Title

Design and synthesis of herboxidiene derivatives that potently inhibit in vitro splicing

Journal

Organic & Biomolecular Chemistry, 19(6)

ISSN

1477-0520

Authors

Ghosh, Arun K
Allu, Srinivasa Rao
Reddy, Guddeti Chandrashekar
et al.

Publication Date

2021-02-18

DOI

10.1039/d0ob02532a

Peer reviewed

HHS Public Access

Author manuscript

Org Biomol Chem. Author manuscript; available in PMC 2022 March 10.

Published in final edited form as:

Org Biomol Chem. 2021 February 18; 19(6): 1365–1377. doi:10.1039/d0ob02532a.

Design and synthesis of herboxidiene derivatives that potently inhibit *in vitro* splicing†

Arun K. Ghosh[a], **Srinivasa Rao Allu**[a], **Guddeti Chandrashekar Reddy**[a], **Adriana Gamboa Lopez**[b], **Patricia Mendez**[b], **Melissa S. Jurica**[b]

[a]Department of Chemistry and Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, USA.

[b]Department of Molecular Cell and Developmental Biology and Center for Molecular Biology of RNA, University of California, Santa Cruz, California 95064, USA

Abstract

Herboxidiene is a potent antitumor agent that targets the SF3B subunit of the spliceosome. Herboxidiene possesses a complex structural architecture with nine stereocenters and design of potent less complex structures would be of interest as a drug lead as well as a tool for studying SF3B1 function in splicing. We investigated a number of C-6 modified herboxidiene derivatives in an effort to eliminate this stereocenter and, also to understand the importance of this functionality. The syntheses of structural variants involved a Suzuki–Miyaura cross-coupling reaction as the key step. The functionalized tetrahydrofuran core has been constructed from commercially available optically active tri-*O*-acetyl-D-glucal. We investigated the effect of these derivatives on splicing chemistry. The C-6 alkene derivative showed very potent splicing inhibitory activity similar to herboxidiene. Furthermore, the C-6 *gem*-dimethyl derivative also exhibited very potent *in vitro* splicing inhibitory activity comparable to herboxidiene.

Information transfer from genes to proteins is a critical event for life. For nearly all human genes, precursor messenger RNA (pre-mRNA) splicing is an essential step in the process. Splicing removes intron sequences from gene transcripts to create functional messenger RNA (mRNA) for protein translation.[1,2] Recent studies have shown that defects in pre-mRNA splicing and acquired mutations of pre-mRNA splicing factors are associated with many human cancers.[3,4]

Splicing is carried out by a large and highly dynamic ribo-nucleoprotein complex called the spliceosome, which is assembled from uridine-rich small nuclear RNAs (snRNA) and dozens of specific proteins.[5,6] Splicing involves complex and highly regulated multi-step reactions.[7,8] Significant progress has been made in recent years in terms of understanding

†Electronic supplementary information (ESI) available: Experimental procedues and NMR spectra of compounds. See DOI: 10.1039/d0ob02532a

akghosh@purdue.edu; Fax: +1 765 4961612; Tel: +1 765 4945323.

Conflicts of interest

There are no conflicts to declare.

bio-chemical activity, protein composition, and structures of several distinct forms of the spliceosome as they occur during the reaction pathways.[9,10]

A core component of spliceosome is the SF3B1 protein, which is the largest subunit of the spliceosome factor 3b (SF3B) complex.[11,12] SF3B1 is an important pre-mRNA splicing factor with a role in the earliest stages of spliceosome assembly.[13] It participates in a series of structural and compositional rearrangements of spliceosome snRNAs and proteins that ultimately position the intron into the spliceosome's active site.[14] Increasing evidence points to mutations in SF3B1 of the spliceosome and their involvement in various types of human cancers, including haematological malignancies and solid tumors.[15,16] The pre-mRNA splicing factor SF3B1 has emerged as an outstanding target for anticancer drug development.[17,18] Currently, a number of natural products and their derivatives have been shown to potently inhibit spliceosome function by binding to the SF3B subunit of U2 snRNP.[19,20] These include, herboxidiene (**1**, Fig. 1) pladienolide B (**2**), a semisynthetic derivative E71O7 (**3**), FR901464 (**4**), and spliceostatin A (**5**). These compounds display potent *in vitro* splicing inhibition by interfering with the SF3B subunit of spliceosome.[21–24] The semisynthetic derivative of pladienolide B and semisynthetic derivative, E71O7 have undergone advanced human clinical trials.[25] Herboxidiene is a very potent inhibitor of spliceosome. It exhibits potent antitumor properties and it induces both G1 and G2/M cell cycle arrest in a human normal fibroblast cell line WI-38.[26]

As a result of its important structural and biological properties, herboxidiene attracted considerable synthetic attention. Several total syntheses of herboxidiene have been reported by a number of research groups.[26–30] Also, synthesis and biological evaluation of structural variants of herboxidiene have been reported by us and others. As outlined in Fig. 2, 5(*R*)-hydroxy herboxidiene **6** is a natural product which showed slightly lower splicing inhibition than herboxidiene. Interestingly, compound **7**, the C5 epimer also showed comparable inhibitory activity.[31] The C6 desmethyl herboxidiene **8** was synthesized, however, this compound exhibited slightly lower splicing inhibitory activity as well as anticancer activity in human cells.[29,32] The corresponding carba analog **9** showed substantial reduction in splicing inhibitory activity.[32] An interesting pladienolide-herboxidiene hybrid **10** was designed. However, it was significantly less potent.[29] Furthermore, a carbohydrate-based herboxidiene-pladienolide hybrid molecule **11** has been designed and synthesized to modulate splicing.[33]

Herboxidiene contains nine stereocenters and a number of sensitive functionalities. Therefore, it is imperative to elucidate structure–activity relationships of herboxidiene and design less complex and more potent herboxidiene derivatives. In our continuing interests in chemistry and biology of splicing inhibitors, we have designed a number of novel C6-modified, herboxidiene derivatives. Herein, we report enantioselective syntheses of C-6 modified methylene, (*R*)-methyl, cyclopropyl, and *gem*-dimethyl derivatives of herboxidiene and their *in vitro* evaluation of splicing inhibitory activity.

Results and discussion

We recently showed that incorporation of hydroxyl group at C-5 and removal of the C-6 methyl group resulted in only a small reduction of splicing activity. In an effort to probe the importance of C-6 methyl group, we have designed four C-6 modified derivatives including C-6 methylene derivative **12**, C-6 (*R*)-methyl derivative **13**, cyclopropyl derivative **14**, and *gem*-dimethyl derivative **15**. Our synthetic strategy for these derivatives is shown in Scheme 1. Our plan was to carry out Suzuki–Miyaura cross-coupling reaction of vinyl iodides **16** and pinacol boronate **17** to provide herboxidiene derivatives **12–15**. The pinacol boronate **17** was then be synthesized in optically active form using our previously reported procedure.[30,31] Various vinyl iodides **16a–d** were constructed from the known,[34,35] optically active aldehyde derivative **18**, which was obtained from commercially available, optically active triacetoxy-D-glucal **19** as the key starting material.

For the synthesis of C-6 vinyl derivative **12**, the synthesis of requisite vinyl iodide **16a** is shown in Schemes 2 and 3. Commercially available triacetoxy-D-glucal **19** was converted to acetaldehyde derivative **18** in multigram scale as reported in the literature.[35,36] Aldehyde derivative **18** was converted to enone **20** in a four-step sequence involving, (1) reaction of aldehyde with ethylene glycol in the presence of a catalytic amount of p-TSA in toluene, heated at reflux for 3 h; (2) treatment of the resulting di-*t*-butylsilyl acetal with $n\text{Bu}_4\text{N}^+\text{F}^-$ in THF at 0 °C to 23 °C for 12 h to give diol; (3) selective protection of primary alcohol as TBDPS ether with TBDPSCl in the presence of imidazole in CH_2Cl_2 at 0 °C to 23 °C for 3 h; and (4) oxidation of the resulting allylic alcohol with Dess–Martin periodinane to provide **20** in 60% yield over 4-steps. Catalytic hydrogenation of **20** over 10% Pd-C in EtOAc at 23 °C under a hydrogen-filled balloon afforded the corresponding ketone. Wittig olefination of the resulting ketone with methylenetriphenylphosphorane, generated from this reaction of Ph_3PCH_3Br and ^{t}BuOK in THF at 0 °C to 23 °C afforded the corresponding methylene derivative. Reaction of the resulting alkene derivative with $n\text{Bu}_4\text{N}^+\text{F}^-$ in THF at 0 °C to 23 °C for 12 h furnished alcohol **21** in 87% yield over 3-steps. To install the vinyl iodide, we first converted alcohol **21** to its acetylene derivative by oxidation with Dess–Martin periodinane at 0 °C to 23 °C for 6 h followed by reaction of the resulting aldehyde with Bestmann–Ohira reagent[36] in MeOH in the presence of K_2CO_3 at 0 °C to 23 °C for 4 h to provide alkyne derivative **22** in 66% yield over 2-steps. The 1,3-dioxalane functionality of **22** was converted to the corresponding methyl ester in a three-step sequence involving, (1) treatment of 1N aqueous HCl in THF at 23 °C for 36 h; (2) oxidation of the resulting aldehyde with $NaClO_2$ in aqueous *t*-butanol in the presence of 2-methyl-2-butene and NaH_2PO_4 at 0 °C to 23 °C for 5 h and (3) esterification of the resulting carboxylic acid with MeOH using EDC in the presence of a catalytic amount of DMAP in CH_2Cl_2 at 0 °C to 23 °C for 12 h to afford methyl ester **23** in 68% yield over 3-steps.

Methyl ester **23** was converted to vinyl iodide derivatives **16a** and **16b** as shown in Scheme 3. Reaction of alkyne derivative **23** with a catalytic amount of $HgSO_4$ in the presence of 3 M H_2SO_4 in aqueous THF at 23 °C for 3 h resulted in methyl ketone derivative **24** in 84% yield.[37] Treatment of ketone **24** with $CrCl_2$ and CHI_3 in THF at 23 °C for 12 h using protocol described by Takai and co-workers,[38] afforded vinyl iodide **16a** in 79% yield

as a *E*/*Z* mixture (20 : 1 by ^{1}H NMR analysis) which was used directly for the coupling reaction. Similarly, methyl ketone **24** was also converted to vinyl iodide derivative **16b**. Catalytic hydrogenation of **24** over 10% Pd-C in EtOAc under hydrogen-filled balloon at 23 °C provided 1 : 1 mixture of diastereomeric methyl ketones **25** and **26** in 97% yield. These diastereomers were separated by silica gel column chromatography using 25% ethyl acetate in hexanes. The C6 (*R*)-diastereomer **26** was converted to vinyl iodide **16b** by exposure to $CrCl_2$ and CHI_3 in THF at 23 °C as described above.

The synthesis of vinyl iodides **16c** and **16d** was carried out from alkene derivative **21** as shown in Scheme 4. Simmons–Smith cyclopropanation[39] of **21** was sluggish. We decided to carry out dichlorocyclopropanation.[40,41] Thus, exposure of alkene **21** to $CHCl_3$ and 50% aqueous NaOH in the presence of a catalytic amount of benzyltriethylammonium chloride at 80 °C for 48 h furnished dichlorocyclopropane derivative **27** in 80% yield. Reduction of **27** with $LiAlH_4$ in THF at 0 °C to 64 °C for 24 h afforded cyclopropane derivative **28** in 90% yield. Oxidation of alcohol **28** with Dess–Martin periodinane and reaction of the resulting aldehyde with Ohira–Bestmann reagent[36] in MeOH furnished alkyne derivative **29**. The alkyne was converted to keto ester **30**. Deprotection of 1,3-dioxolane with 1N HCl provided aldehyde which was oxidized with $NaClO_2$ to carboxylic acid. Esterification of the resulting carboxylic acid with MeOH using EDC and a catalytic amount of DMAP as described for compound **23**, furnished the corresponding methyl ester. Reaction of the resulting alkyne with a catalytic amount of $HgSO_4$ in the presence of 3 M H_2SO_4 in aqueous THF provided methyl ketone derivative **30** in 70% yield over 4-steps starting from alkyne **29**. Treatment of methyl ketone **30** with $CrCl_2$ and CHI_3 in THF as described above, resulted in vinyl iodide **16c** in 82% yield. For installation of C-6 *gem*-dimethyl group, cyclopropane derivative, we planned to hydrogenate the cyclopropane derivative over PtO_2.[42] Thus, cyclopropane derivative **30** was subjected to catalytic hydrogenation over PtO_2 in acetic acid under hydrogen-filled balloon at 40 °C for 30 min to provide cyclopropane ring opened product **31** in 85% yield. Methyl ketone functionality of **31** was transformed into vinyl iodide **16d** as described above to provide **16d** in 75% yield. Various vinyl iodides **16a-d** so synthesized were immediately used for Suzuki–Miyaura cross-coupling reaction[43,44] with boronate **17**.

Synthesis of herboxidiene C-6 methylene derivative **12** is shown in Scheme 5. Suzuki–Miyaura cross-coupling[43,44] of pinacol boronate **17** and vinyl iodide **16a** was carried out with a catalytic amount of $Pd(PPh_3)_4$ (5 mol%) and Cs_2CO_3 in THF at 23 °C to 55 °C for 5 h to provide coupling product **32** in 47% yield. For regio- and stereoselective installation of epoxide functionality at C14–C15, the TBS-ether of **32** was removed by exposure to 0.2 M HCl in methanol at 23 °C for 45 min. Directed epoxidation[45] of the resulting alcohol was carried out with a catalytic amount of $VO(acac)_2$ in the presence of *t*-BuOOH in CH_2Cl_2 at −20 °C for 36 h to provide the epoxide **33** in 62% yield over 2-steps. Methyl ester hydrolysis with Me_3SnOH in dichloroethane at 80 °C for 24 h using the protocol described by Nicolaou and co-workers,[46,47] provided herboxidiene C-6 methylene derivative **12** in 85% yield. For the synthesis of herboxidiene C-6 (*R*)-methyl derivative **13**, Suzuki–Miyaura cross-coupling of pinacol boronate **17** and vinyl iodide **16b** provided coupling product **34** in 51% yield. Removal of TBS group followed by directed epoxidation as described above, afforded epoxide **29** in 58% yield over 2-steps. Me_3SnOH promoted hydrolysis of

the methyl ester as described above, furnished herboxidiene C-6 (*R*)-methyl derivative **7** in 84% yield. The syntheses of herboxidiene C-6 cyclopropyl and *gem*-dimethyl derivatives **14** and **15** is shown are Scheme 6. Suzuki–Miyaura cross-coupling of pinacol boronate **17** and vinyl iodides **16c** and **16d** provided coupling products **36** and **38** in 44% and 47% yield, respectively. Removal of TBS group followed by directed epoxidation as described above, afforded epoxide derivatives **37** and **39** in 57% and 66% yield, respectively over 2-steps. Me_3SnOH promoted hydrolysis of the methyl ester as described above, resulted in C-6 cyclopropyl and *gem*-dimethyl derivatives **14** and **15** in 82% and 87% yield, respectively.

We carried out dose response analysis for all synthetic derivatives in an *in vitro* splicing system.[23] For splicing efficiency, we measured spliced product produced after incubating an RNA splicing substrate in extracts prepared from the nuclei of human cells, which also contained herboxidiene derivative or DMSO as a control. IC_{50} values were determined as the concentration required to reduce *in vitro* splicing efficiency by half compared to DMSO control (Fig. 3). Of the four C-6 modified herboxidiene derivatives evaluated, C-6 alkene derivative **12** exhibited the most potent *in vitro* splicing inhibitory activity. The IC_{50} value of alkene **12** is 0.4 μM, which within the variability of the assay is comparable to the 0.3 μM IC_{50} that we previously measured for herboxidiene.[27] Derivative **13** with a C-6 (*R*)-methyl group shows good IC_{50} value of 2.5 μM, however, it is about 6-fold less potent than herboxidiene. Essentially, both C-6 methyl isomers shows potent splicing inhibitory activity. In an effort to remove the C-6 stereocenter, we therefore designed both C-6 cyclopropyl derivative **14** and *gem*-dimethyl derivative **15**. The C-6 cyclopropyl derivative **14** exhibited an average IC_{50} value of 5.2 μM. Interestingly, the *gem*-dimethyl derivative **15** showed 3-fold reduction in potency compared to herboxidiene.

These results were nicely supported in terms of spliceosome assembly. Spliceosome assembly intermediates were visualized as the stereotypical change in mobility of radiolabeled splicing substrate in native gels. The herboxidiene derivatives disrupted the transition from **A** to **B** complex at the same concentration at which they inhibited splicing efficiency (Fig. 4). This result is consistent with the requirement for the inhibitor target SF3B in the spliceosome assembly pathway.

Conclusion

In summary, we have designed, synthesized, and evaluated the splicing properties of C-6 modified derivatives of herboxidine. These compounds were designed to investigate the importance of the C-6 substituent, possible replacement of the C-6 stereocenter with a new substituent, and improve splicing inhibitory activity. The syntheses of derivatives involved the Suzuki–Miyaura cross-coupling reaction of a pinacol boronate derivative with an appropriate vinyl iodide partner. All vinyl iodide derivatives were synthesized in optically active form using triacetoxy-D-glucal as the key starting material. The C-6 cyclopropane derivatives were synthesized by cyclopropanation of the C-6 methylene derivative with dichlorocarbene followed by LAH reduction. Reductive opening of the cyclopropane ring provided the corresponding C-6-*gem*-dimethyl derivatives. We evaluated all synthetic derivatives in *in vitro* spliceosome inhibitory assay. The C-6 alkene derivative **12** showed very potent splicing inhibitory activity comparable to herboxidiene, indicating

that C-6(*S*)-methyl group may not be critical to herboxidiene splicing inhibitory activity. The C-6 (*R*)-methyl derivative **13** resulted in about 6-fold loss in potency. Interestingly, the C-6 *gem*-dimethyl derivative **15**, which eliminated C-6 stereochemistry, exhibited very potent splicing inhibition with slight loss in activity compared to herboxidiene. The corresponding cyclopropyl derivative **14** showed 5-fold loss of activity compared to its *gem*-dimethyl derivative. The present work indicated that both C-6 alkene and the corresponding *gem*-dimethyl derivative are able to maintain comparable splicing inhibitory activity as herboxidiene. Further design and synthesis of novel derivatives are in progress.

Experimental section

All reactions were carried out under an inert atmosphere, either nitrogen or argon, using magnetic stirring and oven-dried glassware. All solvents were anhydrous and distilled prior to use. Dichloromethane and triethylamine were distilled from calcium hydride. Tetrahydrofuran, diethyl ether, and benzene were distilled from sodium/benzophenone. All other solvents were HPLC grade. Flash column chromatography was performed using EM Science 60–200 mesh silica gel. Thin-layer chromatography was performed using 60 F-254 E. Merck silica gel plates. ^{1}H- and ^{13}C-NMR were recorded using Bruker AV-400 MHz, Avance DRX-500, Varian Mercury-Vx-300, and Gemini-2300 spectrometers and use Me_4Si as an internal standard. Optical rotations were recorded on a PerkinElmer 341 polarimeter. A Thermo Finnigan LCQ classic mass was used for HRMS analysis. The purity of test compounds was determined by HRMS and HPLC analysis.

(2*R*,6*S*)-6-((1,3-Dioxolan-2-yl)methyl)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2*H*-pyran-3(6*H*)-one (20)

To a solution of aldehyde **18** (4.0 g, 12.81 mmol) in toluene (40 mL) at 23 °C were added ethylene glycol (2.0 mL) and p-TSA (441 mg, 2.56 mmol). The reaction was then connected to Dean–Stark apparatus and refluxed for 3 h. After this period, the reaction mixture was cooled to 23 °C, and quenched with saturated $NaHCO_3$ solution. The resulting solution was extracted with EtOAc (2×), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% EtOAc in hexanes) to give desired product as an amorphous solid (4.1 g, 90%).

To a solution of di-*t*-butylsilyl acetate (2.7 g, 7.58 mmol) in THF (30 mL) was added TBAF (1 M in THF, 11.4 mL, 11.37 mmol) at 0 °C. The resulting reaction mixture was allowed to warm to 23 °C and stirred for 12 h. The reaction mixture was quenched with H_2O and extracted with EtOAc (3×). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude was used for the next step without any further purification.

To a solution of above diol compound (1.5 g, 6.94 mmol) in CH_2Cl_2 (25 mL) at 0 °C were added imidazole (566 mg, 8.33 mmol) and TBDPSCl (1.9 g, 6.94 mmol). The reaction mixture was allowed to warm to 23 °C and stirred for 3 h. The reaction was diluted with H_2O and extracted with CH_2Cl_2 (2×), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (20% EtOAc in hexanes) to give allylic the alcohol as a colorless oil (2.5 g, 80%).

To a solution of the above allylic alcohol (1.2 g, 2.64 mmol) in CH_2Cl_2 (20 mL) were added DMP (2.2 g, 5.28 mmol) and $NaHCO_3$ (666 mg, 7.93 mmol) at 0 °C. The resulting reaction mixture was allowed to warm to 23 °C and stirred for 4 h. After this period, the reaction mixture was quenched with saturated $Na_2S_2O_3$ followed by saturated $NaHCO_3$ solution. The mixture was stirred vigorously for 20 min and extracted with CH_2Cl_2 (2×). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% EtOAc in hexanes) to give enone **20** as an amorphous solid (1.0 g, 60% over 4 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (ddd, J = 6.2, 3.1, 10.8 Hz, 4H), 7.45–7.33 (m, 6H), 7.04 (dd, J = 10.3, 1.5 Hz, 1H), 6.10 (dd, J = 10.3, 2.4 Hz, 1H), 5.11 (dd, J = 6.2, 3.6 Hz, 1H), 4.60 (ddd, J = 7.5, 5.6, 2.0 Hz, 1H), 4.16–4.05 (m, 3H), 4.04–3.96 (m, 2H), 3.92–3.82 (m, 2H), 2.14 (ddd, J = 14.0, 7.8, 3.6 Hz, 1H), 1.98 (ddd, J = 14.1, 6.1, 5.4 Hz, 1H), 1.02 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.5, 151.3, 135.6, 133.6, 133.5, 129.5, 127.5, 126.9, 101.3, 81.5, 70.3, 64.9, 64.7, 63.0, 38.9, 26.6, 19.2; $[\alpha]_D^{20} = -18.6$ (c 0.22, $CHCl_3$); HRMS (m/z) (ESI M + Na^+) calc. for $C_{26}H_{32}O_5SiNa$ 475.1919, found 475.1923.

((2*S*,6*R*)-6-((1,3-Dioxolan-2-yl)methyl)-3-methylenetetrahydro-2*H*-pyran-2-yl)methanol (21)

To a solution of enone **20** (960 mg, 2.12 mmol) in EtOAc (10 mL) was added Pd-C (100 mg, 10 wt%). The resulting reaction mixture was stirred under a hydrogen-filled balloon for 12 h. After this period, the reaction mixture was filtered through Celite, washed with EtOAc, and concentrated under reduced pressure. The crude was used for the next step without any further purification.

To a suspension of $Ph_3P^+CH_3Br^-$ (2.2 g, 6.14 mmol) in dry THF (10 mL) was added potassium *tert*-butoxide (5.1 mL, 1 M, 5.12 mmol) at 0 °C and the reaction mixture was continued to stir for another 30 min. A solution of ketone (930 mg, 2.05 mmol) in THF (10 mL) was added at 0 °C and the resulting mixture was continued to stir for another 1 h. The reaction was quenched with NH_4Cl solution and extracted with EtOAc (2×). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude was used for the next step without any further purification.

To a solution of alkene compound (800 mg, 1.77 mmol) in dry THF (10 mL) at 0 °C was added a solution of TBAF (1.0 M in THF, 2.65 mL, 2.65 mmol). The resulting reaction mixture was allowed to warm to 23 °C and stirred for 12 h. The reaction mixture was diluted with H_2O and extracted with EtOAc (2×). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (30% EtOAc in hexanes) to give alcohol **21** as a colorless oil (348 mg, 87% over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.04 (dd, J = 5.9, 4.1 Hz, 1H), 4.84 (d, J = 0.8 Hz, 1H), 4.69 (s, 1H), 4.04–3.95 (m, 2H), 3.95–3.84 (m, 4H), 3.83–3.73 (m, 2H), 2.42 (ddd, J = 13.9, 4.8, 2.7 Hz, 1H), 2.37–2.23 (m, 2H), 1.98 (ddd, J = 14.1, 8.3, 4.1 Hz, 1H), 1.80 (dddd, J = 14.1, 10.1, 5.4, 3.3 Hz, 2H), 1.50 (tdd, J = 12.9, 11.4, 4.9 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.4, 107.5, 102.3, 77.9, 74.0, 64.9, 64.7, 63.0, 40.4, 33.7, 32.8; $[\alpha]_D^{20} = +5.3$ (c 0.28, $CHCl_3$); HRMS (m/z) (ESI M + Na^+) calc. for $C_{11}H_{18}O_4Na$ 237.1106, found 237.1103.

(2*S*,6*R*)-6-((1,3-Dioxolan-2-yl)methyl)-2-ethynyl-3-methylenetetrahydro-2*H*-pyran (22)

To a solution of alcohol **21** (1.0 g, 4.67 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added DMP (2.97 g, 7.0 mmol). The reaction temperature was allowed to warm to 23 °C and stirred for 6 h. After this period, the reaction mixture was quenched with saturated $Na_2S_2O_3$ followed by saturated $NaHCO_3$ solution. The mixture was stirred vigorously for 30 min and extracted with CH_2Cl_2 (2×). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude was used for the next step without any further purification.

To a solution of aldehyde in MeOH (10 mL) at 0 °C were added Bestmann–Ohira reagent (1.08 g, 5.60 mmol) and K_2CO_3 (973.8 mg, 7.0 mmol). The flask was wrapped with aluminium foil. The suspension was warmed to 23 °C and stirred for 4 h. The reaction was then diluted with EtOAc, quenched with saturated $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc (2×). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% EtOAc in hexanes) to give alkyne **22** as a colorless oil (646 mg, 66% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.39 (d, J = 1.4 Hz, 1H), 5.08 (dd, J = 6.8, 3.6 Hz, 1H), 4.96 (s, 1H), 4.69 (s, 1H), 4.03–3.94 (m, 2H), 3.94–3.84 (m, 2H), 3.81–3.73 (m, 1H), 2.66 (d, J = 2.2 Hz, 1H), 2.54 (ddd, J = 13.9, 4.4, 2.5 Hz, 1H), 2.36–2.25 (m, 1H), 2.10–2.03 (m, 1H), 1.87–1.81 (m, 1H), 1.73 (ddd, J = 14.1, 6.9, 4.6 Hz, 1H), 1.55–1.46 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.9, 110.6, 101.8, 79.9, 75.6, 75.0, 70.7, 64.8, 64.7, 40.3, 33.6, 31.7; $[\alpha]_D^{20} = -36.0$ (c 0.13, $CHCl_3$).

Methyl 2-((2*R*,6*S*)-6-ethynyl-5-methylenetetrahydro-2*H*-pyran-2-yl)acetate (23)

To a solution of alkyne **22** (140 mg, 0.67 mmol) in THF (1.5 mL) at 23 °C was added 1N HCl (1.5 mL), and the resulting mixture was stirred for 36 h. After this period, the reaction mixture was quenched with saturated $NaHCO_3$ solution and the aqueous layer was extracted with CH_2Cl_2 (2×). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude aldehyde was used for the next step without any further purification.

To a solution of above crude aldehyde (106 mg, 0.64 mmol) in *tert*-butanol and water (2 : 1, 6.0 mL) at 0 °C were added 2-methyl-2-butene (450 mg, 6.43 mmol), $NaClO_2$ (80% purity, 175 mg, 1.93 mmol) and $NaH_2PO_4{\cdot}H_2O$ (264 mg, 1.93 mmol). The reaction was allowed to warm to 23 °C. The solution was continued to stir until the aldehyde disappeared (5 h). The biphasic mixture was separated and the aqueous layer was extracted with EtOAc (2×), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude acid was used for the next step without any further purification.

The above crude acid (110 mg, 0.61 mmol) was dissolved in CH_2Cl_2 (5 mL) at 0 °C. To this solution was added EDC·HCl (141 mg, 0.73 mmol), DMAP (90 mg, 0.73 mmol) and MeOH (59 mg, 1.84 mmol). The reaction temperature was allowed to warm to 23 °C and stirred for 12 h. After this period, the reaction was diluted with H_2O, extracted with CH_2Cl_2 (2×), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (20% EtOAc in hexanes) to afford methyl ester

23 as a colorless oil (89 mg, 68% over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (d, J = 1.4 Hz, 1H), 4.97 (s, 1H), 4.72 (d, J = 1.0 Hz, 1H), 4.02 (dtd, J = 11.2, 6.5, 2.0 Hz, 1H), 3.71 (s, 3H), 2.74–2.68 (m, 1H), 2.66 (d, J = 2.2 Hz, 1H), 2.58–2.52 (m, 1H), 2.45 (dd, J = 15.8, 6.3 Hz, 1H), 2.38–2.27 (m, 1H), 1.87 (ddt, J = 12.9, 4.6, 2.2 Hz, 1H), 1.49 (tdd, J = 13.1, 11.3, 4.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 141.4, 111.0, 79.6, 75.8, 74.6, 70.9, 51.7, 40.7, 32.8, 31.5; $[\alpha]_D^{20} = -6.1\ (c\ 0.13,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{11}H_{14}O_3Na$ 217.0844, found 217.0840.

Methyl 2-((2*R*,6*S*)-6-acetyl-5-methylenetetrahydro-2*H*-pyran-2-yl)acetate (24)

To a solution of methyl ester **23** (65 mg, 0.34 mmol) in THF (3 mL) was added $HgSO_4$ (20 mg, 0.07 mmol in 1 mL of 3 M H_2SO_4) at 23 °C. This solution was stirred at 23 °C until the starting material disappeared (3 h). The reaction mixture was diluted with EtOAc and neutralized with saturated $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc (2×), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (20% EtOAc in hexanes) to afford methyl ketone **24** as a colorless oil (60 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.89 (s, 1H), 4.62 (s, 1H), 4.35 (s, 1H), 4.07–3.95 (m, 1H), 3.69 (s, 3H), 2.63 (dd, J = 15.4, 7.6 Hz, 1H), 2.53–2.43 (m, 2H), 2.35 (ddd, J = 13.0, 4.2, 2.1 Hz, 1H), 2.20 (s, 3H), 1.86 (ddt, J = 12.9, 5.0, 2.5 Hz, 1H), 1.52 (ddd, J = 17.7, 12.1, 6.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.5, 171.4, 140.8, 109.8, 84.7, 73.4, 51.8, 40.9, 32.4, 32.4, 26.7; $[\alpha]_D^{20} = -42.3\ (c\ 0.13,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{11}H_{16}O_4Na$ 235.0950, found 235.0948.

Methyl 2-((2*R*,6*S*)-6-((*E*)-1-iodoprop-1-en-2-yl)-5-methylenetetrahydro-2*H*-pyran-2-yl)acetate (16a)

A suspension of $CrCl_2$ (203 mg, 1.70 mmol) in THF (3 mL) was stirred at 23 °C. To this suspension was added a mixture of ketone **24** (35 mg, 0.17 mmol) and CHI_3 (195 mg, 0.51 mmol) in THF (3 mL) with the use of a cannula. The resulting dark brown solution was stirred for 12 h. After this period, EtOAc and H_2O were added to this mixture. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% EtOAc in hexanes) to afford vinyl iodide **16a** as a yellow oil (25 mg, 79% brsm, E/Z: 20 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (s, 1H), 4.83 (s, 1H), 4.57 (d, J = 1.1 Hz, 1H), 4.41 (s, 1H), 4.07–3.93 (m, 1H), 3.68 (s, 3H), 2.59 (dd, J = 15.4, 7.0 Hz, 1H), 2.50–2.39 (m, 2H), 2.39–2.26 (m, 1H), 1.87–1.82 (m, 1H), 1.81 (d, J = 1.0 Hz, 3H), 1.52–1.40 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 145.7, 143.8, 110.0, 83.5, 80.2, 73.9, 51.7, 40.9, 32.9, 32.3, 21.9; $[\alpha]_D^{20} = -14.0\ (c\ 0.1,\ CHCl_3)$.

Methyl 2-((2*R*,5*R*,6*S*)-6-acetyl-5-methyltetrahydro-2*H*-pyran-2-yl)acetate (26)

To a solution of methyl ketone **24** (200 mg, 0.94 mmol) in EtOAc (10 mL) was added Pd-C (20 mg, 10 wt%). The reaction mixture was stirred under a hydrogen-filled balloon for 12 h. After this period, the reaction mixture was filtered through Celite, washed with EtOAc (20 mL), and concentrated under reduced pressure. The residue was purified by silica gel

chromatography (25% EtOAc in hexanes) and separated diastereomeric mixture of (1 : 1) methyl ketones **25** and **26** as a colorless oil (195 mg, combined yield 97%). Data for 26: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (d, J = 2.4 Hz, 1H), 3.87–3.77 (m, 1H), 3.69 (s, 3H), 2.65 (dd, J = 15.1, 7.8 Hz, 1H), 2.48 (dd, J = 15.1, 5.2 Hz, 1H), 2.18 (dd, J = 9.1, 4.9 Hz, 1H), 2.12 (s, 3H), 1.81 (ddd, J = 13.0, 11.4, 4.7 Hz, 1H), 1.70 (s, 1H), 1.59–1.41 (m, 2H), 0.87 (d, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 210.1, 171.6, 85.4, 75.1, 51.6, 41.2, 30.0, 29.2, 27.1, 25.4, 12.1; $[\alpha]_D^{20} = -36.2\ (c\ 1.0,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{11}H_{18}O_4Na$ 237.1106, found 237.1104.

Methyl 2-((2*R*,5*R*,6*S*)-6-((*E*)-1-iodoprop-1-en-2-yl)-5-methyltetrahydro-2*H*-pyran-2-yl) acetate (16b)

Compound **16b** was prepared from **26** (70 mg, 0.33 mmol) by following the same procedure outlined for **16a** to give a colorless oil. Yield (45 mg, 82% brsm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14–6.12 (m, 1H), 3.92 (s, 1H), 3.85–3.77 (m, 1H), 3.67 (s, 3H), 2.58 (dd, J = 14.9, 7.4 Hz, 1H), 2.43 (dd, J = 15.0, 5.5 Hz, 1H), 1.92 (ddt, J = 9.2, 4.7, 2.4 Hz, 1H), 1.86–1.75 (m, 1H), 1.71 (s, 3H), 1.69–1.61 (m, 1H), 1.51–1.41 (m, 2H), 0.75 (d, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 145.7, 83.1, 77.0, 75.0, 51.7, 41.4, 30.1, 29.0, 25.7, 21.9, 11.6; $[\alpha]_D^{20} = -49.1\ (c\ 1.0,\ CHCl_3)$.

((4*S*,6*R*)-6-((1,3-Dioxolan-2-yl)methyl)-1,1-dichloro-5-oxaspiro [2.5]octan-4-yl)methanol (27)

A solution of alcohol **21** (1.0 g, 4.67 mmol) in $CHCl_3$ (100 mL) was added a solution of 50% aqueous NaOH (30 mL) and benzyltriethylammonium chloride (128 mg, 0.56 mmol) at 23 °C. The resulting reaction mixture was heated to 80 °C for 48 h. The organic layer was separated and washed with H_2O, then brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (10% EtOAc/DCM) to give dichloro cyclopropane **27** as a colorless oil (801 mg, 80% brsm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.02 (t, J = 4.9 Hz, 1H), 3.98 (ddd, J = 13.3, 8.7, 3.7 Hz, 3H), 3.91–3.71 (m, 4H), 3.55 (dd, J = 11.4, 7.6 Hz, 1H), 2.50 (s, 1H), 2.05–1.90 (m, 3H), 1.88–1.74 (m, 2H), 1.60–1.53 (m, 1H), 1.51 (d, J = 7.8 Hz, 1H), 1.31 (d, J = 7.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 102.4, 71.4, 65.6, 65.0, 64.8, 63.4, 40.5, 31.8, 29.4, 28.7, 28.2; $[\alpha]_D^{20} = +32.17\ (c\ 0.23,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{12}H_{18}Cl_2O_4Na$ 319.0474, found 319.0479.

((4*S*,6*R*)-6-((1,3-Dioxolan-2-yl)methyl)-5-oxaspiro[2.5]octan-4-yl) methanol (28)

To a solution of dichloro cyclopropane **27** (200 mg, 0.68 mmol) in dry THF (5 mL) at 0 °C was added a solution of LAH (1.0 M in THF, 6.8 mL, 6.76 mmol). The reaction was allowed to warm to 23 °C and then heated at reflux for 24 h. The reaction was quenched with NH_4Cl and extracted with EtOAc (3×). The combined organic extracts were washed with H_2O, then brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (20% EtOAc/DCM) to give cyclopropane **28** as a colorless oil (141 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.02 (dd, J = 5.8, 4.3 Hz, 1H), 4.02–3.89 (m, 2H), 3.89–3.78 (m, 3H), 3.75–3.62 (m, 1H), 3.32–3.18 (m, 2H), 2.21 (s, 1H), 2.11–1.91 (m, 2H), 1.78 (ddd, J = 14.1, 5.8, 4.1 Hz, 1H), 1.71–1.59 (m, 2H), 1.52

(tdd, J = 13.0, 11.2, 4.1 Hz, 1H), 0.95 (ddd, J = 13.4, 4.0, 2.8 Hz, 1H), 0.69–0.61 (m, 1H), 0.45 (dd, J = 9.1, 4.7 Hz, 1H), 0.26–0.18 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 102.5, 79.7, 74.7, 64.9, 64.7, 61.7, 40.6, 35.2, 31.8, 19.2, 9.0, 8.6; $[\alpha]_D^{20} = +11.1\ (c\ 0.1,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{12}H_{20}O_4Na$ 251.1265, found 251.1261.

(4*S*,6*R*)-6-((1,3-Dioxolan-2-yl)methyl)-4-ethynyl-5-oxaspiro[2.5] octane (29)

Compound **29** was prepared from **28** (130 mg, 0.57 mmol) by following the same procedure outlined for **22** to give a colorless oil. Yield (90 mg, 71% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.05 (dd, J = 6.9, 3.6 Hz, 1H), 4.56 (s, 1H), 4.00–3.93 (m, 2H), 3.91–3.80 (m, 2H), 3.69–3.59 (m, 1H), 2.32 (d, J = 2.2 Hz, 1H), 2.10–1.94 (m, 2H), 1.78–1.59 (m, 3H), 1.59–1.46 (m, 1H), 1.09 (ddd, J = 13.5, 3.9, 2.9 Hz, 1H), 1.01–0.95 (m, 1H), 0.65 (dd, J = 8.5, 4.5 Hz, 1H), 0.27–0.24 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 101.9, 79.4, 75.3, 73.5, 72.9, 64.8, 64.7, 40.6, 33.1, 31.4, 19.8, 8.9; $[\alpha]_D^{20} = -38.3\ (c\ 0.18,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{13}H_{18}O_3Na$ 245.1160, found 245.1157.

Methyl 2-((4*S*,6*R*)-4-acetyl-5-oxaspiro[2.5]octan-6-yl)acetate (30)

Compound **30** was prepared from **29** (50 mg, 0.24 mmol) by following the same procedure outlined for **23** and **24** to give a colorless oil. Yield (36 mg, over 4 steps 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.06 (d, J = 0.8 Hz, 1H), 3.90 (dddd, J = 11.1, 7.7, 5.2, 2.2 Hz, 1H), 3.68 (s, 3H), 2.64 (dd, J = 15.1, 7.9 Hz, 1H), 2.49 (dd, J = 15.1, 5.2 Hz, 1H), 2.09 (s, 3H), 2.08–2.01 (m, 1H), 1.73–1.64 (m, 1H), 1.53 (tdd, J = 13.0, 11.3, 4.1 Hz, 1H), 0.99 (ddd, J = 13.6, 4.0, 2.8 Hz, 1H), 0.72 (dd, J = 7.3, 2.6 Hz, 1H), 0.33–0.18 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 208.1, 171.6, 84.9, 74.1, 51.7, 41.2, 34.7, 30.6, 27.5, 18.2, 9.1, 8.5; $[\alpha]_D^{20} = -74.2\ (c\ 0.07,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{12}H_{18}O_4Na$ 249.1108, found 249.1105.

Methyl 2-((4*S*,6*R*)-4-((*E*)-1-iodoprop-1-en-2-yl)-5-oxaspiro[2.5] octan-6-yl)acetate (16c)

Compound **16c** was prepared from **30** (30 mg, 0.13 mmol) by following the same procedure outlined for **16a** to give a colorless oil. Yield (18 mg, 82% brsm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.11 (s, 1H), 4.32 (s, 1H), 3.99–3.87 (m, 1H), 3.70 (s, 3H), 2.64 (dd, J = 15.1, 7.0 Hz, 1H), 2.48 (dd, J = 15.1, 6.0 Hz, 1H), 2.08 (dd, J = 26.6, 13.5 Hz, 1H), 1.75 (s, 3H), 1.74–1.63 (m, 1H), 1.52 (ddd, J = 24.3, 12.9, 4.1 Hz, 1H), 1.09–1.01 (m, 1H), 0.48 (dd, J = 8.5, 4.7 Hz, 1H), 0.38–0.29 (m, 1H), 0.23 (dt, J = 9.8, 5.1 Hz, 1H), 0.15 (dt, J = 9.2, 4.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 144.8, 84.6, 80.2, 74.7, 51.6, 41.2, 34.8, 31.0, 23.4, 20.4, 9.3, 8.5; $[\alpha]_D^{20} = +7.4\ (c\ 1.3,\ CHCl_3)$.

Methyl 2-((2*R*,6*S*)-6-acetyl-5,5-dimethyltetrahydro-2*H*-pyran-2-yl) acetate (31)

To a solution of cyclopropane methyl ketone **30** (30 mg, 0.13 mmol) in AcOH (2 mL) was added PtO_2 (45 mg, 0.20 mmol). The reaction mixture was stirred at 40 °C under a hydrogen-filled balloon for 30 min. After this period, the reaction mixture was filtered through Celite, washed with EtOAc (20 mL), and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% EtOAc in hexanes) to give *gem*-dimethyl ketone **31** as an oil (26 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (td,

J = 8.1, 4.0 Hz, 1H), 3.68 (s, 3H), 3.51 (s, 1H), 2.61 (dd, *J* = 15.0, 7.8 Hz, 1H), 2.48 (dd, *J* = 15.0, 5.1 Hz, 1H), 2.10 (s, 3H), 1.60–1.42 (m, 4H), 0.97 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.6, 171.5, 89.8, 74.4, 51.6, 41.1, 39.2, 32.6, 27.9, 27.5, 26.8, 19.9; $[\alpha]_D^{20} = -73.8\left(c\ 0.93,\ CHCl_3\right)$; HRMS (*m*/*z*) (ESI M + Na^+) calc. for $C_{12}H_{20}O_4Na$ 251.1265, found 251.1263.

Methyl 2-((2*R*,6*S*)-6-((*E*)-1-iodoprop-1-en-2-yl)-5,5-dimethyltetrahydro-2*H*-pyran-2-yl)acetate (16d)

Compound **16d** was prepared from **31** (40 mg, 0.18 mmol) by following the same procedure outlined for **16a** to give a light yellow oil. Yield (19 mg, 75% brsm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (s, 1H), 3.76 (dd, *J* = 13.1, 6.6 Hz, 1H), 3.70 (s, 1H), 3.67 (s, 3H), 2.60 (dd, *J* = 15.0, 6.8 Hz, 1H), 2.45 (dd, *J* = 15.0, 6.0 Hz, 1H), 1.80 (s, 3H), 1.54–1.46 (m, 4H), 0.87 (s, 3H), 0.83 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.0, 171.6, 146.0, 89.4, 79.8, 74.8, 51.5, 41.1, 39.2, 33.3, 27.9, 27.7, 23.1, 20.1; $[\alpha]_D^{20} = +13.3\left(c\ 0.06,\ CHCl_3\right)$.

Methyl 2-((2*R*,6*S*)-6-((2*E*,4*E*,6*S*,8*E*,10*S*,11*R*,12*R*)-12-((*tert*-butyldimethylsilyl)oxy)-11-methoxy-6,8,10-trimethyltrideca-2,4,8-trien-2-yl)-5-methylenetetrahydro-2*H*-pyran-2-yl)acetate (32)

A mixture of vinyl iodide **16a** (25 mg, 0.07 mmol) and pinacol boronate **17** (38 mg, 0.08 mmol) in THF (3.0 mL) was stirred under argon. To this solution was added $Pd(PPh_3)_4$ (4.3 mg, 0.003 mmol) and Cs_2CO_3 (487 mg, 1.49 mmol). The resulting mixture was heated at 55 °C for 5 h. The reaction mixture was cooled to 23 °C. The mixture was diluted with H_2O and the aqueous layer was extracted with EtOAc (2×). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (5% EtOAc in hexanes) to give triene compound **32** as an oil (24 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (dd, *J* = 15.1, 10.9 Hz, 1H), 5.98 (d, *J* = 10.9 Hz, 1H), 5.56 (dd, *J* = 15.1, 7.5 Hz, 1H), 5.12 (d, *J* = 9.0 Hz, 1H), 4.78 (s, 1H), 4.60 (d, *J* = 1.4 Hz, 1H), 4.28 (s, 1H), 3.96 (dtd, *J* = 11.1, 6.5, 2.0 Hz, 1H), 3.82 (p, *J* = 6.2 Hz, 1H), 3.67 (s, 3H), 3.43 (s, 3H), 2.77 (t, *J* = 5.4 Hz, 1H), 2.66–2.52 (m, 2H), 2.50–2.29 (m, 4H), 2.02 (dd, *J* = 13.2, 6.9 Hz, 1H), 1.90 (dd, *J* = 13.4, 7.6 Hz, 1H), 1.82 (ddd, *J* = 5.9, 5.0, 2.5 Hz, 1H), 1.73 (d, *J* = 0.8 Hz, 3H), 1.58 (d, *J* = 1.1 Hz, 3H), 1.51–1.39 (m, 1H), 1.12 (d, *J* = 6.3 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H), 0.89 (d, *J* = 4.7 Hz, 12H), 0.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 144.4, 140.6, 133.2, 131.4, 130.8, 127.9, 123.6, 109.3, 89.7, 84.7, 73.4, 70.1, 60.7, 51.5, 47.4, 41.0, 35.0, 33.7, 32.8, 32.2, 25.9, 20.1, 19.9, 18.0, 16.1, 15.4, 13.6, −4.7; $[\alpha]_D^{20} = -25.0\left(c\ 0.06,\ CHCl_3\right)$; HRMS (*m*/*z*) (ESI M + Na^+) calc. for $C_{32}H_{56}O_5SiNa$ 571.3789, found 571.3793.

Methyl 2-((2*R*,6*S*)-6-((*S*,2*E*,4*E*)-7-((2*R*,3*R*)-3-((2*R*,3*R*,4*R*)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)-5-methylenetetrahydro-2*H*-pyran-2-yl) acetate (33)

To a stirred solution of compound **32** (50 mg, 0.09 mmol) in MeOH (2 mL) at 0 °C was added 0.2 M HCl in MeOH (1 mL). The ice-bath was removed and the stirring was continued for 1 h. The reaction was quenched with saturated $NaHCO_3$ solution. The aqueous

layer was extracted with EtOAc (2×). The combined organic layers were dried over Na_2SO_4, and concentrated under reduced pressure. The crude was used for the next step without any further purification.

To a mixture of alcohol (35 mg, 0.08 mmol) and $VO(acac)_2$ (5.4 mg, 0.02 mmol) in CH_2Cl_2 (2 mL) at −78 °C was added ^{t}BuOOH (63 μl, 5–6 M in PhH, 0.34 mmol). The resulting mixture was stirred at −20 °C for 36 h. The reaction was quenched by adding Me_2S (0.5 mL). The resulting mixture was stirred at 23 °C until the color turned green. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (20–30% EtOAc/hexanes) to yield the epoxy ester **33** as a colorless oil (23 mg, 62% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (dd, J = 14.9, 11.0 Hz, 1H), 5.95 (d, J = 10.8 Hz, 1H), 5.45 (dd, J = 15.0, 8.7 Hz, 1H), 4.77 (s, 1H), 4.56 (s, 1H), 4.28 (s, 1H), 4.02–3.91 (m, 1H), 3.85 (s, 1H), 3.67 (s, 3H), 3.53 (s, 3H), 2.96 (t, J = 5.3 Hz, 1H), 2.65–2.52 (m, 3H), 2.50–2.38 (m, 3H), 2.32 (t, J = 11.3 Hz, 1H), 1.92–1.79 (m, 2H), 1.73 (s, 3H), 1.58–1.51 (m, 1H), 1.44 (dd, J = 11.9, 4.1 Hz, 1H), 1.29 (s, 3H), 1.18 (d, J = 6.4 Hz, 3H), 1.04 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 144.3, 139.2, 134.2, 127.6, 124.9, 109.2, 87.5, 84.7, 73.4, 68.2, 65.9, 61.2, 61.2, 51.5, 46.7, 40.9, 35.2, 35.0, 32.7, 32.2, 21.9, 18.9, 16.5, 13.5, 11.8; $[\alpha]_D^{20} = -19.3\left(c\ 0.16,\ CHCl_3\right)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{26}H_{42}O_6Na$ 473.2874, found 473.2880.

2-((2*R*,6*S*)-6-((*S*,2*E*,4*E*)-7-((2*R*,3*R*)-3-((2*R*,3*R*,4*R*)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)-5-methylenetetrahydro-2*H*-pyran-2-yl)acetic acid (12)

To a stirred solution of epoxy ester **33** (8.0 mg, 0.02 mmol) in $(CH_2Cl)_2$ (1 mL) was added Me_3SnOH (32 mg, 0.18 mmol). The reaction mixture was then heated at 80 °C for 24 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with $NaHSO_4$ (0.01 M), then brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (5% MeOH/CH_2Cl_2) to give the acid **12** as an oil (6.6 mg, 85% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 6.37 (dd, J = 15.0, 10.9 Hz, 1H), 5.99 (d, J = 10.4 Hz, 1H), 5.51 (dd, J = 15.0, 9.0 Hz, 1H), 4.80 (s, 1H), 4.59 (d, J = 1.4 Hz, 1H), 4.33 (s, 1H), 3.98 (dt, J = 11.1, 5.6 Hz, 1H), 3.82 (p, J = 6.4 Hz, 1H), 3.55 (s, 3H), 3.01 (dd, J = 6.3, 4.2 Hz, 1H), 2.68 (d, J = 9.4 Hz, 1H), 2.56–2.33 (m, 5H), 1.94 (dd, J = 13.4, 4.4 Hz, 1H), 1.89–1.82 (m, 1H), 1.75 (d, J = 0.9 Hz, 3H), 1.53 (ddd, J = 9.5, 6.9, 4.2 Hz, 1H), 1.43 (dd, J = 11.3, 4.7 Hz, 1H), 1.38 (t, J = 7.1 Hz, 1H), 1.32 (s, 3H), 1.14 (d, J = 6.5 Hz, 3H), 1.08 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 7.0 Hz, 3H); ^{13}C NMR (201 MHz, CD_3OD) δ 144.8, 139.1, 134.0, 130.9, 128.4, 127.6, 125.0, 108.1, 87.1, 84.8, 73.8, 68.4, 66.3, 61.2, 60.4, 46.6, 35.0, 35.0, 32.5, 31.9, 21.1, 18.4, 15.3, 12.4, 10.0; $[\alpha]_D^{20} = -16.6\left(c\ 0.12,\ CHCl_3\right)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{25}H_{40}O_6Na$ 459.2734, found 459.2729.

Methyl 2-((2*R*,5*R*,6*S*)-6-((2*E*,4*E*,6*S*,8*E*,10*S*,11*R*,12*R*)-12-((*tert*-butyldimethylsilyl)oxy)-11-methoxy-6,8,10-trimethyltrideca-2,4,8-trien-2-yl)-5-methyltetrahydro-2*H*-pyran-2-yl) acetate (34)

Compound **34** was prepared from **16b** (20 mg, 0.06 mmol) by following the same procedure outlined for **32** to give a yellow oil. Yield (17 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.20 (dd, J = 15.0, 11.0 Hz, 1H), 5.99 (d, J = 11.0 Hz, 1H), 5.54 (dt, J = 12.4, 6.2 Hz, 1H), 5.13 (d, J = 9.2 Hz, 1H), 3.88–3.76 (m, 3H), 3.68 (s, 3H), 3.44 (d, J = 2.0 Hz, 3H), 2.78 (dd, J = 5.8, 5.0 Hz, 1H), 2.63 (dd, J = 14.8, 7.0 Hz, 1H), 2.59–2.50 (m, 1H), 2.49–2.32 (m, 2H), 2.04 (dd, J = 13.3, 6.3 Hz, 1H), 1.89 (dt, J = 13.7, 7.6 Hz, 2H), 1.83–1.75 (m, 1H), 1.70 (dt, J = 6.2, 4.3 Hz, 1H), 1.63 (s, 3H), 1.58 (d, J = 1.2 Hz, 3H), 1.52–1.42 (m, 2H), 1.12 (d, J = 6.3 Hz, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.8 Hz, 12H), 0.77 (d, J = 3.4 Hz, 3H), 0.06 (d, J = 2.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 139.5, 134.0, 131.6, 130.7, 123.8, 123.2, 89.8, 82.4, 74.8, 70.1, 60.8, 51.5, 47.5, 41.4, 35.0, 33.6, 30.4, 29.2, 25.9, 20.1, 19.90, 18.0, 16.1, 15.3, 13.8, 11.6, −4.7; $[\alpha]_D^{20} = -12.5\ (c\ 0.16,\ CHCl_3)$.

Methyl 2-((2*R*,5*R*,6*S*)-6-((*S*,2*E*,4*E*)-7-((2*R*,3*R*)-3-((2*R*,3*R*,4*R*)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)-5-ethyltetrahydro-2*H*-pyran-2-yl) acetate (35)

Compound **35** was prepared from **34** (15 mg, 0.03 mmol) by following the same procedure outlined for **33** to give a light yellow oil. Yield (6.1 mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.23 (dd, J = 15.0, 11.0 Hz, 1H), 5.97 (d, J = 11.0 Hz, 1H), 5.44 (dd, J = 14.9, 8.5 Hz, 1H), 3.91–3.75 (m, 3H), 3.68 (s, 3H), 3.55 (s, 3H), 2.98 (t, J = 5.4 Hz, 1H), 2.66–2.53 (m, 3H), 2.48–2.37 (m, 2H), 1.92–1.76 (m, 3H), 1.75–1.65 (m, 2H), 1.58 (s, 3H), 1.54 (dd, J = 6.3, 3.3 Hz, 1H), 1.49–1.44 (m, 1H), 1.28 (s, 3H), 1.18 (d, J = 6.4 Hz, 3H), 1.05 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.9 Hz, 3H), 0.77 (d, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 137.9, 135.0, 125.0, 122.9, 87.5, 82.4, 74.8, 68.2, 65.9, 61.3, 61.2, 51.5, 46.8, 41.4, 35.1, 34.7, 30.4, 29.6, 29.4, 25.8, 21.8, 18.9, 16.5, 13.8, 11.6; $[\alpha]_D^{20} = -42.8\ (c\ 0.07,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{26}H_{44}O_6Na$ 475.3048, found 475.3041.

2-((2*R*,5*R*,6*S*)-6-((*S*,2*E*,4*E*)-7-((2*R*,3*R*)-3-((2*R*,3*R*,4*R*)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)-5-methyltetrahydro-2*H*-pyran-2-yl)acetic acid (13)

Compound **13** was prepared from **35** (5.2 mg, 0.01 mmol) by following the same procedure outlined for **12** to give a colorless oil. Yield (4.2 mg, 84% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 6.28 (dd, J = 15.0, 11.0 Hz, 1H), 6.00 (d, J = 11.1 Hz, 1H), 5.41 (dd, J = 15.0, 8.8 Hz, 1H), 3.87–3.74 (m, 2H), 3.54 (d, J = 5.3 Hz, 3H), 3.35 (dd, J = 3.2, 1.6 Hz, 1H), 2.99 (dd, J = 6.4, 4.1 Hz, 1H), 2.65 (d, J = 9.4 Hz, 1H), 2.53–2.38 (m, 3H), 1.93–1.82 (m, 3H), 1.71 (d, J = 17.8 Hz, 2H), 1.64 (s, 3H), 1.54–1.42 (m, 3H), 1.27 (s, 3H), 1.23–1.20 (m, 1H), 1.12–1.10 (m, 3H), 1.04 (d, J = 6.3 Hz, 3H), 0.85–0.82 (m, 3H), 0.78 (d, J = 7.1 Hz, 3H); ^{13}C NMR (201 MHz, $CDCl_3$) δ 139.0, 133.6, 125.0, 124.0, 87.6, 82.8, 75.6, 68.6, 65.9, 61.3, 61.0, 47.5, 41.4, 36.0, 35.5, 30.3, 29.7, 29.1, 26.0, 22.3, 19.6, 16.3, 14.04, 11.6, 11.2; $[\alpha]_D^{20} = -35.5\ (c\ 0.09,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{25}H_{42}O_6Na$ 461.2890, found 461.2884.

Methyl 2-((4*S*,6*R*)-4-((2*E*,4*E*,6*S*,8*E*,10*S*,11*R*,12*R*)-12-((*tert*-butyldimethylsilyl)oxy)-11-methoxy-6,8,10-trimethyltrideca-2,4,8-trien-2-yl)-5-oxaspiro[2.5]octan-6-yl)acetate (36)

Compound **36** was prepared from **16c** (17 mg, 0.05 mmol) by following the same procedure outlined for **32** to give a colorless oil. Yield (12 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14–6.04 (m, 1H), 5.78 (d, J = 10.9 Hz, 1H), 5.51 (dd, J = 15.1, 7.5 Hz, 1H), 5.11 (d, J = 9.2 Hz, 1H), 4.14 (s, 1H), 3.94–3.78 (m, 2H), 3.66 (s, 3H), 3.43 (s, 3H), 2.78 (dd, J = 12.5, 7.4 Hz, 1H), 2.64 (dt, J = 9.4, 7.7 Hz, 1H), 2.53 (ddd, J = 9.2, 6.9, 5.0 Hz, 1H), 2.46 (ddd, J = 15.0, 6.2, 3.9 Hz, 1H), 2.35 (dq, J = 14.0, 7.0 Hz, 1H), 2.12–1.96 (m, 2H), 1.94–1.81 (m, 1H), 1.65 (d, J = 0.8 Hz, 3H), 1.56 (t, J = 5.7 Hz, 3H), 1.52–1.45 (m, 1H), 1.12 (d, J = 6.3 Hz, 3H), 1.07–0.99 (m, 1H), 0.97–0.91 (m, 3H), 0.90–0.86 (m, 12H), 0.47 (d, J = 8.2 Hz, 1H), 0.34–0.24 (m, 1H), 0.22 – −0.07 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 140.2, 132.0, 131.4, 130.8, 128.0, 123.5, 89.7, 85.0, 74.2, 70.1, 60.8, 51.5, 47.5, 41.3, 35.0, 33.6, 31.0, 25.9, 20.4, 20.1, 20.0, 18.0, 16.1, 15.4, 14.9, 9.1, 8.6, −4.7; $[\alpha]_D^{20} = -10.8\ (c\ 0.12,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{35}H_{58}O_5SiNa$ 585.3946, found 585.3938.

Methyl 2-((4*S*,6*R*)-4-((*S*,2*E*,4*E*)-7-((2*R*,3*R*)-3-((2*R*,3*R*,4*R*)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)-5-oxaspiro[2.5]octan-6-yl)acetate (37)

Compound **37** was prepared from **36** (13 mg, 0.02 mmol) by following the same procedure outlined for **33** to give a colorless oil. Yield (6.2 mg, over 2 steps 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.17 (ddd, J = 16.4, 10.9, 5.9 Hz, 1H), 5.79 (d, J = 10.8 Hz, 1H), 5.45 (dd, J = 15.1, 8.7 Hz, 1H), 4.17 (s, 1H), 4.00–3.83 (m, 2H), 3.70 (s, 3H), 3.57 (s, 3H), 3.00 (t, J = 5.4 Hz, 1H), 2.69–2.36 (m, 5H), 2.07 (ddd, J = 13.4, 12.5, 10.2 Hz, 1H), 1.89 (dt, J = 13.6, 5.0 Hz, 1H), 1.76–1.65 (m, 3H), 1.65 (d, J = 0.9 Hz, 2H), 1.59–1.45 (m, 3H), 1.33–1.25 (m, 3H), 1.24–1.19 (m, 3H), 1.11–1.02 (m, 3H), 0.97–0.85 (m, 3H), 0.56–0.42 (m, 1H), 0.39–0.23 (m, 1H), 0.21–0.06 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 138.9, 133.2, 127.7, 125.0, 87.6, 85.0, 74.3, 68.3, 66.0, 61.3, 61.3, 51.6, 46.8, 41.4, 35.3, 35.0, 31.1, 29.7, 22.0, 20.3, 19.0, 16.6, 14.8, 11.8, 9.1, 8.8.

2-((4*S*,6*R*)-4-((*S*,2*E*,4*E*)-7-((2*R*,3*R*)-3-((2*R*,3*R*,4*R*)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)-5-oxaspiro[2.5]octan-6-yl)acetic acid (14)

Compound **14** was prepared from **37** (5 mg, 0.13 mmol) by following the same procedure outlined for **12** to give a colorless oil. Yield (3.9 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.17–6.08 (m, 1H), 5.88 (d, J = 11.2 Hz, 1H), 5.41 (dd, J = 15.0, 9.2 Hz, 1H), 4.10 (s, 1H), 3.91 (dd, J = 12.2, 6.4 Hz, 2H), 3.58 (d, J = 3.4 Hz, 3H), 3.12–3.08 (m, 1H), 2.64–2.46 (m, 3H), 2.37 (dd, J = 12.4, 7.8 Hz, 1H), 2.16–2.05 (m, 1H), 1.95 (dd, J = 13.5, 4.1 Hz, 1H), 1.64 (s, 3H), 1.62 (s, 2H), 1.59 (d, J = 4.1 Hz, 2H), 1.57 (s, 2H), 1.26 (d, J = 1.9 Hz, 3H), 1.17 (d, J = 6.5 Hz, 3H), 1.04 (d, J = 6.7 Hz, 3H), 0.80 (d, J = 7.0 Hz, 3H), 0.60–0.50 (m, 1H), 0.37–0.28 (m, 1H), 0.25–0.16 (m, 1H), 0.11 (dd, J = 9.2, 4.6 Hz, 1H); $[\alpha]_D^{20} = +16.3\ (c\ 0.11,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{26}H_{43}O_6$ 451.3072, found 451.2302.

Methyl 2-((2*R*,6*S*)-6-((2*E*,4*E*,6*S*,8*E*,10*S*,11*R*,12*R*)-12-((*tert*-butyldimethylsilyl)oxy)-11-methoxy-6,8,10-trimethyltrideca-2,4,8-trien-2-yl)-5,5-dimethyltetrahydro-2*H*-pyran-2-yl)acetate (38)

Compound **38** was prepared from **16d** (18 mg, 0.05 mmol) by following the same procedure outlined for **32** to give a colorless oil. Yield (16 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.21–6.14 (m, 1H), 5.81 (d, J = 10.8 Hz, 1H), 5.51 (dd, J = 15.1, 7.5 Hz, 1H), 5.12 (d, J = 8.5 Hz, 1H), 3.83 (dd, J = 12.3, 6.2 Hz, 1H), 3.75 (dd, J = 12.6, 6.1 Hz, 1H), 3.66 (s, 3H), 3.53 (s, 1H), 3.43 (s, 3H), 2.79–2.76 (m, 1H), 2.65–2.51 (m, 2H), 2.49–2.31 (m, 2H), 2.01 (dd, J = 13.0, 7.1 Hz, 1H), 1.90 (dd, J = 13.3, 7.6 Hz, 1H), 1.72 (d, J = 0.9 Hz, 3H), 1.58 (d, J = 1.3 Hz, 3H), 1.55–1.41 (m, 4H), 1.12 (d, J = 6.1 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.92–0.86 (m, 15H), 0.79 (s, 3H), 0.06 (d, J = 3.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 139.9, 133.6, 131.5, 130.8, 127.8, 123.8, 90.1, 89.8, 74.5, 70.1, 60.8, 51.4, 47.5, 41.3, 39.5, 35.0, 33.7, 33.7, 28.0, 28.0, 25.9, 20.4, 20.1, 20.0, 18.0, 16.1, 15.4, 14.5, −4.7; $[\alpha]_D^{20} = +9.2\ (c\ 0.13,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{33}H_{60}O_5SiNa$ 587.4115, found 587.4110.

Methyl 2-((2*R*,6*S*)-6-((*S*,2*E*,4*E*)-7-((2*R*,3*R*)-3-((2*R*,3*R*,4*R*)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)-5,5-dimethyltetrahydro-2*H*-pyran-2-yl) acetate (39)

Compound **39** was prepared from **38** (18 mg, 0.02 mmol) by following the same procedure outlined for **33** to give a colorless oil. Yield (9.8 mg, 66% yield, over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (dd, J = 15.0, 10.8 Hz, 1H), 5.79 (d, J = 10.9 Hz, 1H), 5.41 (dd, J = 15.1, 8.7 Hz, 1H), 3.85 (d, J = 4.3 Hz, 1H), 3.76 (dt, J = 13.0, 6.4 Hz, 1H), 3.66 (s, 3H), 3.57–3.51 (m, 4H), 2.97 (t, J = 5.4 Hz, 1H), 2.64–2.52 (m, 3H), 2.48–2.36 (m, 2H), 1.88 (dd, J = 13.5, 4.8 Hz, 1H), 1.72 (d, J = 1.0 Hz, 3H), 1.58–1.43 (m, 5H), 1.28 (s, 3H), 1.18 (d, J = 6.4 Hz, 3H), 1.04 (d, J = 6.7 Hz, 3H), 0.90–0.87 (m, 6H), 0.78 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 138.4, 134.7, 127.4, 125.1, 90.1, 87.5, 74.5, 68.2, 66.0, 61.2, 51.5, 46.8, 41.3, 39.5, 35.2, 35.0, 33.6, 27.9, 22.0, 20.5, 18.9, 16.5, 14.4, 11.8; $[\alpha]_D^{20} = -4.5\ (c\ 0.20,\ CHCl_3)$; HRMS ($m/z$) (ESI M + Na^+) calc. for $C_{27}H_{46}O_6Na$ 489.3191, found 489.3188.

2-((2*R*,6*S*)-6-((*S*,2*E*,4*E*)-7-((2*R*,3*R*)-3-((2*R*,3*R*,4*R*)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)-5,5-dimethyltetrahydro-2*H*-pyran-2-yl)acetic acid (15)

Compound **15** was prepared from **39** (9.8 mg, 0.02 mmol) by following the same procedure outlined for **12** to give a colorless oil. Yield (8.5 mg, 87% yield). ^{1}H NMR (800 MHz, CD_3OD) δ 6.30 (dd, J = 15.0, 10.9 Hz, 1H), 5.86 (d, J = 10.9 Hz, 1H), 5.44 (dt, J = 15.2, 7.6 Hz, 1H), 3.84–3.80 (m, 1H), 3.79–3.76 (m, 1H), 3.61–3.52 (m, 4H), 2.99 (dd, J = 6.3, 4.2 Hz, 1H), 2.67 (d, J = 9.4 Hz, 1H), 2.54–2.41 (m, 3H), 1.93 (dt, J = 20.6, 10.3 Hz, 1H), 1.74 (s, 3H), 1.59–1.48 (m, 5H), 1.30 (s, 4H), 1.22–1.18 (m, 1H), 1.13 (d, J = 6.5 Hz, 3H), 1.06 (d, J = 6.7 Hz, 3H), 0.91 (s, 3H), 0.87 (d, J = 7.0 Hz, 3H), 0.83 (s, 3H); ^{13}C NMR (201 MHz, CD_3OD) δ 138.4, 134.3, 127.4, 125.1, 90.0, 87.1, 74.8, 68.4, 66.4, 61.2, 60.4, 48.0, 46.7, 41.2, 39.3, 35.0, 35.0, 33.3, 27.7, 27.2, 21.3, 19.6, 18.4, 15.3, 13.4,

10.1; $[\alpha]_D^{20} = -20.0\ (c\ 0.27,\ CHCl_3)$; HRMS (*m*/*z*) (ESI M + Na^+) calc. for $C_{26}H_{44}O_6Na$ 475.3030, found 475.3034.

In vitro splicing assays

10 nM radiolabeled pre-mRNA substrate was incubated at 30 °C for 30 minutes in 50% HeLa cell nuclear extract supplemented with 60 mM potassium glutamate, 2 mM magnesium acetate, 2 mM ATP, 5 mM creatine phosphate, 0.05 mg mL^{-1} tRNA, and increasing concentrations of compounds dissolved in DMSO or DMSO alone. RNA was then and separated by denaturing polyacrylamide gel electrophoresis. Radiolabeled RNA was visualized by phosphorimaging, and individual bands were quantified with ImageQuant software (Molecular Dynamics). Splicing efficiency is calculated as the amount of mRNA relative to total substrate RNA and normalized to a DMSO control reaction. Values from three independent measurements were graphed and IC_{50} values were determined by nonlinear regression using the *[Inhibitor] vs. normalized response* model in GraphPad Prism version 9.0.0 for Mac, GraphPad Software, San Diego, California USA, http://www.graphpad.com.

Native gel analysis

Aliquots of *in vitro* splicing reactions were taken at different time intervals, and splicing complexes were separated native agarose gel electrophoresis as detailed in.[35] Radiolabeled RNA was visualized by phosphorimaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Financial support by the National Institutes of Health (GM122279) is gratefully acknowledged. The authors would like to thank the Purdue University Center for Cancer Research, which supports the shared NMR and mass spectrometry facilities.

Notes and references

1. Padgett RA, Grabowski PJ, Konarska MM, Seiler S and Sharp PA, Annu. Rev. Biochem, 1986, 55, 1119–1150. [PubMed: 2943217]
2. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP and Burge CB, Nature, 2008, 456, 470–476. [PubMed: 18978772]
3. Dvinge H, Kim E, Abdel-Wahab O and Bradley RK, Nat. Rev. Cancer, 2016, 16, 413–430. [PubMed: 27282250]
4. Daguenet E, Dujardin G and Valcarcel J, EMBO Rep., 2015, 16, 1640–1655. [PubMed: 26566663]
5. Jurica MS and Moore MJ, Mol. Cell, 2003, 12, 5–14. [PubMed: 12887888]
6. Will CL and Luhrmann R, Cold Spring Harbor Perspect. Biol, 2011, 3, a003707.
7. Staley JP and Woolford JL, Curr. Opin. Cell Biol, 2009, 21, 109–118. [PubMed: 19167202]
8. Wahl MC, Will CL and Luhrmann R, Cell, 2009, 136, 701–718. [PubMed: 19239890]
9. Wilkinson ME, Charenton C and Nagai K, Annu. Rev. Biochem, 2020, 89, 359–388. [PubMed: 31794245]

10. Kastner B, Will CL, Stark H and Luhrmann R, Cold Spring Harbor Perspect. Biol, 2019, 11, a032417.
11. Cretu C, Schmitzova J, Ponce-Salvatierra A, Dybkov O, De Laurentiis EI, Sharma K, Will CL, Urlaub H, Luhrmann R and Pena V, Mol. Cell, 2016, 64, 307–319. [PubMed: 27720643]
12. Brosi R, Hauri HP and Kramer A, J. Biol. Chem, 1993, 268, 17640–17646. [PubMed: 8349644]
13. Roybal GA and Jurica MS, Nucleic Acids Res., 2010, 38, 6664–6672. [PubMed: 20529876]
14. Fica SM and Nagai K, Nat. Struct. Mol. Biol, 2017, 24, 791–799. [PubMed: 28981077]
15. Tang AD, Soulette CM, van Baren MJ, Hart K, Hrabeta-Robinson E, Wu CJ and Brooks AN, Nat. Commun, 2020, 11, 1438. [PubMed: 32188845]
16. Inoue D, Bradley RK and Abdel-Wahab O, Genes Dev., 2016, 30, 989–1001. [PubMed: 27151974]
17. Lee SC, Dvinge H, Kim E, Cho H, Micol JB, Chung YR, Durham BH, Yoshimi A, Kim YJ, Thomas M, Lobry C, Chen CW, Pastore A, Taylor J, Wang X, Krivtsov A, Armstrong SA, Palacino J, Buonamici S, Smith PG, Bradley RK and Abdel-Wahab O, Nat. Med, 2016, 22, 672–678. [PubMed: 27135740]
18. Bonnal S, Vigevani L and Valcárcel J, Nat. Rev. Drug Discovery, 2012, 11, 847–859. [PubMed: 23123942]
19. Kotake Y, Sagane K, Owa T, Mimori-Kiyosue Y, Shimizu H, Uesugi M, Ishihama Y, Iwata M and Mizui Y, Nat. Chem. Biol, 2007, 3, 570–575. [PubMed: 17643112]
20. Kaida D, Motoyoshi H, Tashiro E, Nojima T, Hagiwara M, Ishigami K, Watanabe H, Kitahara T, Yoshida T, Nakajima H, Tani T, Horinouchi S and Yoshida M, Nat. Chem. Biol, 2007, 3, 576–583. [PubMed: 17643111]
21. Effenberger KA, Anderson DD, Bray WM, Prichard BE, Ma N, Adams MS, Ghosh AK and Jurica MS, J. Biol. Chem, 2014, 289, 1938–1947. [PubMed: 24302718]
22. Effenberger KA, Urabe VK, Prichard BE, Ghosh AK and Jurica MS, RNA, 2016, 22, 350–359. [PubMed: 26742993]
23. Corrionero A, Minana B and Valcarcel J, Genes Dev., 2011, 25, 445–459. [PubMed: 21363963]
24. Folco EG, Coil KE and Reed R, Genes Dev, 2011, 25, 440–444. [PubMed: 21363962]
25. Horiguchi T, Shirasaki M and Tanida S, Takeda Kenkyushoho, 1996, 55, 149–159.
26. Banwell M, McLeod M, Premaj R and Simpson G, Pure Appl. Chem, 2000, 72, 1631–1634.
27. Zhang Y and Panek JS, Org. Lett, 2007, 9, 3141–3413. [PubMed: 17629295]
28. Murray TJ and Forsyth CJ, Org. Lett, 2008, 10, 3429–3431. [PubMed: 18642829]
29. Lagisetti C, Yermolina MV, Sharma LK, Palacios G, Prigaro BJ and Webb TR, ACS Chem. Biol, 2014, 9, 643–648. [PubMed: 24377313]
30. Ghosh AK and Li J, Org. Lett, 2011, 13, 66–69. [PubMed: 21126066]
31. Ghosh AK, Ma N, Effenberger KA and Jurica MS, Org. Lett, 2014, 16, 3154–3157. [PubMed: 24869489]
32. Ghosh AK, Lv K, Ma N, Cárdenas E, Effenberger KA and Jurica MS, Org. Biomol. Chem, 2016, 14, 5263–5271. [PubMed: 27188838]
33. Dhar S, La Clair JJ, León B, Hammons JC, Yu Z, Kashyap MK, Castro JE and Burkart MD, J. Am. Chem. Soc, 2016, 138, 5063–5068. [PubMed: 27058259]
34. Hoberg JO, Carbohydr. Res, 1997, 300, 365–367.
35. Ghosh AK, Veitschegger AM, Nie S, Relitti N, MacRae AJ and Jurica MS, J. Org. Chem, 2018, 83, 5187–5198. [PubMed: 29696980]
36. Müller S, Liepold B, Roth GJ and Bestmann HJ, Synlett, 1996, 1996, 521–522.
37. Yates P, Grewal RS, Hayes PC and Sawyer JF, Can. J. Chem, 1988, 66, 2805–2815.
38. Takai K, Nitta K and Utimoto K, J. Am. Chem. Soc, 1986, 108, 7408–7410.
39. Simmons HE and Smith RD, J. Am. Chem. Soc, 1958, 80, 5323–5324.
40. Doering WE and Hoffmann AK, J. Am. Chem. Soc, 1954, 76, 6162–6165.
41. Makosza M and Wawrzyniewicz M, Tetrahedron Lett, 1969, 10, 4659–4662.
42. Oppolzer W and Godel T, Helv. Chim. Acta, 1984, 67, 1154–1167.
43. Miyaura N and Suzuki-Miyaura A, Chem. Rev, 1995, 95, 2457–2483.

44. Suzuki-Miyaura A, J. Organomet. Chem, 1999, 576, 147–168.
45. Itoh T, Jitsukawa K, Kaneda K and Teranishi S, J. Am. Chem. Soc, 1979, 101, 159–169.
46. Nicolaou KC, Baran PS, Zhong Y-L, Choi H-S, Yoon WH, He Y and Fong KC, Angew. Chem., Int. Ed, 1999, 38, 1669–1675.
47. Nicolaou KC, Baran PS, Zhong Y-L, Choi H-S, Fong KC, He Y and Yoon WH, Org. Lett, 1999, 1, 883–886. [PubMed: 10823217]

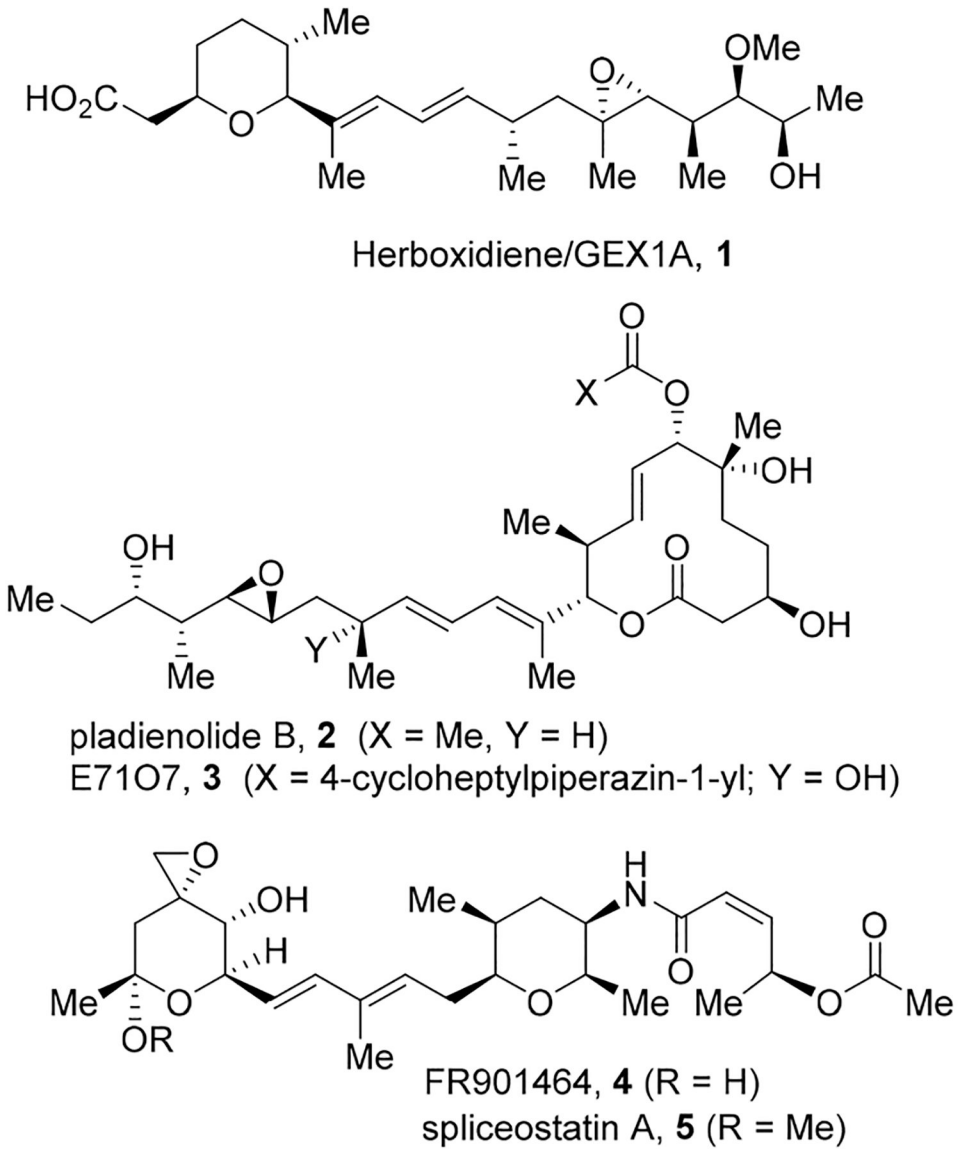


Fig. 1.
Structures of herboxidiene, pladienolide B, E71O7, FR901464, and spliceostatin A.

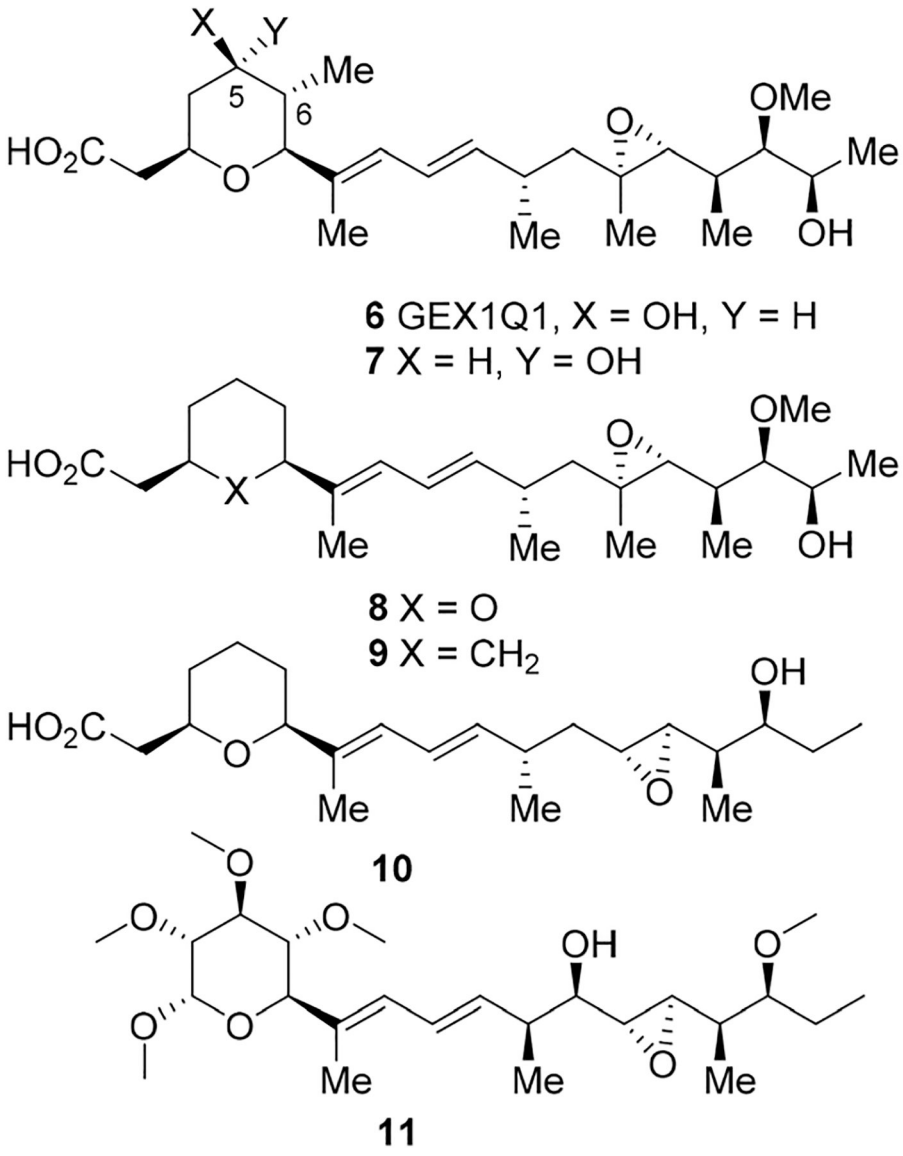


Fig. 2.
Structures of herboxidiene derivatives **6–11**.

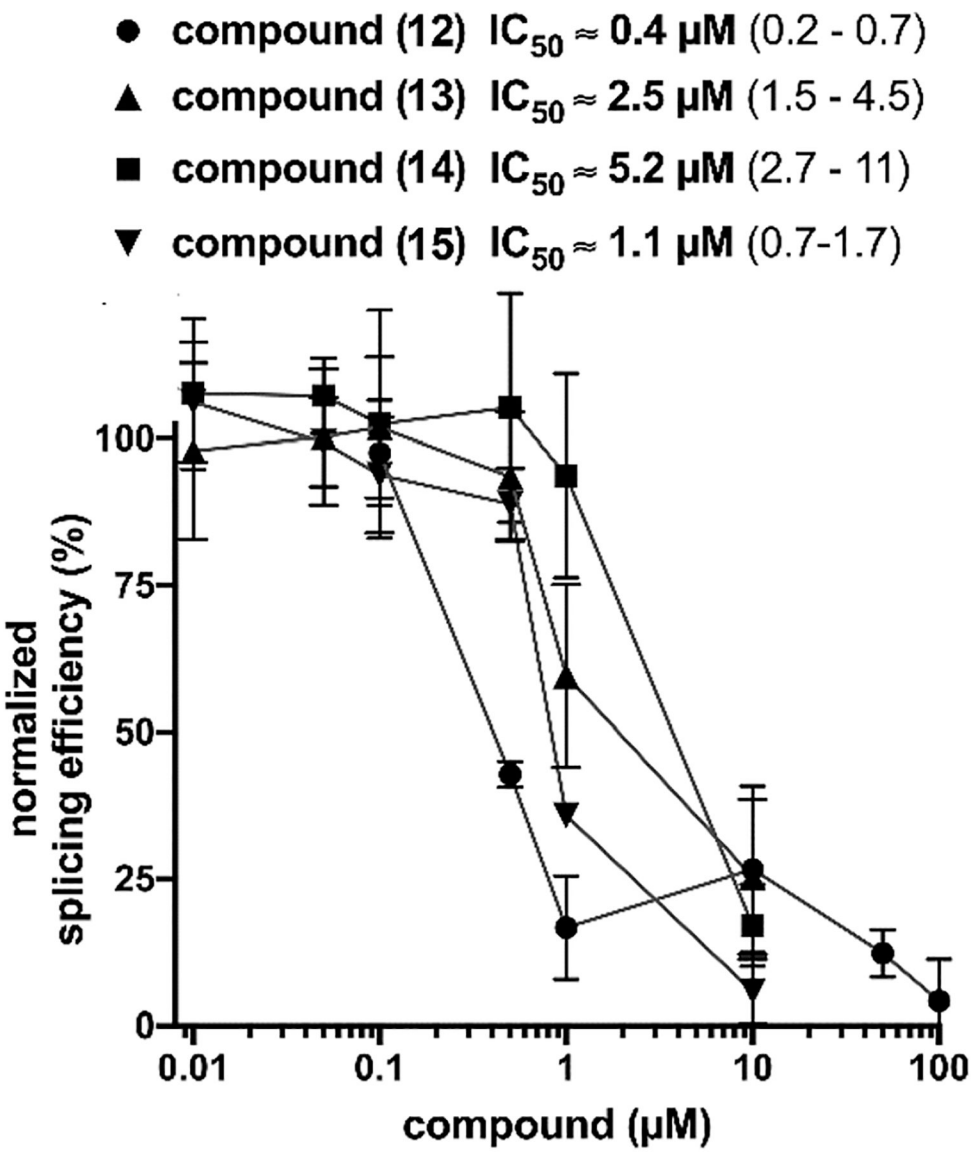


Fig. 3.

Impact of herboxidiene derivatives on *in vitro* splicing. Quantification of normalized splicing efficiency *vs.* inhibitor concentration for *in vitro* splicing reactions with compounds **12–15**. IC_{50} values are estimated from nonlinear regression fit of triplicate measurements with 95% confidence interval values indicated in parentheses.

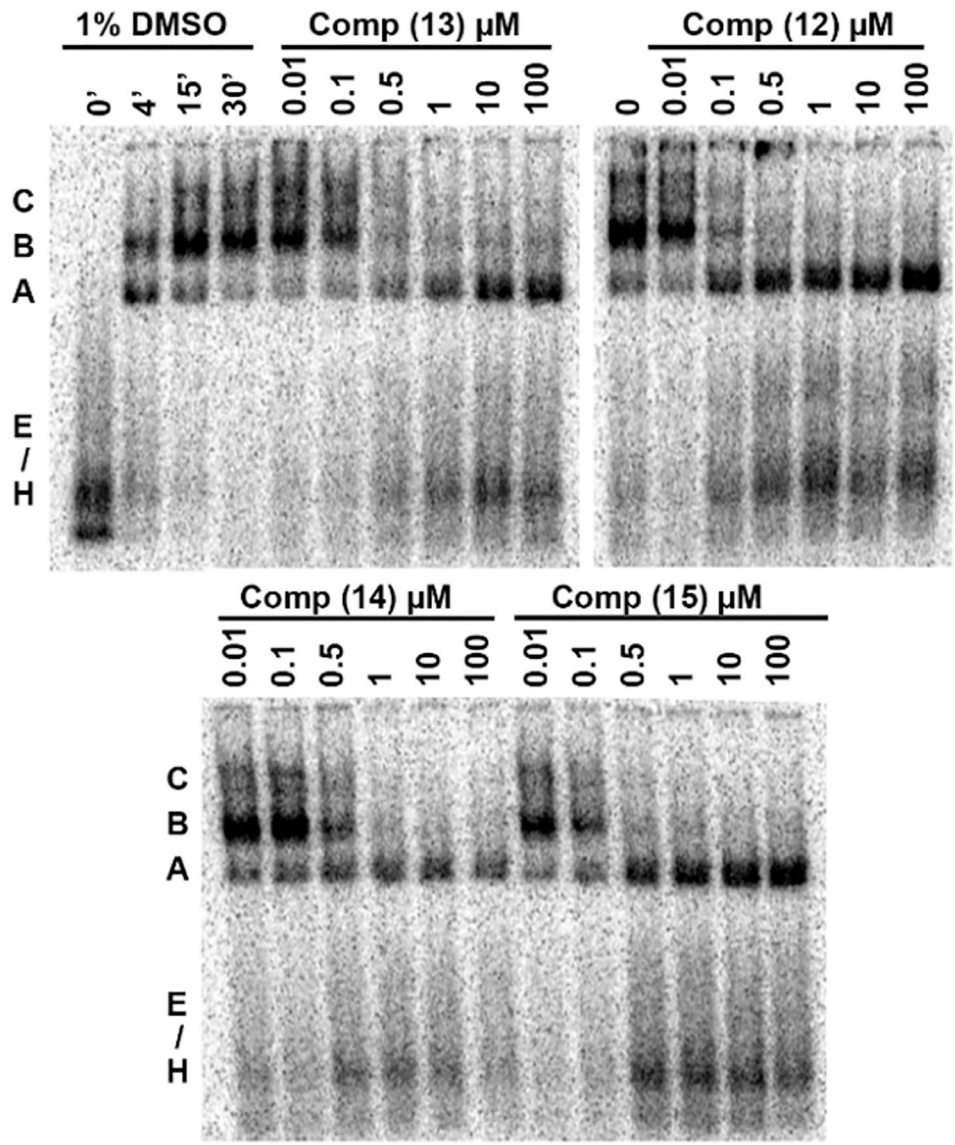
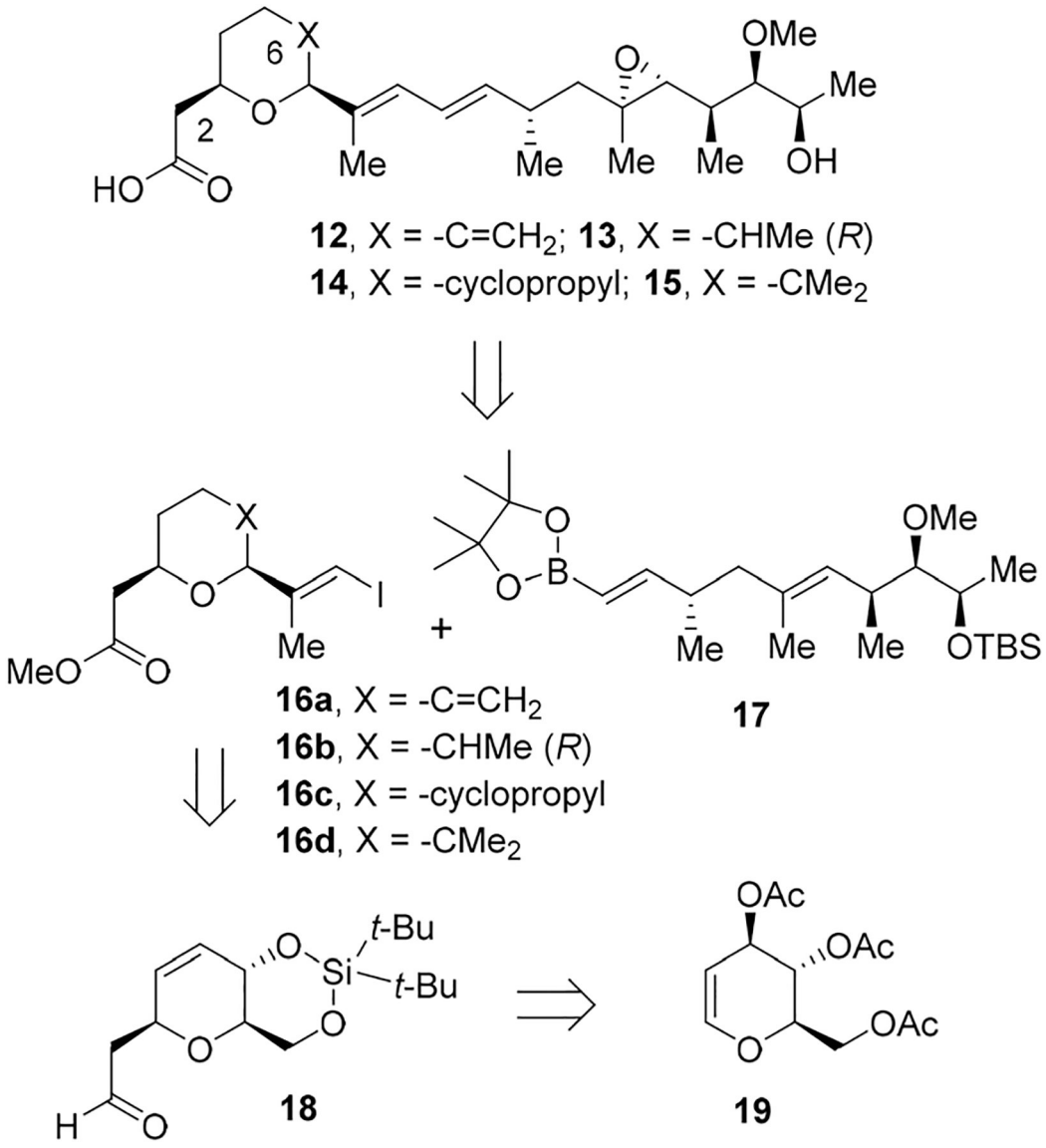
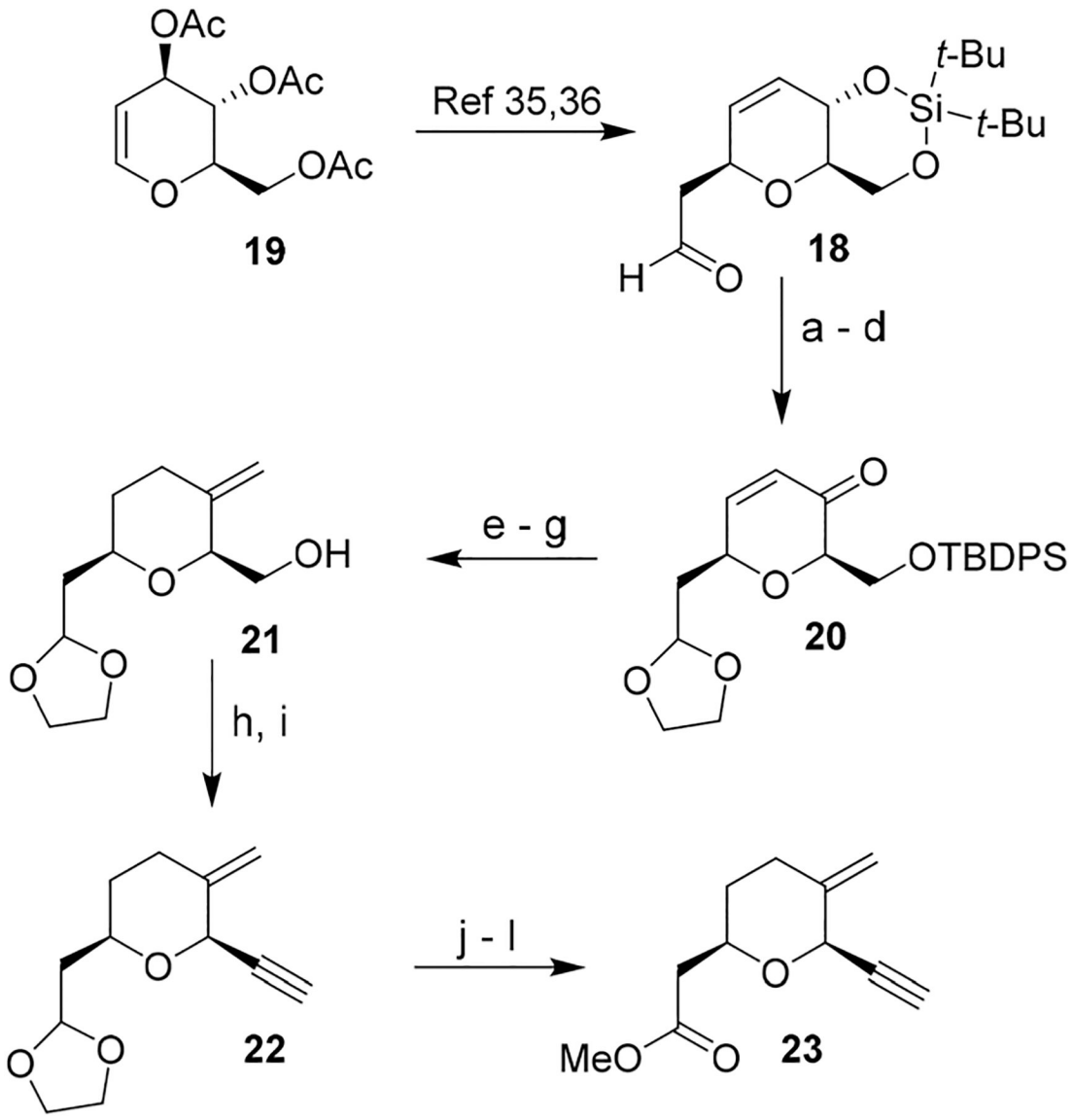


Fig. 4.

Native gel analysis of spliceosome assembly. The first five lanes show a time course of splicing reactions in 1% DMSO using a radiolabeled pre-mRNA substrate which were separated under native conditions. The following lanes show 30 minutes time points of splicing reactions incubated with the indicated compound concentration. The identity of splicing complexes is denoted with assembly occurring in the following order: H/E → A → B → C.

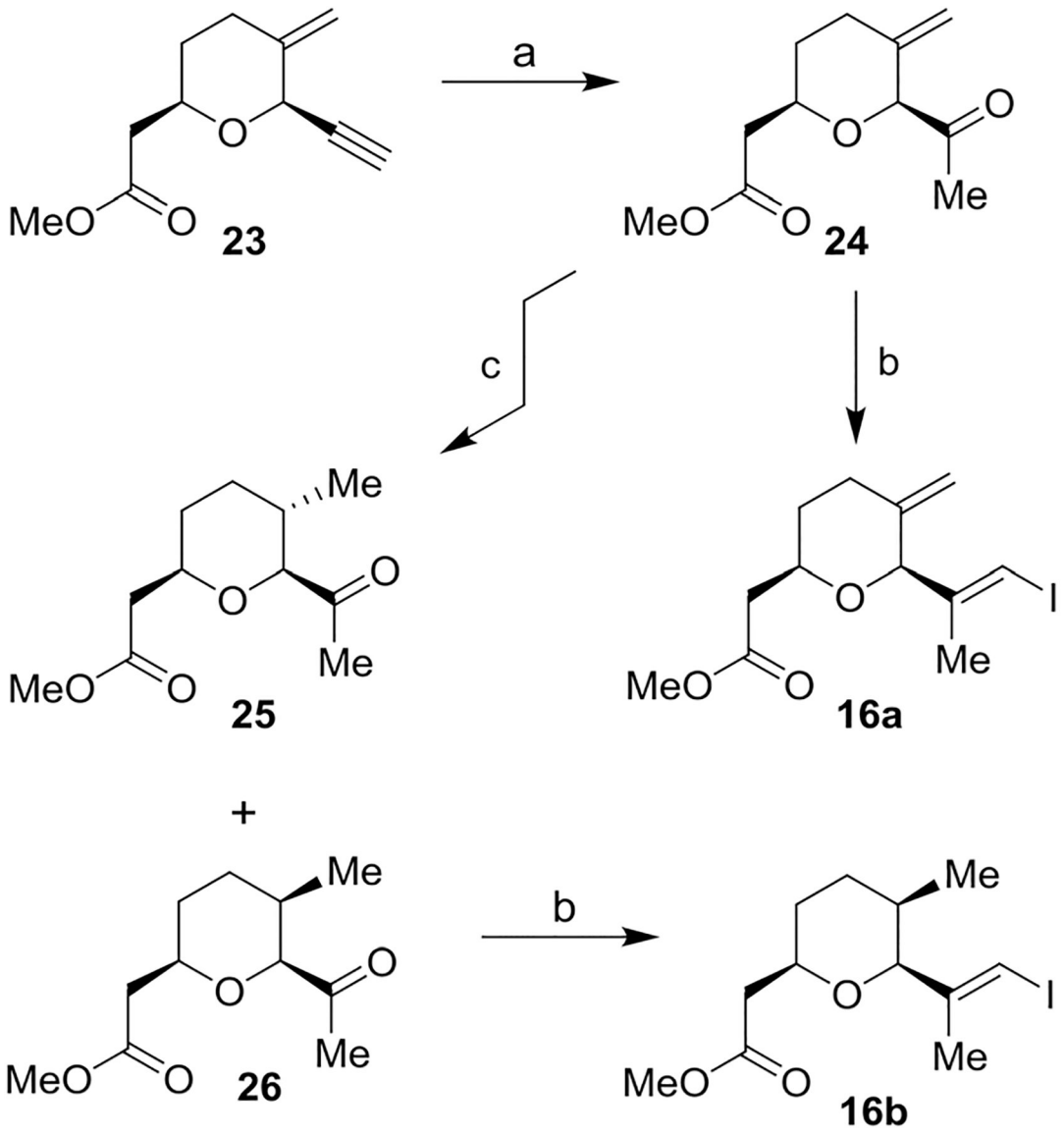


Scheme 1.
Synthetic strategies of C-6 modified derivatives of herboxidiene.



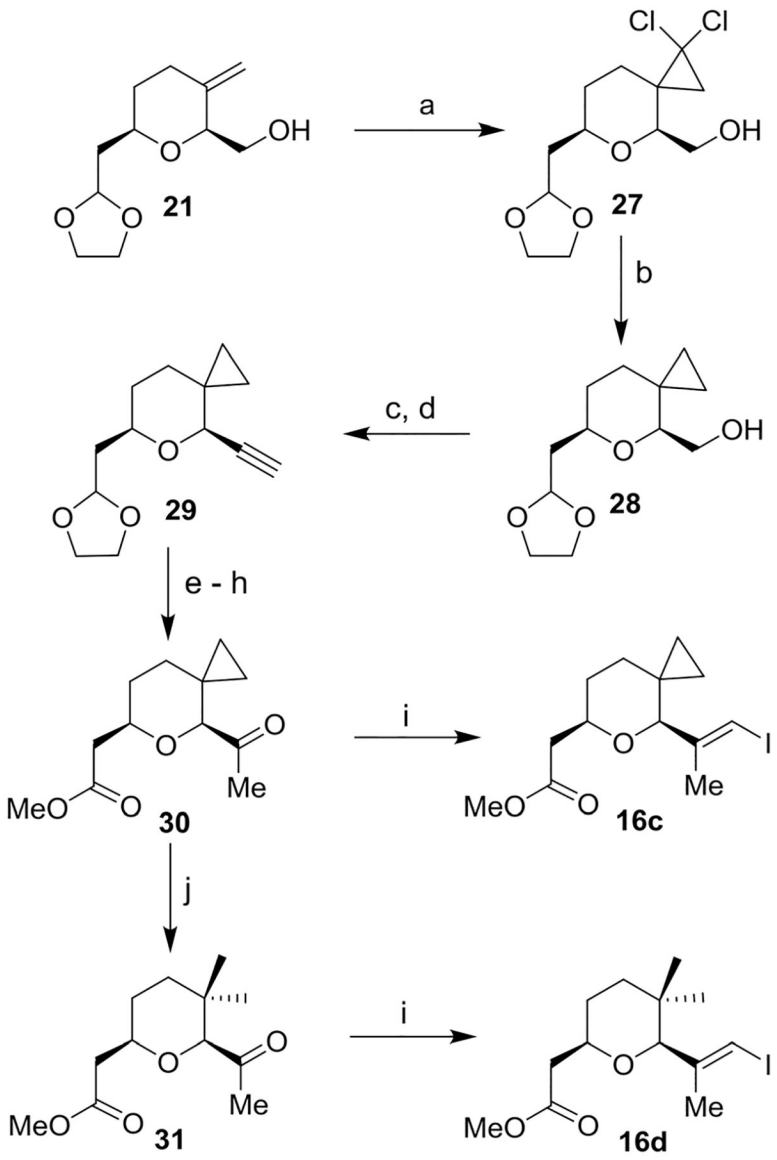
Scheme 2.

Reagents and conditions: (a) Ethylene glycol, PTSA, toluene, reflux, 3 h, 90%; (b) TBAF, THF, 0 °C to 23 °C, 12 h; (c) TBDPSCl, imidazole, DCM, 0 °C to 23 °C, 3 h, (d) DMP, $NaHCO_3$, DCM, 23 °C, 4 h, 60% over four steps; (e) H_2 balloon, Pd/C, EtOAc, 23 °C, 12 h; (f) Ph_3PCH_3Br, KO*t*Bu, THF, 0 °C to 23 °C, 1 h; (g) TBAF, THF, 0 °C to 23 °C, 12 h, 87% over three steps; (h) DMP, DCM, 0 °C to 23 °C, 6 h; (i) Bestmann–Ohira reagent, K_2CO_3, MeOH, 0 °C to 23 °C, 4 h, 66% over two steps; (j) 1N HCl, THF, 23 °C, 36 h; (k) $NaClO_2$, NaH_2PO_4, *t*BuOH/H_2O, 0 °C to 23 °C, 5 h; (l) EDC-HCl, DMAP, MeOH, DCM, 0 °C to 23 °C, 12 h.



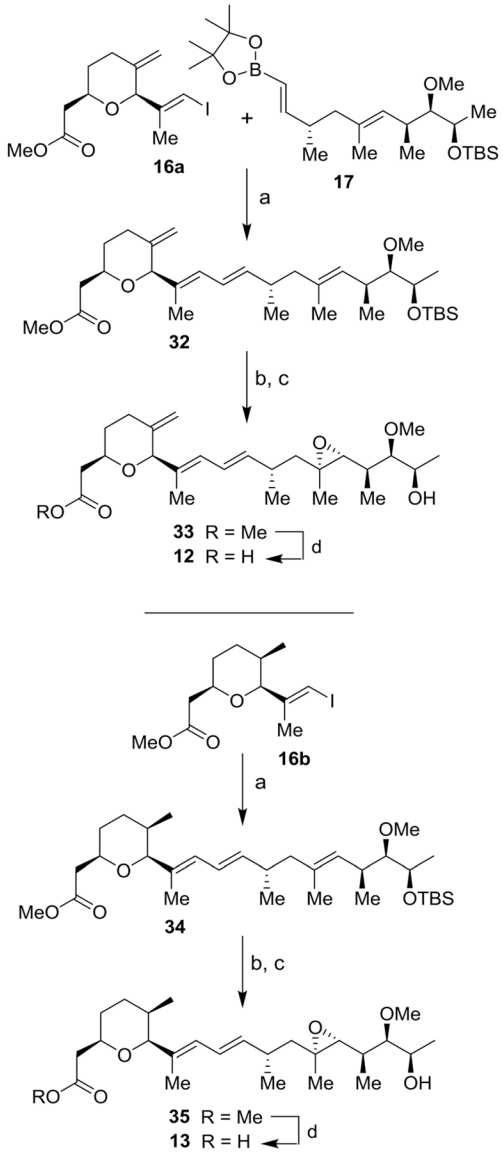
Scheme 3.

Reagents and conditions: (a) $HgSO_4$, H_2SO_4, aq. THF, 23 °C, 3 h, 84%; (b) $CrCl_2$, CHl_3, THF, 23 °C, 12 h, 79% (brsm) for 16a; 82% (brsm) for 16b; (c) H_2, 10% Pd-C, EtOAc, 23 °C, 12 h (97%).



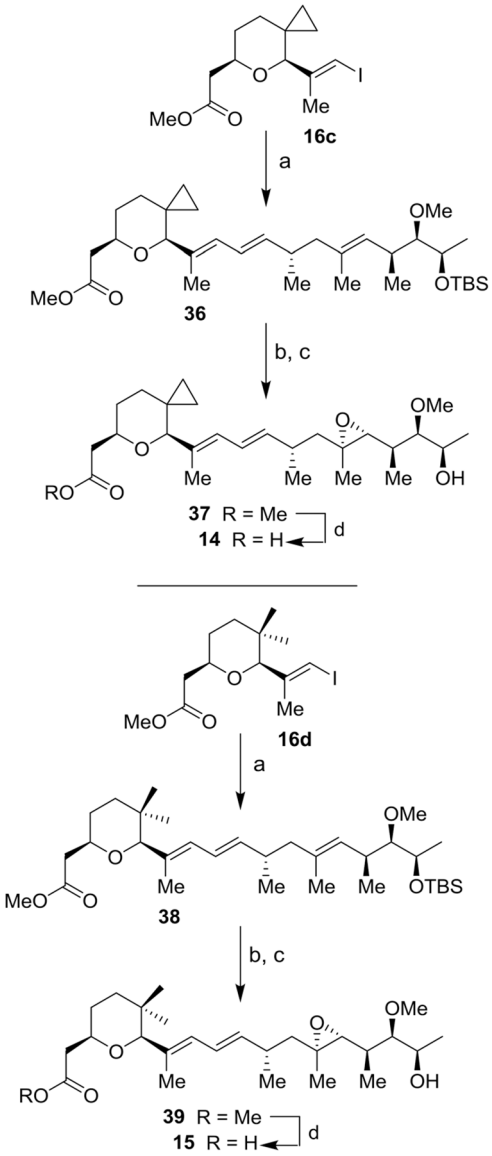
Scheme 4.

Reagents and conditions: (a) $Pd(PPh_3)_4$ (5 mol%), Cs_2CO_3, THF, 23 °C to 55 °C, 5 h, 47% for 32; 51% for 34; (b) 0.16 M HCl, MeOH, 23 °C, 45 min; (c) $Vo(acac)_2$, *t*-BuOOH, DCM, −20 °C, 36 h, 62% over two steps for 33; 58% over two steps for 35; (d) Me_3SnOH, DCE, 80 °C, 24 h, 85% for 12; 84% for 13.



Scheme 5.

Reagents and conditions: (a) Benzyltriethyl, ammonium chloride, 50% aq. NaOH, $CHCl_3$, 80 °C, 80% brsm; (b) LAH, THF, 64 °C, 24 h, 90%; (c) DMP, $NaHCO_3$, DCM, 0 °C to 23 °C; (d) Ohira–Bestmann, K_2CO_3, MeOH, over two steps, 71%; (e) 1N HCl, THF, 23 °C, 36 h; (f) 2-methyl-2-butene, $NaClO_2$, NaH_2PO_4, $^tBuOH/H_2O$, 0 °C to 23 °C; (g) EDC-HCl, DMAP, MeOH, DCM, 0 °C to 23 °C, 66% over three steps; (h) $HgSO_4$, H_2SO_4, aq. THF, 23 °C, 3 h, 70%; (i) $CrCl_2$, CHI_3, THF, 23 °C, 12 h, 82% (brsm) for 16c and 75% (brsm) for 16d; (j) H_2, PtO_2, AcOH, 40 °C, 30 min (85%).



Scheme 6.

Reagents and conditions: (a) $Pd(PPh_3)_4$ (5 mol%), Cs_2CO_3, THF, 23 °C to 55 °C, 5 h, 44% for 36; 47% for 38; (b) 0.16 M HCl, MeOH, 23 °C, 1 h; (c) $Vo(acac)_2$, *t*-BuOOH, DCM, −20 °C, 36 h, 57% over two steps for 37; 66% over two steps for 39; (d) Me_3SnOH, DCE, 80 °C, 24 h, 82% for 14; 87% for 15.