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Tridemethylisovelleral, a potent cytotoxic agent

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Abstract—The synthesis and in vitro cytotoxicity toward various tumor cell lines of (±)-tridemethylisovelleral, an analogue of the bioactive fungal sesquiterpene (+)-isovelleral retaining the bicyclo[4,1,0]hept-2-en-1,2-dicarbaldehyde system but lacking the three methyl groups, is reported. The cytotoxicity of tridemethylisovelleral toward several tumor cell lines was found to be comparable with those of established antitumor drugs, and significantly higher than that of isovelleral.

1. Introduction

(+)-Isovelleral (**2**) (see Fig. 1) is an extremely pungent marasmane sesquiterpene with potent cytotoxic and antimicrobial activities[1] that was originally isolated from the fruit bodies of *Lactarius vellereus*. It is formed enzymatically in seconds from an inactive precursor (stearoylvelutinal) as a response to injury to the fruit body (Scheme 1), and is the active principle in a binary chemical defense system developed by evolution to protect the fruit bodies from parasites and predators.[2]

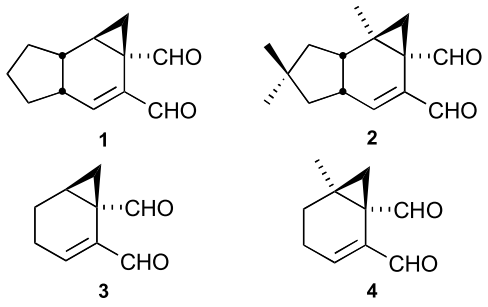

Figure 1.

The biological activities of isovelleral (**2**) depend on the electrophilic unsaturated 1,4-dialdehyde moiety, present in numerous other terpenoids claimed to be part of the natural defense systems,[3] as the transformation of either of the aldehyde functions to an alcohol or keto group or the reduction of the carbon–carbon double bond is associated with loss of activity.[4] Also the cyclopropane ring and its environment appears to be important for the biological activity of **2**, as suggested by the difference in antimicrobial activity and cytotoxicity toward mammalian cells of the two bicyclic analogues **3** and **4**;[5] **3** is at least 10 times more potent than **4**.[6] The reason for this difference is believed to be that a nucleophilic attack on the cyclopropane ring is involved in the biological effects,[6] and there is less steric hindrance for such an attack in **3**.[7] In addition, the cyclopentane ring of isovelleral (**2**) seems to play a role, as **2** is considerably more potent than **4**.[6] In order to confirm these suggestions, and to prepare a more potent analogue of the natural product, tridemethylisovelleral (**1**) was synthesized and assayed.

2. Results and discussion

Although the original goal was to prepare the analogue of **2** lacking only the methyl attached to the cyclopropane ring, we decided to omit also the geminal methyls of the cyclopentane ring as it is unlikely that they will moderate the reactivity of the dialdehyde moiety. However, it should be remembered that the geminal

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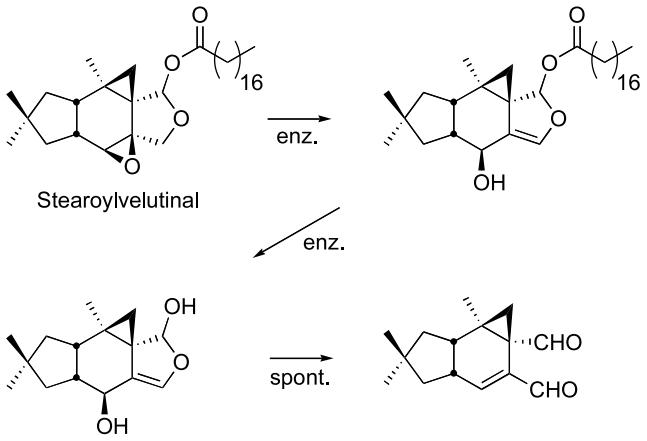
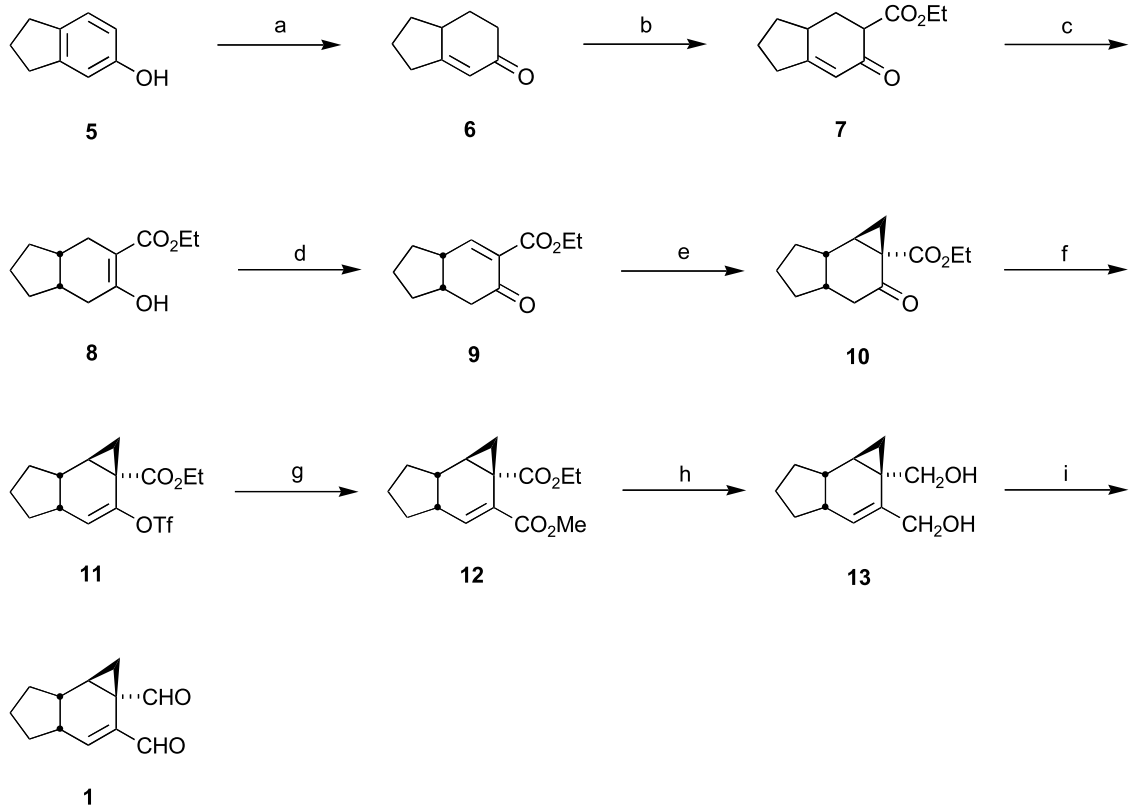


Scheme 1. Conversion of stearoylvelutinal to isovelleral (**2**) as a response to injury.

methyl groups may provide for some increase in steric hindrance for reagents that have to react with the cyclopropane ring and in that way cause even a decrease in activity. The strategy selected was to start from commercially available 5-indanol (**5**), and to introduce the two aldehyde groups and the cyclopropane ring later. α,β-Unsaturated ketones can easily be made from aromatic methyl ethers through Birch reduction followed by acidic hydrolysis.[8]

Commercially available 5-indanol was converted into its methyl ether and was immediately reduced with lithium in ammonia to the bicyclic ketone **6** (Scheme 2). The introduction of the ester group of **7** was achieved by the reaction of the enolate ion of **6** with ethylcyanoformate,[9] yielding the β-keto ester as a 3:1 diastereomeric mixture of epimeric esters. No migration of the double bond was observed. The *cis* bicyclic framework of tridemethylisovelleral (**1**) was obtained by hydrogenation of the bicyclic enone, a reaction that, as expected,[10] was stereoselective. Both epimers of **7** gave the same product **8**, and the reaction was run with the 3:1 diastereomeric mixture obtained in the preceding step. The introduction of the conjugated double bond in **9** was accomplished by treatment of the enol ester **8** with phenylselenyl chloride in the presence of pyridine followed by oxidation with hydrogen peroxide and subsequent pyrolysis.[11] Using the Corey–Chaykovsky reagent (methylsulfoxonium ylid),[12] the cyclopropane ring was introduced in a stereoselective manner giving only one cyclopropane diastereomer. From this point, Heathcock's route to racemic isovelleral (**2**)[13] was essentially followed. β-Keto ester (**10**) was deprotonated with LDA and the enolate was trapped as the corresponding enol triflate (**11**). Carbonylation with palladium acetate under a carbon monoxide[14] atmosphere gave the diester **12**. Subsequently, the ester groups were reduced to diol **13** and then reoxidized using Swern conditions[15] to give the desired dialdehyde **1** in racemic form.



Scheme 2. Reagents and conditions: (a) i—MeI, K_2CO_3, DMF, 55 °C; ii—Li, NH_3, EtOH, THF, −78 °C; iii—10% HCl, MeOH, 85% from 5-indanol; (b) i—DIPA, *n*-BuLi, ethylcyanoformate, THF, −78 °C 90%; (c) HCO_2NH_4, 10% Pd/C, MeOH, reflux, 76%; (d) i—DIPA, *n*-BuLi, PhSeCl, THF, −78 °C ii—35% H_2O_2, CH_2Cl_2, 86%; (e) NaH (oil free), Me_3SOI, DMF, −15 °C, 69%; (f) LDA, $PhNTf_2$, THF, 85%; (g) $Pd(OAc)_2$, PPh_3, CO, MeOH, Et_3N, DMF, 20 °C, 90%; (h) DIBAL-H, THF, 65%; (i) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 °C, 80%.

The cytotoxic activity of tridemethylisovelleral (**1**) was tested against a series of tumor cell lines,[16] and the IC_{50} values obtained are given in Table 1. For comparison, the activities of isovelleral (**2**) as well as of several established antitumor drugs are also given. The cells were plated in 96-wells flat bottom microtiter plates and preincubated in RPMI 1640 medium supplemented with 10% FCS for 48 h at 37 °C. The test compounds were dissolved in DMSO and a dilution series in the medium was prepared and added to the cells. After 5 days, the incubation was terminated by washing the plate twice with PBS and fixing the cells with 10% trichloroacetic acid in PBS, whereafter the cells were stained and the result was recorded in an automated microplate reader. A detailed description of the test procedure is published elsewhere.[16]

It is noteworthy that **1** is very potent toward all cell lines, also those that are considered less sensitive to chemotherapy (e.g., the lung cancer cell line H226), and the concentrations required for inhibition are approximately 0.1 nmol/ml. Although the lack of selectivity makes **1** useless as a drug by itself, but the possibility to disguise it as a prodrug that is activated selectively by tumor cells is attractive. Such efforts are currently in progress. Both enantiomers of isovelleral (**2**) have previously been shown to possess similar antimicrobial and cytotoxic activities,[17] and it is therefore relevant to compare with racemic tridemethylisovelleral (**1**). In comparison with the natural product **2**, the cytotoxicity of **1** is between 4 and 11 times higher in these assays. Assuming that the effect of the geminal methyls of the cyclopentane ring of **2** is insignificant, this would confirm that the cyclopentane ring is important for the biological activities of the isovelleraloids. The cyclopentane ring increases the rigidity of **1** and **2** compared to **3** and **4**, thereby altering the dihedral angle between the two aldehyde groups. In addition, the methyl adjacent to the cyclopropane ring in isovelleral (**2**) will also affect the conformation slightly, by its steric interaction with the cyclopentane ring. This can be observed in Figure 2, where the lowest energy conformers of **1** and **2** are shown. The dihedral angle between the two aldehyde groups is 52° in **1** and 36° in **2**. As the aldehyde groups are anticipated to take part in the events that lead to the biological activity, conformational effects on the dialdehyde moiety may be important.[18]

3. Conclusions

The demethylated analogue **1** of the potent fungal unsaturated dialdehyde isovelleral (**2**) was shown to inhibit the growth of different tumor cell lines at around 0.1 nmol/ml. Although the lack of selectivity of **2** renders it useless for direct pharmaceutical applications, the unsaturated 1,4-dialdehyde functionality can be disguised chemically in ways that are (a) chemically relatively stable and (b) transformed back to the unsaturated dialdehyde in the presence of certain chemical or enzymatic conditions. This makes tridemethylisovelleral

Table 1. Cytotoxicity of tridemethylisovelleral (**1**), isovelleral (**2**) and several antitumor drugs towards seven tumor cell lines[a]

	Cell line[b]						
Compound	MCF7	EVSA-T	WiDr	IGROV	M19	A498	H226
1	0.17	0.07	0.18	0.06	0.17	0.21	0.23
2	0.56	0.28	1.08	0.66	0.66	1.87	1.78
DOX[c]	0.02	0.01	0.02	0.11	0.03	0.17	0.37
CPT[c]	2.21	1.34	3.06	0.54	1.77	7.13	10.35
5-FU[c]	5.77	3.65	1.73	2.28	3.40	1.10	2.61
MTX[c]	0.04	0.01	<0.01	0.02	0.05	0.08	5.03
ETO[c]	4.41	0.54	0.25	0.99	0.86	2.23	6.68

[a] IC_{50} values of test compounds (nmol/ml) in vitro using SRB as a cell viability test.[16]
[b] Tumor cell line: human breast adenocarcinoma (MCF-7), human breast carcinoma (EVSA-T), human colon colorectal adenocarcinoma (WiDr), human ovary carcinoma (IGROV-1), human skin melanoma (M19-MEL, human kidney carcinoma (A498), and human lung mesothelioma.
[c] Established cytotoxic drugs: doxorubicin (DOX), cisplatin (CPT), 5-fluorouracil (5-FU), methotrexate (MTX), and etoposide (ETO).

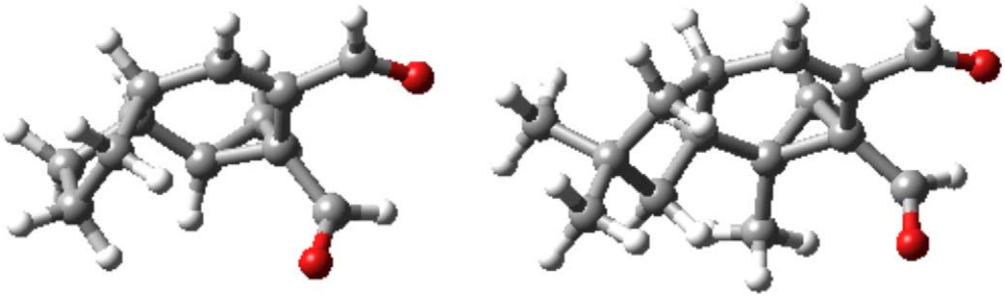


Figure 2. The lowest energy conformations of tridemethylisovelleral (**1**) (left) and isovelleral (**2**) (right).

(**1**) a suitable base for a prodrug that for example is liberated in a tumor tissue that overexpresses a certain enzyme, or that is specifically activated in an insect or another pest. Such work is currently in progress in our laboratory.

4. Experimental

Materials were obtained from commercial suppliers and were used without further purification unless otherwise noted. THF was dried by refluxing over sodium/benzophenone ketyl immediately prior to use. CH_2Cl_2 and triethylamine were distilled from calcium hydride prior to use. DMF and DMSO were distilled under reduced pressure and kept over 4Å MS. MeOH was dried by distilling from magnesium/iodine. All moisture and air-sensitive reactions were carried out under an atmosphere of dry nitrogen using oven-dried glassware. HRESIMS spectra were recorded with a Micromass Q-TOF Micro spectrometer and HREIMS spectra (direct inlet, 70 eV) were recorded with a JEOL SX102 spectrometer. NMR spectra (in $CDCl_3$) were recorded with a Bruker DRX 400 spectrometer at 400 MHz (^{1}H) and at 100 MHz (^{13}C) and with a Bruker DRX 500 spectrometer at 500 MHz (^{1}H) and at 125 MHz (^{13}C). Chemical shifts are given in ppm relative to TMS using the residual $CHCl_3$ peak in $CDCl_3$ solution as internal standard (7.26 and 77.0 ppm, respectively, relative to TMS). Organic extracts were dried over $MgSO_4$. All flash chromatography was performed on 60 Å 35–70 μm Matrex silica gel (Grace Amicon). TLC analyses were made on silica gel 60 F_{254} (Merck) plates and visualized with anisaldehyde/sulfuric acid and heating. The cytotoxicity was assayed according to Ref. 16. Calculations were performed using MacroModel v8.6 (Force field: MMFFs; solvent: water (using the analytical gereralized born/surface area (GB/SA) model); minimization method: TNCG; conformational search: MonteCarlo (MCMM); steps: 2000).

4.1. 1,2,3,6,7,7a-Hexahydroinden-5-one (6)

To a solution of 12.6 g (0.09 mol) of 5-indanol (**5**) in 70 ml of DMF was added 9.0 ml (0.14 mol) of MeI and 21.0 g (0.15 mol) of anhydrous K_2CO_3. The resulting solution was stirred at 55 °C for 4 h under a nitrogen atmosphere. The mixture was cooled to room temperature, diluted with 70 ml of ether and 110 ml of water and extracted with ether. The organic layers were washed with 5% aqueous NaOH, dried over K_2CO_3 and concentrated. The methyl ether was obtained as an orange oil (13.9 g). To a stirred solution of 13.9 g of the crude methyl ether in 80 ml of THF, 80 ml of EtOH and 500 ml of liquid ammonia, 2.8 g (0.4 mol) lithium in small pieces was carefully added at −78 °C under an inert atmosphere. The stirring was continued at −78 °C until the blue color had disappeared whereafter the ammonia was allowed to evaporate. Water was added to dissolve lithium salts and the aqueous layer was extracted three times with ether. The combined extracts were washed with brine, dried, and concentrated. The residue, obtained as a yellow oil, was dissolved in a mixture of 400 ml of MeOH and 120 ml of 10% aqueous HCl, and stirred for 3 h at room temperature. The solution was concentrated and the residue was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried. Evaporation of the solvent afforded an oil, which was purified by distillation (88–90 °C, 1 mmHg). Ketone **6** was obtained as 10.9 g (85% from 5-indanol (**5**)) of a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.23 (1H, dq, J = 11.8 and 7.2 Hz), 1.59 (1H, m), 1.69 (1H, m), 1.89 (1H, m), 2.06 (1H, J = 13.2, 6.7 Hz), 2.22 (1H, m), 2.31 (1H, dd, J = 14.3 and 4.8 Hz), 2.40–2.55 (3H, m), 2.63 (1H, dd, J = 19.3 and 9.1 Hz), 5.86 (1H, s); ^{13}C NMR ($CDCl_3$) δ 24.3, 29.7, 32.3, 33.2, 37.9, 43.5, 122.7, 176.1, 200.5; HRMS (EI) $[M]^+$ calcd for $C_9H_{12}O$, 136.0888; found, 136.0889.

4.2. 6-Oxo-2,3,3a,5,7-hexahydroindene-5-carboxylic acid ethyl ester (7)

A measure of 3.36 ml (2.5 M in hexanes) of *n*-BuLi was added at −78 °C to a solution of 1.23 ml (8.8 mmol) of dry diisopropylamine in 20 ml of THF. The mixture was stirred at −78 °C for 30 min. A solution of 1.09 g (8.0 mmol) of **6** in 4 ml of THF was added over 20 min at −78 °C. The resulting mixture was stirred at −78 °C for 1 h whereafter 800 μL (8.0 mmol) of ethylcyanoformate was added in one aliquot. After 1 h at −78 °C the reaction was quenched with water. The mixture was allowed to warm to room temperature and extracted with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to afford an oil which was purified by flash chromatography (petroleum ether/ethyl acetate 8:2) to give 1.50 g of **7** (90%) as a 3:1 diastereomeric mixture of epimeric esters (NMR data are given for the major isomer): ^{1}H NMR ($CDCl_3$) δ 1.30 (3H, t, J = 7.1 Hz), 1.33 (1H, q, J = 7.3 Hz), 1.72 (1H, m), 1.95 (1H, m), 2.03 (1H, ddd, J = 12.8 and 1.2, 1.1 Hz), 2.13 (1H, m), 2.41 (1H, dt, J = 12.8 and 4.4 Hz), 2.45–2.73 (3H, m), 3.35 (1H, dd, J = 14.1 and 4.6 Hz), 4.22 (2H, dq, J_1 = 7.1 and 1.7 Hz), 5.95 (1H, m); ^{13}C NMR δ 14.6, 24.0, 32.3, 32.8, 33.1, 42.6, 54.4, 61.5, 122.1, 171.3, 176.0, 194.7; HRMS (EI) $[M]^+$ calcd for $C_{12}H_{16}O_3$, 208.1099; found, 208.1101.

4.3. 6-Oxo-2,3,3a,5,7,7a,8-octahydroindane-5-carboxylic acid ethyl ester (8)

A measure of 1.89 g (30.0 mmol) of ammonium formate was added to a mixture of 1.26 g (6.0 mmol) of **7** and 63 mg of 10% Pd/C in 60 ml of dry MeOH at room temperature under a nitrogen atmosphere. The resulting solution was refluxed for 10 min. The reaction mixture was filtered through celite and washed with MeOH. The solvent was removed under reduced pressure and the residue was dissolved in a mixture of water and ether. The layers were separated and the aqueous layer was extracted with ether. The combined organic layers were dried and concentrated. Flash chromatography (heptane/ethyl acetate 98:2) of the residue yielded 946 mg **8** (75%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.31 (3H, t, J = 7.1 Hz), 1.39 (2H, m), 1.54 (1H, m), 1.75 (3H, m), 2.05–2.17 (4H, m), 2.40 (2H, m), 4.21 (2H, dq, J = 7.1 and 0.9 Hz), 12.21 (1H, s); ^{13}C NMR

δ 14.7, 22.7, 25.0, 31.3, 31.8, 31.9, 36.8, 37.0, 60.6, 96.5, 172.4, 172.9; HRMS (EI) $[M-H]^+$ calcd for $C_{12}H_{17}O_3$, 209.1178; found, 209.1174.

4.4. 6-Oxo-2,3,7,7a,8-hexahydroindene-5-carboxylic acid ethyl ester (9)

A measure of 2.20 ml (2.5 M in hexanes) of *n*-BuLi was added at −78 °C to a solution of 0.77 ml (5.5 mmol) of dry diisopropylamine in 20 ml of THF. The mixture was stirred at −78 °C for 30 min. A solution of 1.01 g (5.0 mmol) of **8** in 5 ml of dry THF was added over 5 min. The resulting mixture was stirred at −78 °C for 40 min, then a solution of 920 mg (5.0 mmol) of phenylselenyl chloride in 5 ml of THF was added in one aliquot. The ice bath was removed, and the solution was washed with aqueous 1 M HCl, water, aqueous saturated $NaHCO_3$ solution, and then dried on Na_2SO_4. After filtration and evaporation of the solvent, the afforded oil was diluted in CH_2Cl_2 (40 ml) and the solution was cooled to 0 °C, at which time 0.33 ml of 35% aqueous H_2O_2 was slowly added. An additional 0.33 ml of 35% aqueous H_2O_2 was added after 10 min, and again after 20 min. After an additional 10 min, H_2O was added and the organic layer was separated, washed with saturated $NaHCO_3$, dried over $MgSO_4$, and evaporated. The crude oil was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 7:3) to give 854 mg of **6** (86%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.30 (3 H, dt, $J = 7.1$, 0.5 Hz), 1.40 (1H, m), 1.65–1.75 (3H, m), 1.85 (1H, m), 2.05 (1H, m), 2.48 (1H, t, $J = 8.6$ Hz), 5.57 (1H, m), 2.61 (1H, t, $J = 5.6$ Hz), 2.92 (1H, m), 4.24 (2H, q, $J = 7.1$ Hz), 7.43 (1H, d, $J = 3.8$ Hz); ^{13}C NMR δ 14.6, 24.7, 31.1, 31.5, 38.3, 40.6, 41.9, 61.5, 131.8, 158.5, 165.0, 195.4; HRMS (EI) $[M]^+$ calcd for $C_{12}H_{16}O_3$, 208.1099; found, 208.1097.

4.5. 7-Oxo-2,3,8,8a-octahydro-cyclopropindene-6-carboxylic acid ethyl ester (10)

A flask containing 0.40 g (16.5 mmol, oil free) of fresh NaH and 3.37 g (16.5 mmol) of trimethylsulfoxonium iodide followed by 50 ml of dry DMF, which was added slowly via a syringe and the hydrogen generated was ventilated. The mixture was stirred at room temperature until it became clear and all hydride was consumed. The flask was cooled in an acetone-ice bath to −15 °C and a solution 3.26 g (15.7 mmol) of **9** in 5 ml of DMF was added in one portion to the flask via a syringe and the solution turned orange. After 7 min TLC indicated complete consumption of starting material **9**, and the reaction was quenched by addition of 150 ml of H_2O. The mixture was extracted with 3×100 ml Et_2O, and the combined extracts were washed with large amounts of water, dried, and concentrated. After purification by flash chromatography (heptane/ethyl acetate), **10** was obtained as 2.4 g (69%) of a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.20 (1H, d, $J = 1.7$ Hz), 1.27 (3H, t, $J = 7.1$ Hz), 1.43 (1H, m), 1.60–1.80 (5H, m), 1.89 (1H, m), 1.99 (1H, m), 2.06 (1H, dd, $J_1 = 9.5$ Hz, $J_2 = 15.72$ Hz), 2.17 (1H, m), 2.27 (1H, q, $J = 15.6$ and 5.0 Hz), 2.38 (1H, m), 4.18 (2H, q, $J = 7.2$ Hz); ^{13}C NMR δ 14.5, 20.3, 22.5, 31.2, 31.4, 31.8, 35.4, 36.3, 37.7, 40.4, 61.8, 170.7, 208.3; HRMS (EI) $[M]^+$ calcd for $C_{13}H_{18}O_3$, 222.1256; found, 222.1254.

4.6. 2-Trifluoromethanesulfonyloxy-3a,4,5,6,6a,6b-hexahydro-1*H*-cyclprop[*e*]indene-1a-carboxylic acid ethyl ester (11)

To an ice cold solution of 240 µl (1.69 mmol) of diisopropylamine in 1.25 ml of THF was added dropwise 800 µl (1.9 M in cyclohexane) of *n*-BuLi under a nitrogen atmosphere. After 10 min the solution was cooled to −78 °C and 311 mg (1.40 mmol) of **10** dissolved in 0.63 ml of THF was added dropwise. The reaction mixture was stirred for 40 min at −78 °C when 536 mg (1.50 mmol) of *N*-phenyltrifluoromethanesulfonimide in 1.5 ml of THF was added. The resulting solution was allowed to reach room temperature and stirred for 1 h. The reaction mixture was then diluted with 10 ml saturated aqueous $NaHCO_3$ and extracted with 10 ml of ether. The organic phase was washed with water, brine, dried and concentrated. The crude product was purified with flash chromatography (heptane/ethyl acetate 4:1, 1% EtOH) to give 468 mg (94%) of **11**: ^{1}H NMR ($CDCl_3$) δ 1.17 (1H, dd, $J = 7.5$ and 4.5 Hz), 1.29 (3H, t, $J = 7.1$ Hz), 1.45–1.65 (4H, m), 1.77 (1H, m), 1.86 (1H, dt, $J = 9.3$ and 1.9 Hz), 1.93 (1H, m), 1.99 (1H, dd, $J = 9.3$ and 4.5 Hz), 2.43 (1H, dq, $J = 8.5$ and 2.0 Hz), 2.57 (1H, m), 4.21 (1H, dq, $J = 10.8$ and 7.1 Hz), 4.23 (1H, dq, $J = 10.8$ and 7.1 Hz), 5.42 (1H, d, $J = 3.6$); HRMS (EI) $[M]^+$ calcd for $C_{14}H_{17}F_3O_5S$, 354.0749; found, 354.0761.

4.7. 3a,4,5,6,6a,6b-Hexahydro-1*H*-cycloprop[*e*]indene-1a,2-dicarboxylic acid 1a-ethyl ester 2-methyl ester (12)

A measure of 468 mg (1.32 mmol) of **11**, 400 µl (2.91 mmol) of triethylamine, 2.5 ml (55 mmol) of MeOH, 15 mg (0.066 mmol) of palladium acetate, and 21 mg (0.079 mmol) of triphenylphosphine were dissolved in 6 ml of DMF. CO was bubbled through the solution and the reaction mixture was stirred for 2 h under a CO atmosphere at room temperature. The mixture was diluted with 60 ml of ether, washed with 60 ml of water and 60 ml brine, dried, and concentrated. Flash chromatography (heptane/ethyl acetate 4:1, 1% EtOH) gave 314 mg (1.19 mmol, 90 %) of 12: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd, $J = 7.1$ and 4.3 Hz), 1.21 (3H, t, $J = 7.1$ Hz), 1.45 (1H, dt, $J = 8.6$ and 12.6 Hz), 1.43–1.55 (3H, m), 1.65 (1H, m), 1.74 (1H, m), 1.83 (1H, dd, $J = 9.3$ and 4.3 Hz), 1.87 (1H, m), 2.42 (2H, m), 3.75 (3H, s), 4.07 (1H, dq, $J = 10.8$ and 7.1 Hz), 4.18 (1H, dq, $J_1 = 10.8$ and 7.1 Hz), 6.46 (1H, d, $J = 3.3$ Hz); ^{13}C NMR δ 13.9, 22.9, 23.0, 23.9, 27.6, 32.0, 32.8, 36.2, 37.2, 51.3, 60.4, 129.8, 138.8, 167.2, 173.1; HRMS (ESI) $[M]^+$ calcd for $C_{15}H_{20}O_4$, 264.1362; found, 264.1369.

4.8. (2-Hydroxymethyl-3a,4,5,6,6a,6b-hexahydro-1*H*-cycloprop[*e*]inden-1a-yl)-methanol (13)

A measure of 1 ml (1 mmol) of DIBAL-H (1 M in hexane) was added dropwise to a solution of 53 mg (0.21 mmol) of **12** in 2 ml of THF at −78 °C. The

reaction mixture was allowed to warm to room temperature and stirred for 30 min. The solution was then cooled with an ice-bath to 0 °C and quenched with a saturated aqueous potassium sodium tartrate solution. The mixture was extracted with ether and the ether extract was washed with brine, dried, and concentrated. Flash chromatography (heptane/ethyl acetate 4:1) afforded 25.2 mg (0.13 mmol, 65%) of the diol **13** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.64 (1H, dd, $J = 5.7$ and 4.6 Hz), 0.89 (1H, dd, $J = 8.7$ and 4.3 Hz), 1.19 (1H, m), 1.32 (2H, m), 1.45 (1H, m), 1.55 (1H, m), 1.64 (1H, m), 1.81 (1H, m), 2.35 (2H, m), 3.45 (1H, d, $J = 11.9$ Hz), 3.50 (2H, br s), 3.72 (1H, dd, $J = 11.9$ and 2.7 Hz), 4.25 (2H, m), 5.17 (1H, m); ^{13}C NMR ($CDCl_3$) δ 19.9, 24.2, 24.9, 30.7, 32.3, 33.4, 36.6, 37.4, 67.2, 6.3, 128.7, 139.2; HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{18}O_2Na$, 217.1191; found, 217.1204.

4.9. 3a,4,5,6,6a,6b-Hexahydro-1*H*-cycloprop[*e*]indene-1a,2-dicarbaldehyde (1)

To a solution of 92 μl (6.33 mmol) of DMSO in 2 ml of CH_2Cl_2 was added 218 μl (2.54 mmol) of oxalyl chloride at −78 °C. The solution was stirred for 15 min before a solution of 123 mg (0.63 mmol) of **13** in 2 ml of CH_2Cl_2 was added dropwise during 5 min. After stirring for 45 min at −78 °C, 2.12 ml (0.64 mmol) of triethylamine was added. The solution was stirred for an additional 30 min at −78 °C before equilibrating to room temperature. After 30 min stirring at room temperature the reaction mixture was diluted with 20 ml of water and 20 ml of ether. The phases were separated and the aqueous phase was extracted with an additional 10 ml of ether. The combined ether extracts were dried and concentrated. Flash chromatography (heptane/ethyl acetate 8:1) gave 96 mg (0.50 mmol, 80%) of the dialdehyde **1** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.88 (1H, dd, $J = 7.0$ and 4.1 Hz), 1.28 (1H, m), 1.60–1.72 (4H, m), 1.92 (1H, m), 1.98 (1H, m), 2.08 (1H, dd, $J = 9.2$ and 4.1 Hz), 2.53 (1H, dq, $J = 8.2$ and 1.7 Hz), 2.63 (1H, tt, $J = 8.6$ and 3.0 Hz), 6.50 (1H, d, $J = 2.9$ Hz), 9.51 (1H, s), 9.78 (1H, s); ^{13}C NMR ($CDCl_3$) δ 24.6, 24.6, 29.5, 31.9, 32.6, 33.0, 36.8, 38.4, 139.7, 153.6, 193.0, 199.9. HRMS (CI) $[M+H]^+$ calcd for $C_{12}H_{15}O_2$, 191.1072; found, 191.1071.

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