC (1:3 acetone/$CH_2Cl_2$): $R_f$ = 0.28 (CAM stain); NMR data provided in Table S1; FTIR (film) $\nu_{max}$ 3447, 2963, 2930, 2875, 1739, 1457, 1374, 1239, 1176, 1089, 1021 $cm^{-1}$; HR-ESI-MS *m/z* calcd. for $C_{31}H_{50}IO_9Na$ $[M+Na]^+$: 589.3345, found 589.3347; $[\alpha]^{25}_D$ = +8.8 ° (c = 1.0, $CH_2Cl_2$).

**Table 2.S1.** NMR data for 17*S*-FD-895 (**1**) in $C_6D_6$

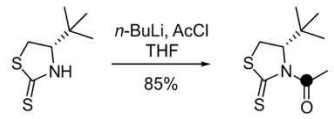
| Position | $\delta_C$ | $\delta_H$, mult (*J* in Hz) |
|---|---|---|
| 1 | 171.8 | |
| 2α | 38.2 | 2.29, dd (14.8, 3.9) |
| 2β | | 2.19, dd (14.8, 3.0) |
| 3 | 69.0 | 3.49, td (11.1, 3.5) |
| 3-OH | | 3.63, d (11.2) |
| 4α | 30.0 | 1.56, m |
| 4β | | 1.23, dt (19.1, 10.3) |
| 5α | 35.5 | 1.55, m |
| 5β | | 1.22, dt (19.1, 10.3) |
| 6 | 72.5 | |
| 6-OH | | 1.75, s |
| 7 | 78.8 | 5.26, d (1.5) |
| 8 | 140.3 | 5.62, dd (15.2, 10.0) |
| 9 | 126.0 | 5.83, dd (10.5, 9.1) |
| 10 | 40.8 | 2.39, dd (10.4, 6.8) |
| 11 | 82.2 | 5.24, d (2.4) |
| 12 | 131.0 | |
| 13 | 131.4 | 6.11, d (10.2) |
| 14 | 126.1 | 6.26, dd (15.2, 10.8) |
| 15 | 137.6 | 5.80, dd (10.5, 9.1) |
| 16 | 41.2 | 2.35, m |
| 17 | 73.0 | 3.42, q (3.7) |
| 17-OH | | 1.55, bs |
| 18 | 57.3 | 2.56, dd (3.8, 2.2) |
| 19 | 59.3 | 3.01, dd (8.3, 2.3) |
| 20 | 38.9 | 1.33, m |
| 21 | 83.4 | 3.15, m |
| 22α | 23.5 | 1.63, m |
| 22β | | 1.40, dt (14.0, 6.9) |
| 23 | 9.7 | 0.85, t (7.5) |
| 24 | 24.4 | 1.00, s |
| 25 | 16.1 | 0.70, d (6.7) |
| 26 | 11.5 | 1.59, d (1.3) |
| 27 | 16.9 | 1.12, d (7.0) |
| 28 | 10.5 | 0.88, d (6.9) |
| 29 | 168.7 | |
| 30 | 20.4 | 1.61, s |
| 31 | 57.4 | 3.23, s |

**E. Procedures for the synthesis of $^{13}$C1-17*S*-FD-895.** The following procedures are modified to deliver 1 g of $^{13}$C1-17*S*-FD-895. $^{13}$C NMR spectra and HR-ES-MS data are provided for all isotopically-labeled compounds. $^{1}$H NMR spectra were the same as unlabeled materials and are not reported. Copies of selected NMR spectra are provided at the end of this file.




**Scheme 2.S1.** Black sphere denotes position of $^{13}$C labeling.

**Synthesis of $^{13}$C1-(*S*)-1-(4-(*tert*-butyl)-2-thioxothiazolidin-3-yl)ethan-1-one**




**Reagents:**

*n*-BuLi, 2.5 M in hexanes (Acros Organics): used without further purification

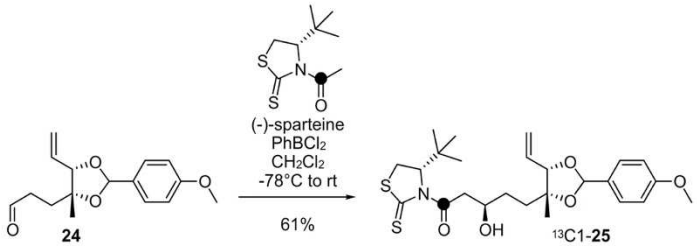
Acetyl chloride (1-$^{13}$C, 99% $^{13}$C): used without further purification

**$^{13}$C1-(*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one.** *n*-BuLi (2.5 M, 9.77 mL, 24.4 mol) was added dropwise to a 500 mL flask containing a solution of (*S*)-4-(*tert*-butyl)thiazolidine-2-thione (4.44, 24.3 mol) in anhydrous THF (180 mL) at -78 °C. The mixture was stirred at -78 °C for 30 min. Acetyl chloride (1-$^{13}$C, 99% $^{13}$C) (1.89 mL, 25.5 mol) was added dropwise, and the mixture was stirred at -78 °C for 1.5 h. The mixture was then warmed to rt, stirred for 1 h, re-cooled to 0 °C and quenched with satd. $NH_4Cl$ (10 mL). The phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$ (2 × 200 mL). The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator. Pure $^{13}$C1-(*S*)-1-(4-(*tert*-butyl)-2-thioxothiazolidin-3-yl)ethan-1-one (4.01 g, 85%) was obtained by flash chromatography, eluting with a gradient of heptane to $CH_2Cl_2$.

**$^{13}$C1-(*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one**: $^{13}$C NMR ($CDCl_3$, 125 MHz) δ 205.3, 170.3*, 72.0, 38.0, 30.4, 26.8, 26.8; LC-MS $[M+1]^+$: 219.1.

* denotes $^{13}$C-labeled carbon.

**Synthesis of $^{13}$C1-25**




**Reagents:**

Dichlorophenylborane, 97% (Acros Organics): used without further purification

(-)-Sparteine ,98% (TCI Chemicals), S0461: used without further purification

(*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one: dried *via* azeotropic removal of toluene by rotary evaporation

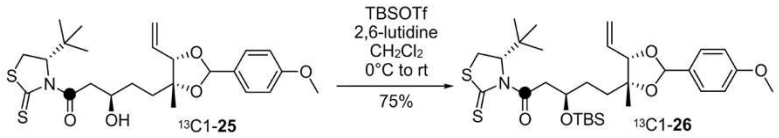
**$^{13}$C1-(3*R*)-1-((*R*)-5-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)-3-hydroxy-5-((4*R*,5*S*)-2-(4-methoxy-phenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentan-1-one ($^{13}$C1-25).** $^{13}$C1-(*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one (3.89 g, 17.9 mmol) was added to a flame dried 2 L flask and dissolved in anhydrous $CH_2Cl_2$ (300 mL). An Ar atmosphere was introduced and dichlorophenylborane (2.00 mL, 15.9 mmol) was added at rt and stirred for 15 min. (-)-Sparteine (7.30 mL, 31.8 mmol) was added neat, at which point the mixture turns cloudy but clears up upon further stirring. After stirring at rt for 30 min the mixture was cooled to -78 °C, and aldehyde **24** (3.66 g, 13.3 mmol) in a solution of anhydrous $CH_2Cl_2$ (30 mL) was added dropwise over 15 min. The mixture was stirred at -78 °C for 1 h and slowly warmed to 0 °C over 3 h, at which point NMR analyses indicated complete consumption of starting material. The mixture was quenched with satd. $NaHCO_3$ (65 mL), and the organic phase was separated. The aqueous phase was washed with $CH_2Cl_2$ (100 mL), and the organic phases were combined, dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator to yield a crude oil. Pure **$^{13}$C1-25** (4.27 g, 61%) was obtained

as a yellow oil by flash chromatography over neutral silica gel, eluting with a gradient of hexanes to 1:2 EtOAc/hexanes.

**$^{13}$C1-25**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 205.2, 205.2, 173.0*, 173.0*, 172.4, 172.0, 160.8, 160.6, 159.6, 135.8, 133.9, 133.8, 133.8, 133.0, 131.2, 128.7, 128.4, 128.2, 128.1, 127.6, 118.1, 118.0, 117.9, 114.2, 114.1, 113.9, 113.5, 102.8, 102.3, 93.7, 88.0, 86.5, 86.3, 83.4, 82.3, 81.8, 72.1, 72.0, 72.0, 70.6, 68.8, 68.8, 68.8, 54.8, 54.8, 47.3, 45.8, 45.7, 45.5, 37.9, 37.9, 33.2, 31.3, 31.1, 30.9, 30.8, 29.8, 29.8, 29.4, 26.7, 22.8, 22.2, 22.0; HR-ESI-MS *m/z* calcd. for $C_{25}H_{35}NO_5S_2Na$ $[M+Na]^+$: 517.5412, found 517.5415.

* denotes $^{13}$C-labeled carbons.

**TBS protection of $^{13}$C1-25 to $^{13}$C1-26**




**Reagents:**

2,6-Lutidine, redistilled, 99% (Chem-Impex Int.): used without further purification

TBSOTf, 99% (Chem-Impex Int.): used without further purification

**$^{13}$C1-(3*S*)-1-((*R*)-5-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)-3-((*tert*-butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentan-1-one ($^{13}$C1-26)**.

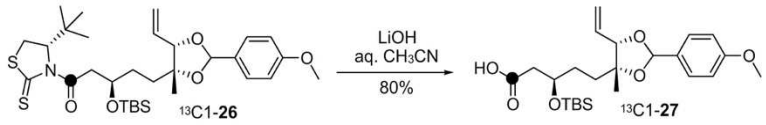
**$^{13}$C1-25** (4.00 g, 8.12 mmol) was dissolved in anhydrous $CH_2Cl_2$ (300 mL) followed by addition of 2,6-lutidine (5.12 mL, 40.8 mmol). The mixture was purged with Ar and cooled to 0 °C. TBSOTf (6.52 mL, 28.4 mmol) was added dropwise, and the mixture was warmed to rt and stirred overnight, at which point NMR analyses indicated complete consumption of starting material. The reaction was quenched with addition of solid $NaHCO_3$ (2 g) and stirred for 15 min. The mixture was filtered and concentrated under rotary evaporation to yield a yellow crude oil. Pure **$^{13}$C1-26** (3.64 g, 75%) was obtained as a yellow oil by flash chromatography, eluting with a gradient of hexanes to 1:9 EtOAc/hexanes.

**$^{13}$C1-26**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 205.1, 205.0, 170.9*, 170.9*, 170.8, 160.8, 160.6, 133.9, 133.7, 133.6, 133.0, 131.3, 128.7, 128.4, 128.2, 127.9, 127.7, 127.5, 118.0, 117.9, 114.1, 113.9, 102.7, 102.4, 88.0, 87.9, 86.1, 83.6, 83.5, 82.4, 82.2, 72.2, 72.1, 70.3, 69.5, 69.4, 54.8, 54.8, 53.3, 46.4, 46.1, 37.9, 37.8, 34.0, 32.8, 32.0, 31.5, 29.9, 29.8, 28.9, 26.8, 26.2, 26.2, 25.9, 22.7, 22.2,

18.4, 18.3, -3.4, -4.2, -4.2, -4.3, -4.3; HR-ESI-MS *m/z* calcd. for $C_{31}H_{49}NO_5S_2SiNa$ $[M+Na]^+$: 631.2612, found 630.2611.

* denotes $^{13}C$-labeled carbons.

**Saponification of $^{13}$C1-26 to $^{13}$C1-27**




**Reagents:**

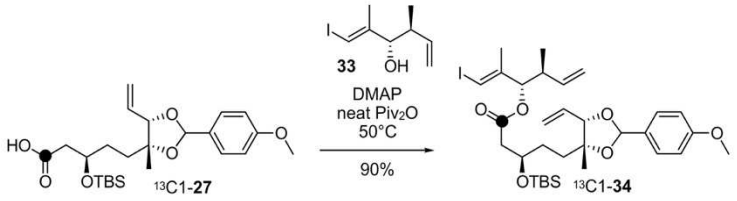
LiOH•$H_2O$, 98% (Alfa Aesar): used without further purification

**$^{13}$C1-(3*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentanoic acid ($^{13}$C1-27)** LiOH•$H_2O$ (424 mg, 17.8 mmol) was added to a solution of **$^{13}$C1-26** (3.60 g, 5.92 mmol) in 20% aq. $CH_3CN$ (500 mL). The mixture was stirred at rt overnight, at which point the deep yellow color dissipates into a light brown solution. The mixture was diluted with $H_2O$ (500 mL) and $Et_2O$ (500 mL). The aqueous phase was collected, and the organic phase was back extracted with $H_2O$ (2 × 500 mL). The aqueous phases were combined, and the pH was adjusted to 6.5 with 1 M HCl. The mixture was extracted into EtOAc (3 × 700 mL), and the organics were combined, dried over $Na_2SO_4$, filtered and concentrated by rotary evaporation. Pure **$^{13}$C1-27** (2.13 g, 80%) was obtained as a colorless oil by flash chromatography, eluting with a gradient of hexanes to 1:2 EtOAc/hexanes.

**$^{13}$C1-27**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 177.4*, 177.4*, 160.8, 160.6, 133.6, 133.5, 132.9, 131.2, 128.5, 128.4, 128.2, 128.0, 127.7, 127.7, 127.6, 118.0, 117.9, 114.0, 114.0, 93.7, 87.9, 86.3, 86.0, 83.3, 82.0, 81.6, 70.0, 69.9, 54.8, 54.8, 32.6, 31.8, 31.4, 30.2, 28.8, 26.6, 26.1, 26.0, 22.6, 22.1, 21.9, 18.3, 18.2, -4.3, -4.4, -4.4, -4.6, -4,6, -4.7; HR-ESI-MS *m/z* calcd. for $C_{24}H_{38}O_6SiNa$ $[M+Na]^+$: 474.2254, found 474.2257.

* denotes $^{13}$C-labeled carbons.

**Esterification of $^{13}$C1-27 and alcohol 33 to $^{13}$C1-34**




**Reagents:**

DMAP, 98% (Sigma-Aldrich): used without further purification

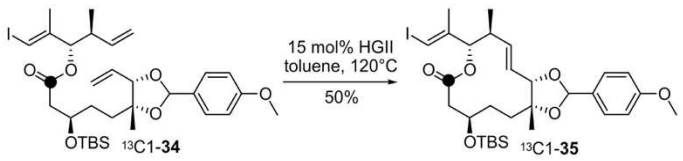
Pivalic anhydride, 99% (Alfa Aesar): used without further purification

**$^{13}$C1-(3*S*,4*S*,*E*)-1-Iodo-2,4-dimethylhexa-1,5-dien-3-yl-(3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentanoate ($^{13}$C1-34).** DMAP (54.4 mg, 0.444 mmol) and pivalic anhydride (2.25 mL, 11.1 mmol) were sequentially added to acid **$^{13}$C1-27** (2.00 g, 4.44 mmol) and alcohol **33** (1.23 g, 4.88 mmol). The mixture was purged with Ar and stirred neat at 50 °C for 8 h. Pivalic anhydride was removed from the mixture under airflow. The crude material in hexanes was then loaded directly onto silica gel and eluted with a gradient of hexanes to 1:9 $Et_2O$/hexanes. Pure **$^{13}$C1-34** (2.73 g, 90%) was obtained as a clear oil.

**$^{13}$C1-34**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 170.0*, 170.0*, 160.8, 160.6, 144.9, 144.9, 139.7, 137.7, 137.6, 132.9, 131.3, 128.6, 128.4, 128.2, 128.1, 127.6, 118.0, 117.9, 115.8, 115.8, 114.0, 114.0, 102.7, 102.3, 87.9, 86.0, 83.3, 82.1, 82.0, 81.9, 80.4, 80.4, 69.9, 69.7, 54.8, 54.8, 42.9, 42.7, 40.4, 40.4, 32.9, 31.8, 31.3, 29.0, 26.2, 26.1, 22.8, 22.2, 20.3, 18.3, 18.3, 16.4, 16.4, -4.4, -4.4, -4.4, -4.5; HR-ES-MS *m/z* calcd. for $C_{32}H_{49}NO_5S_2SiNa$ $[M+Na]^+$: 708.2203, found 708.2199.

* denotes $^{13}$C-labeled carbons.

**Ring-Closing Metathesis of $^{13}$C1-34 to $^{13}$C1-35**




**Reagents:**

2nd Generation Hoyveda Grubbs catalyst, 97% (Sigma-Aldrich): used without further purification

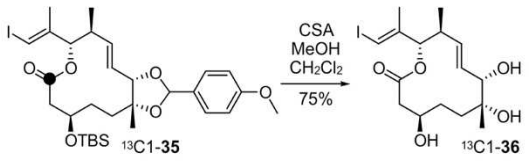
**$^{13}$C1-(3a*S*,6*S*,7*S*,11*R*,13a*R*,*E*)-11-((*tert*-Butyldimethylsilyl)oxy)-7-((*E*)-1-iodoprop-1-en-2-yl)-2-(4-methoxyphenyl)-6,13a-dimethyl-3a,6,7,10,11,12,13,13a-octahydro-9*H*-[1,3]dioxolo[4,5-*f*][1]oxacyclododecin-9-one ($^{13}$C1-35).** Ester **$^{13}$C1-34** (2.50 g, 3.65 mmol) was dissolved into anhydrous degassed toluene (280 mL). The mixture was purged with Ar and heated to reflux. 2nd Generation Hoyveda Grubbs catalyst (282 mg, 0.452 mmol) as an Ar purged solution in anhydrous degassed toluene (280 mL) was dropwise added to the solution of boiling toluene. After stirring for 20 min the mixture turned from a clear green color into a black solution and was further stirred at reflux for 5 h. The mixture was then cooled to rt and concentrated on a rotary evaporator. The crude black semi-solid was then suspended in hexanes and filtered through a pad of Celite eluting with hexanes. The elutants were concentrated on a rotary evaporator. Pure **$^{13}$C1-35** (1.20 g, 50%) was obtained as an off-white semi-solid by flash chromatography, eluting with a gradient of hexanes to 1:6 $Et_2O$/hexanes.

**$^{13}$C1-35**: $^{13}$C NMR (125 MHz, $C_6D_6$) Isomer A δ 168.2*, 160.8, 144.3, 136.4, 131.5, 131.2, 128.4, 128.4, 128.2, 128.0, 127.7, 127.5, 114.0, 101.6, 85.2, 84.0, 83.6, 80.0, 72.1, 54.8, 43.9, 40.4, 35.1, 31.9, 26.0, 22.8, 19.0, 18.2, 16.4, -4.5; Isomer B δ 168.2*, 160.6, 144.3, 137.2, 132.4, 128.4, 128.1, 128.0, 127.7, 127.6, 114.0, 102.7, 86.0, 84.0, 83.6, 80.0, 72.2, 54.8, 43.7, 40.6, 35.0, 32.5, 26.2,

26.0, 19.0, 18.2, 16.4, -4.5, -4.5; HR-ES-MS *m/z* calcd. for $C_{30}H_{45}IO_6SiNa$ $[M+Na]^+$: 680.1902, found 680.1899.

* denotes $^{13}C$-labeled carbon.

**Deprotection of $^{13}$C1-35 to $^{13}$C1-36**




**Reagents:**

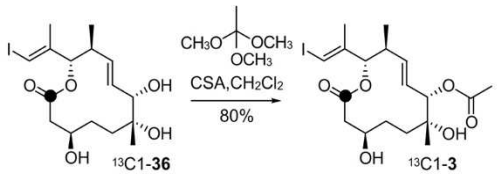
(1*S*)-(+)-10-Camphorsulfonic acid, 98% (TCI Chemicals): used without further purification

**(4*R*,7*R*,8*S*,11*S*,12*S*,*E*)-4,7,8-Trihydroxy-12-((*E*)-1-iodoprop-1-en-2-yl)-7,11-dimethyloxacyclododec-9-en-2-one ($^{13}$C1-36).** **$^{13}$C1-35** (1.20 g, 1.83 mmol) were dissolved in 1:3 MeOH/$CH_2Cl_2$ (50 mL) in a 250 mL flask and (1*S*)-(+)-10-camphorsulfonic acid (1.10 mg, 4.72 mmol) was added as a solid in one portion. The mixture was stirred for 5 h, at which point TLC indicated complete conversion of starting material. Satd. $NaHCO_3$ solution (50 mL) was added, and the mixture was extracted into $CH_2Cl_2$ (3 × 200 mL). The organics were collected and concentrated on a rotary evaporator. Pure **$^{13}$C1-36** (628 mg, 75%) was obtained as a white solid by flash chromatography, eluting with a gradient of $CH_2Cl_2$ to 1:2 acetone/$CH_2Cl_2$.

**$^{13}$C1-36**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 171.6*, 143.6, 135.4, 131.2, 127.2, 83.9, 79.7, 76.7, 72.9, 69.0, 40.6, 37.9, 35.7, 30.0, 24.3, 16.0; HR-ES-MS *m/z* calcd. for $C_{16}H_{25}IO_5Na$ $[M+Na]^+$: 448.0586, found 448.0589.

* denotes $^{13}$C-labeled carbon.

**Selective Acetylation of $^{13}$C1-36 to $^{13}$C1-3**




**Reagents:**

(1*S*)-(+)-10-Camphorsulfonic acid, 98% (TCI Chemicals): used without further purification

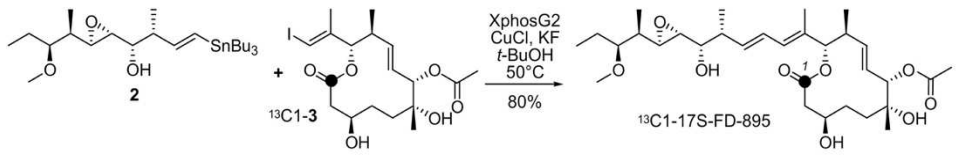
Trimethyl orthoformate, 99% (Sigma-Aldrich): used without further purification

**$^{13}$C1-(2*S*,3*S*,6*S*,7*R*,10*R*,*E*)-7,10-Dihydroxy-2-((*E*)-1-iodoprop-1-en-2-yl)-3,7-dimethyl-12-oxooxacyclododec-4-en-6-yl acetate ($^{13}$C1-3).** Triol **$^{13}$C1-36** (700 mg, 1.65 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (1.91 g, 8.25 mmol) were dissolved in anhydrous $CH_2Cl_2$ (5 mL) in a 20 mL scintillation vial and cooled to 0 °C. Trimethyl orthoformate (1.54 mL, 0.626 mmol) was added neat to the mixture and stirred at 0 °C for 1 h, at which point satd. $NH_4Cl$ (5 mL) was added. The mixture was extracted into $CH_2Cl_2$ (150 mL), and the organics were concentrated on a rotary evaporator. Pure core **$^{13}$C1-3** (701 mg, 80%) was obtained as a white semi-solid by flash chromatography, eluting with a gradient of $CH_2Cl_2$ to 1:3 acetone/$CH_2Cl_2$.

**$^{13}$C1-3**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 171.7*, 169.0, 143.8, 139.8, 126.9, 84.4, 80.0, 79.0, 73.2, 69.3, 41.1, 38.4, 35.8, 30.2, 24.7, 20.8, 19.1, 16.1; HR-ESI-MS *m/z* calcd. for $C_{18}H_{27}IO_6Na$ $[M+Na]^+$: 490.0712, found 490.0713.

* denotes $^{13}$C-labeled carbon.

**Synthesis of $^{13}$C1-17*S*-FD-895 by Stille coupling of core $^{13}$C1-3 to 2**




**Reagents:**

CuCl, anhydrous, beads, 99.99% (Sigma-Aldrich): used without further purification

KF, anhydrous, powder, 99.9% (Sigma-Aldrich): used without further purification

XPhos Pd G2 (Sigma-Aldrich): used without further purification

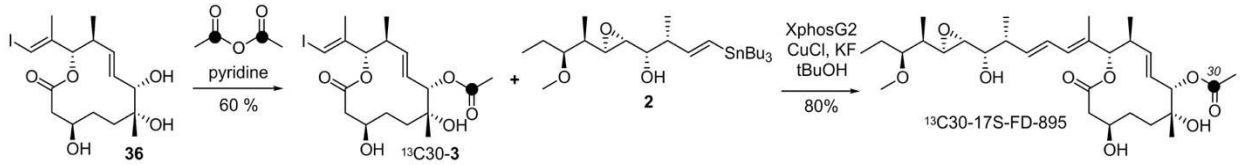
*t*-BuOH, anhydrous, 99.5% (Sigma-Aldrich): used without further purification

**$^{13}$C1-17*S*-FD-895.** Vinylstannane **2** (1.27 g, 2.25 mmol) and core **$^{13}$C1-3** (700 mg, 1.50 mmol) were combined in a 100 mL flask and dried *via* rotary evaporation of benzene. To the mixture was then sequentially added CuCl (150 mg, 0.150 mmol), KF (89.2 mg, 0.150 mmol) and XPhos Pd G2 (126 mg, 0.160 mmol) and anhydrous *t*-BuOH (50 mL). The reaction vessel was purged under Ar, heated to 50 °C and stirred overnight, at which point the solution turns into a gray cloudy mixture. The mixture was then filtered through a plug of Celite and eluted with acetone (50 mL). The elutants were concentrated on a rotary evaporator. Pure **$^{13}$C1-17*S*-FD-895** (680 mg, 80%) was obtained as a white semi-solid by flash chromatography over neutral silica gel eluting with a gradient of hexanes to 1:2 acetone/hexanes.

**$^{13}$C1-17*S*-FD-895**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 171.7*, 168.7, 140.3, 137.5, 131.3, 131.0, 126.0, 126.0, 83.3, 82.2, 78.8, 73.0, 72.5, 69.0, 59.3, 57.4, 57.3, 41.1, 40.8, 38.9, 38.2, 35.5, 30.0, 24.4, 23.5, 20.4, 16.9, 16.1, 11.5, 10.5, 9.7; HR-ESI-MS *m/z* calcd. for $C_{18}H_{27}IO_6Na$ $[M+Na]^+$: 590.3401, found 590.3403.

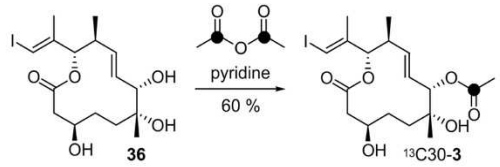
* denotes $^{13}C$-labeled carbon.

**F. Procedures for the synthesis of $^{13}$C30-17*S*-FD-895.** A two step procedure was used to convert triol **36** and side chain **2** to $^{13}$C30-17*S*-FD-895.




**Scheme 2.S2.** Black sphere denotes position of $^{13}$C labeling.

**Selective acetate isotopic labeling of triol 36 to $^{13}$C30-3**




**Reagents:**

Acetic anhydride (1,1 $^{13}$C2, 99%) (Cambridge Isotopes): used without further purification

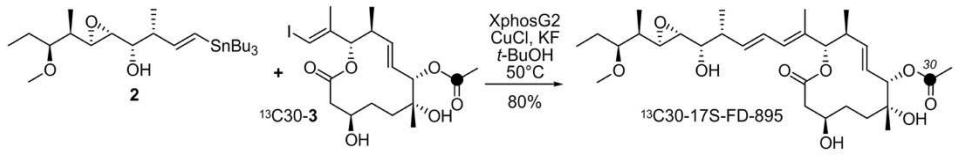
Pyridine, 99% (Fischer Scientific): freshly distilled over $CaH_2$

**$^{13}$C30-(2*S*,3*S*,6*S*,7*R*,10*R*,*E*)-7,10-Dihydroxy-2-((*E*)-1-iodoprop-1-en-2-yl)-3,7-dimethyl-12-oxooxacyclododec-4-en-6-yl acetate ($^{13}$C30-3).** Triol **36** (150 mg, 0.354 mmol) was dissolved in pyridine (2 mL). Acetic anhydride (1,1 $^{13}$C2, 99%) (334 µL, 3.54 mmol) was added neat, and the mixture was stirred for 3 h. Satd. $NaHCO_3$ (1 mL) was added. $Na_2SO_4$ was added, and the organics were filtered and concentrated on a rotary evaporator. Pure **$^{13}$C30-3** (97.7 mg, 60%) was obtained as a white semi-solid by flash chromatography, eluting with a gradient of $CH_2Cl_2$ to 1:3 acetone/$CH_2Cl_2$.

**$^{13}$C30-3**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 171.7, 169.0*, 143.8, 139.8, 126.9, 84.4, 80.0, 79.0, 73.2, 69.3, 41.1, 38.4, 35.8, 30.2, 24.7, 20.8, 19.1, 16.1; HR-ESI-MS *m/z* calcd. for $C_{18}H_{27}IO_6Na$ $[M+Na]^+$: 489.0745, found 489.0742; $[\alpha]^{25}_D$ = -67.5 ° (c = 1.0, $CH_2Cl_2$).

* denotes $^{13}$C-labeled carbon.

**Synthesis of $^{13}$C30-17*S*-FD-895 by Stille coupling of core $^{13}$C30-3 to 2**




**Reagents:**

CuCl, anhydrous, beads, 99.99% (Sigma-Aldrich): used without further purification

KF, anhydrous, powder, 99.9% (Sigma-Aldrich): used without further purification

XPhos Pd G2 (Sigma-Aldrich): used without further purification

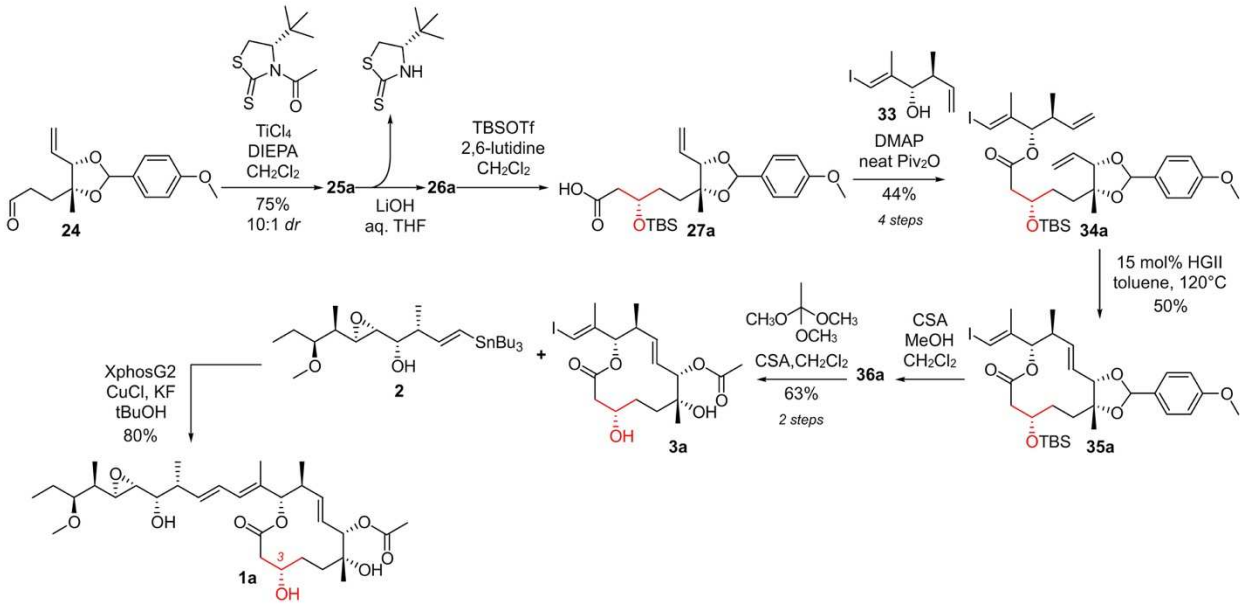
*t*-BuOH, anhydrous, 99.5% (Sigma-Aldrich): used without further purification

**$^{13}$C30-17*S*-FD-895.** Vinylstannane **2** (0.127 g, 0.225 mmol) and core **$^{13}$C30-3** (70.0 mg, 0.150 mmol) were combined in a 100 mL flask and dried *via* rotary evaporation of benzene. To the mixture was then sequentially added CuCl (15.0 mg, 0.150 mmol), KF (8.92 mg, 0.150 mmol) and XPhos Pd G2 (12.6 mg, 0.0160 mmol) and anhydrous *t*-BuOH (5 mL). The reaction vessel was purged under Ar, heated to 50 °C and stirred overnight, at which point the solution turns into a gray cloudy mixture. The mixture was then filtered through a plug of Celite and eluted with acetone (20 mL). The elutants were concentrated on a rotary evaporator. Pure **$^{13}$C30-17*S*-FD-895** (68.0 mg, 80%) was obtained as a white semi-solid by flash chromatography over neutral silica gel eluting with a gradient of hexanes to 1:2 acetone/hexanes.

**$^{13}$C30-17*S*-FD-895**: $^{13}$C NMR (125 MHz, $C_6D_6$) δ 171.7, 168.7*, 140.3, 137.5, 131.3, 131.0, 126.0, 126.0, 83.3, 82.2, 78.8, 73.0, 72.5, 69.0, 59.3, 57.4, 57.3, 41.1, 40.8, 38.9, 38.2, 35.5, 30.0, 24.4, 23.5, 20.4, 16.9, 16.1, 11.5, 10.5, 9.7; HR-ESI-MS *m/z* calcd. for $C_{18}H_{27}IO_6Na$ $[M+Na]^+$: 590.3401, found 590.3400.

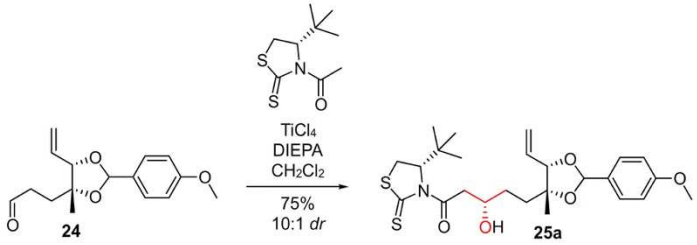
* denotes $^{13}C$-labeled carbon.

**G. Procedures for the synthesis of 3*S*, 17*S*-FD-895 (1a).** An eight step sequence was used to prepare 3*S*, 17*S*-FD-895 from aldehyde **24** and side chain **2**.




**Scheme 2.S3.** Red denotes the region of isomer installation.

**Synthesis of alcohol 25a**




**Reagents:**

$TiCl_4$, 97% (Alfa Aesar): used without further purification

$Et_2i$-PrN, 95% (Fischer Scientific): redistilled over $CaH_2$

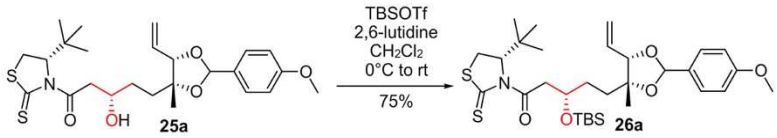
(*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one: dried *via* azeotropic removal of toluene by rotary evaporation

**(3*S*)-1-((*R*)-5-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)-3-hydroxy-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentan-1-one (25a).** (*S*)-1-(4-(*tert*-Butyl)-2-thioxothiazolidin-3-yl)ethan-1-one (1.17 g, 5.37 mmol) was dissolved in dry $CH_2Cl_2$ (80 mL) and purged with an Ar atmosphere. $TiCl_4$ (525 μL, 4.78 mmol) was added at rt and stirred for 15 min, at which point the mixture turns cloudy orange. $Et_2i$-PrN (862 μL, 4.95 mmol) was added neat, and the mixture turns black. After stirring at rt for 30 min, the mixture was cooled to -78 °C and **24** (1.10 g, 3.98 mmol) in a solution of anhydrous $CH_2Cl_2$ (10 mL) was added dropwise over 15 min. The mixture was stirred at -78 °C for 1 h and slowly warmed to 0 °C over 3 h, at which point NMR analyses indicated complete consumption of starting material. The mixture was quenched with satd. $NaHCO_3$ (10 mL), and the organic phase was separated. The aqueous phase was washed with $CH_2Cl_2$ (100 mL), and the combined organic phases were dried over $Na_2SO_4$, filtered and concentrated on a rotary evaporator to yield a crude yellow oil. Pure alcohol **25a** (1.47

g, 75%) was obtained as a yellow oil by flash chromatography over neutral silica gel eluting with a gradient of hexanes to 1:2 EtOAc/hexanes.

Alcohol **25a**: TLC (1:3 EtOAc/hexanes): $R_f$ = 0.23 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 8.10 (d, *J* = 9.0 Hz, 2H), 7.60 (d, *J* = 8.7 Hz, 1H), 7.55 (d, *J* = 8.7 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 8.7 Hz, 1H), 6.60 (d, *J* = 8.9 Hz, 2H), 6.25 (s, 1H), 5.94 (s, 1H), 5.85 (m, 1H), 5.66 (ddd, *J* = 16.9, 10.5, 6.3 Hz, 1H), 5.85 (dt, *J* = 6.3, 1.2 Hz, 1H), 5.32 (ddt, *J* = 17.2, 3.0, 1.6 Hz, 1H), 5.25 (dt, *J* = 17.1, 1.4 Hz, 1H), 5.09 (ddt, *J* = 10.4, 5.7, 1.5 Hz, 1H), 5.00 (dt, *J* = 10.5, 1.3 Hz, 1H), 4.92 (m, 1H), 4.21 (dt, *J* = 6.6, 1.3 Hz, 1H), 4.15 (m, 1H), 4.12 (dt, *J* = 6.7, 1.3 Hz, 1H), 3.69 (dd, *J* = 17.5, 2.6 Hz, 1 H), 3.61 (dd, *J* = 17.3, 2.8 Hz, 1 H), 3.27 (m, 2H), 3.15 (s, 3H), 2.37 (ddd, *J* = 14.0, 10.2, 7.4 Hz, 1H), 2.13 (ddd, *J* = 17.6, 10.5, 6.8 Hz, 1H), 1.95 (m, 2H), 1.83 (m, 1H), 1.70 (m, 2H), 1.23 (s, 3H), 1.20 (s, 3H), 1.08 (m, 2H), 1.01 (s, 9H), 0.75 (s, 3H), 0.72 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 205.2, 205.1, 174.9, 172.8, 172.6, 164.9, 164.0, 160.8, 160.6, 133.9, 133.8, 132.0, 132.1, 126.8, 127.7, 127.5, 122.6, 120.0, 118.0, 117.9, 114.2, 114.0, 113.9, 102.6, 102.4, 88.0, 86.3, 84.7, 83.7, 82.5, 77.9, 71.9, 68.8, 54.9, 54.8, 37.8, 37.8, 33.7, 31.2, 30.7, 29.8, 29.5, 28.5, 26.7, 23.2, 22.5; HR-ESI-MS *m/z* calcd. for $C_{25}H_{35}NO_5S_2Na$ $[M+Na]^+$: 516.6689, found 516.6690; $[\alpha]^{25}_D$ = +37.2 ° (c = 1.0, $CH_2Cl_2$).

**TBS protection of 25a to 26a**




**Reagents:**

2,6-Lutidine, redistilled, 99% (Chem-Impex Int.): used without further purification

TBSOTf, 99% (Chem-Impex Int.): used without further purification

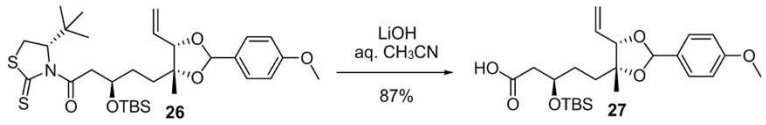
**(3*S*)-1-((*R*)-5-(*tert*-butyl)-2-thioxothiazolidin-3-yl)-3-((*tert*-butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentan-1-one (26a)**.

Adduct **25a** (1.00 g, 2.03 mmol) was dissolved in anhydrous $CH_2Cl_2$ (75 mL) followed by addition of 2,6-lutidine (1.28 mL, 10.2 mmol). The mixture was purged with Ar and cooled to 0 °C. TBSOTf (1.63 mL, 7.10 mmol) was added dropwise, and the mixture was warmed to rt and stirred overnight, at which point NMR analyses indicated complete consumption of starting material. The reaction was quenched with addition of solid $NaHCO_3$ (1 g) and stirred for 15 min. The mixture was filtered and concentrated under rotary evaporation to yield a yellow crude oil. Pure adducts **26a** (910 mg, 75%) was obtained as a yellow oil by flash chromatography, eluting with a gradient of hexanes to 1:9 EtOAc/hexanes.

Adducts **26a**: TLC ($CH_2Cl_2$): $R_f$ = 0.40 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 7.61 (d, *J* = 8.9 Hz, 2H), 7.55 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H) 6.29 (s, 1H), 5.94 (s, 1H), 5.90 (m, 1H), 5.86 (m, 1H), 5.32 (dt, *J* = 17.1, 1.6 Hz, 2H), 5.12 (dt, *J* = 10.6, 1.5 Hz, 1H), 5.10 (d, *J* = 10.5, 1.5 Hz, 2H), 5.09 (d, *J* = 7.9 Hz, 1H), 5.06 (d, *J* = 7.9 Hz, 1H), 4.59 (tt, *J* = 6.7, 4.4 Hz, 1H), 4.49 (tt, *J* = 6.4, 4.9 Hz, 1H), 4.22 (dt, *J* = 6.4, 1.2 1H), 4.13 (dt, *J* = 6.4, 1.2 Hz,

1H), 4.04 (dd, *J* = 17.2, 6.7 Hz, 1H), 4.01 (dd, *J* = 17.2, 6.8 Hz, 1H), 3.44 (dd, *J* = 11.9, 5.2 Hz, 1H), 3.40 (dd, *J* = 11.9, 5.2 Hz, 1H), 3.30 (s, 3H), 3.28 (d, *J* = 1.4 Hz, 1H), 3.26 (s, 3H), 3.25 (d, *J* = 2.8 Hz, 1H), 2.64 (dd, *J* = 12.6, 7.6 Hz, 1H), 2.62 (dd, *J* = 13.2, 8.3 Hz, 1H), 2.05 (ddd, *J* = 11.8, 5.4, 0.8 Hz, 2H), 2.00 (m, 1H), 1.79 (m, 1H), 1.64 (m, 1H), 1.54 (m, 1H), 1.24 (s, 3H), 1.21 (s, 3H), 1.02 (s, 9H), 0.99 (s, 9H), 0.77 (s, 9H), 0.75 (s, 9H), 0.21 (s, 3H), 0.17 (s, 3H), 0.15 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 205.3, 205.2, 171.2, 171.2, 160.8, 160.6, 134.0, 133.8, 133.0, 131.9, 131.3, 128.6, 128.4, 128.2, 127.5, 118.0, 117.9, 114.0, 113.9, 102.6, 102.3, 87.9, 86.2, 83.6, 82.4, 72.2, 72.2, 70.1, 69.9, 58.4, 58.4, 46.2, 46.0, 37.9, 37.9, 33.8, 33.7, 33.6, 32.2, 31.6, 30.1, 30.1, 29.4, 26.8, 26.2, 26.1, 25.2, 22.7, 22.2, 18.4, 18.4, -4.1, -4.2, -4.3, -4.3; HR-ESI-MS *m/z* calcd. for $C_{31}H_{49}NO_5S_2SiNa$ $[M+Na]^+$: 630.2689, found 630.2688; $[\alpha]^{25}_D$ = +49.4 ° (c = 1.0, $CH_2Cl_2$).

**Saponification of 26a to 27a**




**Reagents:**

LiOH•$H_2O$, 98% (Alfa Aesar): used without further purification

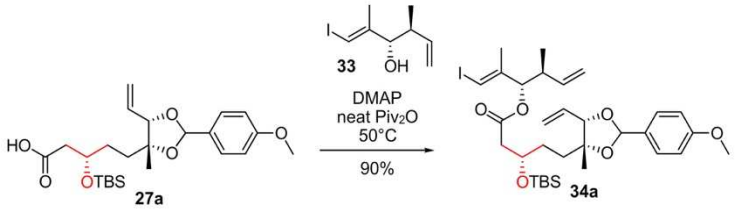
**(3*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentanoic acid (27a)** LiOH•$H_2O$ (106 mg, 4.45 mmol) was added to a solution of **26a** (900 mg, 1.48 mmol) in 20% aq. $CH_3CN$ (50 mL). The mixture was stirred at rt overnight, at which point the deep yellow color dissipates into a light brown solution. The mixture was diluted with $H_2O$ (50 mL) and $Et_2O$ (50 mL). The aqueous phase was collected, and the organic phase was back extracted with $H_2O$ (2 × 50 mL). The aqueous phases were combined, and the pH was adjusted to 6.5 with 1 M HCl. The mixture was extracted into EtOAc (3 × 100 mL), and the organics were combined, dried over $Na_2SO_4$, filtered and concentrated by rotary evaporation. Pure acid **27a** (533 mg, 87%) was obtained as a colorless oil by flash chromatography, eluting with a gradient of hexanes to 1:2 EtOAc/hexanes.

*Note 1*: *NMR spectral data was complicated due to the presence of minor amounts of carboxylate salts.*

Acid **27a**: TLC (1:1 EtOAc/hexanes): $R_f$ = 0.54 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 7.58 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.11 (d, *J* = 8.6, 6.7 Hz, minor), 6.87 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.24 (s, 1H), 6.20 (s, minor), 5.95 (s, 1H), 5.93 (s, minor), 5.82 (m, 1H), 5.32 (ddt, *J* = 17.1, 5.3, 1.6 Hz, 1H), 5.18 (m, minor), 5.12 (ddd, *J* = 7.6, 2.0, 1.3 Hz, 1H),

5.10 (m, 1H), 5.08 (m, minor), 4.20 (dt, $J$ = 6.6, 1.3 Hz, 1H), 4.16 (dt, $J$ = 6.5, 1.3 Hz, minor), 4.11 (dt, $J$ = 6.7, 1.1 Hz, 1H), 4.08 (m, minor), 3.30 (s, 3H), 3.27 (s, 3H), 3.26 (m, minor), 2.78 (dd, $J$ = 15.0, 9.5 Hz, minor), 2.47 (dd, $J$ = 14.9, 7.6 Hz, 1H), 2.39 (dd, $J$ = 15.0, 7.2 Hz, 1H), 2.31 (dd, $J$ = 15.0, 5.8 Hz, minor), 2.27 (dd, $J$ = 13.3, 4.9 Hz, 1H), 2.24 (dd, $J$ = 13.5, 5.0 Hz, 1H), 1.85 (m, 2H), 1.63 (m, 2H), 1.49 (s, minor), 1.34 (s, minor), 1.20 (s, 3H), 1.18 (s, 3H), 1.02 (s, 9H), 0.99 (s, minor), 0.98 (s, minor), 0.97 (s, 9H), 0.16 (s, minor), 0.15 (s, 3H), 0.13 (s, minor), 0.11 (s, 3H), 0.10 (s, 3H), 0.09 (s, minor), 0.05 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 178.2, 177.8, 160.8, 160.6, 160.4, 159.7, 136.1, 133.9, 133.7, 132.9, 131.3, 128.4, 128.2, 128.0, 127.6, 127.4, 118.0, 117.7, 114.1, 114.0, 114.0, 107.8, 102.3, 87.7, 86.1, 83.4, 82.4, 82.3, 70.2, 70.1, 69.8, 54.8, 54.8, 54.7, 42.8, 42.7, 42.6, 33.3, 32.1, 32.0, 31.7, 31.4, 29.0, 28.8, 27.3, 26.1, 26.1, 23.1, 22.6, 21.1, 18.4, 18.3, 18.3, -4.3, -4.4, -4.6; HR-ESI-MS *m/z* calcd. for $C_{24}H_{38}O_6SiNa$ $[M+Na]^+$: 473.2287, found 473.22889; $[\alpha]^{25}_D$ = +10.0 ° (c = 0.8, $CH_2Cl_2$).

**Esterification of 27a and alcohol 33 to 34a**




**Reagents:**

DMAP, 98% (Sigma-Aldrich): used without further purification

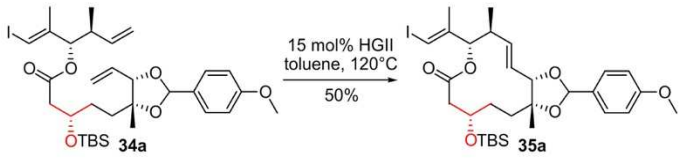
Pivalic anhydride, 99% (Alfa Aesar): used without further purification

**(3*S*,4*S*,*E*)-1-iodo-2,4-dimethylhexa-1,5-dien-3-yl-(3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-5-((4*R*,5*S*)-2-(4-methoxyphenyl)-4-methyl-5-vinyl-1,3-dioxolan-4-yl)pentanoate (34a).** DMAP (13.6 mg, 0.111 mmol) and pivalic anhydride (563 μL, 2.78 mmol) were sequentially added to acid **27a** (500 mg, 4.44 mmol) and alcohol **33** (308 mg, 1.22 mmol). The mixture was purged with Ar and stirred neat at 50 °C for 8 h. Pivalic anhydride was removed from the mixture under airflow. The crude material in hexanes was then loaded directly onto silica gel and eluted with a gradient of hexanes to 1:9 $Et_2O$/hexanes. Pure esters **34a** (683 mg, 90%) were obtained as a clear oil.

Esters **34a**: TLC (1:4 $Et_2O$/hexanes): $R_f$ = 0.40, 0.38 (CAM stain); $^1H$ NMR (500 MHz, $C_6D_6$) δ 7.58 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.27 (s, 1H), 6.23 (d, *J* = 0.9 Hz, 1H), 6.21 (d, *J* = 0.9 Hz, 1H), 5.94 (s, 1H), 5.84 (m, 1H), 5.80 (m, 1H), 5.65 (m, 1H), 5.58 (ddd, *J* = 17.0, 10.3, 8.1 Hz, 1H), 5.32 (dt, *J* = 17.2, 1.6 Hz, 1H), 5.20 (dd, *J* = 18.8, 8.9 Hz, 1H), 5.10 (m, 1H), 4.96 (m, 2H), 4.20 (m, 1H), 4.12 (m, 1H), 3.27 (s, 3H), 3.26 (s, 3H), 2.51 (dd, *J* = 15.3, 6.9 Hz, 1H), 2.43 (dd, *J* = 15.3, 6.7 Hz, 1H), 2.36 (m, 1H), 2.33 (dd, *J* = 15.3, 5.7 Hz, 1H), 2.23 (m, 1H), 1.89 (m, 2H), 1.72 (d, *J* = 1.2 Hz, 3H), 1.70 (d, *J* = 1.2 Hz, 3H), 1.67 (m, 1H), 1.51 (m, 1H) 1.69 (s, 3H), 1.23 (s, 3H), 1.21 (s, 3H), 1.01 (s, 9H), 0.98

(s, 9H), 0.66 (d, $J$ = 6.7 Hz, 3H), 0.65 (d, $J$ = 6.8 Hz, 3H), 0.14 (s, 3H), 0.14 (s, 3H), 0.10 (s, 3H), 0.08 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 170.2, 160.8, 160.6, 144.8, 144.7, 139.9, 139.8, 134.0, 134.0, 132.9, 131.1, 128.4, 128.2, 128.0, 127.7, 127.6, 115.9, 114.0, 114.0, 102.5, 102.5, 87.9, 87.8, 83.5, 82.3, 54.8, 43.0, 40.6, 33.6, 32.1, 26.2, 22.6, 20.0, 18.3, 118.3, 16.4, 16.4, -4.4, -4.4; HR-ES-MS *m/z* calcd. for $C_{32}H_{49}NO_5S_2SiNa$ $[M+Na]^+$: 707.2203, found 707.2201; $[\alpha]^{25}_D$ = -38.1 ° (c = 1.0, $CH_2Cl_2$).

**Ring Closing Metathesis of 34a to 35a**




**Reagents:**

2nd Generation Hoyveda Grubbs catalyst, 97% (Sigma-Aldrich): used without further purification

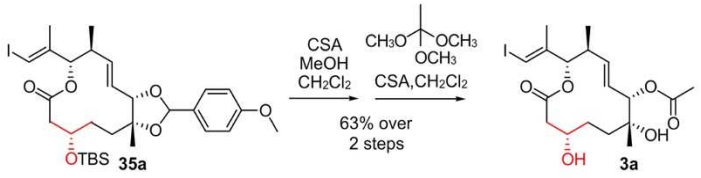
**(3a*S*,6*S*,7*S*,11*R*,13a*R*,*E*-11)-((*tert*-Butyldimethylsilyl)oxy)-7-((*E*)-1-iodoprop-1-en-2-yl)-2-(4-methoxyphenyl)-6,13a-dimethyl-3a,6,7,10,11,12,13,13a-octahydro-9*H*-[1,3]dioxolo[4,5-*f*][1]oxacyclododecin-9-one (35a).** Ester **34a** (625 mg, 913 mmol) was dissolved into anhydrous degassed toluene (70 mL). The mixture was purged with Ar and heated to reflux. 2nd Generation Hoyveda Grubbs catalyst (70.5 mg, 0.113 mmol) as an Ar purged solution in anhydrous degassed toluene (70 mL) was dropwise added to the solution of **34a** in boiling toluene. After stirring for 20 min the mixture turned from a clear green into a black solution and was stirred at reflux for 5 h. The mixture was then cooled to rt and concentrated on a rotary evaporator. The crude black solid was then suspended in hexanes and filtered through a pad of Celite eluting with hexanes. The elutants were concentrated on a rotary evaporator. Pure lactones **35a** (300 mg, 50%) were obtained as a white solid by flash chromatography, eluting with a gradient of hexanes to 1:6 $Et_2O$/hexanes.

*Note 1: NMR spectra data reflect the predominant acetal diastereomer.*

Lactones **35a**: TLC (1:2 $Et_2O$/hexanes): $R_f$ = 0.38 (CAM stain); $^1H$ NMR (500 MHz, $C_6D_6$) δ 7.65 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.25 (d, *J* = 1.3 Hz, 1H), 6.21 (s, 1H), 5.71 (dd, *J* = 15.2, 9.7 Hz, 1H), 4.97 (d, *J* = 10.7 Hz, 1H), 4.90 (dd, *J* = 15.2, 9.6 Hz, 1H), 4.42 (p, *J* = 5.2 Hz, 1H), 4.12 (d, *J* = 9.8 Hz, 1H), 3.23 (s, 3H), 2.40 (dd, *J* = 13.6, 11.2 Hz, 1H), 2.20 (dd, *J* = 12.6,

5.0 Hz, 1H), 2.16 (m, 1H), 1.95 (td, *J* = 13.6, 3.1 Hz, 1H), 1.61 (d, *J* = 1.2 Hz, 3H), 1.53 (m, 2H), 1.31 (s, 3H), 1.24 (m, 2H), 0.92 (s, 9H), 0.49 (d, *J* = 6.8 Hz, 3H), 0.02 (s, 3H), -0.02 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 169.5, 160.6, 144.3, 132.6, 128.4, 128.2 128.0, 127.7, 127.5, 114.0, 84.3, 68.5, 54.8, 40.7, 40.4, 28.9, 27.5, 25.9, 21.5, 19.0, 18.2, 15.8, -4.9; HR-ES-MS *m/z* calcd. for $C_{30}H_{45}IO_6SiNa$ $[M+Na]^+$: 679.1902, found 679.1903; $[\alpha]^{25}_D$ = -12.7 ° (c = 0.5, $CH_2Cl_2$).

**Two step conversion of 35a to core 3a.**




**Reagents:**

(1*S*)-(+)-10-Camphorsulfonic acid, 98% (TCI Chemicals): used without further purification

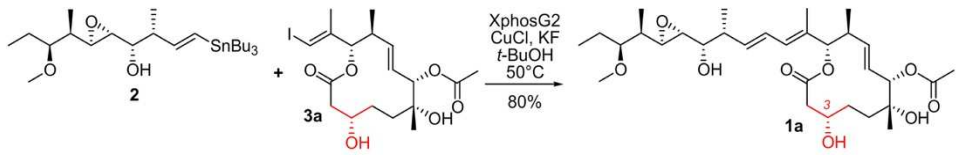
Trimethyl orthoformate, 99% (Sigma-Aldrich): used without further purification

**(2*S*,3*S*,6*S*,7*R*,10*R*,*E*)-7,10-Dihydroxy-2-((*E*)-1-iodoprop-1-en-2-yl)-3,7-dimethyl-12-oxooxacyclododec-4-en-6-yl acetate (3a).** Macrocycles **35a** (247 mg, 0.377 mmol) were dissolved in 1:3 MeOH/$CH_2Cl_2$ (30 mL) in a 1 L flask and (1*S*)-(+)-10-camphorsulfonic acid (345 mg, 1.49 mmol) was added as a solid in one portion. The mixture was stirred for 5 h, at which point TLC analyses indicated complete conversion of starting material. The solvent was removed under rotary evaporation, and the resulting crude was taken up in anhydrous $CH_2Cl_2$ (50 mL) in a 100 mL flask and cooled to 0 °C. Trimethyl orthoformate (40.0 μL, 0.313 mmol) was added neat, and the mixture was stirred at 0 °C for 1 h, at which point satd. $NaHCO_3$ (1 mL) was added. The mixture was extracted into $CH_2Cl_2$ (15 mL), and the organics were concentrated on a rotary evaporator. Pure core **3a** (89.0 mg, 63% over two steps) was obtained as a film by flash chromatography, eluting with a gradient of $CH_2Cl_2$ to 1:3 acetone/$CH_2Cl_2$.

Core **3a**: TLC (1:8 acetone/$CH_2Cl_2$): $R_f$ = 0.30 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 6.22 (d, *J* = 1.4 Hz, 1H), 5.85 (dd, *J* = 15.2, 9.9 Hz, 1H), 5.47 (dd, *J* = 15.2, 10.0 Hz, 1H), 5.20 (d, *J* = 9.8 Hz, 1H), 5.11 (d, *J* = 10.6 Hz, 1H), 4.22 (m, 1H), 2.39 (dd, *J* = 13.4, 11.2 Hz, 1H), 2.30 (dd, *J* =13.4, 5.4 Hz, 3H), 2.42 (m, 1H), 2.12 (bs, 1H), 1.80 (t, *J* = 9.1 Hz, 2H), 1.67 (m, 1H), 1.65 (s,

3H), 1.62 (d, $J$ = 1.1 Hz, 3H), 1.30 (m, 1H), 1.09 (s, 3H), 0.53 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 169.4, 169.2, 144.1, 139.6, 139.5, 126.9, 84.2, 84.0, 79.7, 79.0, 73.4, 67.5, 41.2, 39.8, 30.6, 27.4, 24.8, 20.7, 19.0, 16.1; HR-ESI-MS *m/z* calcd. for $C_{18}H_{27}IO_6Na$ $[M+Na]^+$: 489.0745, found 489.0742; $[\alpha]^{25}_D$ = -31.6 ° (c = 1.0, $CH_2Cl_2$).

**Synthesis of 3*S*,17*S*-FD-895 (1a) by Stille coupling of core 3a to 2**




**Reagents:**

CuCl, anhydrous, beads, 99.99% (Sigma-Aldrich): used without further purification

KF, anhydrous, powder, 99.9% (Sigma-Aldrich): used without further purification

XPhos Pd G2 (Sigma-Aldrich): used without further purification

*t*-BuOH, anhydrous, 99.5% (Sigma-Aldrich): used without further purification

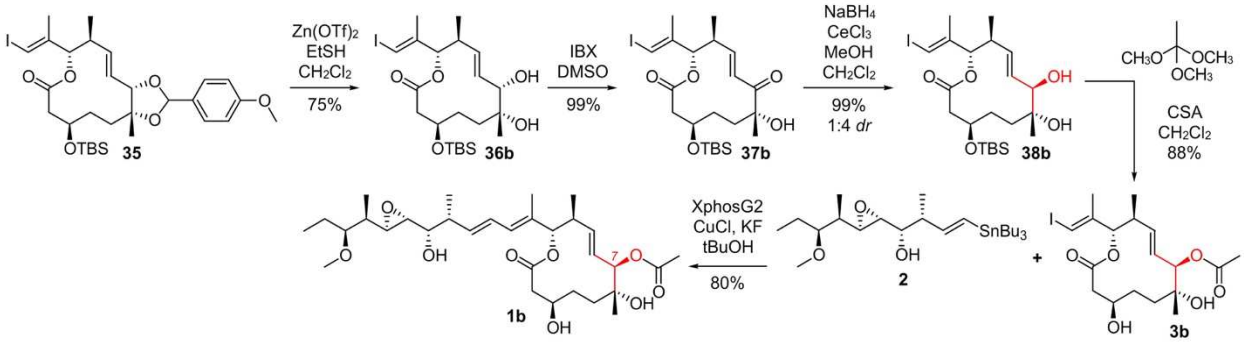
**3*S*,17*S*-FD-895 (1a).** Vinylstannane **2** (127 mg, 0.225 mmol) and core **3a** (70.0 mg, 0.150 mmol) were combined in a 100 mL flask and dried *via* rotary evaporation of benzene. To the mixture was then sequentially added CuCl (15.0 mg, 0.0150 mmol), KF (8.92 mg, 0.0150 mmol) and XPhos Pd G2 (12.6 mg, 0.0160 mmol) and anhydrous *t*-BuOH (15 mL). The reaction vessel was purged under Ar, heated to 50 °C and stirred overnight, at which point the solution turns into a gray cloudy mixture. The mixture was then filtered through a plug of Celite and eluted with acetone (20 mL). The elutants were concentrated on a rotary evaporator. Pure 3*S*-17*S*-FD-895 (**1a**) (68.0 mg, 80%) was obtained as a white semi-solid by flash chromatography over neutral silica gel eluting with a gradient of hexanes to 1:3 acetone/hexanes.

**3*S*,17*S*-FD-895 (1a):** TLC (1:3 acetone/$CH_2Cl_2$): $R_f$ = 0.20 (CAM stain); NMR data provided in Table S2; HR-ESI-MS *m/z* calcd. for $C_{30}H_{50}O_9Na$ $[M+Na]^+$: 589.3441, found 589.3440; $[\alpha]^{25}_D$= +12.4 ° (c = 1.0, $CH_2Cl_2$).

**Table 2.S2.** NMR data for 3*S*,17*S*-FD-895 (**1a**) in $C_6D_6$

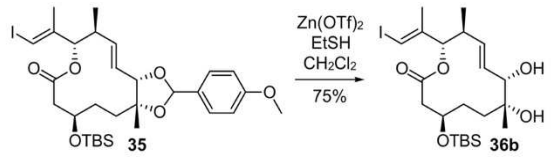
| Position | $\delta_C$, Type | $\delta_H$, mult (*J* in Hz) |
|---|---|---|
| 1 | 169.9 | |
| 2α | 40.2 | 2.51, dd (13.3, 11.3) |
| 2β | | 2.41, dd (13.4, 5.4) |
| 3 | 67.8 | 4.30, m |
| 4α | 27.6 | 1.63, m |
| 4β | | 1.43, m |
| 5α | 30.9 | 1.82, m |
| 5β | | 1.89, m |
| 6 | 73.6 | |
| 7 | 79.3 | 5.28, d (9.7) |
| 8 | 126.5 | 5.94, dd (15.1, 9.7) |
| 9 | 140.5 | 5.63, dd (15.2, 10.0) |
| 10 | 41.3 | 2.48, m |
| 11 | 82.3 | 5.21, d (10.6) |
| 12 | 131.6 | |
| 13 | 131.8 | 6.21, d (10.8) |
| 14 | 126.6 | 6.29, dd (14.9, 10.8) |
| 15 | 137.6 | 5.85, dd (14.8, 8.4) |
| 16 | 41.5 | 2.38, m |
| 17 | 73.0 | 3.48, q (3.7) |
| 18 | 59.7 | 2.60, dd (3.8, 2.2) |
| 19 | 57.7 | 3.05, dd (8.2, 2.2) |
| 20 | 39.2 | 1.34, m |
| 21 | 83.8 | 3.15, m |
| 22α | 23.9 | 1.65, m |
| 22β | | 1.38, m |
| 23 | 10.2 | 0.86, t (7.4) |
| 24 | 24.8 | 1.14, s |
| 25 | 16.5 | 0.75, d (6.7) |
| 26 | 12.0 | 1.63, d (1.2) |
| 27 | 17.3 | 1.15, d (7.0) |
| 28 | 10.9 | 0.90, d (7.0) |
| 29 | 169.4 | |
| 30 | 20.9 | 1.67, s |
| 31 | 57.7 | 3.24, s |

**H. Procedures for the synthesis of 7*R*,17*S*-FD-895 (1b).** A five step sequence was used to convert lactone **35** to core **3b** containing inversion at C7 and coupling it to side chain **2** to afford **1b**.




**Scheme 2.S4.** Red denotes the region of isomer installation.

**Conversion of 35 to diol 36b**




**Reagents:**

$Zn(OTf)_2$, 97% (Alfa Aesar): used without further purification

EtSH, 99% (Alfa Aesar): used without further purification

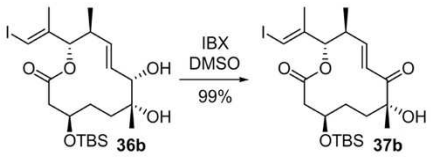
$NaHCO_3$, 98% (Fischer Scientific): used without further purification

**(3*R*,6*R*,7*S*)-(3*S*,4*S*,*E*)-1-iodo-2,4-dimethylhexa-1,5-dien-3-yl-3-((tert-butyldimethyl-silyl)oxy)-6,7-dihydroxy-6-methylnon-8-enoate (36b)**. Zinc triflate (1.60 g, 4.41 mmol) and EtSH (0.950 mL, 13.2 mmol) was added to a solution of **35** (500 mg, 0.882 mmol) in $CH_2Cl_2$ (50 mL) at 0 °C. The reaction was warmed to rt. After 4 h satd. $NaHCO_3$ (10 mL) was added. The phases were separated, and the organic phases were dried with $Na_2SO_4$ and concentrated by a rotary evaporator. Pure diol **36b** (356 mg, 75%) was obtained as colorless oil by flash chromatography, eluting with a gradient from hexanes to 1:4 EtOAc/hexanes.

Diol **36b**: TLC (1:4 EtOAc/hexanes): $R_f$ = 0.30 (CAM stain); $^1$H NMR (500 MHz, $CDCl_3$) δ 6.35 (d, *J* = 1.3 Hz, 1H), 5.62 (dd, *J* = 15.1, 9.7 Hz, 1H), 5.33 (dd, *J* = 15.2, 9.9 Hz, 1H), 5.01 (d, *J* = 10.7 Hz, 1H), 3.72 (m, 1H), 3.69 (d, *J* = 9.8 Hz, 1H), 2.40 (m, 1H), 2.38 (dd, *J* = 13.8, 3.3 Hz, 1H), 2.30 (dd, *J* = 13.8, 4.8 Hz, 1H), 1.81 (s, 3H), 1.68 (d, *J* = 1.2 Hz, 3H), 1.50 (m, 2H), 1.29 (m, 2H), 1.20 (s, 3H), 1.15 (bs, 1H), 0.81 (d, *J* = 6.9 Hz, 3H), 0.80 (s, 9H), -0.02 (s, 3H), -0.04 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 168.8, 143.9, 137.1, 130.2, 128.5, 83.9, 80.6, 73.7, 70.6, 40.6, 36.1

30.4, 29.8, 24.8, 25.9, 18.3, 16.6, -4.6, -4.7; HR-ESI-MS *m/z* calcd. for $C_{24}H_{43}IO_5SiNa$ $[M+Na]^+$: 561.1817, found 561.1819; $[\alpha]^{25}_D$= -28.1 ° (c = 1.0, $CH_2Cl_2$).

**Oxidation of diol 36b to ketone 37b**



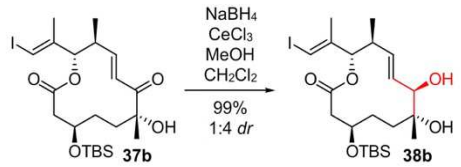

**Reagents:**

IBX, 95%: synthesized from 2-iodobenzoic acid and oxone (*46)*

**(4*R*,7*R*,11*S*,12*S*,*E*)-4-((*tert*-Butyldimethylsilyl)oxy)-7-hydroxy-12-((*E*)-1-iodoprop-1-en-2-yl)-7,11-dimethyloxacyclododec-9-ene-2,8-dione (37b)** Diol **36b** (300 mg, 0.558 mmol) was dissolved in DMSO (3 mL) in a scintillation vial and IBX (389 mg, 1.39 mmol) was added in one portion. The mixture was stirred at rt for 3 hr. EtOAc (50 mL) and $H_2O$ (50 mL) were added, and the phases were separated. The organic phase was washed with $H_2O$ (3 × 25 mL), dried over $Na_2SO_4$ and concentrated by a rotary evaporator. Pure ketone **37b** (290 mg, 99%) was obtained as a colorless oil by flash chromatography, eluting with a gradient of hexanes to 1:4 EtOAc/hexanes.

Ketone **37b**: TLC (1:4 EtOAc/hexanes): $R_f$ = 0.40 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 6.87 (d, *J* = 15.6 Hz, 1H), 6.37 (dd, *J* = 15.6, 9.7 Hz, 1H), 6.19 (d, *J* = 1.2 Hz, 1H), 5.02 (d, *J* = 10.4 Hz, 1H), 4.25 (tt, *J* = 8.3, 4.1 Hz, 1H), 2.34 (dd, *J* = 12.8, 3.6 Hz, 1H), 2.20 (m, 1H), 2.15 (dd, *J* =12.8, 9.1 Hz, 1H), 1.88 (bs, 1H), 1.79 (ddd, *J* = 14.0, 9.2, 6.5 Hz, 1H), 1.65 (m, 1H), 1.63 (d, *J* = 1.7 Hz, 3H), 1.52 (m, 1H), 1.44 (m, 1H), 1.23 (s, 3H), 0.96 (s, 9H), 0.46 (d, *J* = 6.7 Hz, 3H), 0.10 (s, 3H), 0.05 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 202.3, 168.4, 146.7, 143.7, 129.3, 84.3, 79.5, 79.0, 69.0, 44.3, 40.3, 36.9, 32.6, 26.1, 19.1, 18.3, 15.5, -4.3, -4.4; HR-ESI-MS *m/z* calcd. for $C_{24}H_{41}IO_5SiNa$ $[M+Na]^+$: 559.1442, found 559.1441; $[\alpha]^{25}_D$= -46.8 ° (c = 1.0, $CH_2Cl_2$).

**Reduction of ketone 37b to alcohol 38b**




**Reagents:**

$CeCl_3$•7 $H_2O$, 99% (Acros Organics): used without further purification

$NaBH_4$ 98%, (Acros Organics): used without further purification

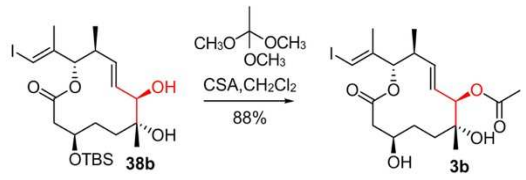
**(4*R*,7*R*,8*R*,11*S*,12*S*,*E*)-4-((*tert*-Butyldimethylsilyl)oxy)-7,8-dihydroxy-12-((*E*)-1-iodoprop-1-en-2-yl)-7,11-dimethyloxacyclododec-9-en-2-one (38b)** $CeCl_3$•7 $H_2O$ (274 mg, 1.11 mmol) was added to a solution of **37b** (215 mg, 0.743 mmol) in MeOH (5 mL) and cooled to -20 °C. $NaBH_4$ (0.817 mmol, 30.8 mg) was added in one portion, and the mixture was stirred for 5 min. The reaction was quenched with satd. $NaHCO_3$ (1 mL), dried over $NaSO_4$, and concentrated by a rotary evaporator. Pure diol **38b** (54.8 mg, 99%) was obtained in a 1:4 *dr* by flash chromatography, eluting with a gradient of hexanes to 1:3 EtOAc/hexanes.

*Note 1: Diol **36b** was the major diastereomeric product and was recycled by oxidation to **37b** and reduction to provide additional **38b**.*

Diol **38b**: TLC (1:4 EtOAc/hexanes): $R_f$ = 0.28 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 6.36 (s, 1H), 5.93 (dd, *J* = 15.6, 2.9 Hz, 1H), 5.30 (dd, *J* = 15.6, 9.3 Hz, 1H), 5.01 (d, *J* = 10.1 Hz, 1H), 3.79 (m, 1H), 3.75 (m, 1H), 2.31 (m, 1H), 2.26 (m, 2H), 1.83 (m, 1H), 1.72 (s, 3H), 1.60 (m, 2H), 1.43 (d, *J* = 5.2 Hz, 1H), 1.29 (m, 1H), 1.18 (s, 3H), 1.01 (s, 9H), 0.65 (d, *J* = 6.7 Hz, 3H), 0.08 (s, 3H), 0.07 (s, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 168.4, 144.8, 132.2, 130.0, 128.6, 83.4, 80.7, 78.3,

74.7, 71.0, 41.7, 40.3, 36.1, 31.6, 26.1, 19.5, 18.4, 16.6, -4.5; HR-ESI-MS *m/z* calcd. for $C_{24}H_{43}IO_5SiNa$ $[M+Na]^+$: 561.1817, found 561.1819; $[\alpha]^{25}_D$= +2.5 ° (c = 1.0, $CH_2Cl_2$).

**Two- step conversion of 38b to core 3b**




**Reagents:**

(1*S*)-(+)-10-Camphorsulfonic acid, 98% (TCI Chemicals): used without further purification

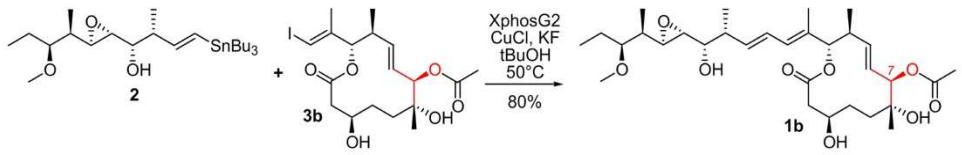
Trimethyl orthoformate, 99% (Sigma-Aldrich): used without further purification

**(2*S*,3*S*,6*R*,7*R*,10*R*,*E*)-7,10-Dihydroxy-2-((*E*)-1-iodoprop-1-en-2-yl)-3,7-dimethyl-12-oxooxacyclododec-4-en-6-yl acetate (3b)** Diol **38b** (41.1 mg, 0.0638 mmol) was dissolved in 1:3 MeOH/$CH_2Cl_2$ (10 mL) in a 20 mL scintillation vial and (1*S*)-(+)-10-camphorsulfonic acid (57.5 mg, 0.248 mmol) was added as a solid in one portion. The mixture was stirred for 5 h, at which point TLC analyses indicated complete conversion of starting material. The solvent was removed under rotary evaporation, and the resulting crude was taken up in anhydrous $CH_2Cl_2$ (10 mL) in a 20 mL scintillation vial and cooled to 0 °C. Trimethyl orthoformate (10.0 µL, 0.0783 mmol) was added neat, and the mixture was stirred at 0 °C for 1 h, at which point satd. $NaHCO_3$ (1 mL) was added. The mixture was extracted into $CH_2Cl_2$ (15 mL), and the organics were concentrated on a rotary evaporator. Pure core **3b** (38.9 mg, 88%) was obtained as a colorless wax by flash chromatography, eluting with a gradient of $CH_2Cl_2$ to 1:3 acetone/$CH_2Cl_2$.

Core **3b**: TLC (1:8 acetone/$CH_2Cl_2$): $R_f$ = 0.27 (CAM stain); $^1$H NMR (500 MHz, $C_6D_6$) δ 6.19 (d, *J* = 1.3 Hz, 1H), 5.87 (dd, *J* = 15.4, 2.4 Hz, 1H), 5.39 (q, *J* = 1.9 Hz, 1H), 5.24 (d, *J* = 10.5 Hz, 1H), 5.24 (m, 1H), 3.54 (bs, 1H), 2.25 (m, 2H), 2.19 (d, *J* = 14.0 Hz, 1H), 1.71 (m, 1H), 1.66 (s, 3H), 1.65 (d, *J* = 1.7 Hz, 3H), 1.61 (m, 1H), 1.50 (m, 1H), 1.17 (bs, 1H), 1.01 (s, 3H), 0.96 (m,

1H), 0.56 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (125 MHz, $C_6D_6$) δ 171.9, 169.2, 144.1, 129.8, 84.2, 80.0, 77.8, 73.7, 69.5, 41.0, 38.8, 36.4, 30.5, 24.7, 20.3, 19.1, 16.5; HR-ESI-MS *m/z* calcd. for $C_{18}H_{27}IO_6Na$ $[M+Na]^+$: 489.0745, found 489.0744; $[\alpha]^{25}_D$ = -14.8 ° (c = 1.0, $CH_2Cl_2$).

**Synthesis of 7*R*,17*S*-FD-895 (1b) by Stille coupling of core 3b to 2**




**Reagents:**

CuCl, anhydrous, beads, 99.99% (Sigma-Aldrich): used without further purification

KF, anhydrous, powder, 99.9% (Sigma-Aldrich): used without further purification

XPhos Pd G2 (Sigma-Aldrich): used without further purification

*t*-BuOH, anhydrous, 99.5% (Sigma-Aldrich): used without further purification

**7*R*,17*S*-FD-895 (1b).** Vinylstannane **2** (42.3 mg, 0.0750 mmol) and core **3b** (23.3 mg, 0.0500 mmol) were combined in a 20 mL scintillation vial and dried *via* rotary evaporation of benzene. To the mixture was then sequentially added CuCl (5.00 mg, 0.0500 mmol), KF (2.97 mg, 0.0500 mmol) and XPhos Pd G2 (4.20 mg, 0.00533 mmol) and anhydrous *t*-BuOH (5 mL). The reaction vessel was purged under Ar, heated to 50 °C and stirred overnight, at which point the solution turns into a gray cloudy mixture. The mixture was then filtered through a plug of Celite and eluted with acetone (20 mL). The elutants were concentrated on a rotary evaporator. Pure 7*R*-17*S*-FD-895 (**1b**) (13.6 mg, 80%) was obtained as a white semi-solid by flash chromatography over neutral silica gel eluting with a gradient of hexanes to 1:4 acetone/hexanes.

**7*R*,17*S*-FD-895 (1b):** TLC (1:8 acetone/$CH_2Cl_2$): $R_f$ = 0.28 (CAM stain); NMR data provided in Table S3; HR-ESI-MS *m/z* calcd. for $C_{30}H_{50}O_9Na$ $[M+Na]^+$: 589.3441, found 589.3440; $[\alpha]^{25}_D$= +22.1 ° (c = 1.0, $CH_2Cl_2$).

**Table 2.S3.** NMR data for 7*R*,17*S*-FD-895 (**1b**) in $C_6D_6$.

| Position | $\delta_C$ | $\delta_H$, mult (*J* in Hz) |
|---|---|---|
| 1 | 172.3 | 4.65, d (9.3) |
| 2α | 39.3 | 2.30, dd (14.7, 3.2) |
| 2β | | 2.36, dd (14.5, 4.2) |
| 3 | 69.6 | 3.59, m |
| 3-OH | | 3.76, d (10.6) |
| 4α | 30.6 | 1.80, m |
| 4β | | 1.69, m |
| 5α | 36.5 | 1.62, m |
| 5β | | 1.00, m |
| 6 | 73.8 | |
| 6-OH | | 1.97, bs |
| 7 | 82.9 | 5.36, d (10.5) |
| 8 | 128.3 | 5.96, dd (15.4, 2.4) |
| 9 | 130.7 | 5.40, ddd (9.8, 5.5, 2.2) |
| 10 | 41.1 | 2.48, tq (10.2, 6.7) |
| 11 | 78.0 | 5.44, m |
| 12 | 131.6 | |
| 13 | 131.6 | 6.18, dd (10.9, 1.5) |
| 14 | 126.4 | 6.29, dd (15.2, 10.9) |
| 15 | 137.8 | 5.81, dd (15.1, 8.5) |
| 16 | 41.5 | 2.38, m |
| 17 | 72.8 | 3.45, t (4.2) |
| 17-OH | | 1.82, bs |
| 18 | 59.6 | 2.58, dd (3.8, 2.3) |
| 19 | 57.6 | 3.04, dd (8.2, 2.2) |
| 20 | 39.0 | 1.33, m |
| 21 | 83.7 | 3.15, m |
| 22α | 23.8 | 1.62, m |
| 22β[1] | | 1.40, dt (13.9, 7.0)<br>1.26, m |
| 23 | 10.0 | 0.85, t (7.4)<br>0.86, t (7.4)[1] |
| 24 | 24.7 | 1.04, s |
| 25 | 16.9 | 0.77, d (6.8) |
| 26 | 11.9 | 1.64, d (1.2) |
| 27 | 17.3 | 1.13, d (7.0)<br>1.13, d (10.6)[1] |
| 28 | 10.8 | 0.89, d (7.0) |
| 29 | 169.3 | |
| 30 | 20.4 | 1.67, s |
| 31 | 57.7 | 3.23, s |

[1] Rotational isomers were observed by $^1$H NMR

**I. Cell Viability Assays.** HCT-116 cells were cultured in McCoy's 5a (Life Technologies) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U $mL^{-1}$ penicillin and 100 μg $mL^{-1}$ streptomycin at 37 °C in an atmosphere of 5% $CO_2$. HCT-116 cells were plated at 5 × 10 cells/well in McCoy's 5a containing 10% FBS. Cells were cultured for 24 h, pretreated with **1** or **1a-1c** in DMSO ranging from 0 to 1000 nM for 72 h (cell media contained □0.5% DMSO), and then washed with PBS (2 × 100 μL). 100 μL of PBS was added to each well, followed by 20 μL of CellTiter Aqueous One Solution (Promega). After 2 h at 37 °C, absorbance readings were taken at 490 nm (test wavelength) and 690 nm (reference wavelength). $GI_{50}$ values were calculated in Prism (GraphPad) using at least three biological replicates.

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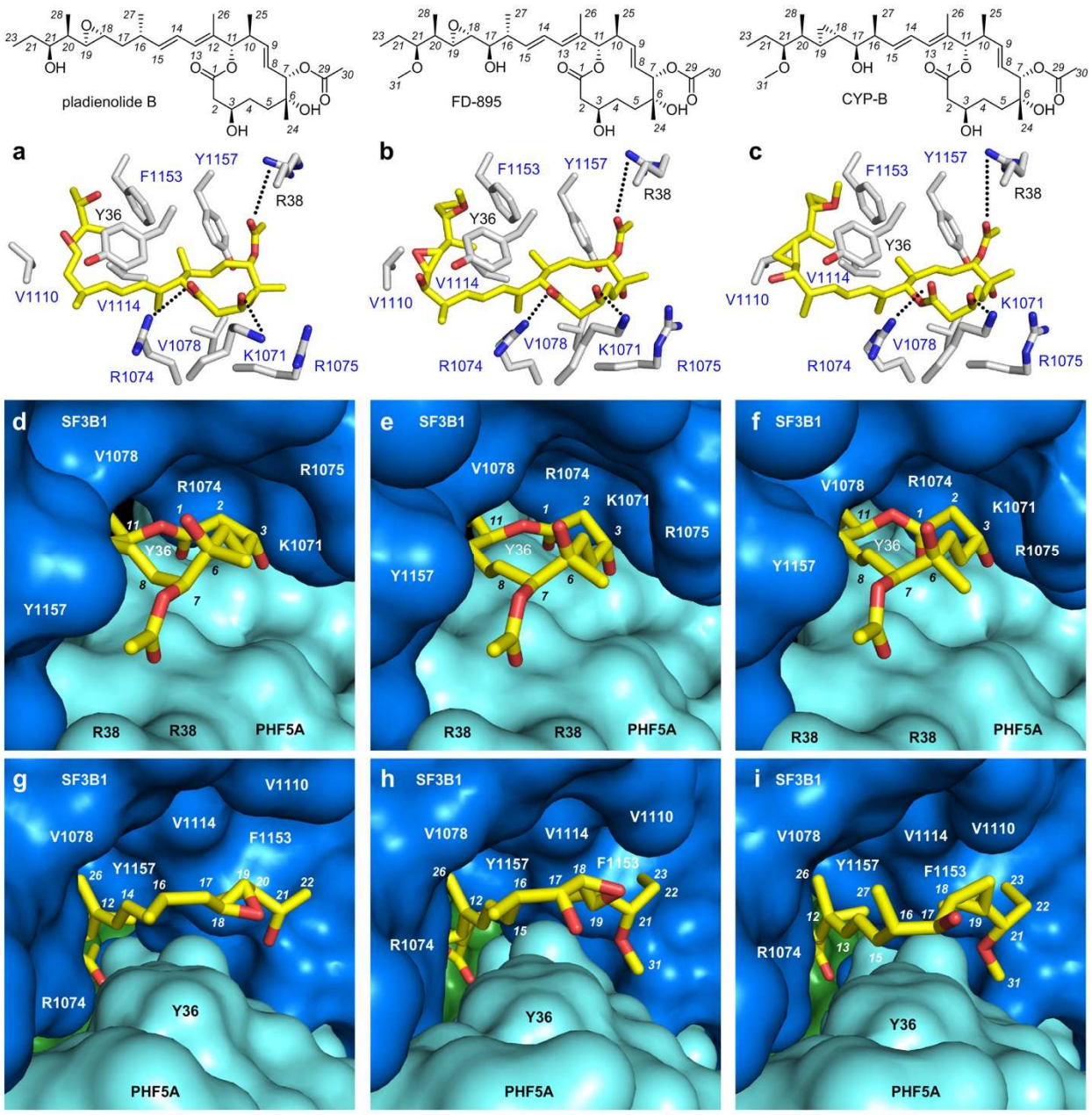


**Figure 2.S1.** X-ray crystal structures depicting the binding of pladienolide B (PDB ID 6EN4), FD-895 (*18*) and CYP (*18*) within the SF3B core. Side-chains of residues observed within 6 Å from **a)** pladienolide B, **b)** FD-895, and **c)** CYPB are shown in grey corresponding to SF3B1 (blue labels) and PHF5A (black labels). Van der Waals surfaces rendered to depict the core of **d)** pladienolide B, **e)** FD-895, and **f)** CYPB (yellow) and side chain of **g)** pladienolide B, **h)** FD-895 (yellow), and **i)** CYPB (yellow) are shown. Surface renderings depicting pladienolide B, FD-895 or CYPB. The structures of pladienolide B bound to the SF3B core are described in (*14*). A discussion on the structures of FD-895 and the cyclopropane analog CYPB are provided in (*18*).

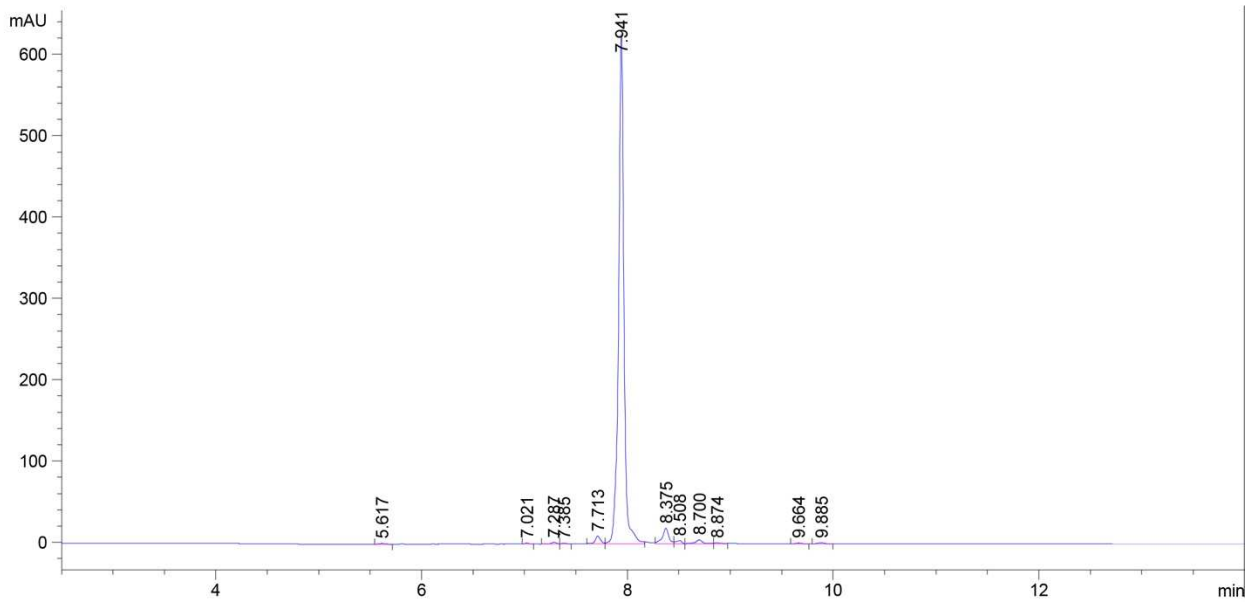


**Fig. 2.S2.** LC-MS trace. A 20-40 µL sample prepared in EtOH or DMSO was injected into an Agilent 1260 liquid chromatograph (LC) system coupled with a Thermo LCQdeca mass spectrometer (MS) using positive ion mode electrospray ionization (ESI) as the ion source. A Phenomenex Kinetex EVO C18 (ID 2.1 mm × length 50 mm, particle size 5.0 µm) was utilized for LC separation using water with 0.1 % formic acid as the mobile phase A and acetonitrile with 0.1 % formic acid as the mobile phase B. The LC flow rate was set at 0.30 mL/min. The LC gradient setting was as follows: 0 min: 5% mobile phase B; 10 min: 95% mobile phase B; 12 min: 95% mobile phase B; 13 min: 5% mobile phase B; and, 18 min: 5% mobile phase B. The total run time was 18 min. The UV detection wavelength was set at 254 nm (17*S*-FD-895 can be observed using detection at 254 nm). MS and HRMS is typically observed as the sodium ion in positive mode (HR-ESI-MS *m/z* calcd. for $C_{31}H_{50}O_9Na$ $[M+Na]^+$: 589.3345, found 589.3347).

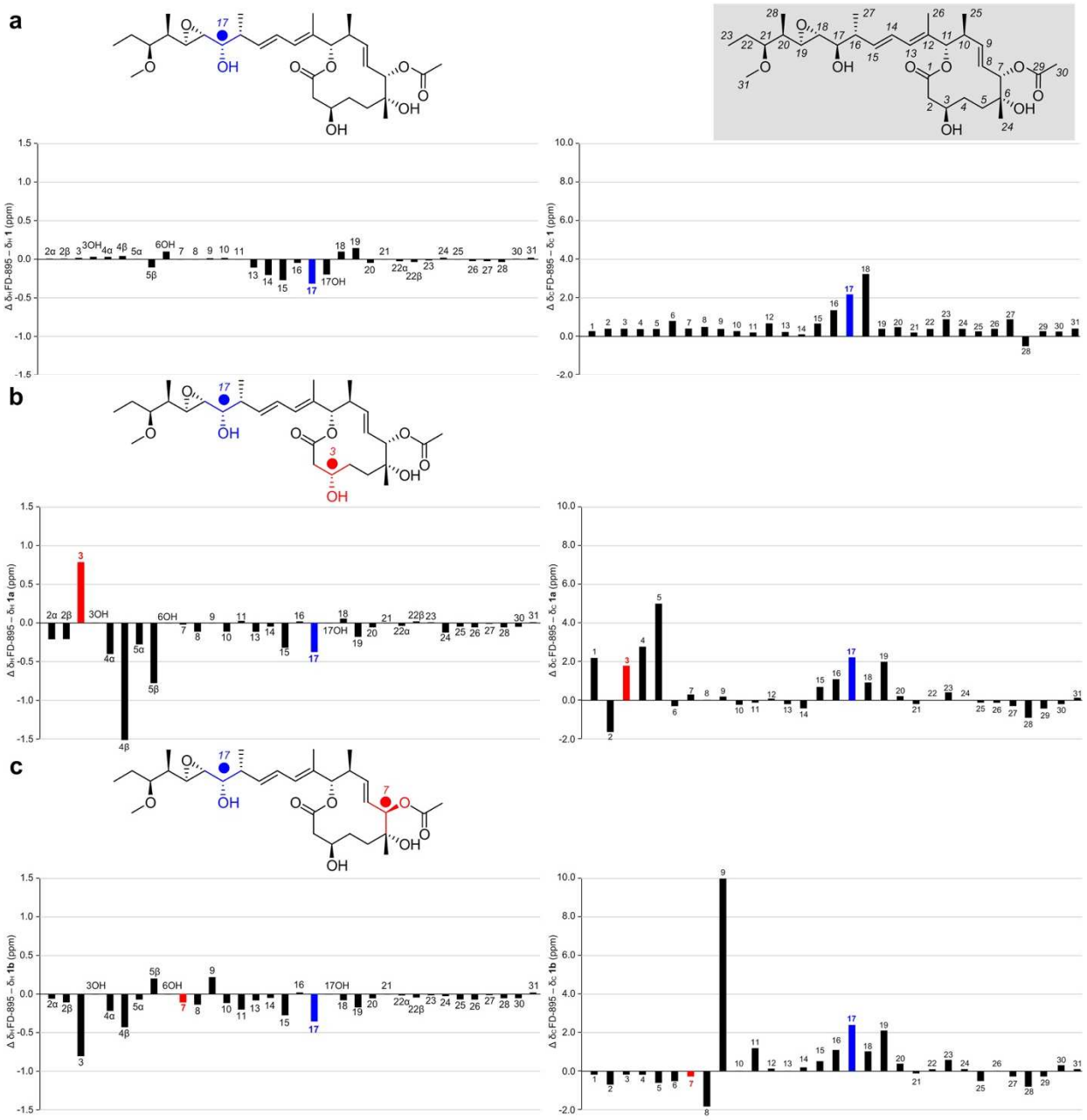


**Fig. 2.S3.** NMR comparison. **a)** Histograms depicting $^{1}$H (left) and $^{13}$C (right) chemical shifts differences between FD-895 (grey insert, upper right) and 17*S*-FD-895 (**1**). **b)** Histograms depicting $^{1}$H (left) and $^{13}$C (right) chemical shifts differences between FD-895 and 3*S*,17*S*-FD-895 (**1a**). **c)** Histograms depicting $^{1}$H (left) and $^{13}$C (right) chemical shifts differences between FD-895 and 7*R*,17*S*-FD-895 (**1a**). Red denotes an inverted center and blue is used to highlight the inverted C17 center.

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## Chapter 3: The Daedal Facets of Splice Modular Optimization

**Abstract:** The spliceosome has been shown to be a promising target for the development of new anticancer thera-peutics. Synthetic and chemical biological efforts directed towards the development of natural product-based splice modulators (SPLMs) have shown that the potency of these compounds derives from their ability to selectively affect the alternate splicing of apoptotic genes in tumor cells. However, questions remain regarding the mechanistic under-standing of splice modulation as well as the selectivity with which SPLMs impact certain genes.

The spliceosome is a complex molecular machine comprised of five small nuclear RNAs (snRNAs) and a number of associated proteins and cofactors that excise introns from pre-RNA to form mature RNA (*1*). Proper function of the spliceosome is essential to the life of higher organisms (*2*), and aberrant splicing is known to be responsible for a number of diseases, including clear-cell renal[2] cell carcinoma, lung cancer, acute myeloid leukemia (AML), and chronic myelomonocytic leukemia (CML) (*3-5*). For these reasons, the spliceosome has been identified as a promising target in the design of next generation cancer therapeutics. Herein, we highlight recent efforts to drug the spliceosome through the advance of medicinally-chemically optimized splice modulators (SPLMs) (*6-9*).

Three classes of natural products have been shown to target the SF3B component of the spliceosome (Figure 3.1) (*6*), the first of which, includes the 12-membered macrolide, FD-895 (**1a**, Fig. 1) (*10*). SPLMs belonging to this class have been referred to as the pladienolides (*11*). Their structures consist of a central diene that joins a macrolide core with an acyclic side chain unit (Fig. 1). The second class of SF3B-targeting SPLMs resembles **1a**, differing in the ring linked to the side chain moiety. This difference is illustrated in the structure of spliceostatin A (**2**) (*12*), which

features a six-membered ring. The third class, illustrated with herboxidiene (**3**) (*13*), contains a six membered core like the spliceostatins and a tail that shares similarities to that contained in the pladienolides.

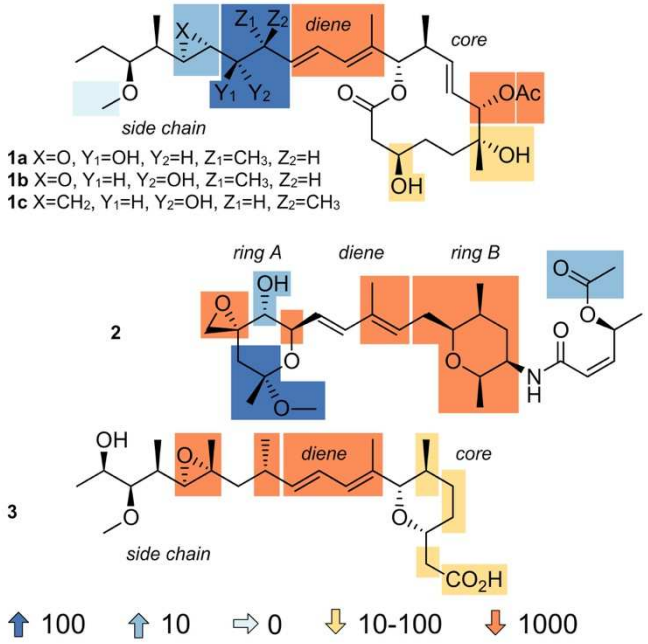


**Figure 3.1** Maps of the structure-activity relationship (SAR) data derived from cytotoxicity analyses (tumor cell $GI_{50}$ values) on synthetic or semi-synthetic derived SPLMs displayed with FD-895 (**1a**), 17S-FD-895 (**1b**), cyclopropane **1c**, spliceostatin A (**2**) and herboxidiene (**3**). Chemical modifications either enhance (blue) or attenuate (orange) activity.

To date, two compounds, E7107 (**4**) (*14-15*) and H3B-8800 (**5**) (*16*) have been explored as potential therapeutics in clinical trials (Figure 3.2), and a third, 17S-FD-895 (**1b**, Figure 3.1) is currently approaching IND filing. All three compounds are derived from the common 12-membered core macrolide observed in **1a-1c**. The first, E7107 (**4**), launched by Eisai Co. Ltd was evaluated in Phase I trials for application to solid tumors. However, the trials were halted due to patient vision loss caused by a presumed side or off-target effect (*14*).

Since the initiation of these trials, natural product semi-syntheses and total syntheses carried out by research groups across the world have revealed many of the key SARs within the three classes of natural products. As illustrated in Fig. 1, only a few modifications have been found to enhance their anti-tumor activity (*6*). Based in part on these findings, H3 Biomedicine advanced an orally active analogue H3B-8800 (**5**), in which the pladienolide side chain, a region that mapped favorably for optimization (Figure 3.2), has been replaced with a pyridyl-unit (Figure 3.2). Through a series of rigorous preclinical analyses, this material has been systematically tested for the treatment of patients with myelodysplastic syndromes, AML, and CML (*17*).

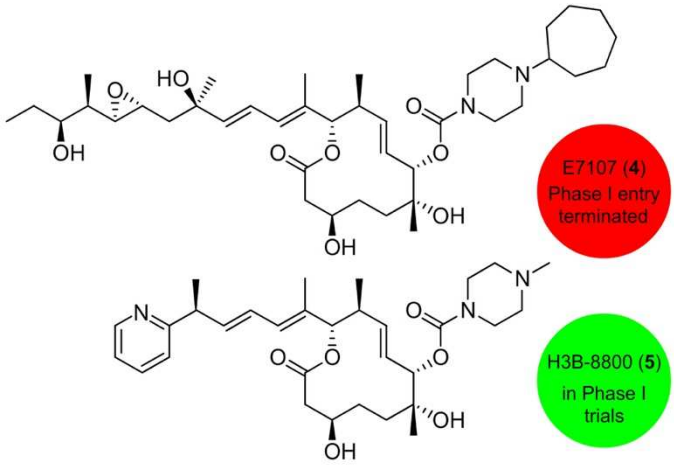


**Figure 3.2** Clinical leads. Structures of analogs that entered (red) or are ongoing (green) clinical trials.

Recent structural studies have revealed the molecular details of the SPLM binding pocket (*17-19)*. These structures, key advances brought forward by the Pena laboratory (*18*), demonstrate that pladienolide B binds to the branch point adenine (BPA) binding pocket of SF3B. Recently, co-crystal structures of **1a** (Figure 3.3a-c) and **1c** (Figure 3.3d-f) with the SF3B core were determined confirming the same binding motif as pladienolide B. As depicted in Fig. 3, two distinct pockets that accommodate either the side chain or macrolide ring define the SPLM binding cavity (*18*, *19*). The side chains of **1a** (Figure 3.3b) and **1c** (Figure 3.3e) occupy a hydrophobic pocket in which

their diene moieties interact with Y36 (PHF5A), while their alkyl termini form a hydrophobic contact with F1153 (SF3B1). This region of the binding pocket is relatively plastic in terms of its structure, as demonstrated by V1110's ability to reposition itself in response to different ligands as evidenced by comparing the structure of epoxide in **1a**•SF3B *versus* cyclopropane in **1c**•SF3B. In contrast, the contacts between the macrolide rings of **1a** (Figure 3.3c) and **1c** (Figure 3.3d-f) and SF3B are largely polar in nature with residues R38 (PHF5A) or K1071 (SF3B1) forming hydrogen bonds with the core.

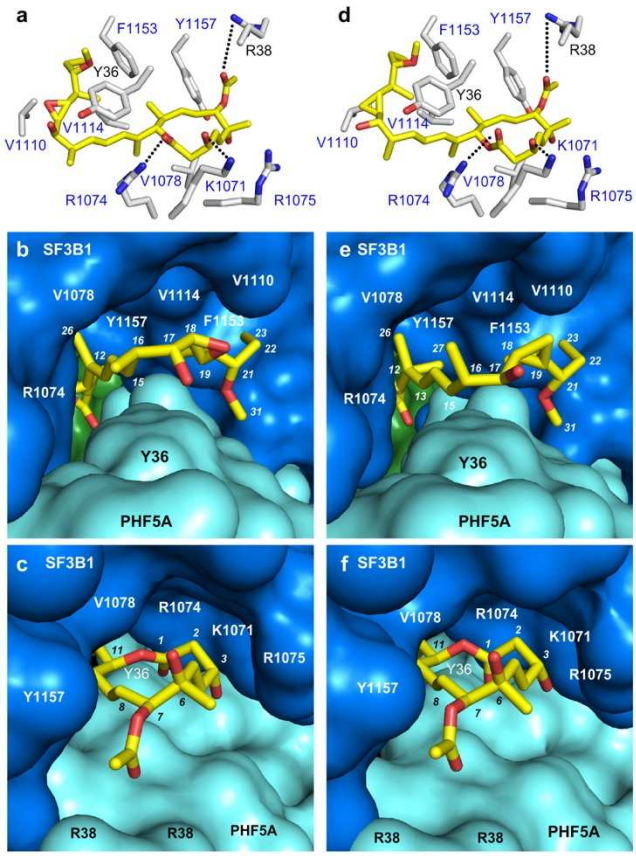


**Figure 3.3** Co-crystal structures of **(a-c)** FD-895 (**1a**) and **(h-i)** cyclopropane **1c** within the SF3B core. **(a,d)** Side chains of residues within 6 Å of the SPLM (yellow) are labeled in grey corresponding to SF3B1 (blue labels) and PHF5A (black labels). **(b,e)** "Connelly" surfaces of the SPLM binding site showing side chains of **1a** and **1c** occupying a pocket formed at the interface between SF3B1 (blue), PHF5A (cyan) and SF3B3 (green). **(c,f)** "Connelly" surfaces in the SPLM binding site depicting the macrolides of **1a** and **1c** positioned at the other end of this tunnel.

Ongoing studies have found that RNA splicing is altered in cancer cells, and SPLMs' ability to regulate aberrant splicing in tumors makes them attractive clinical candidates (*20, 21*). While these data were influential in guiding the indications clinically-evaluated for E7107 (**4**) and H3B-8800 (**5**), the intricate complexity associated with RNA splicing has made it challenging to understand the comprehensive anti-tumor mode of action (MOA) of SPLMs. As shown in Figure 3.4, natural and small molecule-induced splice modulation can occur through multiple and often parallel pathways, yielding different gene products that can influence cell phenotypes. While systems wide tools such as RNA-seq analyses (*22-24*) can be used to gather a global perspective of the effects of SPLMs in cells, the fact that these cells are often not synchronized and hence represent a population of cells in disparate stages of cell life further complicates work to decipher the discrete mechanistic responses to specific modulated splicing events.

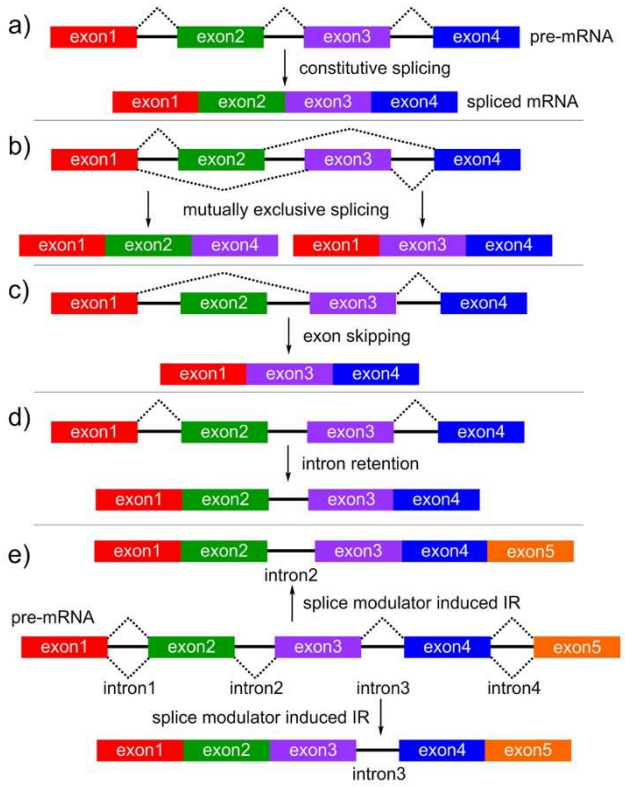


**Figure 3.4.** Types of RNA splicing: **a)** constitutive **b)** mutually exclusive **c)** exon skipping ES, or **d)** intron retention (IR). **e)** A schematic representation of SPLM-induced alternate splicing (AS). In this example, two IR products bearing intron2 (top) and intron3 (bottom) can arise from the same pre-mRNA.

The development of predictive models that reveal the links between the genetic, temporal and cellular selectivity of splice modulation is a critical next step for the future clinical translation of SPLMs. Such models play a key part in the development of valid assays, which can be used to guide lead identification. These assays also provide the tools to identify the specific cellular responses associate with SPLM activity, thereby facilitating the design of leads with minimal off-target splicing effects. Most critically, the information provided by these models offers a therapeutic tool that may one day allow physicians to predict the efficacy of a given therapeutic on a patient-by-patient basis. The fusion of molecular and cellular biological data with home-accessible biomarker development has a critical role in the broader implementation of SPLM-based and related RNA modulatory therapies as commercially viable cancer treatments.

Ongoing clinical and translational processes are focused on the advance of synthetic methods to evaluate analogs of the naturally occurring SPLMs. It is likely that computer-based drug discovery (CADD) approaches will identify new core and side chain motifs that reproduce the interactions between in SPLMs and SF3B, as revealed by X-ray crystal structures such as **1c**•SF3b (Figure 3.3a) and **1d**•SF3b (Figure 3.3b). This complemented with new screening tools opens a new window towards the future clinical translation of splice modulators. The key to this advance lies in unique balance between medicinal chemistry a chemical biology, and provides robust forum to advance the central principals of drug discovery.

Chapter 3, in full, is a reprint of the material as it appears in Daedal Facets of Splice Modulator Optimization, *ACS Med. Chem.Lett.* **9**, 1070-107 (2018). Brian León, Kelsey A. Trieger, Ashay Patel, James J. La Clair and Michael D. Burkart express their consent for inclusion of this published material in Chapter 2 of this dissertation. The dissertation author was an investigator and author on this paper.

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