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Function-oriented synthesis applied to the anti-botulinum natural product toosendanin

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ABSTRACT

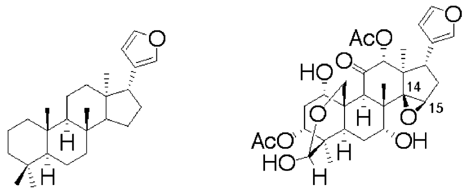
Botulinum neurotoxins (BoNTs) are the etiological agents responsible for botulism, a disease characterized by peripheral neuromuscular blockade and a characteristic flaccid paralysis of humans. The natural product toosendanin is a traditional Chinese medicine which has been reported to have anti-botulinum properties in animal models. To establish what chemical functionalities are necessary for the anti-botulinum properties found within toosendanin, a study was initiated with the goal of using function-oriented synthesis (FOS) as a strategy to begin to unravel toosendanin's powerful anti-botulinum properties. From these studies a new synthetic strategy is put forth allowing access to a 4-acetoxy CD fragment analogue (**14**) of toosendanin, which was achieved from mesityl oxide and acetylacetone in 14 steps. Animal studies on this fragment are also reported.

1. Introduction

Clostridium botulinum is a rod-shaped, gram-positive, sporulating anaerobic bacillus that is widely distributed in the environment.[1] The spores, which are resistant to heat, desiccation, chemicals, radiation, and oxygen, facilitate survival for very long periods. Under anaerobic conditions, and in the correct nutritional environment, spore germination and cell division take place. The global distribution of *C. botulinum* in the environment varies widely. The botulinum neurotoxins (BoNTs) comprise a family of seven immunologically distinct proteins synthesized primarily by strains of the anaerobic bacteria. These toxins (BoNTs A–G) are the most lethal poisons known, with BoNT serotype A having a LD_{50} for a 70 kg human of a mere 0.8 μg by inhalation.[2] Historically associated with food poisoning, these proteinaceous toxins inhibit the release of acetylcholine at neuromuscular junctions by cleavage of SNARE proteins, resulting in progressive flaccid paralysis.[3,4] It is as a result of this potent neurotoxic activity that BoNTs are of substantial concern as potential bioterrorist weapons.[5–7]

2. Result and discussions

We have embarked on a program to identify new small molecule inhibitors of BoNT/A that are non-proteinaceous in nature and that work by a different mechanism other than inhibiting the catalytic light chain of the BoNT/A protease.[8] Limonoids, **1**, are tetranortriterpenoids with a 4,4,8-trimethylfuranylsteroid skeleton (Fig. 1) derived from euphane or tirucallane triterpenoids, and, in general, have intense bitter taste.[9] Meliaceae plants are a rich source of limonoids. Limonoids from the neem tree *Melia azadirachta indica* and the bead tree *M. azedarach* have been well studied, mainly because of their marked insect anti-feedant properties.[10] Along this same vein of research, *Melia toosendan*, which is native to China and known to contain limonoid constituents, has been found to possess effective antihelmintics.[11] A major limonoid constituent found in *M. toosendan* is the compound toosendanin (**2**, Fig. 1), which appears to have multiple modes of action in insects including damage to midgut tissues and inhibition of esterases, cytochrome P450-aldrin epoxidase and proteinase activities.[12]



1 **2**

Figure 1. General structure of a limonoid **1** and toosendanin **2**.

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Toosendanin from a structural diversity standpoint is a fascinating molecule. The core carbon skeleton of toosendanin comprises a cyclopentanoperhydrophenanthrene (**3**, Fig. 2), a molecular framework that consists of four fused, non-planar rings (labeled A–D) and a densely packed core of 14 stereocenters. Interestingly, this framework is commonly found in all mammalian steroids and hormones. What has intrigued us besides toosendanin's anti-parasitic properties are two reports in the 1980s describing toosendanin as also having anti-botulinum properties.[13] Strikingly, these findings have remained dormant, as little research has been conducted upon this molecule from a synthetic standpoint.

To probe toosendanin's anti-botulinum properties two tactics were envisioned. The first being a total synthesis of the molecule that ultimately would allow derivatives to be assessed, and thus a means to explore toosendanin's structure in relationship to its biological activity. A second was what Wender has termed Function-Oriented Synthesis (FOS).[14] Because of the complexity of toosendanin and the insurmountable odds of a total synthesis that would provide large quantities of this natural product or closely related derivatives, we have set a course on investigating this molecule from a FOS standpoint. The underpinnings of FOS are that the function of a biologically active lead structure can be emulated, tuned, or possibly improved by replacement with simpler scaffolds designed to embrace the key activity-determining structural features of the natural product. Because it is unknown which elements and/or structural features impact toosendanin's biological activity, we have decided to break the molecule into two fragments consisting of the AB and CD rings. In this paper, we report our initial work exploring the CD ring of toosendanin and its potential biological function.

We envisioned any synthesis emulating the CD rings of toosendanin would need to incorporate the epoxide moiety, shared by rings C and D at the C-14 and C-15 positions. We hypothesized this functionality to be crucial, as this moiety is not observed within any steroidal skeletons, including mammalian or plant (Fig. 1). While the epoxide was deemed critical, the furan ring was not, as we have found through semi-synthesis efforts (Janda et al. unpublished results) that this ring in a reduced chemical state did not alter the molecules biological activity. Furthermore, this functionality was readily susceptible to both oxidation and reduction conditions, which would clearly hamper any practical synthesis of an abbreviated structure if included within our synthetic scheme (Janda et al. unpublished data).

Overall, there are few de novo synthetic efforts that describe the successful syntheses of similar limonoids or key structural fragments. Selected examples are dumsin[15] and fragments of epoxyazadiradione.[16] Our FOS approach would build on the teachings of Mateos et al. who have recently described a synthesis of the limonoid fragment **4** in ten steps with an overall yield of 44% (Fig. 3).[17]

We envisioned four crucial cyclization steps in the synthesis of a FOS toosendanin CD fragment: the Robinson annulation, an aldol condensation, the Nazarov cyclization, and an epoxidation reaction. Using these series of reactions it was envisioned that at various stages appropriate substituents could be embedded within the CD ring. Importantly, this approach would enable the first synthesis of the CD ring fragment of toosendanin correctly displaying all heteroatom substituents found within the natural product. Finally, it is worth noting that a previous paper focusing on the reaction scope within limonoid construction has considered the possibility of building such a desired fragment; however, no compound bearing the α-acetoxy ketone functionality within ring C was reported.[18]

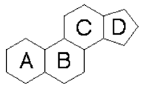

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Figure 2. Toosendanin's core ring system **3**.

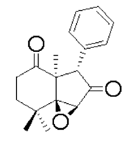

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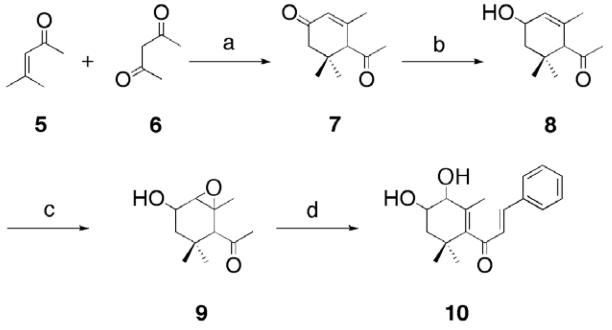
Figure 3. Limonoid fragment synthesized by Mateos et al. **4**.

In developing a synthesis, our starting point featured a Robinson-type annulation of mesityl oxide **5** and acetylacetone **6** using BF_3 as a catalyst, which bestowed α-cyclocitral (Scheme 1).[16,18] It is appropriate to note that α-cyclocitral **7** has been a preparatory material for many liminoid derivatives, particularly for SAR studies of insect anti-feedant development.[16–22] The convenience of starting with **7** is most certainly due to the fact that it can be readily synthesized on a multi-gram scale with inexpensive starting materials. Selective reduction[16] of the conjugated carbonyl of diketone **7** using $NaBH_4/CeCl_3$ at −40 °C furnished the hydroxyketone **8**, which could then be utilized for epoxidation with *m*-chloroperbenzoic acid affording **9**. Aldol condensation of **9** with benzaldehyde provided dihydroxyketone **10** in 89% overall yield from **7**.

With dihydroxyketone **10**, a Nazarov electrocyclization reaction was envisioned. For comparative purposes we note that the Nazarov electrocyclization reaction of **a**, **b**, and **c** (Fig. 4) in the presence of acid grants the corresponding cyclization compounds.[18,22] In lieu of these reports, we attempted the reaction of **d** with acid for the synthesis of a 'non-functionalized' moiety at C3, however, this reaction failed. It is well understood that to accomplish the required cyclization a keto-group at C4, and a phenyl group at C3 are needed; unfortunately these requirements limit diversity, and as stated also dictate the stereochemical outcome.

Thus, the Nazarov electrocyclization reaction of **10** in the presence of acid granted **11a** and **11b** (inseparable), **11c**, and **11d**. We were pleased to observe that the cyclization exclusively formed the *cis*-fused product,[17,18,21,23] placing the core ring system in the desired geometry of toosendanin. Reduction of the unsaturated C-ring by catalytic hydrogenation supplied compounds **12a** and **12b** (inseparable), **12c**, and **12d**, respectively (Scheme 2). We attempted the asymmetric reduction of **11a–d** with a plethora of reagents (e.g., (2*S*,5*S*)-(−)-5-benzyl-3-methyl-2-(5-methyl-2-furyl)-4-imidazolidi-none, binaphthyl derivatives, to name a few) that would have provided the correct C3-stereocenter, but,



Scheme 1. Synthesis of compound **10**. Reagents and conditions: (a) $BF_3 \cdot Et_2O$, 5 °C; (b) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, −40 °C; (c) *m*CPBA, CH_2Cl_2, rt; 12 h, 98% (d) PhCHO, NaOH, EtOH, rt, 13 h, 96%.

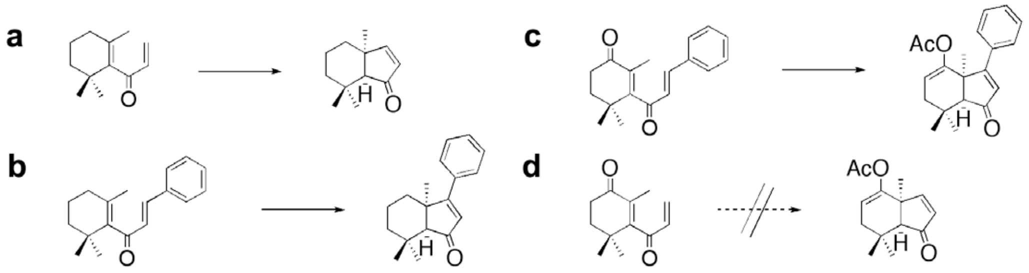


Figure 4. Nazarov electrocyclization reaction of **a**–**d**.

unfortunately the lone reaction that succeeded was palladium–carbon.

The relative stereochemistry of each of these compounds was determined by 2D-ROESY spectrum (Fig. 5). Thus, for **12a** ROESY correlation was seen between H-4 (geminal with the acetoxy group) and Me-7 (axial); H-3 (geminal with the phenyl group) and Me-3a (the angular methyl group); Me-3a and H-7a. For the case with **12b**, we could see a correlation (sv) between H-3 and Me-3a; Me-3a and H-7a; for **12c,** correlation could be seen between H-3 and Me-3a and H-7a; H-4 and Me-7 (axial); H-5 and Me-7 (axial); while for **12d**, it could be seen between H-3 and Me-3a; H-7a, Me-3a and H-4 (geminal with the acetoxy group); H-5 and Me-7 (axial).

The D-ring ketone of **12a** and **12b** was reduced to *sec*-alcohol by treatment with 9-BBN (Scheme 3),[22] subsequent dehydroxylation of this *sec*-alcohol with thionylchloride in pyridine afforded the desired olefin, which upon hydrolysis of the diacetyl functionalities with potassium carbonate gave dihydroxyolefin **13** in 19% overall yield from **12**. Selective protection of dihydroxyolefin **13** using TBSOTf at −78 °C provided the 7-TBS-protected compound in 19% yield. The hydroxyl group at C-6 was oxidized by Dess–Martin's reagent, which on subsequent deprotection of the silyl group with TBAF, followed by acetylation with acetic anhydride in pyridine, and epoxydation of olefin with *m*-chloroperbenzoic acid granted **14** in 14% overall yield from **13** (Scheme 3).

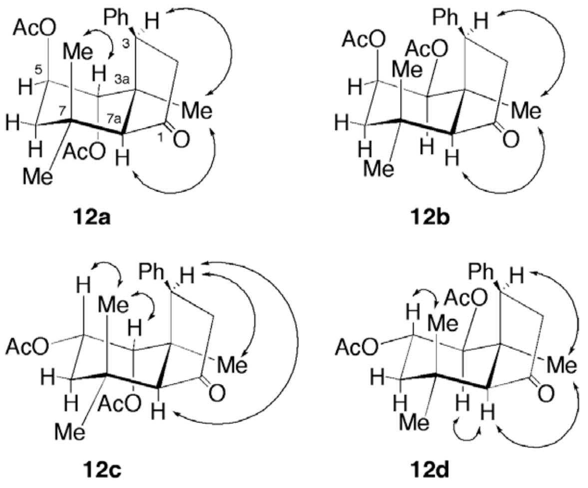
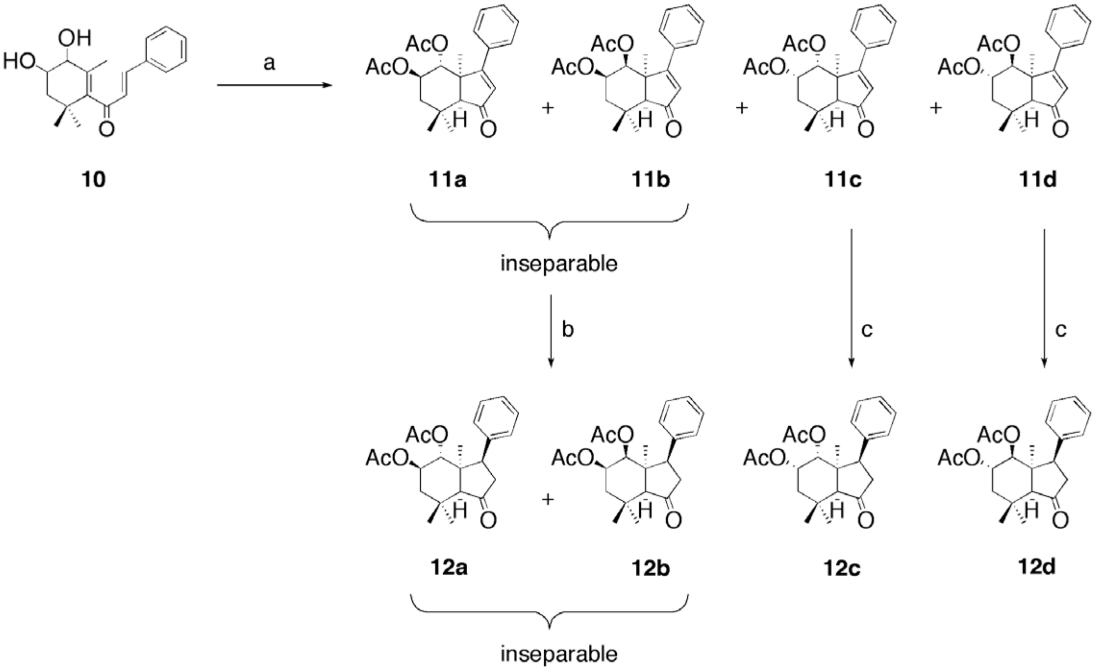
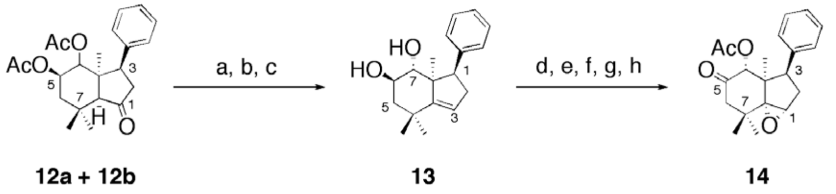


Figure 5. The relative stereochemistry of compounds **12a–d** was determined by 2D-ROESY experiments.



Scheme 2. Synthesis of compounds **12a–d**. Reagents and conditions: (a) 10^{-1} M $HClO_4$/0.5 M Ac_2O/AcOEt, rt, 63 h; (b) Pd–alumina, EtOH, rt, 18 h; (c) Pd–carbon, MeOH, rt, 21 h.



Scheme 3. Synthesis of compound **14**. Reagents and conditions: (a) 9-BBN, THF, rt, 4 h; (b) $SOCl_2$, pyridine, CH_2Cl_2, rt, 12 h; (c) K_2CO_3, MeOH, rt, 86 h, 19% for three steps; (d) TBSOTf, DIPEA, CH_2Cl_2, −78 °C, 2.5 h; (e) Dess-Martin reagent, CH_2Cl_2, rt, 13 h; (f) TBAF, THF, rt, 2.5 h; (g) Ac_2O, pyridine, cat. DMAP, rt, 24 h; (h) *m*CPBA, CH_2Cl_2, rt, 6 h, 14% for five steps.

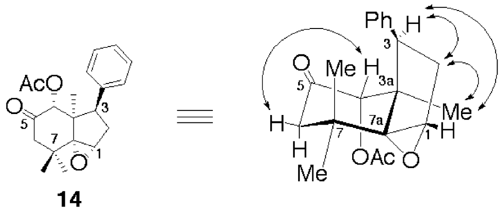


Figure 6. The relative stereochemistry of compound **14** was determined by 2D-ROESY experiment.

To determine **14**'s relative stereochemistry we again looked to the 2D-ROESY spectrum (Fig. 6). We could see a ROESY correlation between H-3 (geminal with the phenyl group) and Me-3a (the angular methyl group), H-4 (geminal with the acetoxy group) and H-6 (equatorial).

To investigate the potential bioactivity of epoxide **14**, we utilized the mouse lethality assay (MLA).[8d] This assay is considered the 'gold standard' for the field and is used to assess any new protein or small molecule that may possess anti-botulinum activity. Before the assay was initiated with **14**, we first investigated potential toxicity issues with this molecule. Gratifyingly, at a concentration of 2.5 mM this compound showed no propensity to be toxic to any of the animals it was administered, $n = 4$. Thus, mice were injected intravenously with **14** followed immediately by ip injection of approximately $10LD_{50}$s of BoNT/A. The mice were observed over a 48 h period and unfortunately all mice succumbed to BoNT/A toxicity. In contrast, the natural product toosendanin at this same concentration extended time to death 7.1 h.

3. Conclusions

The work reported was initiated with a goal of using FOS as a strategy to begin to unravel toosendanins powerful anti-botulinum properties. Thus, a new synthetic approach to a 4-acetoxy CD fragment analogue of Toosendanin was achieved from mesityl oxide and acetylacetone in 14 steps. Unfortunately, this molecule provided no efficacy in vivo. However, we note that our synthetic route should allow access to other CD ring analogues, for example, exchange of benzaldehyde to other aldehydes would allow entry into other unnatural analogues. In addition, an acetyl group functional change would also provide another series of FOS-molecules. These compounds as well as the AB ring synthesis will be reported in due course.

4. Experimental

In general, reagents and solvents were used as purchased without further purification. All flash column chromatography was performed using silica gel 60 (230–400 mesh). Analytical and preparative thin-layer chromatography (TLC) was performed using Merck Kieselgel 60 F254 silica gel plates (0.25, 0.5, or 1 mm). ^1H NMR spectra were recorded on Bruker DRX-600 (600 MHz), DRX-500 (500 MHz), or Varian Inova-400 (400 MHz) spectrometers. ^{13}C NMR spectra were recorded on Bruker DRX-600 (150 MHz) spectrometer. All 2D-ROESY experiments ($\tau_m = 200$ ms) were also recorded on Bruker DRX-600 (600 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) on the δ scale from an internal standard (NMR descriptions: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet). High-resolution mass spectra were recorded on an Agilent ESI-TOF mass spectrometer.

4.1. 1-(5-Hydroxy-1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptan-2-yl)ethanone (9)

m-Chloroperbenzoic acid (*m*CPBA) (77%, 107.5 mg, 0.48 mmol) was added to a solution of **8** (87.3 mg, 0.48 mmol) in CH_2Cl_2 (2 mL) at room temperature, and the mixture was stirred at room temperature for 12 h. Five percentage of Na_2SO_3 aq was added and the resulting heterogeneous mixture was vigorously stirred. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with 5% Na_2CO_3 aq and brine, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with Et_2O–hexane (2:1, v/v) to give **9** (93.3 mg, 98%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ: 4.09 (ddd, $J = 10.2$, 6.3, 2.4 Hz, 1H), 3.17 (d, $J = 2.3$ Hz, 1H), 2.49 (s, 1H), 2.29 (s, 3H), 1.78 (dd, $J = 13.1$, 10.4 Hz, 1H), 1.48–1.41 (m, 1H), 1.47 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 207.2, 66.1, 61.5, 60.5, 60.1, 37.2, 33.7, 32.5, 29.2, 26.9, 24.8; HRMS $[MH]^+$ calcd for $C_{11}H_{19}O_3$, 199.1329; found, 199.1329.

4.2. (*E*)-1-(3,4-Dihydroxy-2,6,6-trimethylcyclohex-1-enyl)-3-phenylprop-2-en-1-one (10)

Under argon atmosphere, NaOH (52.6 mg, 1.3 mmol) was added to a solution of **9** (130.3 mg, 0.66 mmol) and benzaldehyde (66.8 μL, 0.66 mmol) in EtOH (3 mL) at room temperature, and the mixture was stirred at room temperature for 13 h. After evaporation of the solvent, the whole was made acidic by adding 1 N HCl under ice cooling and extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with AcOEt–hexane (5:1, v/v) to give **10** (180.7 mg, 96%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.59–7.54 (m, 2H), 7.48 (d, $J = 16.2$ Hz, 1H), 7.43–7.39 (m, 3H), 6.78 (d, $J = 16.2$ Hz, 1H), 4.04–3.97 (m, 2H), 1.86–1.76 (m, 1H), 1.77 (s, 3H), 1.63–1.53 (m, 1H), 1.23 (s, 3H), 1.06 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 201.2, 146.6, 144.0, 134.3, 130.9, 129.6, 129.0, 128.6, 127.7, 70.4, 66.7, 40.8, 36.3, 29.7, 29.5, 28.0, 19.1; HRMS $[MH]^+$ calcd for $C_{18}H_{23}O_3$, 287.1642; found, 287.1648.

4.3. (3a*RS*,4*RS*,5*RS*,7a*SR*)-3a,7,7-Trimethyl-1-oxo-3-phenyl-3a,4,5,6,7,7a-hexahydro-1*H*-indene-4,5-diyl diacetate (11a), (3a*RS*,4*SR*,5*RS*,7a*SR*)-3a,7,7-trimethyl-1-oxo-3-phenyl-3a,4,5,6,7,7a-hexahydro-1*H*-indene-4,5-diyl diacetate (11b), (3a*RS*,4*RS*,5*SR*,7a*SR*)-3a,7,7-trimethyl-1-oxo-3-phenyl-3a,4,5,6,7,7a-hexahydro-1*H*-indene-4,5-diyl diacetate (11c), and (3a*RS*,4*SR*,5*SR*,7a*SR*)-3a,7,7-trimethyl-1-oxo-3-phenyl-3a,4,5,6,7,7a-hexahydro-1*H*-indene-4,5-diyl diacetate (11d)

Under argon atmosphere, **10** (484.6 mg, 1.7 mmol) was dissolved in 48 mL of 10^{-1} M $HClO_4$/0.5 M Ac_2O/AcOEt reagent.[18] The solution was allowed to stand at room temperature for 63 h. Saturated $NaHCO_3$ aq was added to quench the reaction. The organic layer was separated, and the aqueous phase was extracted with AcOEt. The extract was washed with 5% Na_2CO_3 aq and brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave a solid. The residue was recrystallized from AcOEt–hexane to give a mixture of **11a** and **11b** (311.3 mg, 50%) as a colorless powder. The mother liquor was purified by p-TLC on SiO_2 with AcOEt–hexane (1:4, v/v) to give **11c** (109.0 mg, 17%) as a yellow oil and **11d** (77.8 mg, 12%) as a colorless oil in the order of elution. Compound **11a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52–7.43 (m, 5H), 6.05 (s, 1H), 5.73 (d, J = 3.3 Hz, 1H), 4.91 (ddd, J = 11.1, 4.7, 3.4 Hz, 1H), 2.25 (s, 1H), 1.99 (s, 3H), 1.95–1.87 (m, 1H), 1.93 (s, 3H), 1.61–1.53 (m, 1H), 1.51 (s, 3H), 1.28 (s, 3H), 1.01 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) (mixture of **11a** and **11b**) δ: 208.5, 206.4, 180.7, 179.3, 170.7, 170.2, 170.0, 169.8, 136.3, 134.0, 133.2, 130.2, 130.0, 128.9, 128.2, 127.6, 127.3, 74.6, 71.9, 70.1, 68.4, 64.3, 63.7, 50.9, 50.4, 43.4, 39.2, 34.3, 33.9, 32.7, 32.3, 29.0, 25.9, 25.1, 22.2, 21.1, 20.9, 20.6, 20.0; HRMS $[MH]^+$ calcd for $C_{22}H_{27}O_5$, 371.1853; found, 371.1854. Compound **11b** (data obtained from the spectrum of mixture): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.34 (m, 3H), 7.32–7.28 (m, 2H), 6.08 (s, 1H), 5.50 (d, J = 8.0 Hz, 1H), 5.14 (dt, J = 8.0, 2.1 Hz, 1H), 2.27 (s, 1H), 2.16–2.04 (m, 1H), 1.94 (s, 3H), 1.67 (s, 3H), 1.61–1.53 (m, 1H), 1.34 (s, 3H), 1.29 (s, 3H), 1.17 (s, 3H). Compound **11c**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.37 (m, 5H), 6.27 (s, 1H), 5.65 (d, J = 2.1 Hz, 1H), 5.30 (ddd, J = 12.1, 5.5, 2.3 Hz, 1H), 2.12 (s, 1H), 2.00 (s, 3H), 1.93–1.83 (m, 1H), 1.75 (s, 3H), 1.60 (s, 3H), 1.58–1.51 (m, 1H), 1.40 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 205.5, 174.0, 170.7, 169.5, 134.0, 131.2, 129.8, 128.8, 127.7, 71.7, 68.6, 62.4, 51.6, 36.2, 33.5, 32.7, 28.7, 25.6, 21.0, 20.5; HRMS $[MH]^+$ calcd for $C_{22}H_{27}O_5$, 371.1853; found, 371.1861. Compound **11d**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.33 (m, 5H), 6.26 (s, 1H), 5.32 (d, J = 3.6 Hz, 1H), 5.05 (ddd, J = 9.3, 7.2, 3.6 Hz, 1H), 2.25 (s, 1H), 2.09–2.02 (m, 1H), 2.07 (s, 3H), 1.71 (s, 3H), 1.55–1.50 (m, 1H), 1.54 (s, 3H), 1.34 (s, 3H), 1.14 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 207.0, 177.5, 169.7, 169.3, 135.3, 132.2, 129.6, 128.6, 127.6, 75.9, 72.1, 63.4, 50.6, 41.6, 33.6, 33.0, 29.7, 28.4, 23.7, 21.2, 20.6; HRMS $[MH]^+$ calcd for $C_{22}H_{27}O_5$, 371.1853; found, 371.1856.

4.4. (3*RS*,3a*SR*,4*RS*,5*RS*,7a*SR*)-3a,7,7-Trimethyl-1-oxo-3-phenyloctahydro-1*H*-indene-4,5-diyl diacetate (12a) and (3*RS*,3a*SR*,4*SR*,5*RS*,7a*SR*)-3a,7,7-trimethyl-1-oxo-3-phenyloctahydro-1*H*-indene-4,5-diyl diacetate (12b)

A solution of **11a** and **11b** (34.0 mg, 0.09 mmol) in EtOH (2 mL) was hydrogenated in the presence of Pd–alumina (5 wt%, 34.0 mg) at room temperature, and 1 atom for 18 h. Pd–alumina was filtered off, and the filtrate was evaporated under reduced pressure to leave an oil, which was purified by p-TLC on SiO_2 with Et_2O–hexane (1:1, v/v) to give a mixture of **12a** and **12b** (34.2 mg, 100%) as a colorless oil. Compound **12a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.32–7.19 (m, 5H), 5.37 (q, J = 4.0 Hz, 1H), 4.88 (d, J = 4.4 Hz, 1H), 2.99 (dd, J = 13.1, 8.1 Hz, 1H), 2.86 (dd, J = 18.8, 13.2 Hz, 1H), 2.56 (dd, J = 18.8, 8.2 Hz, 1H), 2.16 (s, 1H), 1.98 (s, 3H), 1.78 (dd, J = 15.3, 3.9 Hz, 1H), 1.68–1.61 (m, 1H), 1.67 (s, 3H), 1.35 (s, 3H), 1.32 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) (mixture of **12a** and **12b**) δ: 214.8, 214.1, 170.5, 170.0, 169.9, 137.5, 136.3, 128.7, 128.2, 128.1, 127.1, 127.0, 71.6, 70.9, 69.2, 68.6, 66.7, 66.5, 50.7, 49.5, 49.0, 47.4, 41.1, 40.9, 40.6, 38.5, 33.7, 32.5, 31.3, 30.3, 29.7, 28.9, 23.2, 23.1, 21.2, 21.0, 20.3; HRMS $[MH]^+$ calcd for $C_{22}H_{29}O_5$, 373.2009; found, 373.2006. Compound **12b** (data obtained from the spectrum of mixture): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.32–7.18 (m, 5H), 5.16–5.09 (m, 1H), 5.04 (d, J = 9.5 Hz, 1H), 3.06–2.90 (m, 2H), 2.58–2.51 (m, 1H), 2.05 (d, J = 1.5 Hz, 1H), 1.92 (s, 3H), 1.65–1.61 (m, 2H), 1.59 (s, 3H), 1.40 (s, 3H), 1.25 (s, 3H), 1.19 (s, 3H).

4.5. (3*RS*,3a*SR*,4*RS*,5*SR*,7a*SR*)-3a,7,7-Trimethyl-1-oxo-3-phenyloctahydro-1*H*-indene-4,5-diyl diacetate (12c)

A solution of **11c** (102.7.0 mg, 0.28 mmol) in MeOH (6 mL) was hydrogenated in the presence of Pd–carbon (10 wt%, 50.5 mg) at room temperature, and 1 atom for 21 h. Pd–carbon was filtered off, and the filtrate was evaporated under reduced pressure to leave an oil, which was purified by p-TLC on SiO_2 with AcOEt–hexane (1:3, v/v) to give **12c** (89.9 mg, 87%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.33–7.29 (m, 2H), 7.28–7.24 (m, 1H), 7.14–7.10 (m, 2H), 5.13 (ddd, J = 12.7, 4.4, 2.2 Hz, 1H), 4.71 (d, J = 1.9 Hz, 1H), 3.23–3.18 (m, 1H), 2.82 (dd, J = 18.8, 12.0 Hz, 1H), 2.56 (ddd, J = 18.7, 10.4, 1.0 Hz, 1H), 2.14 (s, 3H), 1.97–1.89 (m, 2H), 1.86 (s, 3H), 1.55 (s, 3H), 1.53 (s, 3H), 1.33 (dd, J = 12.9, 4.4 Hz, 1H), 1.21 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 213.6, 170.5, 169.0, 135.4, 129.0, 128.3, 127.6, 72.8, 68.9, 62.8, 50.7, 49.1, 42.3, 36.0, 33.7, 31.2, 29.4, 26.2, 21.7, 20.9; HRMS $[MNa]^+$ calcd for $C_{22}H_{28}O_5Na$, 395.1829; found, 395.1829.

4.6. (3*RS*,3a*SR*,4*SR*,5*SR*,7a*SR*)-3a,7,7-Trimethyl-1-oxo-3-phenyloctahydro-1*H*-indene-4,5-diyl diacetate (12d)

A solution of **11d** (135.7 mg, 0.37 mmol) in MeOH (6 mL) was hydrogenated in the presence of Pd–carbon (10 wt%, 60.5 mg) at room temperature, and 1 atom for 21 h. Pd–carbon was filtered off, and the filtrate was evaporated under reduced pressure to leave an oil, which was purified by p-TLC on SiO_2 with AcOEt–hexane (1:3, v/v) to give **12d** (82.5 mg, 61%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.35–7.30 (m, 2H), 7.29–7.20 (m, 3H), 4.87–4.80 (m, 1H), 4.24 (s, 1H), 3.23 (dd, J = 13.9, 8.2 Hz, 1H), 3.04 (dd, J = 17.6, 13.9 Hz, 1H), 2.51 (ddd, J = 17.6, 8.2, 1.6 Hz, 1H), 2.15 (s, 1H), 2.07 (s, 3H), 2.02–1.95 (m, 1H), 1.99 (s, 3H), 1.58–1.48 (m, 1H), 1.56 (s, 3H), 1.28 (s, 3H), 1.19 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 216.7, 169.2, 168.2, 136.3, 128.4, 127.4, 75.5, 72.0, 62.8, 51.3, 46.7, 43.9, 39.3, 34.6, 32.1, 29.7, 28.4, 24.8, 21.7, 21.1; HRMS $[MNa]^+$ calcd for $C_{22}H_{28}O_5Na$, 395.1829; found, 395.1834.

4.7. (1*RS*,6*RS*,7*RS*,7a*SR*)-4,4,7a-Trimethyl-1-phenyl-2,4,5,6,7,7a-hexahydro-1*H*-indene-6,7-diol (13)

To a solution of **12a** and **12b** (935.0 mg, 2.5 mmol) in THF (20 mL) was slowly added 9-BBN (0.5 M, 10 mL, 5.0 mmol). The reaction mixture was stirred under argon atmosphere at room temperature for 4 h, and then MeOH (10 mL) was slowly added under stirring for 3 h. Removal of the solvent afforded a crude product. To a solution of crude product in CH_2Cl_2 (10 mL) at 0 °C under argon atmosphere were added pyridine (0.8 mL, 10 mmol) and $SOCl_2$ (0.4 mL, 5.0 mmol). The reaction mixture was stirred at room temperature for 12 h, and then poured into ice-water. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2. The whole was washed with 5% Na_2CO_3 and brine, and dried over Na_2SO_4. Removal of the solvent afforded a crude product, which was purified by flash chromatography on SiO_2 with

AcOEt–hexane (1:7, v/v) to give diacetoxyolefin. A 1 N aqueous solution of K_2CO_3 (3 mL) was added to a solution of diacetoxyolefin in MeOH (10 mL) and CH_2Cl_2 (2 mL) at room temperature, and the mixture was stirred at room temperature for 86 h. After evaporation of the solvent, the whole was made acidic by adding 2 N HCl under ice cooling and was extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave an oil, which was purified by flash chromatography on SiO_2 with AcOEt–hexane (1:4, v/v) to give **13** (128.6 mg, 19%, three steps from mixture of **12a** and **12b**) as a colorless amorphous substance. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.28–7.16 (m, 5H), 5.65–5.59 (m, 1H), 3.88 (q, J = 3.6 Hz, 1H), 3.14 (dd, J = 7.9, 1.7 Hz, 1H), 3.08 (d, J = 3.6 Hz, 1H), 2.92 (ddd, J = 16.8, 7.9, 1.7 Hz, 1H), 2.43–2.38 (m, 1H), 1.80 (dd, J = 14.6, 3.3 Hz, 1H), 1.51 (s, 3H), 1.33 (s, 3H), 1.27 (dd, J = 14.7, 3.6 Hz, 1H), 1.16 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 154.9, 144.9, 128.4, 128.0, 126.5, 122.2, 72.2, 72.0, 57.0, 55.8, 43.3, 37.3, 33.0, 32.1, 30.9, 23.3; HRMS $[MNa]^+$ calcd for $C_{18}H_{24}O_2Na$, 295.1668; found, 295.1665.

4.8. (1*SR*,3*RS*,3a*RS*,4*RS*,7a*RS*)-4-Acetoxy-1,7a-epoxy-3-phenyl-3a,7,7-trimethyl-1,2,3a,4,7,7a-hexahydro-3*H*-inden-5-one (14)

To a solution of **13** (115.9 mg, 0.43 mmol) in CH_2Cl_2 (5 mL) at −78 °C under argon atmosphere were added diisopropylethylamine (DIPEA) (0.1 mL, 0.86 mmol) and *t*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (98%, 0.2 mL, 0.86 mmol). The reaction mixture was stirred at −78 °C for 2.5 h. After addition of H_2O, the whole was extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave an oil, which was purified by p-TLC on SiO_2 with AcOEt–hexane (1:9, v/v) to give 7-TBS-protected product, which was desired (30.6 mg, 19%) as a colorless oil, 6-TBS-protected product (69.0 mg, 42%) as a colorless oil, and unreacted **13** (25.8 mg, 22%) as a colorless oil in the order of elution. To a solution of 7-TBS-protected product (30.6 mg, 0.08 mmol) in CH_2Cl_2 (3 mL) at room temperature under argon atmosphere was added Dess–Martin reagent (97%, 41.6 mg, 0.1 mmol). The reaction mixture was stirred at room temperature for 13 h. After addition of saturated $NaHCO_3$ aq, the whole was extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave an oil. To a solution of crude oil in THF (2 mL) at room temperature under argon atmosphere was added TBAF (1.0 M, 0.1 mL, 0.12 mmol). The reaction mixture was stirred at room temperature for 2.5 h. Removal of the solvent afforded a crude product, which was purified by p-TLC on SiO_2 with AcOEt–hexane (1:9, v/v) to give 7-hydroxyketone (16.0 mg, 75%, two steps from 7-TBS protected product) as a colorless oil. To a solution of 7-hydroxyketone (16.0 mg, 0.06 mmol) in pyridine (0.5 mL) at room temperature were added Ac_2O (0.25 mL) and catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred at room temperature for 24 h. Removal of the solvent afforded a crude product, which was purified by p-TLC on SiO_2 with AcOEt–hexane (1:7, v/v) to give 7-acetoxyketone (17.8 mg, 96%) as a colorless oil. *m*CPBA (77%, 15.3 mg, 0.07 mmol) was added to a solution of 7-acetoxyketone (17.8 mg, 0.06 mmol) in CH_2Cl_2 (1.0 mL) at room temperature, and the mixture was stirred at room temperature for 6 h. Na_2SO_3 aq (5%) was added and the resulting heterogeneous mixture was vigorously stirred. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with 5% Na_2CO_3 aq and brine, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to leave an oil, which was purified by p-TLC on SiO_2 with AcOEt–hexane (1:5, v/v) to give **14** (18.7 mg, 14%, five steps from **13**) as a colorless amorphous substance: ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.30–7.25 (m, 2H), 7.25–7.19 (m, 1H), 7.17–7.12 (m, 2H), 5.07–5.06 (m, 1H), 3.80 (s, 1H), 2.85 (dd, J = 10.5, 8.3 Hz, 1H), 2.59 (d, J = 13.2 Hz, 1H), 2.42 (d, J = 13.2 Hz, 1H), 2.37–2.27 (m, 2H), 1.36 (s, 3H), 1.23 (s, 3H), 1.16 (s, 3H), 1.00 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 202.4, 169.6, 138.0, 128.6, 128.3, 127.0, 76.2, 70.9, 61.2, 51.3, 50.5, 50.0, 36.5, 30.1, 27.4, 24.3, 19.6, 17.3; HRMS $[MH]^+$ calcd for $C_{20}H_{25}O_4$, 329.1747; found, 329.1750.

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