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Semisynthesis of labdane diterpene metabolites from the nudibranch *Pleurobranchaea meckelii*

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Abstract—Two isomeric labdane aldehyde metabolites (**1** and **2**), first isolated from the skin of the Notaspidean nudibranch *Pleurobranchaea meckelii*, were synthesized in six steps from manool in 19 and 6% overall isolated yields, respectively.

1. Introduction

Marine sea slugs or nudibranchs are generally conspicuously brightly coloured, slow moving invertebrate animals, which do not possess any visible means of physical protection against predation, e.g., a shell or sharp spines.[1] Most nudibranchs have accordingly evolved complex chemical defence strategies to deter potential predators. The most commonly observed form of chemical defence is the sequestration by nudibranchs of toxic secondary metabolites from their diet of other marine invertebrates such as sponges or octocorals.[2] The sequestered metabolites are stored in the nudibranch's dorsal mantle tissues, with the aposematic colouration of the nudibranch acting as a warning to potential predators of the nudibranch's toxic status.[1,2] A less common chemical defence strategy, and one that predominates in many cold water nudibranch species, is de novo biosynthesis of toxic secondary metabolites and their storage in specialized glands in the nudibranch's skin for release as feeding deterrents in the presence of a predator.[3,4]

Nudibranchs belonging to the order Notaspidea, e.g., the Mediterranean nudibranch *Pleurobranchaea meckelii*, secrete an acidic mucus as an additional defence strategy to deter predation.[5,6] An acetone extract of several specimens of *P. meckelli*, collected by SCUBA from the Gulf of Naples, also afforded two isomeric labdane diterpene aldehyde metabolites: labd-13*E*-ene-8β-ol-15-al (**1**) and labd-13*Z*-ene-8β-ol-15-al (**2**).[5] Diterpene metabolites are commonly sequestered by marine opisthobranch molluscs from their marine invertebrate diet.[6] However, a dietary source of **1** and **2** could not be identified and their presence in the skin of *P. meckelli* seemed to suggest that these two compounds may be the products of de novo biosynthesis. A defensive role for **1** and **2** in the chemical ecology of *P. meckelli* was not established experimentally but rather implied from the presence of α,β-unsaturated aldehyde moieties in these two compounds.[5] Bioactivity studies of marine natural products are frequently hampered by the paucities of these compounds that are often available for these studies.[7] The semisynthesis of selected bioactive diterpene marine natural products from commercially available terrestrial plant diterpenes provides one possible solution to the bioactive marine diterpene supply problem and we report here the semisynthesis (Scheme 1) of **1** and **2** from commercially available manool (**3**).

Cimino and co-workers alluded to the instability of **1** and **2** and the implications this instability had for structure elucidation and further chemical ecology studies.[5] The relative instability of many bioactive marine natural products through facile degradation processes, including isomerization on exposure to sunlight and/or chromatographic media, is often problematic. Accordingly, a desirable semisynthetic strategy would be one that can quantitatively deliver a relatively unstable marine natural product for bioactivity studies from a stable precursor without prior chromatographic purification. We identified labd-13*E*-ene-8β,15-diol (**4**) and labd-13*Z*-ene-8β,15-diol (**5**) as suitable stable precursors of **1** and **2**, respectively, with MnO_2 oxidation of the former two compounds providing a facile route to **1** and **2**.

Only two non-stereoselective semisyntheses of **4** and **5** have been reported. Popa et al.[8] synthesized **4** and **5**, and their respective 8α-epimers, via an initial series of successive MnO_2 and Ag_2O oxidations of **3** followed by a regioselective epoxidation of the $\Delta^{8,17}$ olefin in the final oxidation product

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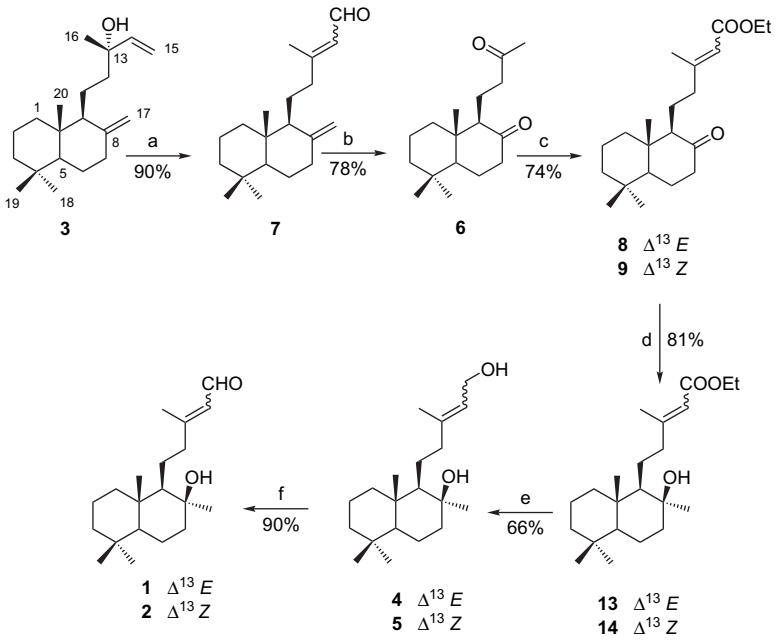


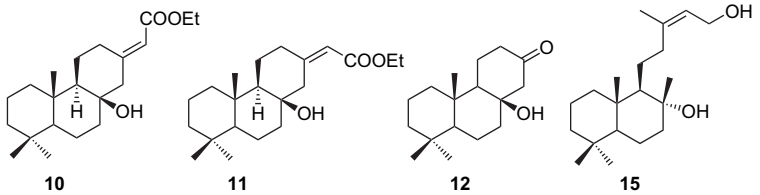
Scheme 1. (a) PCC, CH_2Cl_2; (b) (i) O_3, CH_2Cl_2, −78 °C; (ii) Ph_3P, CH_2Cl_2; (c) triethylphosphonoacetate, NaH, THF, 0 °C; (d) MeMgCl, Et_2O, −20 °C; (e) LAH, THF, 0 °C; (f) MnO_2, CH_2Cl_2.

(methyl labd-8(17),13-dien-15-oate) and LAH reduction of the resultant α- and β-epoxides. The Δ^{13} geometric isomers of **4** and **5** were not separated and the spectroscopic data obtained in support of the chemical structures of the semisynthetic products were limited. A biomimetic cyclization of (*E*,*E*,*E*)-geranylgeranyl acetate in the presence of a mercury triflate/*N*,*N*-dimethylaniline cylization reagent complex afforded racemic **4** in good yield.[9] The semisynthesis reported here therefore represents the first stereoselective synthesis of **4** and **5** and their corresponding aldehyde oxidation products **1** and **2**.

2. Results and discussion

Commercially available (+)-manool (**3**) is a common precursor regularly used for the semisyntheses of both marine and terrestrial natural products.[10–12] Various reported approaches to the synthesis of diketone (**6**) from **3** have included oxidation of the latter compound with either a combination of OsO_4/HIO_5 at pH 6 or $KMnO_4$ followed by subsequent ozonolysis.[13–15] Our preliminary synthesis of **6** from **3** (Scheme 1) was achieved via an initial high yielding oxidative rearrangement of **3**[16] to give a 2:1 mixture (as determined by the aldehyde proton signal intensities in the NMR spectrum of the mixture) of the *E*/*Z* isomers of the α,β-unsaturated aldehyde (**7**). Reductive ozonolysis of *E*/*Z*-**7** afforded **6** in an overall yield (70%) from **3** comparable with yields reported previously for this compound.[13–15]

Regioselective olefination of the more accessible methyl ketone functionality proceeded smoothly via a Horner–Wadsworth–Emmons (HWE) modification of the Wittig reaction.[17] The stereoselectivity of the HWE reaction is diminished when trisubstituted alkenes are prepared from ketones[18] and a 3:1 ratio of ethyl 17-norlabd-13-*E*-en-8-one-15-oate (**8**) and ethyl 17-norlabd-13-*Z*-en-8-one-15-oate (**9**) was obtained in 74% overall yield. The two geometric isomers were readily separated via normal phase HPLC (hexane–EtOAc 6:1) and confirmation of the configuration of the *E* Δ^{13} olefin in **8** and *Z* Δ^{13} olefin in **9** was provided by the ^{13}C chemical shift of the vinylic methyl (C-16) carbon resonance (δ_C 19.0 and 25.1, respectively) and the allylic methylene carbon (C-12) signal (δ_C 40.2 and 32.6, respectively).[19] Two minor tricyclic products from the HWE reaction, ethyl 17-norabiet-13(15)-*E*-en-8β-ol-16-oate (**10**) and ethyl 17-norabiet-13(15)-*Z*-en-8β-ol-16-oate (**11**), were also isolated in 9% combined yield. We have previously shown that these two compounds are formed from 8-hydroxy-13-podocarpanone (**12**), an intramolecular aldol condensation product readily generated from **6** in the presence of excess NaH.[20]



Chemoselective methylation of the C-8 ketone in **8** and **9** proceeded smoothly under standard Grignard conditions. The methyl nucleophile attacked the endocyclic ketone with *Re*-facial selectivity to afford ethyl labd-13*E*-en-8β-ol-15-oate (**13**) and ethyl labd-13*Z*-en-8β-ol-15-oate (**14**), respectively. Interestingly, the yield of **13** and **14** was enhanced when methylmagnesium chloride (81%), as opposed to either methylmagnesium bromide (70%) or methyl lithium (51%), was used in the nucleophilic methyl addition reaction. The 8β-axial orientation of the alcohol substituent in **13** and **14** was supported by threefold spectroscopic evidence, viz. the deshielding of the H_3-20 angular methyl protons (δ_H 0.94), the shielding of C-6 (δ_C 18.6) and a downfield shift of the C-17 methyl carbon resonance (δ_C 30.6) in the ^1H and ^{13}C NMR spectra of these two compounds. The shielding of the C-6 resonance is attributed to a γ-*gauche* effect associated with a β-axially orientated hydroxyl functionality at C-8.[21] Conversely, an 8β-methyl substituent, e.g., in **15** shields the H_3-20 protons (δ_H 0.80), the C-17 methyl carbon resonance is shifted upfield (δ_H 24.0) while the C-6 resonance is shifted downfield δ_C 20.5.[19]

LAH reduction of esters **13** and **14** gave the corresponding diols **4** and **5**, respectively. Although the specific rotation of synthetic (+)-**4** ($[\alpha]_D$ +21) was lower in magnitude than that established by Cimino and co-workers[5] ($[\alpha]_D$ +32), for the reduction product of the nudibranch derived **1**, it was significantly different from the specific rotations reported for labd-13*E*-ene-8β,15-diol isolated from terrestrial plants ($[\alpha]_D$ 0 and +99).[22,23] The negative specific rotation previously reported for *ent*-labd-13*E*-ene-8β,15-diol ($[\alpha]_D$ −32)[24,25] is in agreement with the established labdane stereochemistry of semisynthetic **4** and supports that proposed by Cimino and co-workers for marine-derived **4**.

The target aldehyde **1** was quantitatively procured by stirring a dichloromethane solution of **4** with MnO_2 (10 equiv) overnight. Filtration through Celite gave a 25:1 mixture of **1**:**2** (i.e., >95% *E* isomeric purity from NMR analysis). The ratio of **1**:**2** changed substantially (15:1) after an attempt was made to remove the small amount of **2** in the isomeric mixture using normal phase HPLC (hexane–EtOAc 4:1) and this isomerization was suggestive of the instability of these compounds to silica chromatography. An analogous MnO_2 oxidation of **5** gave **2**:**1** in a 50:1 ratio (i.e., >98% *Z* isomeric purity from NMR analysis) which, after normal phase HPLC, also reverted to a mixture of 11:1 (*Z*/*E*) geometric isomers. The ^1H and ^{13}C NMR data of the 25:1 mixture consisting predominantly of **1** (acquired in $CDCl_3$) were consistent in all respects with the spectroscopic data published for the corresponding *P. meckelii* metabolite.[5] In our hands the ^1H NMR spectrum of the isomeric mixture, containing almost exclusively **2**, showed evidence of initial partial isomerization followed by total degradation (to form an inseparable mixture of products) on standing in a solution of $CDCl_3$ (12 h). Accordingly, the NMR data for the *Z* isomer dominated mixture were obtained in deuterated benzene. We consequently also avoided using chloroform for recording the specific rotations of **1** and **2**. Paradoxically, Cimino and co-workers[5] reported negative specific rotations for chloroform solutions of **1** ($[\alpha]_D$ −35.2) and **2** ($[\alpha]_D$ −28.2) isolated from *P. meckelii* which is not only at variance with the specific rotations we obtained for our semisynthetic **1** ($[\alpha]_D$ +25.5) and **2** ($[\alpha]_D$ +24.1) recorded in benzene but also opposite in sign and magnitude to the specific rotation (recorded in chloroform) of both the reduction product precursor, **4**, derived from **1** isolated from *P. meckelii*,[5] and our semisynthetic **4**. Interestingly, a negative specific rotation ($[\alpha]_D$ −8.5, *c* 0.085, $CHCl_3$) was reported for **15**, the 8α-epimer of **4**.[19] While epimerization at C-8 is an unlikely explanation for the discrepancy between the signs of the specific rotations of the naturally occurring and synthetic **1** and **2**, we believe that this discrepancy may be related to the facile isomerization and instability of the α,β-aldehydes to both chromatographic media and solution in chloroform.

3. Conclusion

Two unstable marine α,β-unsaturated labdane diterpene aldehydes, **1** and **2**, originally isolated from the Mediterranean nudibranch *P. meckelli*, have been successfully synthesized from commercially available **3** via the stable alcohol precursors **4** and **5**. A discrepancy in the sign of the specific rotations has been identified between the naturally occurring and semisynthetic **1** and **2**.

4. Experimental

4.1. General

Melting points were determined using a Reichert hot stage microscope and are uncorrected. Optical rotations were measured using a Perkin–Elmer 141 polarimeter calibrated at the sodium D line (598 nm). IR spectra were recorded on a Perkin Elmer Spectrum 2000 FT-IR spectrometer with the compounds as films (neat) on NaCl discs. NMR spectra were acquired on Bruker 400 MHz Avance and 600 MHz Avance II spectrometers using standard pulse sequences. Chemical shifts are reported in parts per million, referenced to residual solvent resonances ($CDCl_3$ δ_H 7.25, δ_C 77.2, C_6D_6 δ_H 7.15, δ_C 128.02), and coupling constants are reported in hertz. HRFABMS data were obtained on a JEOL SX102 spectrometer. Reactions where exclusion of water was necessary were performed in flame dried glassware under Ar. Just prior to their use Et_2O and THF were distilled from sodium metal/benzophenone ketyl and CH_2Cl_2 from CaH. General laboratory solvents were distilled before use. Reactions were monitored by thin layer chromatography (DC-Plastikfolien Kieselgel 60 F_{254} plates) and visualized under UV light and developed by spraying with either 10% concd H_2SO_4 in methanol or iodine. Kieselgel 60 (230–400 mesh) was used for initial flash chromatographic separations. Semi-preparative HPLC was performed using a Whatman's Magnum 9 Partisil 10 column (10 mm i.d., length 50 cm) with an eluent flow rate of 4 mL min^{-1}.

4.2. Labd-8(17),13*E*-diene-15-al and its 13*Z* isomer (7)

A solution of manool **3** (170 mg, 0.586 mmol) and pyridinium chlorochromate (417 mg, 1.93 mmol, 3.3 equiv) in CH_2Cl_2 (8 mL) was stirred (26 h) at room temperature. Et_2O (8 mL) was added, and the formation of an orange precipitate noted. The solution was then filtered through a Celite plug and the filtrate concentrated in vacuo to yield a dark

brown oil (195 mg). The oil was purified on a silica column (hexane–EtOAc 15:1) to yield an inseparable mixture of the *E* and *Z* isomers of **7** in a 2:1 ratio (154 mg, 90% yield) as a pale yellow oil; ^{1}H and ^{13}C NMR data consistent with literature values;[26] HRFABMS *m/z* 289.2532 $[M+H]^+$ (calcd for $C_{20}H_{33}O_2$, 289.2531).

4.3. 15,16,17-Trinorlabdane-8,13-dione (6)

The isomeric mixture of **7** (100 mg, 0.35 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and cooled to −78 °C. A steady stream of O_3 was bubbled through the solution (10 min) until the solution had turned a pale blue colour whereupon N_2 was bubbled through the solution to remove excess O_3. After the addition of triphenyl phosphine (548 mg, 2.09 mmol, 6 equiv) the solution was stirred at ambient temperature (4 h). The solution was cooled to 0 °C, H_2O_2 (30%, 1 mL) added and stirring continued (0.5 h). The solution was finally washed with water (3×5 mL), and the combined organic extracts dried (anhydrous $MgSO_4$) and concentrated. Subsequent purification of the concentrated organic material on a silica column (EtOAc–hexane 1:1) afforded **6** (72 mg, 0.27 mmol, 78%): colourless oil; ^{1}H and ^{13}C NMR data consistent with literature values;[15,27,28] $[\alpha]_D^{20}$ −28.3 (*c* 1.3, $CHCl_3$) lit.[15] −11; IR (film) ν_{max} 2940, 1709, 1698, 1355, 1164 cm^{-1}; HRFABMS *m/z* 265.2168 $[M]^+$ (calcd for $C_{17}H_{28}O_2$ 265.2168).

4.4. Ethyl 17-norlabd-13-*E*-en-8-one-15-oate (8), ethyl 17-norlabd-13-*Z*-en-8-one-15-oate (9), ethyl 17-norabiet-13(15)-*E*-en-8β-ol-16-oate (10) and ethyl 17-norabiet-13(15)-*Z*-en-8β-ol-16-oate (11)

To a solution of triethylphosphonoacetate (1660 μL, 8.37 mmol, 3 equiv) in anhydrous THF (5 mL) was added NaH (60%, 140 mg, 2.5 equiv) and stirred for 30 min before addition to a 0 °C solution of **6** (737 mg, 2.79 mmol). The solution was warmed to ambient temperature and stirred overnight, whereupon HCl (1 M, 3 mL) was added and the solution concentrated. The resultant yellow oil was taken up in Et_2O (5 mL) and washed with H_2O (3×5 mL). The organic fraction was dried ($MgSO_4$), concentrated and excess triethylphosphonoacetate removed by flash silica chromatography (10:1 hexane–EtOAc). The mixture of HWE-products obtained from flash chromatography (927 mg) was further purified with normal phase HPLC (hexane–EtOAc 14:1) to yield **8** (409 mg, 55%), **9** (175 mg, 19%), **10** (62 mg, 7%) and **11** (18 mg, 2%).

4.4.1. Ethyl 17-norlabd-13-*E*-en-8-one-15-oate (8). Pale yellow oil; $[\alpha]_D^{34}$ −20.2 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 2948, 1713, 1642, 1218, 1146 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.71 (3H, s, H_3-20); 0.84 (3H, s, H_3-19); 0.94 (3H, s, H_3-18); 1.14 (1H, ddd, *J*=13.2, 12.2, 4.9 Hz, H-1a); 1.26 (3H, t, *J*=7.1 Hz, –OCH$_2$C*H*$_3$); 1.23 (1H, m, H-3a); 1.36 (2H, m, H_2-11); 1.44 (1H, m, H-3b); 1.46 (1H, m, H-5); 1.50 (2H, m, H_2-2); 1.64 (1H, dddd, *J*=13.3, 13.1, 13.0, 5.0 Hz, H-6a); 1.74 (1H, m, H-1b); 1.87 (1H, m, H-12a); 1.99 (1H, m, H-9); 2.05 (1H, m, H-6b); 2.13 (3H, d, *J*=1.2 Hz, H_3-16); 2.27 (1H, ddd, *J*=13.3, 7.2, 6.8 Hz, H-7a); 2.18 (1H, m, H-12b); 2.41 (1H, ddd, *J*=13.2, 4.8, 2.1 Hz, H-7b); 4.13 (2H, dq, *J*=7.1, 0.5 Hz, –OC*H*$_2$CH$_3$); 5.61 (1H, dd, *J*=2.1, 1.2 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3 (q, –OCH$_2$*C*H$_3$); 14.7 (q, C-20); 18.7 (q, C-16); 19.0 (t, C-2); 19.6 (t, C-11); 21.7 (q, C-19); 24.1 (q, C-6); 33.5 (q, C-18); 33.7 (s, C-4); 39.3 (t, C-1); 40.2 (t, C-12); 41.9 (t, C-3); 42.6 (t, C-7); 42.6 (s, C-10); 54.3 (d, C-5); 59.4 (t, –O*C*H$_2$CH$_3$); 63.2 (d, C-9); 115.7 (d, C-14); 160.1 (s, C-13); 166.8 (s, C-15); 211.7 (s, C-8); HRFABMS *m/z* 336.2663 $[M]^+$ (calcd for $C_{21}H_{36}O_3$ 336.2664).

4.4.2. Ethyl 17-norlabd-13-*Z*-en-8-one-15-oate (9). Pale yellow oil; $[\alpha]_D^{25}$ −56.6 (*c* 0.9, $CHCl_3$); IR (film) ν_{max} 2945, 1712, 1646, 1244, 1168 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.69 (3H, s, H_3-20); 0.83 (3H, s, H_3-19); 0.95 (3H, s, H_3-18); 1.19 (1H, m, H-1a); 1.25 (3H, t, *J*=6.6 Hz, –OCH$_2$C*H*$_3$); 1.23 (1H, m, H-3a); 1.35 (1H, m, H-11a); 1.43 (1H, m, H-3); 1.48 (1H, m, H-5); 1.52 (2H, m, H_2-2); 1.63 (2H, m, H_2-6); 1.73 (1H, m, H-1b); 1.89 (3H, s, H_3-16); 1.90 (1H, m, H-11b); 2.03 (1H, m, H-6b); 2.13 (1H, m, H-9); 2.11 (1H, m, H-12a); 2.34 (2H, m, H_2-7); 2.85 (1H, m, H-12b); 4.11 (2H, dd, *J*=13.2, 6.4 Hz, –OC*H*$_2$CH$_3$); 5.60 (1H, br s, H-14); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4 (q, –OCH$_2$*C*H$_3$); 14.7 (q, C-20); 19.0 (t, C-2); 20.0 (t, C-11); 21.7 (q, C-19); 24.1 (t, C-6); 25.0 (q, C-16); 32.6 (t, C-12); 33.5 (q, C-18); 33.6 (s, C-4); 39.1 (t, C-1); 41.9 (t, C-3); 42.6 (t, C-7); 42.7 (s, C-10); 54.2 (d, C-5); 59.5 (t, –O*C*H$_2$CH$_3$); 63.6 (d, C-9); 116.3 (d, C-14); 160.5 (s, C-13); 166.4 (s, C-15); 212.0 (s, C-8); HRFABMS *m/z* 336.2664 $[M]^+$ (calcd for $C_{21}H_{36}O_3$ 336.2664).

4.4.3. Ethyl 17-norabiet-13(15)-*E*-en-8β-ol-16-oate (10). White crystals (from Et_2O); mp 110–111 °C; $[\alpha]_D^{34}$ −68.1 (*c* 1.8, $CHCl_3$); IR, ^{1}H and ^{13}C NMR data consistent with literature values;[20] HRFABMS *m/z* 334.2509 $[M]^+$ (calcd for $C_{21}H_{34}O_3$ 334.2508).

4.4.4. Ethyl 17-norabiet-13(15)-*Z*-en-8β-ol-16-oate (11). Yellow oil; $[\alpha]_D^{34}$ +66.3 (*c* 0.8, $CHCl_3$); IR, ^{1}H and ^{13}C NMR data consistent with literature values;[20] HRFABMS *m/z* 335.2582 $[M+H]^+$ (calcd for $C_{21}H_{35}O_3$ 335.2588).

4.5. Ethyl labd-13*E*-en-8β-ol-15-oate (13) and ethyl labd-13*Z*-en-8β-ol-15-oate (14)

This method is representative for the Grignard methylation of either **8** or **9**. A solution of **8** (76 mg, 0.23 mmol) in anhydrous Et_2O (5 mL) was cooled to −20 °C. An ether solution of MeMgCl (3 M, 96 μL, 1.3 equiv) was slowly added in a dropwise fashion with vigorous stirring. The solution was maintained at −20 °C (1 h), warmed to 0 °C (3 h) and excess MeMgCl quenched through the addition of three drops of H_2O. The reaction mixture was further acidified with HCl (1 M, 2 mL) and stirred until the solution had become clear. The Et_2O solution was washed with H_2O (3×5 mL), dried ($MgSO_4$) and concentrated in vacuo to yield a pale yellow oil. Subsequent purification of this oil via normal phase HPLC (hexane–EtOAc 6:1) afforded exclusively **13** with an average yield of 81%.

4.5.1. Ethyl labd-13*E*-en-8β-ol-15-oate (13). Pale yellow oil; $[\alpha]_D^{34}$ +24.8 (*c* 2.2, $CHCl_3$); IR (film) ν_{max} 3498, 2937, 1712, 1454, 1163 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.79 (1H, dd, *J*=4.4, 2.7 Hz, H-5); 0.81 (3H, s, H_3-19); 0.85 (1H, m, H-9); 0.86 (3H, s, H_3-18); 0.87 (1H, m, H-1a);

0.94 (3H, s, H_3-20); 1.12 (1H, m, H-3a); 1.13 (3H, s, H-17); 1.26 (3H, t, J=7.1 Hz, –OCH_2CH_3); 1.36 (1H, m, H-3b); 1.41 (2H, m, H-2a and H-11a); 1.48 (1H, m, H-6a);1.49 (1H, m, H-7a); 1.50 (1H, m, H-6b); 1.56 (1H, m, H-2b); 1.57 (1H, m, H-11b); 1.66 (1H, m, H-1b); 1.75 (1H, m, H-7b); 2.12 (3H, d, J=1.3 Hz, H_3-16); 2.14 (1H, m, H-12a); 2.15 (1H, m, H-12b); 4.14 (2H, q, J=7.1 Hz, –OCH_2CH_3); 5.67 (1H, dd, J=2.3, 1.1 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3 (q, –OCH_2CH_3); 15.1 (q, C-20); 18.1 (t, C-6); 18.3 (t, C-2); 19.0 (q, C-16); 21.6 (q, C-19); 23.6 (t, C-11); 30.6 (q, C-17); 33.2 (s, C-4); 33.4 (q, C-18); 39.0 (s, C-10); 39.2 (t, C-1); 42.0 (t, C-3); 42.3 (t, C-7); 44.6 (t, C-12); 55.9 (d, C-5); 58.8 (d, C-9); 59.5 (t, –OCH_2CH_3); 73.1 (s, C-8); 115.3 (d, C-14); 160.2 (s, C-13); 166.9 (s, C-15); HRFABMS *m/z* 351.2895 $[M+H]^+$ (calcd for $C_{22}H_{39}O_3$ 351.2899).

4.5.2. Ethyl labd-13*Z*-en-8β-ol-15-oate (14). Pale yellow oil; $[\alpha]_D^{34}$ +15.1 (*c* 0.7, $CHCl_3$); IR (film) ν_{max} 3461, 2923, 1709, 1451, 1159 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82 (3H, s, H_3-19); 0.84 (1H, m, H-5); 0.85 (1H, m, H-9); 0.86 (3H, s, H_3-18); 0.94 (1H, ddd, J=14.0, 6.8, 4.0 Hz, H-1a); 0.94 (3H, s, H_3-20); 1.14 (1H, ddd, J=13.9, 6.6, 4.2 Hz, H-3a); 1.24 (3H, s, H_3-17); 1.25 (3H, t, J=7.1 Hz, –OCH_2CH_3); 1.37 (1H, m, H-3b); 1.41 (1H, m, H-11a); 1.49 (2H, m, H-6a and H-7a); 1.51 (2H, m, H-6b and H-11b); 1.60 (1H, m, H-2b); 1.75 (2H, m, H-1b and H-7b); 1.90 (3H, d, J=2.8 Hz, H_3-16); 2.65 (1H, ddd, J=12.4, 5.8, 5.3 Hz, H-12a); 2.75 (1H, ddd, J=12.4, 6.2, 2.7 Hz); 4.12 (2H, q, J=7.1 Hz, –OCH_2CH_3); 5.60 (1H, d, J=1.3 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.4 (q, –OCH_2CH_3); 15.1 (q, C-20); 18.2 (t, C-2); 18.3 (t, C-6); 21.7 (q, C-19); 23.8 (t, C-11); 25.1 (q, C-16); 30.6 (q, C-17); 33.3 (s, C-10); 33.4 (q, C-18); 36.7 (t, C-12); 39.0 (s, C-10); 39.1 (t, C-1); 42.0 (t, C-3); 42.4 (t, C-7); 55.9 (d, C-5); 59.3 (t, –OCH_2CH_3); 59.4 (d, C-9); 115.9 (s, C-14); 159.9 (s, C-13); 166.2 (s, C-15); HRFABMS *m/z* 351.2895 $[M+H]^+$ (calcd for $C_{22}H_{39}O_3$ 351.2899).

4.6. Labd-13*E*-ene-8β,15-diol (4) and labd-13*Z*-ene-8β,15-diol (5)

This method is representative for the LAH reduction of either **13** or **14**. Compound **13** (42.1 mg, 0.12 mmol) was dissolved in anhydrous Et_2O (2 mL) and cooled to 0 °C. LAH (13.5 mg, 0.361 mmol, 3 equiv) was added and stirring continued (0.5 h) after which the solution was warmed to ambient temperature over a period of 2 h. HCl (1 M, 3 mL) was added slowly and the ether solution washed with H_2O (3×2 mL). The organic fraction was then dried ($MgSO_4$) and concentrated to yield a white amorphous powder which was purified by normal phase HPLC (hexane–EtOAc 1:1) to yield **4** (66%).

4.6.1. Labd-13*E*-ene-8β,15-diol (4). White amorphous powder; $[\alpha]_D^{34}$ +20.8 (*c* 1.5, $CHCl_3$), lit.[5] +32; IR (film) ν_{max} 3420, 2921, 1189, 912, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.79 (1H, dd, J=4.3, 2.7 Hz, H-9); 0.81 (3H, s, H_3-19); 0.85 (1H, m, H-5); 0.88 (1H, m, H-1a); 0.86 (3H, s, H_3-18); 0.94 (3H, s, H_3-20); 1.13 (3H, s, H_3-17); 1.14 (1H, m, H-3a); 1.38 (1H, m, H-3b); 1.40 (1H, m, H-11a); 1.42 (1H, m, H-2a); 1.51 (1H, m, H-7a); 1.52 (2H, m, H-6a and H-11b); 1.54 (1H, m, H-6b); 1.59 (1H, dt, J=13.5, 3.4 Hz, H-2b); 1.67 (1H, m, H-1b); 1.69 (3H, s, H_3-16); 1.77 (1H, m, H-7b); 2.02 (1H, m, H-12a); 2.05 (1H, m, H-12b); 4.14 (1H, d, J=6.9 Hz, H_2-15); 5.41 (1H, dt, J=6.9, 1.2 Hz, H-14); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 15.1 (q, C-20); 16.4 (q, C-2); 18.2 (t, C-2); 18.3 (t, C-6); 21.6 (q, C-19); 23.9 (t, C-6); 30.6 (q, C-17); 33.2 (s, C-4); 33.4 (q, C-18); 38.9 (s, C-10); 39.1 (t, C-1); 42.0 (t, C-3); 42.2 (t, C-7); 43.3 (t, C-12); 55.9 (d, C-5); 58.8 (d, C-9); 59.4 (t, C-15); 73.2 (s, C-8); 123.1 (d, C-14); 140.4 (s, C-13); HRFABMS *m/z* 308.2715 $[M]^+$ (calcd for $C_{20}H_{36}O_2$ 308.2715).

4.6.2. Labd-13*Z*-ene-8β,15-diol (5). White amorphous powder; $[\alpha]_D^{34}$ +24.3 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3395, 2930, 1182, 909, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz,) δ 0.79 (1H, dd, J=2.7, 1.6 Hz, H-9); 0.81 (3H, s, H_3-19); 0.84 (1H, m, H-5); 0.86 (3H, s, H_3-18); 0.89 (1H, m, H-1a); 0.93 (3H, s, H_3-20); 1.14 (1H, m, H-3a); 1.17 (3H, s, H_3-17); 1.31 (1H, m, H-11a); 1.39 (1H, m, H-3b); 1.41 (1H, m, H-2a); 1.45 (1H, m, H-11b); 1.48 (1H, m, H-7a); 1.50 (1H, m, H-6a); 1.52 (1H, m, H-6b); 1.60 (1H, dt, J=13.4, 3.1 Hz, H-2b); 1.69 (1H, ddd, J=12.3, 4.3, 3.0 Hz, H-1b); 1.76 (1H, m, H-7b); 1.77 (3H, d, J=0.9 Hz, H_3-16); 2.05 (1H, m, H-12a); 2.08 (1H, m, H-12b); 4.13 (2H, d, J=6.8 Hz, H_2-15); 5.37 (1H, t, J=7.1 Hz, H-14); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 15.1 (q, C-20); 18.2 (t, C-2); 18.3 (t, C-6); 21.6 (q, C-19); 23.6 (q, C-16); 24.5 (t, C-11); 30.6 (q, C-17); 33.3 (s, C-4); 33.4 (q, C-18); 36.0 (t, C-12); 38.9 (s, C-10); 39.1 (t, C-1); 42.0 (t, C-3); 42.2 (t, C-7); 55.9 (d, C-5); 59.28 (t, C-15); 59.32 (d, C-9); 73.1 (s, C-8); 123.6 (d, C-14); 140.9 (s, C-13); HRFABMS *m/z* 308.2716 $[M]^+$ (calcd for $C_{20}H_{36}O_2$ 308.2715).

4.7. Labd-13*E*-ene-8β-ol-15-al (1) and labd-13*Z*-ene-8β-ol-15-al (2)

This method is representative of the MnO_2 oxidation of both **4** and **5**. The allylic alcohol **4** (40.8 mg, 0.136 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) and finely powdered activated MnO_2 (86.9 mg, 1.36 mmol, 10 equiv) was added. The solution was stirred at ambient temperature overnight (16 h) and then filtered through Celite 545 to give **1** as a white amorphous powder (>95% purity from NMR) in 90% overall yield.

4.7.1. Labd-13*E*-ene-8β-ol-15-al (1). White amorphous powder; $[\alpha]_D^{34}$ +25.5 (*c* 1.4, C_6H_6); IR (film) ν_{max} 3481, 2921, 1668, 1454, 1382 cm^{-1}; ^{1}H NMR (C_6D_6, 600 MHz) δ 0.41 (1H, dd, J=4.6, 2.6 Hz, H-9); 0.63 (1H, dd, J=12.2, 2.4 Hz, H-5); 0.65 (1H, m, H-1a); 0.85 (3H, s, H_3-19); 0.86 (3H, s, H_3-17); 0.88 (3H, s, H_3-18); 0.95 (3H, s, H_3-20); 1.10 (1H, ddd, J=14.2, 6.6, 3.8 Hz, H-3a); 1.18 (2H, m, H-2a and H-7a); 1.20 (1H, m, H-11a); 1.23 (1H, m, H-6a); 1.33 (1H, m, H-6b); 1.37 (1H, ddd, J=11.8, 3.8, 1.4 Hz, H-3b); 1.43 (1H, m, H-11b); 1.48 (1H, dd, J=3.5, 1.0 Hz, H-1b); 1.49 (1H, m, H-2b); 1.53 (1H, ddd, J=13.5, 3.5, 3.3 Hz, H-7a); 1.60 (3H, d, J=1.3 Hz, H_3-16); 1.80 (1H, m, H-12a); 1.84 (m, 1H, H-12b); 5.93 (1H, ddd, J=7.8, 2.5, 1.3 Hz, H-14); 9.91 (1H, d, J=7.8 Hz, H-15); ^{13}C NMR (C_6D_6, 150 MHz) δ 15.3 (q, C-20); 17.0 (q, C-16); 18.61 (t, C-2); 18.62 (t, C-6); 21.9 (q, C-19); 23.5 (t, C-11); 30.8 (q, C-17); 33.4 (s, C-4); 33.7 (q, C-18); 39.2 (s, C-10); 39.3 (t, C-1); 42.4 (t, C-3); 42.6 (t, C-7);

44.4 (t, C-12); 56.0 (d, C-5); 58.8 (d, C-9); 72.4 (s, C-8); 127.3 (d, C-14); 162.1 (s, C-13); 190.0 (d, C-15); HRFABMS *m/z* 306.2559 $[M]^+$ (calcd for $C_{20}H_{34}O_2$ 306.2559).

4.7.2. Labd-13*Z*-ene-8β-ol-15-al (2). Pale yellow oil; $[\alpha]_D^{34}$ +24.1 (*c* 0.9, C_6H_6); IR (film) ν_{max} 3410, 2915, 1661, 1447, 1381 cm^{-1}; ^1H NMR (C_6D_6, 600 MHz) δ 0.43 (1H, dd, *J*=4.4, 2.6 Hz, H-9); 0.63 (1H, dd, *J*=12.1, 2.3 Hz, H-5); 0.68 (1H, ddd, *J*=13.2, 6.4, 3.4 Hz, H-1a); 0.83 (3H, s, H_3-19); 0.88 (3H, s, H_3-17); 0.89 (3H, s, H_3-18); 0.93 (3H, s, H_3-20); 1.10 (1H, ddd, *J*=13.5, 6.6, 4.0 Hz, H-3a); 1.17 (1H, m, H-7a); 1.20 (1H, m, H-2a); 1.25 (1H, m, H-11a); 1.32 (1H, m, H-6a); 1.35 (1H, m, H-6b); 1.36 (1H, m, H-3b); 1.46 (1H, m, H-11b); 1.47 (1H, m, H-7b); 1.48 (1H, m, H-2b); 1.49 (3H, d, *J*=1.3 Hz, H_3-16); 1.52 (1H, m, H-1b); 2.17 (1H, ddd, *J*=10.9, 6.2, 5.8 Hz, H-12a); 2.19 (1H, ddd, *J*=10.9, 6.0, 5.8 Hz, H-12b); 5.81 (1H, d, *J*=7.9 Hz, H-14); 10.08 (1H, d, *J*=7.9 Hz, H-15); ^{13}C NMR (C_6D_6, 150 MHz) δ 15.3 (q, C-20); 18.57 (t, C-2); 18.60 (t, C-6); 21.9 (q, C-19); 24.5 (q, C-16); 25.1 (t, C-11); 30.9 (q, C-17); 33.4 (s, C-4); 33.6 (q, C-18); 36.3 (t, C-12); 39.2 (s, C-10); 39.4 (t, C-1); 42.3 (t, C-7); 42.6 (t, C-3); 55.9 (d, C-5); 59.3 (d, C-9); 72.3 (s, C-8); 128.3 (d, C-14); 162.9 (s, C-13); 189.2 (d, C-15); HRFABMS *m/z* 306.2559 $[M]^+$ (calcd for $C_{20}H_{34}O_2$ 306.2559).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.09.039.

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