UC Santa Cruz
UC Santa Cruz Previously Published Works

Title
Design, synthesis and in vitro splicing inhibition of desmethyl and carba-derivatives of herboxidiene

Permalink
https://escholarship.org/uc/item/5549r8bw

Journal
Organic & Biomolecular Chemistry, 14(23)

ISSN
1477-0520

Authors
Ghosh, Arun K
Lv, Kai
Ma, Nianchun
et al.

Publication Date
2016-06-21

DOI
10.1039/c6ob00725b

Peer reviewed

Design, Synthesis and *in Vitro* Splicing Inhibition of Desmethyl and Carba-Derivatives of Herboxidiene

Arun K. Ghosh[a,*], **Kai Lv**[a], **Nianchun Ma**[a], **Emilio L. Cárdenas**[a], **Kerstin A. Effenberger**[b], and **Melissa S. Jurica**[b]

[a]Department of Chemistry and Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907, USA

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

Abstract

Herboxidiene is a potent inhibitor of spliceosomes. It exhibited excellent anticancer activity against multiple human cancer cell lines. Herein, we describe an enantioselective synthesis of a desmethyl derivative and the corresponding carba-derivatives of herboxidiene. The synthesis involved Suzuki coupling of vinyl iodide with boronate as the key reaction. For the synthesis of carbo-derivatives, the corresponding optically active cyclohexane-1,3-dicarbonyl derivatives were synthesized using an enantioselective desymmetrization of meso-anhydride. The biological properties of these derivatives were evaluated in an *in vitro* splicing assay.

Graphical Abstract
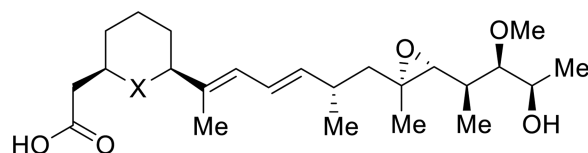
6-Desmethyl herboxidiene **5** (X = O)
6-Desmethyl carba-herboxidiene **6** (X =CH$_2$)

Enantioselective syntheses of desmethyl and carba-derivatives of herboxidiene and their biological evaluation in splicing assay are reported

Keywords

Herboxidiene; spliceosome; Suzuki coupling; desmethyl; derivatives; design; synthesis; splicing

Introduction

Pre-messenger RNA (pre-mRNA) splicing is an essential step in gene expression.[1,2] In the process, noncoding sequences (introns) are removed and the coding sequences (exons) are joined to form mature mRNA. An intricate ribonuclear cellular machinery called spliceosome is assembled to carry out splicing events.[3,4] The mechanism of pre-mRNA splicing involves multiple interactions between pre-mRNA, small ribonucleoproteins and spliceosome proteins.[5] Regulation of this process is highly complex, and not surprisingly, many different human diseases can be caused by errors in RNA splicing or its regulation.[6–8] Incidentally, in an *in vivo* cellular scenario, mRNA is more available to temporal manipulation than DNA, and as a consequence, therapeutic inhibition of splicesome has become an exciting area of anticancer drug development.[9,10]

A number of natural products including pladienolide B, (**1**, Figure 1), FR901464 (**2**), spliceostatin A (**3**) and herboxidiene (**4**) display potent *in vitro* splicing inhibition by interacting with the SF3b subunit of spliceosome.[11–14] A modified derivative of pladienolide B advanced to human clinical trials.[15] FR901464 and its derivative spliceostatin A exhibited potent anticancer activity against numerous human cancer cell lines.[16,17] A number of modified, less complex derivatives have also displayed potent anticancer activity.[18–22] Herboxidiene, another natural product, has been shown to induce both G1 and G2/M cell cycle arrest in a human normal fibroblast cell line WI-38.[23] Total syntheses as well as syntheses of structural variants of herboxidiene have been reported by us and others.[24–27] Herboxidiene is quite complex and contains nine chiral centers. In addition, it contains a number of sensitive functionalities. Elucidation of structure-activity studies is critical in the design of a less complex derivative. In this context, a number of structural derivatives of herboxidiene have been synthesized and evaluated for their splicing activity.[28,29,30] In our continuing interests in chemistry and biology of herboxidiene, we have now examined the importance of the C6 methyl as well as the pyran ring oxygen of herboxidiene. For these studies, we have synthesized the desmethyl and the corresponding carba-analog and its diastereomer and evaluated their biological properties. Herein, we report stereoselective syntheses of herboxidiene derivatives and biological results of our investigation.

Results and discussion

Our synthetic strategy for desmethyl herboxidiene and the corresponding carbo analogs involved Suzuki cross-coupling of vinyl boronate **7** with respective vinyl iodides **8** and **9** (Scheme 1) Vinyl boronate **7** has been synthesized previously in our laboratory.[27,28] The synthesis of desmethyl derivative and its biological activity have been recently reported by Lagisetti and co-workers.[30] For our synthesis of desmethyl derivative **5**, we planned to synthesize vinyl iodide **8** from optically active *cis*-2,6-disubstituted tetrahydropyran derivative **10**, which can be prepared from triacetoxy-D-glucal as the key starting material. For the synthesis of carba-analog **6**, the corresponding optically active vinyl iodide **9** would be synthesized from cyclohexane-1,3-dicarboxylic acid **11**.

For the synthesis of desmethyl derivative **5**, the synthesis of requisite vinyl iodide **8** is shown in Scheme 2. Commercially available triacetoxy-D-glycal **13** was converted to dihydropyran

derivative **14** in three steps as described in the literature.[31] The mixture of diastereomers was converted to TBS-protected tetrahydropyran derivative as follows. Catalytic hydrogenation of **14** over 10% Pd/C in ethyl acetate under a hydrogen-filled balloon for 3 h provided the corresponding saturated derivative. Treatment of the resulting alcohol with thiocarbonyldiimidazole in toluene at reflux afforded the thiocarbamate in 96% yield. Reduction[32] of the thiocarbamate with tri-n-butyltin hydride in the presence of a catalytic amount of AIBN in toluene at reflux afforded the corresponding deoxygenated product in 74% yield. The resulting diastereomeric mixture was treated with NaH in THF at 23 °C for 4 h furnished a 3:1 mixture of diastereomers.[33] The mixture was separated by silica gel chromatography to provide **10** in 63% yield. Tetrahydropyran derivative **10** was converted to acetylene derivative **15** in a three-step sequence involving: (1) removal of the silyl group by treatment with TBAF in THF at 23 °C for 30 min; (2) oxidation of the resulting alcohol to the corresponding aldehyde using $SO_3.Py$ at 23 °C for 2 h and (3) reaction of the resulting aldehyde with the Ohira-Bestmann reagent in MeOH in the presence of K_2CO_3 to provide alkyne derivative **15** in 67% yield over 3-steps. Treatment of the alkyne derivative **15** with a catalytic amount of $HgSO_4$ in the presence of 3M H_2SO_4 as described by Yates and co-workers,[34] provided ketone derivative **16** in 69% yield. This ketone was converted to vinyl iodide **8** by exposure to $CrCl_2$ and CHI_3 in THF at 23 °C for 4 h using protocol described by Takai and co-workers.[35] The resulting vinyl iodide was used immediately for the coupling reaction.

Synthesis of desmethyl derivative **5** is shown in Scheme 3. Reaction of vinyl iodide **8** and boronate **7** in the presence of a catalytic amount of $Pd(PPh_3)_4$ (5 mol%) and Cs_2CO_3 in THF at 55 °C for 5 h resulted in the coupling product **17** in 56% yield. Methyl ester **17** was converted to desmethyl derivative **5** in a three-step sequence that involved, (1) removal of TBS-ether by exposure to 1M HCl in methanol at 23 °C for 45 min; (2) streatment of the resulting alcohol to a catalytic amount of $VO(acac)_2$ in the presence of t-BuOOH at −15 °C for 48 h to provide the epoxide; and (3) saponification of the methyl ester with K_2CO_3 in aqueous methanol to provide **5** in 26% yield over 3-steps.

For synthesis of the carba-derivative, the corresponding vinyl iodide was synthesized as shown in Scheme 4. Cyclohexane-1,3-dicarbonylic acid **11** was converted to 3-oxabicyclo[3.3.1] nonane-2,4-dione by exposure to acetic anhydride at 140 °C for s10 h to provide **18** in 45% yield. Enantioselective desymmetrization of meso-anhydride was carried out as described by Rovis and co-workers.[36] Thus, methylzinc bromide, prepared from $ZnBr_2$ and methylmagnesium bromide, was first reacted with $[Rh(nbd)Cl]_2$ and (*S*)-*tert*-butylphosphine oxazoline. Reaction of anhydride **18** with this reagent at 0 °C to 23 °C for 30 h provided methyl ketone **19** in 58% yield. Enantioselectivity was determined to be 91% ee by chiral HPLC analysis of the benzamide derivative. The depicted stereochemistry was assigned based upon comparison of optical rotation of related compounds.[36,37] Diazo compound **20** was synthesized according to the procedure of Nicolaou and co-workers.[38,39] Carboxylic acid **19** was smoothly converted to diazo compound **20** by activation with CH_3SO_2Cl in the presence of Et_3N at 0 °C followed by reaction of the resulting mesylate with diazomethane in ether at 0 °C for 30 min. Arndt-Eistert homologation[40] was achieved by reaction of diazo compound **20** with silver benzoate in the presence of Et_3N at 23 °C for

1 h to provide the methyl ester **21** in 49% yield. Methyl ketone **21** was converted to vinyl iodide using the Takai procedure[35] as described above to provide vinyl iodide **9** in 44% yield. This vinyl iodide was immediately used for the coupling reaction with boronate **7**.

Synthesis of the carba-derivative **6** is shown in Scheme 5. Coupling of vinyl iodide **9** with boronate **7** in the presence of a catalytic amount of $Pd(PPh_3)_4$ (5 mol %) as described above, furnished the corresponding coupling product. This was exposed to 1 M aqueous HCl solution in MeOH at 23 °C which removed the silyl group and provided alcohol **22** in 61% yield over 2-steps. Epoxidation of **22** with $VO(acac)_2$ and t-BuOOH followed by saponification of the methyl ester with K_2CO_3 in methanol afforded carba-derivative **6** in 40% yield over 2-steps.

Our synthesis has allowed for the preparation of the enantiomeric vinyl iodide and the corresponding diastereomeric carba-derivative. As shown in Scheme 6, enantioselective desymmetrization of *meso*-anhydride **18** using (*S*)-*tert*-butylphosphine oxazoline as described above furnished methyl ketone **23** in 58% yield and 91% ee. Carboxylic acid derivative **23** was converted to methyl ester **24** by mesylation, reaction of the resulting mesylate with diazomethane followed by Arndt-Eistert homologation with silver benzoate in the presence of Et_3N provided the methyl ester **24**. This was converted to vinyl iodide as described above to provide vinyl iodide **25**. Suzuki coupling of vinyl iodide **25** with boronate **7** using $Pd(PPh_3)_4$ (5 mol%) resulted in the corresponding coupling product. Deprotection of TBS-group with aqueous HCl solution in MeOH furnished alcohol coupling product **26** in 49% yield over 2-steps. Epoxidation of **26** with $VO(acac)_2$-catalyzed epoxidation followed by saponification of the methyl ester afforded carba-derivative **27** in 40% yield over 2-steps.

The biological properties of synthetic derivatives were evaluated in an *in vitro* splicing system as previously described[27]. Based on dose response analysis, we calculate the IC_{50} as the concentration required to reduce *in vitro* splicing efficiency by half compared to DMSO control (Figure 2A). The IC_{50} of the 6-desmethyl herboxidiene **5** derivative is 0.35 μM, which within the variability of the assay is comparable to the 0.3 μM IC_{50} that we previously measured for herboxidiene.[27] The carba- derivative **6** potency is decreased by an order of magnitude with an IC_{50} value of 6 μM. Inhibitor activity is further reduced with diastereomoeric derivative **27**, which has an IC_{50} >100 μM. These results were further supported in the context of spliceosome assembly, as measured by native gel analysis (Figure 2B). Similar to other SF3B1 inhibitors, active herboxidiene analogs disrupt the transition from A to B complex in the spliceosome assembly pathway.

Conclusion

In conclusion, we have described the design and synthesis of 6-desmethyl herboxidiene and two corresponding carbo-derivatives. The purpose of the derivatives was to examine the importance of these groups or functionalities to spliceosome inhibitory activity and compare the activity with herboxidiene. Furthermore, we have synthesized the corresponding diastereomeric carbo-derivative to examine the effect of stereochemistry on splicing activity. The synthesis of 6-desmethyl derivative involved the Suzuki coupling between a vinyl iodide

and boronate derivative. The corresponding 6-desmethyl vinyl iodide was synthesized from readily available methyltetrahydropyranyl acetate derivative **10** in optically active form. The corresponding carbo-derivatives were synthesized by asymmetric desymmetrization of *meso*-3-oxabicyclo[3.3.1]-nonane-2,4-dione as the key step. We have evaluated these derivatives in *in vitro* splicesome inhibitory assays. The 6-desmethyl derivative **5** shows the same activity as herboxidiene, which indicates that the methyl group at this position is not important for interactions with the SF3B subunit of the spliceosome. In contrast, the corresponding carbo-derivatives showed lower splicing inhibitory activity compared to 6-desmethyl derivative **5**, indicating that the tetrahydropyran ring oxygen is important to potency. The diastereomeric cyclohexane derivative **27** showed significantly reduced activity over derivative **6**, indicating the importance of stereochemistry as well. Further design of novel herboxidiene derivatives is in progress.

Experimental Section

All reactions were carried out under an inert atmosphere, either N_2 or Ar, 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 or better. 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 -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 Perkin-Elmer 341 polarimeter. A Thermo Finnigan LCQ Classic mass was used for MS analyses. The purity of test compounds was determined by HRMS and HPLC analysis. All test compounds showed 95% purity.

Methyl 2-((2*R*,6*S*)-6-(((*tert*-butyldimethylsilyl)oxy) methyl)tetrahydro-2*H*-pyran-2-yl)acetate (10)

To a stirred solution of **14** (diastereomers, 1.2:1; α:β, 1 g, 3.4 mmol) in EtOAc (30 mL) was added 10% Pd/C (10% wt/wt, 100 mg). After being stirred at 23 °C for 3 h the mixture was filtered through a pad of celite. The volatiles were removed and the crude mixture was purified by column chromatography (40% EtOAc/Hexanes) to give the secondary alcohol as a colorless oil (704 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79 (m, 1H), 3.69 (ddd, *J* = 25.1, 17.8, 7.3 Hz, 1H), 3.65 (m, 2H), 3.60 (s, 3H), 3.17 (bs, 1H), 2.42 (m, 1H), 2.29 (ddd, *J* = 15.2, 8.5, 4.8 Hz, 1H), 1.99 (m, 1H), 1.69 (m, 1H), 1.56 (ddq, *J* = 12.3, 8.2, 4.0, 3.5 Hz, 1H), 1.43 (m, 1H), 1.28 (m, 1H), 0.81 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 79.4, 73.5, 69.7, 65.3, 51.4, 40.5, 36.9, 31.3, 26.2, 25.6, –5.7; LRMS-ESI (m/z): 341.3, [M+Na]$^+$.

To a stirred solution of secondary alcohol (300 mg, 0.942 mmol) in toluene (5 mL) was added (thiocarbonyl)diimidazole (419 mg, 2.36 mmol). The resulting mixture was refluxed for 1 h. After consumption of the starting material, the volatiles were removed under reduced pressure. The crude residue was purified by column chromatography (40% EtOAc/

Hexanes) to give the corresponding thiocarbamate as a colorless oil (387 mg, 96%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.37 (s, 1H), 7.69 (s, 1H), 7.04 (s, 1H), 5.70 (q, J = 3.4 Hz, 1H), 4.27 (s, 1H), 4.10 (m, 2H), 3.94 (dd, J = 10.7, 6.7 Hz, 1H), 3.83 (ddd, J = 10.6, 7.7, 5.0 Hz, 1H), 3.72 (s, 3H), 3.09 (s, 1H), 2.61 (dt, J = 14.9, 7.4 Hz, 1H), 2.45 (ddd, J = 15.5, 5.5, 2.9 Hz, 1H), 2.17 (m, 1H), 2.04 (s, 1H), 1.25 (t, J = 7.1 Hz, 1H), 0.87 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 182.98, 171.19, 136.77, 130.74, 117.78, 79.43, 74.95, 73.84, 63.01, 51.66, 40.40, 27.67, 25.72, 23.42, 18.06, −5.66; LRMS-ESI (m/z): 451.3, [M+Na]$^+$.

A stirred solution of AIBN (53.6 mg, 0.034 mmol) and Bu$_3$SnH (0.26 mL, 0.98 mmol) in toluene (12 mL) was warmed to reflux. Dropwise addition of the thiocarbamate (146 mg, 0.653 mmol) in toluene (3 mL) was carried out in three (1 mL) portions over 30 minutes. The resulting mixture was refluxed for an additional 30 minutes. Upon completion, the mixture was cooled and the volatiles were removed under reduced pressure. The crude mixture was purified by column chromatography (2% EtOAc/Hexanes) to yield the TBS-protected alcohol as a colorless oil (146.5 mg, 74%)

The diastereomeric mixture of TBS-protected alcohols was epimerized under basic conditions. To a stirred solution of TBS-protected alcohol (135 mg, 0.446 mmol) in THF (10 mL) was added NaH (60% in oil, 26.7 mg, 0.67 mmol). The mixture was stirred at 23 °C for 8 h. The reaction was quenched with saturated NH$_4$Cl and extracted with EtOAc. The resulting organic layer was washed with brine and purified by column chromatography (2% EtOAc/Hexanes) to provide **10** (85 mg, 63%). [α]$^{20}_D$ −11.1 (c 0.18, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.76 (m, 1H), 3.67 (s, 3H), 3.64 (m, 1H), 3.46 (m, 1H), 3.42 (m, 1H), 2.53 (dd, J = 15.1, 7.4 Hz, 2H), 1.84 (m, 2H), 1.67 – 1.43 (m, 2H), 1.17 (dddd, J = 27.5, 23.9, 12.7, 4.2 Hz, 2H), 0.87 (d, J = 5.0 Hz, 9H), 0.86 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 171.7, 78.4, 74.1, 66.7, 51.4, 41.4, 31.2, 27.6, 25.8, 22.9, 18.2, −5.3; HRMS-ESI (m/z) calc. for C$_{15}$H$_{30}$NaO$_4$Si [M + Na]$^+$ 325.1811, found 325.1803.

Methyl 2-((2*R*, 6*S*)-6-ethynyltetrahydro-2H-pyran-2-yl)acetate (15)

To the solution of methyl ester **10** (168 mg, 0.49 mmol) in THF (5 mL) was added TBAF (0.98 mL, 1 M in THF, 0.98 mmol). After being stirred at 23 °C for 15 min, it was quenched with aqueous NaHCO$_3$ and extracted with EtOAc. The combined organic phase was washed with brine, dried (NA$_2$SO$_4$) and concentrated to provide crude alcohol which was used directly for the next reaction.

To the solution of the above alcohol in a mixture (3:1) of CH$_2$Cl$_2$ and DMSO (4 mL) was added Et$_3$N (0.68 mL, 4.88 mmol) and SO$_3$.Py (233 mg, 1.462 mmol). After being stirred at 23 °C for 2 h, the reaction was quenched with aqueous NaHCO$_3$, and extracted with EtOAc. The combined organic phase was washed with water, brine, dried (Na$_2$SO$_4$) and concentrated. The residue was purified by column chromatography (hexane:EtOAc, 2:1) to give an aldehyde.

To the solution of above aldehyde (61 mg, 0.33 mmol) in MeOH (4 mL) at 23 °C, was added Bestmann Reagent (1.53 mL, 10% in CH$_3$CN, 0.65 mmol) and K$_2$CO$_3$ (136 mg, 0.98 mmol). After being stirred at 23 °C for 2.5 h, the reaction was quenched with aqueous NaHCO$_3$ and extracted with ether. The combined organic phase was washed with water,

brine, dried Na_2SO_4 and concentrated. Purifiaction of the residue by silica gel column chromatography (hexane : EtOAc, 10:1) afforded alkyne derivative **15** (59 mg, 66% over 3-steps). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.14 (dt, J = 11.1, 2.1 Hz, 1H), 3.82–3.76 (m, 1H), 3.67 (s, 3H), 2.64 (dd, J = 15.7, 6.9, 1H), 2.44 (d, J = 2.1, 1H), 2.41 (dd, J = 15.7, 6.3, 1H), 1.88-1.80 (m, 2H), 1.68-1.53 (m, 3H), 1.33-1.22 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 83.0, 74.6, 72.4, 67.9, 51.6, 41.0, 32.0, 30.3, 22.9; HRMS-ESI (m/z) calc. for $C_{10}H_{14}NaO_3$ [M+Na][+] 205.0841, found 205.0837.

Methyl 2-((2*R*,6*S*)-6-acetyltetrahydro-2H-pyran-2-yl)acetate (16)

To the solution of alkyne **15** (58 mg, 0.32 mmol) in THF (1.5 mL) was added a solution of $HgSO_4$ (19 mg, 0.064 mmol) in 3 M H_2SO_4 (1 mL). After being stirred at 23 °C for 1.5 h, ether was added. The reaction mixture was cooled to 0 °C and $NaHCO_3$ powder was carefully added. The mixture was extracted with ether and the combined extracts was washed with aqueous $NaHCO_3$ solution, brine, dried (Na_2SO_4) and concentrated. Purification of the residue by silica gel column chromatography provided methyl ketone **16** (44 mg, 69%) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.88–3.81 (m, 1H), 3.80 (dd, J = 11.7, 2.5 Hz, 1H), 3.69 (s, 3H), 2.61 (dd, J = 15.3, 8.0, 1H), 2.47 (dd, J = 15.1, 5.3, 1H), 2.15 (s, 3H), 1.95-1.82 (m, 2H), 1.72-1.54 (m, 2H), 1.34-1.22 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.2, 171.5, 82.9, 74.4, 51.6, 41.2, 30.5, 27.1, 25.6, 22.9; HRMS-ESI (m/z) calc. for $C_{10}H_{16}NaO_4$ [M+Na][+] 223.0947, found 223.0943.

Methyl 2-((2R,6S)-6-((E)-1-iodoprop-1-en-2-yl)tetrahydro-2H-pyran-2-yl)acetate (8)

To a mixture of $CrCl_2$ (258 mg, 2.1 mmol) in THF (5 mL) was added dropwise the solution of the ketone **16** (42 mg, 0.21 mmol) and CHI_3 (248 mg, 0.63 mmol) in THF (4 mL). After being stirred at 23 °C under argon for 4 h, the mixture was quenched by water and extracted with EtOAc. The combined organic phase was washed with water, brine, dried over anhydrous Mg_2SO_4 and concentrated. The residue was purified column chromatography (hexane/EtOAc, 40:1) to give the vinyl iodide **8** (47 mg, 69%) as colorless oil which should be used for the next step immediately. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 6.08 (s, 1H), 3.83 (m, 2H), 3.70 (s, 3H), 2.61 (dd, J = 16, 8 Hz, 1H), 2.47, (dd, J = 16, 8 Hz, 1H). 2.16 (s, 3H), 1.89 (m, 2H), 1.62 (m, 2H), 1.28 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 142.9, 88.5, 81.2, 72.6, 53.3, 31.5, 27.1, 22.5, 20.9, 14.1; LRMS-ESI (m/z): 325.4, [M+H][+].

Methyl 2-((2R,6S)-6-((2E,4E,6S,8E,10S,11R,12R)-12-((tert-butyldimethylsilyl)oxy)-11-methoxy-6,8,10-trimethyltrideca-2,4,8-trien-2-yl)tetrahydro-2H-pyran-2-yl)acetate (17)

A mixture of vinyl iodide **8** (22 mg, 0.068 mmol), boronate **7** (35 mg, 0.075 mmol), $Pd(PPh_3)_4$ (4 mg, 0.003 mmol) and Cs_2CO_3 (444 mg, 1.36 mmol) in THF (4 mL) was stirred at 55 °C under argon for 4 h. It was quenched with water, and then extracted with ether. The combined organic phase was washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified column chromatography (hexane/EtOAc, 30:1) to give the TBS-protected **17** as a colorless oil (20 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) 6.17 (dd, J = 14.9 Hz 11.2 Hz, 1H), 5.97 (d, J = 10.3 Hz, 1H), 5.57 (dd, J = 7.4 Hz, 15.0 Hz, 1H), 5.12 (d, J = 9.0 Hz, 1H), 3.83-3.80 (m, 2H), 3.78-3.71 (m, 1H), 3.65 (s, 3H), 3.43 (s, 3H), 2.77 (t, J = 5.1Hz, 1H), 2.58-2.53 (m, 2H), 2.46-2.35 (m, 2H), 2.04-2.00 (m, 1H),

1.90-1.82 (m, 2H), 1.71 (s, 3H), 1.65-1.60 (m, 4H) 1.57 (s, 3H), 1.11 (d, J = 6.3 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) 171.8, 140.3, 135.9, 131.4, 130.8, 124.6, 123.8, 89.7, 82.2, 74.2, 70.0, 60.7, 51.4, 47.4, 41.4, 34.9, 33.6, 30.9, 29.4, 25.8, 23.4, 20.1, 19.8, 18.0, 16.0, 15.2, 13.2, −4.6.

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

To a solution of compound **17** (20 mg, 0.023 mmol) in THF (2 mL) was added 1N HCl in MeOH (0.2 mL) at 23 °C. The mixture was stirred for 1 h at the same temperature, and concentrated. The residue was purified column chromatography (hexane : EtOAc = 4 : 1) to give the TBS-deprotected product (14 mg, 89%) as a colorless oil which was used for the next step immediately.

To a solution of above deprotected product (14 mg, 0.033 mmol) and VO(acac)$_2$ (2 mg, 0.007 mmol) in CH$_2$Cl$_2$ (1 mL) was added t-BuOOH (5.5 M in decane, 20 μL, 0.11 mmol) at −78 °C. After being stirred at −15 – −20 °C for 48 h, it was quenched with Me$_2$S and stirred at 23 °C for 30 min. It was concentrated and used for next reaction directly. To a solution of the above crude product in MeOH (1 mL) and water (0.2 mL) was added K$_2$CO$_3$ (29 mg, 0.21 mmol). After being refluxed for 2 h, it was cooled, treated with aq. NaHSO$_4$ (0.01 M, 50 mL) and extracted with EtOAc. The combined organic phase was washed with brine, dried (Na$_2$SO$_4$) and concentrated. The residue was purified by column chromatography (CH$_2$Cl$_2$: MeOH = 15 : 1) to give the acid **5** (4.1 mg, 29% for 2 steps) as a semi solid. $[\alpha]^{20}_D$ = −21.0 (c 0.15, MeOH); ^{1}H NMR (400 MHz, CD$_3$OD) 6.26 (dd, J = 10.9, 15.0 Hz, 1H), 5.94 (d, J = 10.8 Hz, 1H), 5.43 (dd, J = 8.9, 15.0 Hz, 1H), 3.88-3.77 (m, 3H), 3.52 (s, 3H), 2.97 (dd, J = 4.1, 6.2 Hz, 1H), 2.64 (d, J = 9.5 Hz, 1H), 2.50-2.33 (m, 3H), 1.90-1.84 (m, 2H), 1.70 (s, 3H), 1.69-1.34 (m, 6H), 1.26 (s, 3H), 1.24-1.15 (m, 2H), 1.10 (d, J = 6.4 Hz, 1H), 1.03 (d, J = 6.7 Hz, 1H), 0.81 (d, J = 6.9 Hz, 1H); HRMS-ESI (m/z) calc. for C$_{24}$H$_{40}$NaO$_6$ [M + Na]$^+$ 447.2723, found 447.2724.

(1R,5S)-3-oxabicyclo[3.3.1]nonane-2,4-dione (18)

1,3-Cyclohexanedicarboxylic acid (mixture of cis and trans; 5.00 g, 29 mmol) in acetic anhydride (50 mL) was stirred for 10 h at 140 °C. The solvent was removed under vacuum, and the residual white solid was dissolved in CH$_2$Cl$_2$, filtered, and concentrated. The residue was distilled under vacuum to afford compound **18** (2.01 g, 45%) as a white solid. ^{1}H NMR (400 MHz, CDCl$_3$) 3.05 (s, 2H), 2.24 (d, J = 13.6 Hz, 1H), 2.11 (d, J = 13.6 Hz, 2H), 1.85-1.71 (m, 4H), 1.53-1.45 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) 169.8, 36.3, 28.4, 27.1, 19.9.

(1S,3R)-3-acetylcyclohexane-1-carboxylic acid (19)

To a stirred solution of ZnBr$_2$ (1317 mg, 5.7 mmol) in THF (15 mL) and Et$_2$O (15 mL) was added dropwise a solution of MeMgBr in THF (1.9 mL, 3 M, 5.7 mmol) at 0 °C over 5 min under argon and left to stir at the same temperature. After 30 min, the mixture was stirred for 1 h at 23 °C, and stopped stirring, settled to precipitate for 1 h.

To a stirred solution of [Rh(nbd)Cl]$_2$ (20 mg, 0.043 mmol) and (*R*)-*tert*-butyphosphino-oxazoline (35 mg, 0.089 mmol) in THF (8 mL) in a separate flask was added the above pre-prepared methylzinc bromide solution (25 mL, decanted from the precipitate via syringe) and a solution of **18** (450 mg, 2.92 mmol) in THF (8 mL) at 0 °C. The mixture was stirred at 23 °C for 30 hrs, quenched by 1 M HCl (15 ml), diluted by H$_2$O (20 mL), extracted by Et$_2$O (3 × 20 mL), dried over anhydrous MgSO4, filtered and concentration. The residue was purified over silica gel chromatography (Hexane : Ethyl acetate = 2 : 1) to give compound **19** (290 mg, 58%, 91% ee) as a colorless oil. Chiral HPLC analysis was performed using a chiralcel OD-H column eluting with Hexane : *i*-PrOH = 99 : 1 0.5 mL/min. [α]$^{20}_D$ = +3.4 (c 1.00, CH$_2$Cl$_2$); ^{1}H NMR (400 MHz, CDCl$_3$) 2.40-2.33 (m, 2H), 2.28-2.09 (m, 4H), 2.08-1.90 (m, 3H), 1.52-1.13 (m, 4H); ^{13}C NMR (100 MHz, CDCl$_3$) 210.8, 181.2, 50.2, 42.2, 29.9, 28.0, 27.7, 27.5, 24.7; HRMS-ESI (m/z) calc. for C$_9$H$_{14}$NaO$_3$ [M + Na]$^+$ 193.0841, found 193.0835.

1-((1S, 3R)-3-acetylcyclohexyl)-2-diazoethan-1-one (20)

To a stirred solution of **19** (60 mg, 0.35 mmol) in THF (6 mL) was added Et$_3$N (294 μL, 2.1 mmol) and MsCl (81 μL, 1.03 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. A fresh prepared solution of CH$_2$N$_2$ in Et$_2$O (excess, 6 mL) was added to the mixture at 0 °C. The mixture was quenched by drops of acetic acid, diluted by H$_2$O (8 mL), extracted by Et$_2$O (3 × 10 mL), dried over anhydrous MgSO$_4$, filtered and concentration. The residue was purified over silica gel chromatography (Hexane : Ethyl acetate : Et$_3$N = 2 : 1: 0.001) to give the corresponding diazo **20** (56 mg, 82%) as a colorless oil which should be used for the next step immediately.

Methyl 2-((1S,3R)-3-acetylcyclohexyl)acetate (21)

To a stirred solution of diazo **20** (46 mg, 0.24 mmol) in anhydrous MeOH was added a solution of silver benzoate (10 mg, 0.04 mmol) in dry Et$_3$N (130 μL, 0.92 mmol) at 23 °C under argon. The mixture was stirred for 1h and concentrated. The residue was purified over silica gel chromatography (Hexane : Ethyl acetate = 7 : 1) to give compound **21** (23 mg, 49%) as a colorless oil. [α]$^{20}_D$ = −8.0 (c 1.00, CH$_2$Cl$_2$); ^{1}H NMR (400 MHz, CDCl$_3$) 3.65 (s, 3H), 2.43-2.35 (m, 1H), 2.22 (d, *J* = 6.9 Hz, 2H), 2.12 (s, 3H), 1.91-1.64 (m, 5H), 1.38-0.86 (m, 4H); ^{13}C NMR (100 MHz, CDCl$_3$) 211.4, 173.0, 51.3, 51.0, 41.5, 34.1 34.0, 32.1, 28.0, 27.9, 25.2; HRMS-ESI (m/z) calc. for C$_{11}$H$_{14}$NaO$_3$ [M + Na]$^+$ 221.1151, found 221.1148.

Methyl 2-((1S,3R)-3-((E)-1-iodoprop-1-en-2-yl)cyclohexyl)acetate (9)

To a mixture of CrCl$_2$ (61 mg, 0.5 mmol) in THF (2 mL) was added dropwise the solution of the ketone **21** (10 mg, 0.05 mmol) and CHI$_3$ (59 mg, 0.15 mmol) in THF (2 mL). After being stirred at 23 °C under argon for 4 h, the mixture was quenched by water and extracted with EtOAc. The combined organic phase was washed with water, brine, dried over anhydrous Mg$_2$SO$_4$ and concentrated. The residue was purified column chromatography (hexane/EtOAc, 40:1) to give the vinyl iodide **9** (9 mg, 56%) as colorless oil which should be used for the next step immediately.

Methyl 2-((1S,3R)-3-((2E,4E,6S,8E,10S,11R,12R)-12-hydroxy-11-methoxy-6,8,10-trimethyltrideca-2,4,8-trien-2-yl)cyclohexyl)acetate (22)

A mixture of vinyl iodide **9** (15 mg, 0.046 mmol), boronate **7** (21 mg, 0.046 mmol), Pd(PPh$_3$)$_4$ (3 mg, 0.002 mmol) and Cs$_2$CO$_3$ (299 mg, 0.92 mmol) in THF (2 mL) was stirred at 55 °C under argon for 4 h. It was quenched with water, and then extracted with ether. The combined organic phase was washed with water, brine, dried (Na$_2$SO$_4$) and concentrated. The residue was purified column chromatography (hexane/EtOAc, 30:1) to give a colorless oil (20 mg, 81%).

To a solution of above oil (15 mg, 0.028 mmol) in THF (2 mL) was added 1N HCl in MeOH (0.2 mL) at 23 °C. The mixture was stirred for 1 h at the same temperature, and concentrated. The residue was purified column chromatography (hexane : EtOAc = 4 : 1) to give the de-protected product **22** (7.1 mg, 61% from **9**) as a colorless oil. [α]^{20}$_D$ = −13.6 (c 0.25, CH$_2$Cl$_2$); ^{1}H NMR (800 MHz, CDCl$_3$) 6.21 (dd, J = 15.3, 10.8 Hz, 1H), 5.79 (d, J = 10.4 Hz, 1H), 5.49 (dd, J = 7.4, 15.0 Hz, 1H), 4.99 (d, J = 9.6 Hz, 1H), 3.74 (brs, 1H), 3.69 (s, 3H), 3.54 (s, 3H), 2.74-2.72 (m, 1H), 2.68-2.66 (m, 1H), 2.40 (t, J = 7.1 Hz, 1H), 2.25-2.20 (m, 2H), 2.05 (dd, J = 6.8, 13.1 Hz, 1H), 1.97-1.92 (m, 2H), 1.85-1.74 (m, 2H), 1.72 (s, 3H), 1.64 (s, 3H), 1.36-1.35 (m, 1H), 1.21 (d, J = 6.5 Hz), 1.00 (d, J = 6.9 Hz, 1H), 0.97 (d, J = 7.2 Hz, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) 173.4, 140.9, 138.3, 133.7, 129.6, 124.6, 123.1, 89.7, 67.9, 61.4, 51.4, 47.6, 46.9, 42.0, 37.9, 35.0, 34.9, 34.7, 32.7, 31.1, 26.0, 20.4, 20.1, 16.5, 16.3, 14.8; HRMS-ESI (m/z) calc. for C$_{26}$H$_{44}$NaO$_4$ [M + Na]$^+$ 443.3137, found 443.3118.

2-((1S,3R)-3-((S,2E,4E)-7-((2R,3R)-3-((2R,3R,4R)-4-hydroxy-3-methoxypentan-2-yl)-2-methyloxiran-2-yl)-6-methylhepta-2,4-dien-2-yl)cyclohexyl) acetic acid (6)

To a solution of the **22** (7 mg, 0.016 mmol) and VO(acac)$_2$ (0.88 mg, 0.003 mmol) in CH$_2$Cl$_2$ (1 mL) was added t-BuOOH (5.5 M in decane, 13.3 μL, 0.07 mmol) at −78 °C. After being stirred at −15-−20 °C for 48 h, it was quenched with Me$_2$S and stirred at 23 °C for 30 min. It was concentrated and used for next reaction directly. To a solution of the above crude product in MeOH (1 mL) and water (0.2 mL) was added K$_2$CO$_3$ (14.7 mg, 0.11 mmol). After being refluxed for 2 h, it was cooled, treated with aq. NaHSO$_4$ (0.01 M, 50 mL) and extracted with EtOAc. The combined organic phase was washed with brine, dried (Na$_2$SO$_4$) and concentrated. The residue was purified by column chromatography (CH$_2$Cl$_2$: MeOH = 15 : 1) to give the acid **6** (2.3 mg, 32% for 2 steps) as a semi solid. [α]^{20}$_D$ = −6.0 (c 0.10, CH$_2$Cl$_2$); ^{1}H NMR (800 MHz, CD$_3$OD) 6.30 (dd, J = 10.2, 15.1 Hz, 1H), 5.80 (d, J = 10.6 Hz, 1H), 5.37 (dd, J = 9.0, 15.0 Hz, 1H), 3.82 (t, J = 6.3 Hz, 1H), 3.57 (s, 1H), 3.01 (dd, J = 4.1, 6.3 Hz, 1H), 2.67 (d, J = 9.5 Hz, 1H), 2.46-2.42 (m, 1H), 2.25-2.18 (m, 2H), 2.00-1.93 (m, 2H), 1.87-1.75 (m, 3H), 1.72 (s, 3H), 1.71-1.68 (m, 2H), 1.55-1.51 (m, 1H), 1.42-1.38 (m, 2H), 1.30 (s, 3H), 1.20-1.17 (m, 2H), 1.13 (d, J = 6.5 Hz, 1H), 0.85 (d, J = 6.9 Hz, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) 175.5, 140.9, 136.8, 125.9, 123.0, 87.1, 68.5, 66.5, 61.3, 60.5, 53.3, 47.0, 46.8, 41.7, 37.8, 35.0, 34.9, 32.3, 31.0, 29.3, 25.8, 21.2, 18.4, 15.3, 13.6, 9.8; HRMS-ESI (m/z) calc. for C$_{25}$H$_{42}$NaO$_5$ [M + Na]$^+$ 445.2930, found 445.2917.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Reference

1. Roybal GA, Jurica MS. Nucleic Acids Res. 2010; 38:6664–6672. [PubMed: 20529876]

2. Maniatis T, Reed R. Nature. 2002; 416:499–506. [PubMed: 11932736]

3. Staley JP, Woolford JL Jr. Curr. Opin. Cell Biol. 2009; 21:109–118. [PubMed: 19167202]

4. Raymond B. Nat. Chem. Biol. 2007; 3:533–535. [PubMed: 17710096]

5. Sharp PA. Cell. 1994; 77:805–815. [PubMed: 7516265]

6. Douglas AGL, Wood MJA. Brief. Funct. Gen. 2011; 10:151–164.

7. Pagani F, Baralle FE. Nat. Rev. Genet. 2004; 5:389–396. [PubMed: 15168696]

8. Brinkman BM. Clin. Biochem. 2004; 37:584–594. [PubMed: 15234240]

9. Van Alphen RJ, Weimer EA, Burger H, Eskens FA. Br. J. Cancer. 2009; 100:228–232. [PubMed: 19034274]

10. Venables JP. Cancer Res. 64:7647–7654. [PubMed: 15520162]

11. Kotake Y, Sagane K, Owa T, Mimori-Kiyosue Y, Shimizu H, Uesugi M, Ishihama Y, Iwata M, Mizui Y. Nat. Chem. Biol. 2007; 3:570–575. [PubMed: 17643112]

12. Kaida D, Motoyoshi H, Tashiro E, Nojima T, Hagiwara M, Ishigami K, Watanabe H, Kitahara T, Yoshida T, Nakajima H, Tani T, Horinouchi S, Yoshida M. Nat. Chem. Biol. 2007; 3:576–583. [PubMed: 17643111]

13. Effenberger KA, Anderson DA, Bray WM, Prichard BE, Ma N, Adams MS, Ghosh AK, Jurica MS. J. Biol. Chem. 2014; 289:1938–1947. [PubMed: 24302718]

14. Sakai Y, Tsujita T, Akiyama T, Yoshida T, Mizukami T, Akinaga S, Horinouchi S, Yoshida M. J. Antibiot. 2002; 55:863–872. [PubMed: 12523819]

15. Eskens FALM, Ramos FJ, Burger H, O'Brien JP, Piera A, de Jonge MJA, Mizui Y, Wiemer EAC, Carreras MJ, Baselga J, Tabernero J. Clin.. Cancer Res. 2013; 19:6296–6304. [PubMed: 23983259]

16. Nakajima H, Hori Y, Terano H, Okuhara M, Manda T, Matsumoto S, Shimomura K. J. Antibiot. 1996; 49:1204–1211. [PubMed: 9031665]

17. Motoyoshi H, Horigome M, Ishigami K, Yoshida T, Horinouchi S, Yoshida M, Watanabe H, Kitahara T. Biosci, Biotechnol. Biochem. 2004; 68:2178–2182. [PubMed: 15502365]

18. Thompson CF, Jamison TF, Jacobsen EN. J. Am. Chem. Soc. 2001; 123:9974–9983. [PubMed: 11592876]

19. Motoyoshi H, Horigome M, Watanabe H, Kitahara T. Tetrahedron. 2006; 62:1378–1389.

20. Albert BJ, Sivaramakrishnan A, Naka T, Czaicki NL, Koide K. J. Am. Chem. Soc. 2007; 129:2648–2659. [PubMed: 17279752]

21. Ghosh AK, Chen ZH, Effenberger KA, Jurica MS. J. Org. Chem. 2014; 79:5697–5709. [PubMed: 24873648]

22. Webb TR, Joyner AS, Potter PM. Drug Discovery Today. 2013; 18:43–49. [PubMed: 22885522]

23. Horiguchi T, Shirasaki M, Tanida S. Takeda Kenkyushoho. 1996; 55:149–159.

24. Banwell M, McLeod M, Premaj R, Simpson G. Pure Appl. Chem. 2000; 72:1631–1634.

25. Zhang Y, Panek JS. Org. Lett. 2007; 9:3141–3413. [PubMed: 17629295]

26. Murray TJ, Forsyth CJ. Org. Lett. 2008; 10:3429–3431. [PubMed: 18642829]

27. Ghosh AK, Li J. Org. Lett. 2011; 13:66–69. [PubMed: 21126066]

28. Ghosh AK, Ma N, Effenberger KA, Jurica MS. Org. Lett. 2014; 16:3154–3157. [PubMed: 24869489]

29. Effenberger KA, Urabe VK, Prichard BE, Ghosh AK, Jurica MS. RNA. 2016; 22:1–10.

30. Lagisetti C, Yermolina MV, Sharma LK, Palacios G, Prigaro BJ, Webb TR. ACS Chem. Biol. 2014; 9:634–648.

31. Gerard B, Marie J-C, Pandya BA, Lee MD IV, Liu H, Marcaurelle LA. J. Org. Chem. 2011; 76:1898–1901. [PubMed: 21341742]

32. Clive DLJ, Wang J. J. Org. Chem. 2002; 67:1192–1198. [PubMed: 11846662]

33. Edmunds AJF, Trueb W. Tetrahedron Lett. 1997; 38:1009–1012.

34. Yates P, Grewal RS, Hayes PC, Saywer JF. Can. J. Chem. 1988; 66:2805–2815.

35. Takai K, Nitta K, Utimoto K. J. Am. Chem. Soc. 1986; 108:7408–7410.

36. Cook MJ, Rovis T. J. Am. Chem. Soc. 2007; 129:9302–9303. [PubMed: 17622150]

37. Shintani R, Fu GC. Angew. Chem. Int. Ed. 2002; 41:1057–1059.

38. Nicolaou KC, Baran PS, Zhong Y-L, Choi H-S, Yoon WH, He Y, Fong KC. Angew. Chem. Int. Ed. 1999; 38:1669–1675.

39. Nicolaou KC, Baran PS, Zhong Y-L, Choi H-S, Fong KC, He Y, Yoon WH. Org. Lett. 1999; 1:883–886. [PubMed: 10823217]

40. Winum J-Y, Kamal M, Leydet A, Roque J-P, Montero J-L. Tetrahedron Lett. 1996; 37:1781–1782.
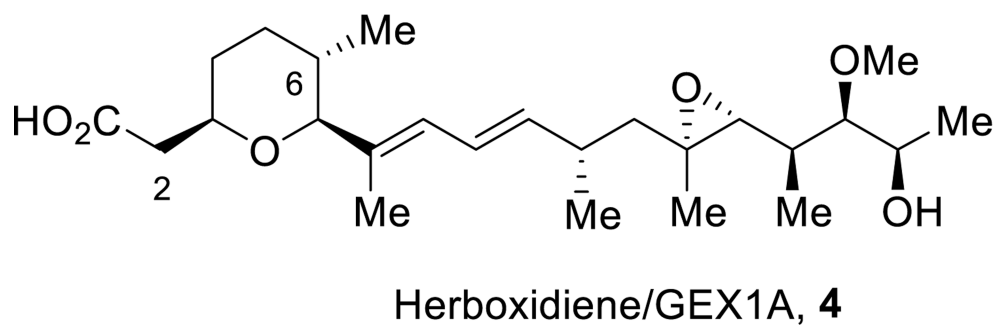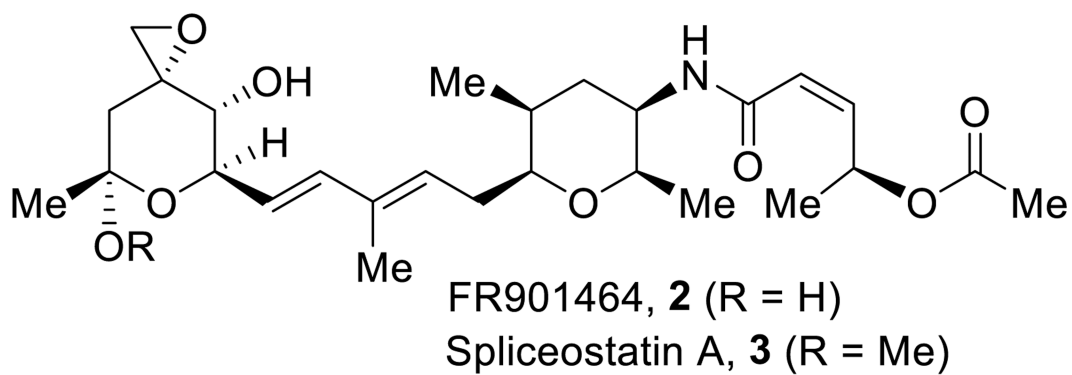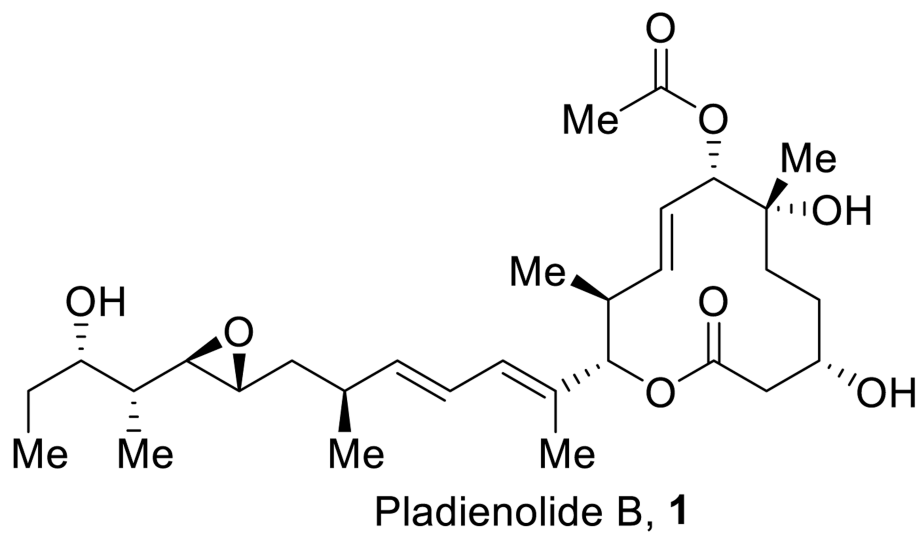
Figure 1.
Structures of pladienolide B, FR901464, spliceostatin A and herboxidiene.
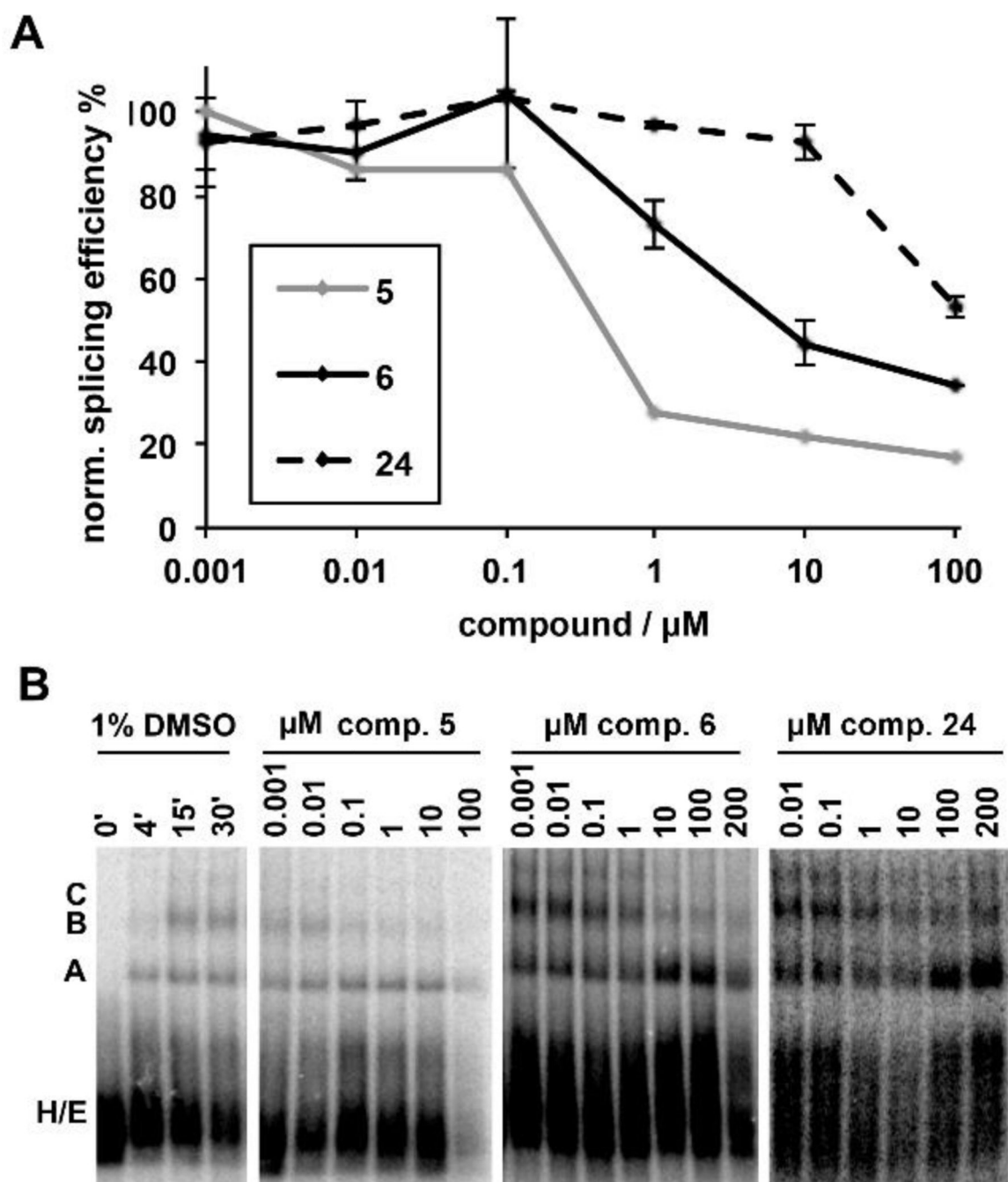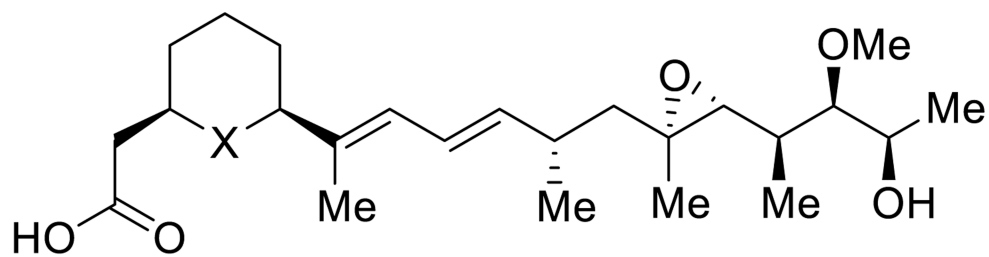
Figure 2.
Impact of herboxidiene derivatives on *in vitro* splicing. **A.** Quantification of normalized splicing efficiency *vs.* inhibitor concentration for *in vitro* splicing reactions. **B.** Native gel analysis of spliceosome assembly. The first five lanes show a time course of splicing reactions in 1% DMSO using a radiolabeled pre-mRNA substrate which were separated under native conditions. The following lanes show 30 minute 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.

6-Desmethyl herboxidiene **5** (X = O)

6-Desmethyl carba-herboxidiene **6** (X =CH$_2$)

8 (X = O)

9 (X = CH$_2$)

7

10

or

11
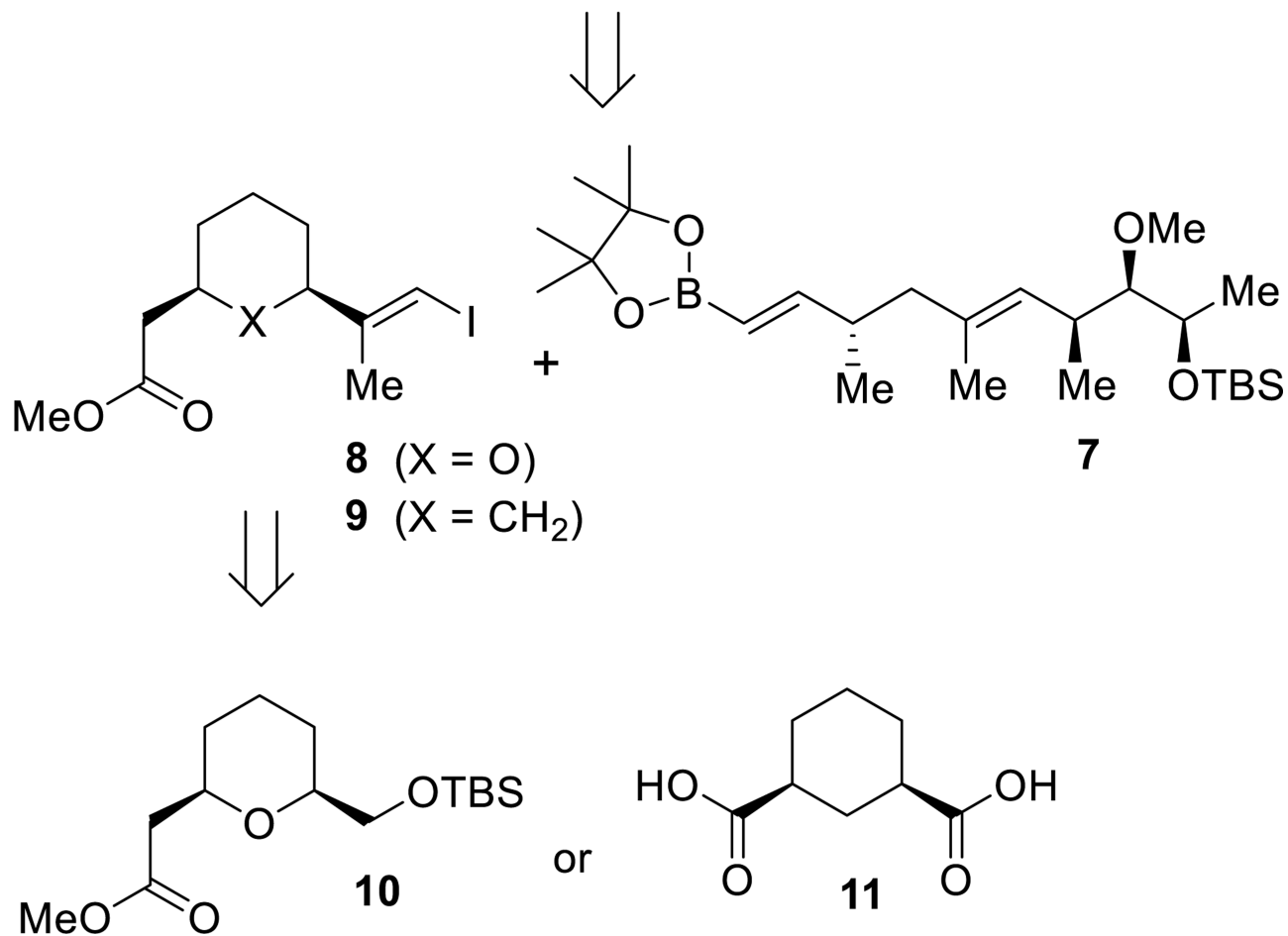
Scheme 1.
Synthetic strategies of desmethyl and carba-derivatives of herboxidiene.
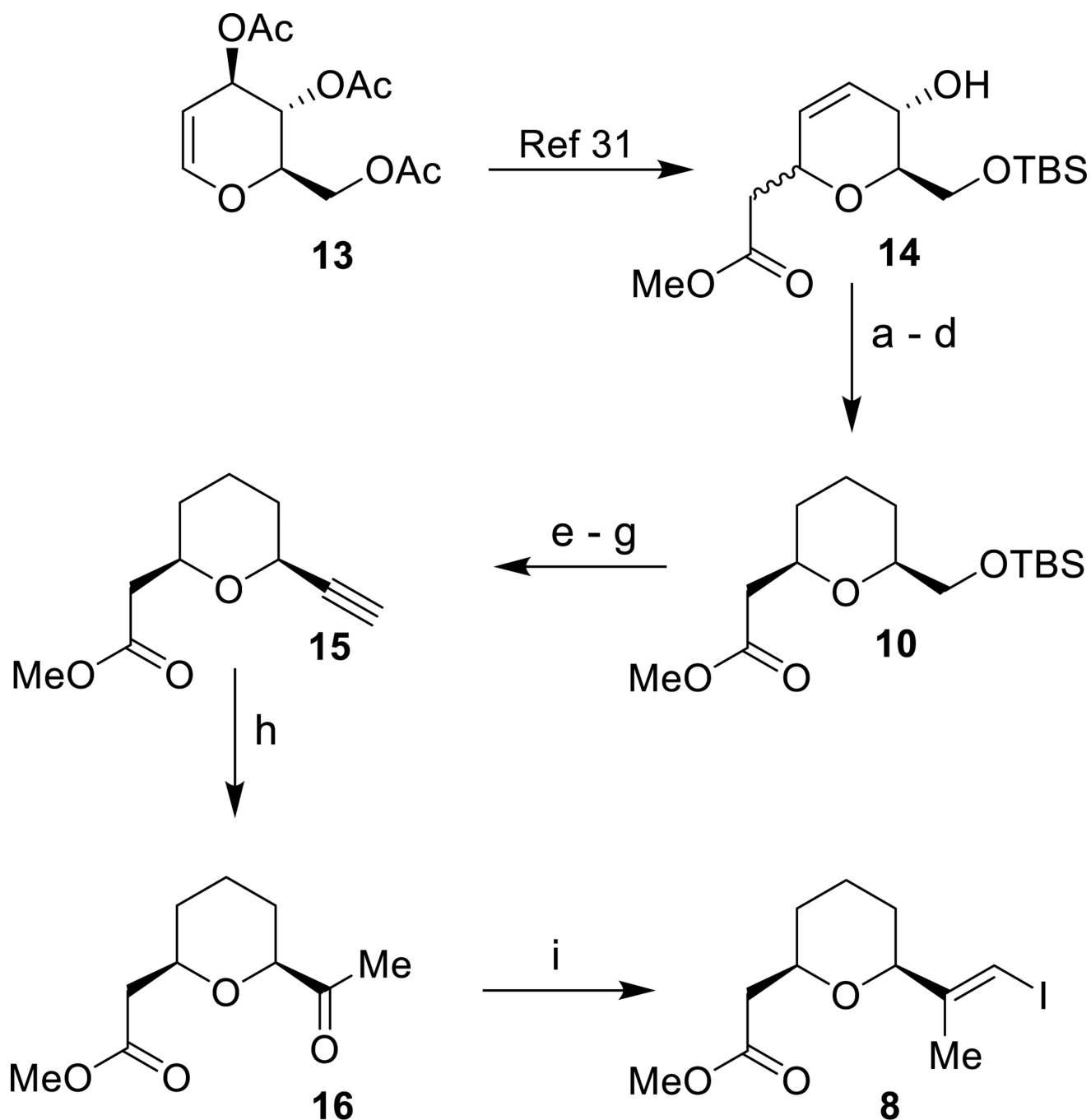
Scheme 2.

Reagents and conditions: (a) H$_2$, 10% Pd-C, EtOAc, 23 °C, 3 h (64%); (b) (thiocarbonyl)diimidazole, PhMe, 115 °C, 1 h (96%); (c) nBu$_3$SnH, AIBN, PhMe, 115 °C 1 h (74%); (d) NaH, THF, 23 °C, 6 h (63%); (e) nBu$_4$N$^+$F$^-$, THF, 23 °C, 30 min; (f) SO$_3$.Py, CH$_2$Cl$_2$, DMSO, 23 °C, 2 h; (g) MeCO(N$_2$)P(O)Me$_3$, K$_2$CO$_3$, MeOH, 23 °C, 2.5 h (67% from **10**); (h) HgSO$_4$, 3M H$_2$SO$_4$ solution 23 °C, 1.5 h (69%); (i) CrCl$_2$, CHI$_3$, THF, 23 °C, 4 h (69%).
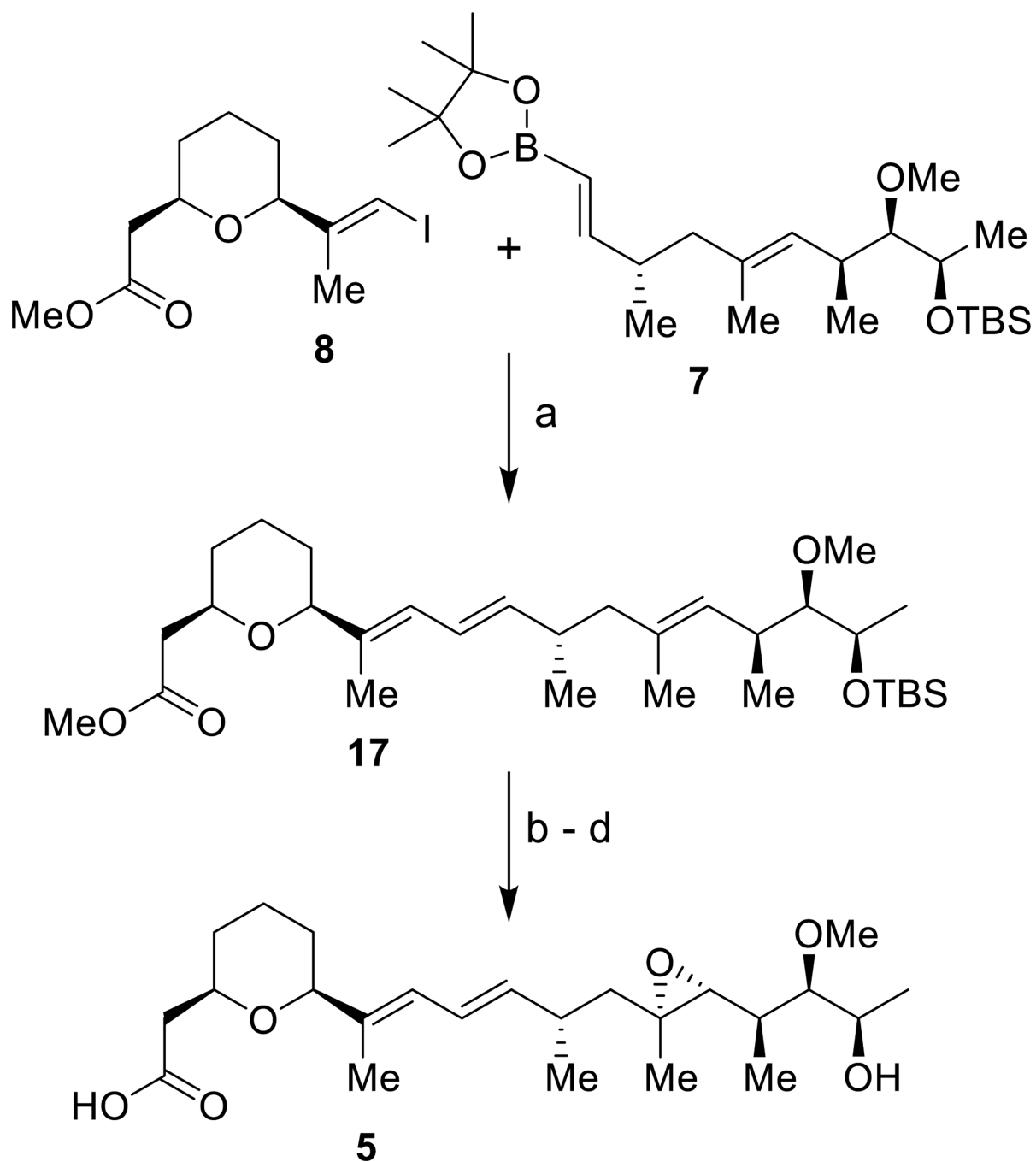
Scheme 3.
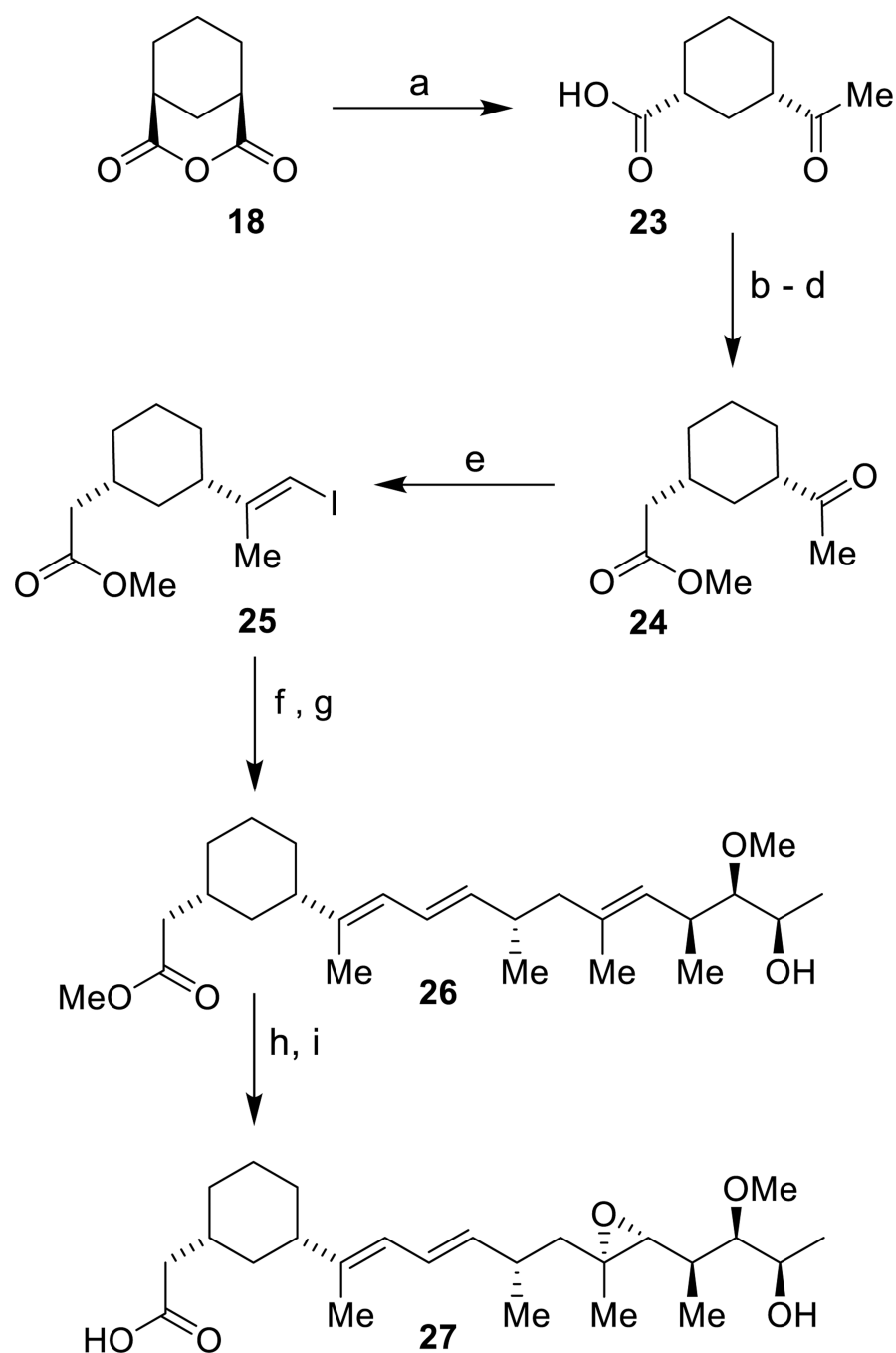Reagents and conditions: (a) Pd (PPh$_3$)$_4$ (5 mol %), Cs$_2$CO$_3$, THF, 55 °C, 5 h (56%); (b) 1 N HCl in MeOH, 23 °C, 45 min; (c) VO(acac)$_2$, t-BuOOH, −15 °C, 48 h; (d) K$_2$CO$_3$, MeOH, 23 °C (26% from **17**).
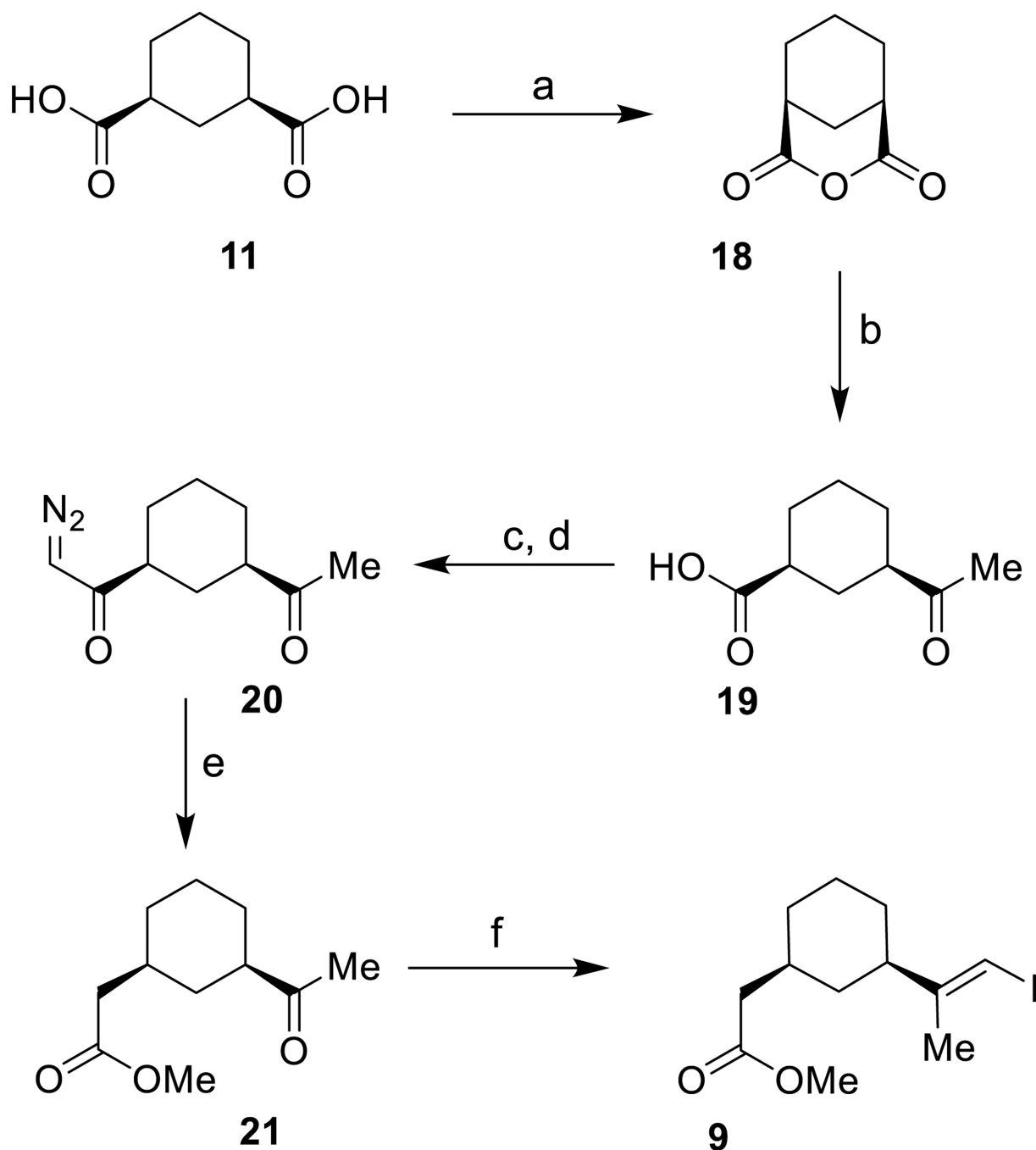
Scheme 4.
Reagents and conditions: (a) Ac_2O, 140 °C, 10 h, (45%); (b) $[Rh(nbd)Cl]_2$, (*R*)-*t*-Bu-Phos, $ZnBr_2$, MeMgBr, THF (58%); (c) CH_3SO_2Cl, Et_3N, 0 °C, 30 min; (d) CH_2N_2, Et_2O, 0 °C, 30 min (82% for 2-steps); (e) $Ag(PhCO_2)$, Et_3N, 23 °C, 1 h (49%); (f) $CrCl_2$, CHI_3, THF, 23 °C, 4 h (56%).
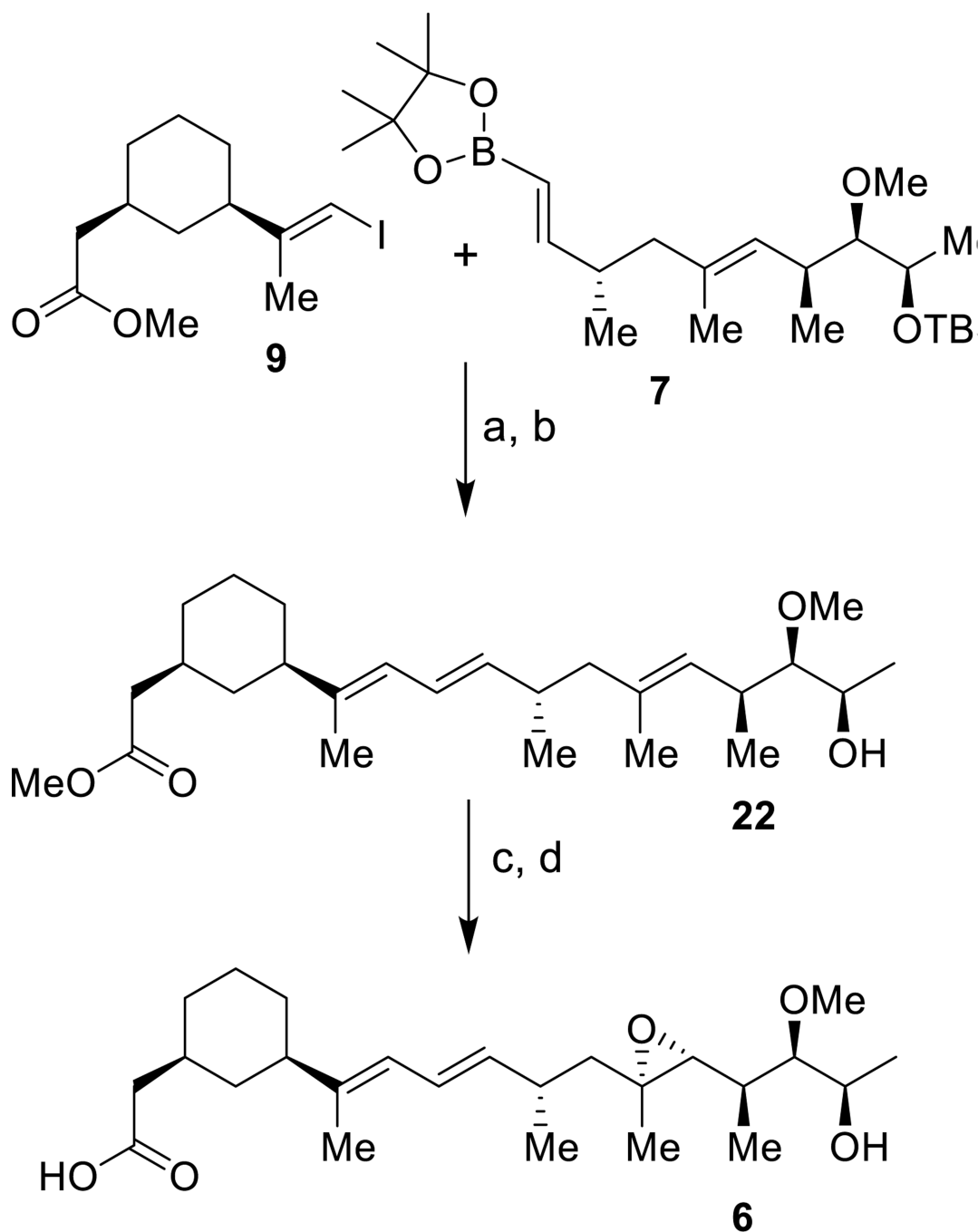
Scheme 5.
Reagents and conditions: (a) Pd(PPh$_3$)$_4$ (5 mol %), Cs$_2$CO$_3$, THF, 55 °C, 4 h (81%); (b) 1N HCl in MeOH, 23 °C, 1 h (61% for 2-steps); (c) VO(acac)$_2$, *t*-BuOOH, CH$_2$Cl$_2$, −20 °C, 48 h; (d) K$_2$CO$_3$, MeOH, 23 °C, 2 h (32% for 2-steps).

Scheme 6.
Reagents and conditions: (a) [Rh(nbd)Cl]$_2$, S-t-Bu-Phos, ZnBr$_2$, MeMgBr, THF (48%); (b) CH$_3$SO$_2$Cl, Et$_3$N, 0 °C, 30 min; (c) CH$_2$N$_2$, Et$_2$O, 0 °C, 30 min (78% for 2-steps); (d) Ag(PhCO$_2$), Et$_3$N, 23 °C, 1 h (59%) ; (e) CrCl$_2$, CHI$_3$, THF, 23 °C, 4 h (44%); (f) boronate **7**, Pd(PPh$_3$)$_4$ (5 mol %), Cs$_2$CO$_3$, THF, 55 °C, 4 h (72%); (g) 1 N HCl in MeOH, 23 °C, 1 h (87%); (h) VO(acac)$_2$, t-BuOOH, CH$_2$Cl$_2$, −20 °C, 48 h; (i) K$_2$CO$_3$, MeOH, 23 °C, 2 h (40% for 2-steps)