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Chemoenzymatic preparation of musky macrolactones

Tancredi Fortunati, Mariantonietta D'Acunto, Tonino Caruso, Aldo Spinella *

Dipartimento di Chimica e Biologia, Università di Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Salerno, Italy

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ABSTRACT

A chemoenzymatic approach to some musk macrolactones has been explored by the optimization of macrolactonization catalyzed by *Candida antarctica* lipase B (Novozym 435). This fast and high yield optimized methodology represents a large improvement to previously reported results. The methodology was applied to the preparation of 16-hexadecanolide, exaltolide, ambrettolide and (15*R*)-15-hexadecanolide.

1. Introduction

Macrocyclic musks are an important group of natural compounds used in perfumery. Actually, due to the expensive recovery from natural source, they are produced industrially by chemical synthesis.[1] From the structural point of view the main macrocyclic musks are C15–17 ketones and C15, C16 lactones. In this paper we have explored a chemoenzymatic approach to some musk macrolactones, in order to study a green approach to their preparation. In recent years, biocatalytic methodologies have attracted organic chemists as a green alternative to conventional chemical synthesis.[2–4] In this context, enzymatic processes in organic solvents[5] are particularly appealing and several studies have been conducted in order to optimize the application of biocatalysis to organic synthesis. In particular, lipases in organic solvents catalyze esterification and transesterification reactions and this ability has been widely used for the kinetic resolution of racemates.[6] Transesterification catalyzed by lipase can also be used for ring closure of hydroxyesters to macrolactones. In 1984, Gatfield was the first to apply this approach to the synthesis of pentadecanolide.[7] Then, in 1987, Yamada et al. described the successful lipase catalyzed preparation of macrocyclic lactones from several ω-hydroxyacid methyl esters. In particular, 16-hexadecanolide (**2**) was prepared in 80% yield using lipases from *Pseudomonas* sp. in dry benzene for 72 h.[8] However, in spite of the advantages offered by this approach some drawbacks such as the long reaction time required for maximal yields and the cost of the enzyme made this methodology scarcely used and few synthetic applications have been reported in literature. Hence, we decided to reinvestigate the enzymatic macrolactonization process, examining different types of lipase and reaction conditions, in order to improve its application.

2. Results and discussion

We started with the preparation of 16-hexadecanolide (**2**), an important macrocyclic musk lactone isolated from the aroma of orchids *Epidendrum aromaticum* and *Cattleya aurantiaca*.[9]

We first considered the reaction environment because this is one of the most important factor limiting enzyme activity in organic solvents. It is well known that hydrophobic solvents are the best choice for enzymes in organic media (in our work all reactions were conducted in cyclohexane), however, they should be nearly but not completely anhydrous because some water is needed for the catalytic function of the enzyme.[10] In fact, water plays a key role in biocatalysis in organic environments, since it is involved in noncovalent interactions essential for maintaining the active conformation of the enzyme. However, while the addition of water to solid enzyme preparations in organic solvents can enhance the enzyme activity by increasing the flexibility of the active site, excess of water may facilitate the aggregation of the enzyme causing a decrease in its activity. Usually the water in the system is described in terms of water activity (a_w) where the a_w of a 'sealed' system is often given by the ratio of its vapor pressure (P_w) and vapor pressure of pure water ($P°_w$): $a_w = P_w/P°_w$. Conditions, with a fixed value of a_w, are generally set by adjusting a_w value through the use of salt hydrates that facilitate the exchange of water with

* Corresponding author. Fax: +39 089969603; e-mail address: spinella@unisa.it (A. Spinella).

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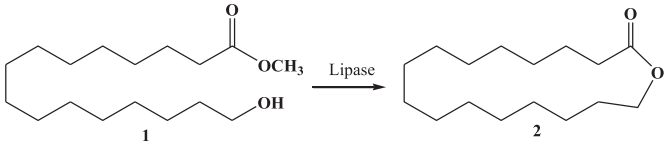
the system.[11] We decided to use a mixture of salt $Na_2HPO_4 \cdot 7H_20/2H_20$. This condition was achieved by placing the solvent and the enzyme in a closed chamber in indirect contact with a saturated aqueous solution of salts (at least for 12 h). We started a screening to evaluate, which enzyme is most efficient for the catalysis of the lactonization reaction. Initially the reactions were carried out adding the ester to the conditioned lipase in cyclohexane at 40 °C. Several commercially available lipases were tested (using comparable amounts in term of units) for the preparation of 16-hexadecanolide (Scheme 1) and the results are summarized in Table 1. Lipase from *Pseudomonas cepacea*, was quite efficient although a long time was required to obtain a significant quantity of lactone (entry 1). Other lipases (entries 2–5) gave unsatisfactory results. Subsequently, we extended the investigation to other commercial enzymes, considering some immobilized enzymes because immobilization is often reported to improve the efficiency of catalytic activity. A very high efficiency was observed with lipase from *Candida antarctica* (CALB) immobilized on acrylic resin (commercially known as Novozym 435®). High yields of lactone were obtained after just a few minutes, increasing up to a maximum value (79%) after 1 h (entry 6). However, this good result was the only one in the class of immobilized enzymes tested. Other immobilized lipases (entries 7 and 8) gave only small amounts of hexadecanolide. These results showed that lactonization activity is not a common feature of all lipases. Thus, we chose lipase from *Candida antarctica* (CALB) immobilized on acrylic resin. In our work we generally used 1.25 g immobilized enzyme (CALB) for 1 mmol of hydroxy ester but we found that good results (74% yield after 1 h) can be obtained lowering this ratio to 0.3. This evidence is very important because it allows a reduction in the amount of catalyst, which ultimately decreases the costs.



Scheme 1. Synthesis of 16-hexadecanolide (2).

Optimum concentration to lactonize methyl 16-hydroxy hexadecanoate in our study, was 1 mM. In fact, high concentrations of hydroxyester appeared to favour inter-molecular reactions and were not favourable for the formation of monolactone. We tested the effect of temperature performing reactions at 40 °C and 65 °C. Examining the influence of temperature, several reactions were conducted at different times in order to optimize also this parameter. For short times, we did not note marked differences, while after 1 h the reaction at 40 °C was by far the most efficient. In fact, the yield in hexadecanolide at 40 °C in 1 h, reached 79% while at 65 °C monolactone yield was only 41%. Some experiments were carried out under microwave heating in order to observe possible effects due to heating mode on the enzymatic process. However, microwave-assisted reactions carried out at the same temperature (40 °C) and for the same reaction time as the conventional heating reactions, did not show any improvement (the conversions, after 1 h and 3 h, were respectively 76% and 70%). This is in accordance with the reported data obtained comparing microwave and conventional heating in lipase-catalyzed transesterification reactions.[12]

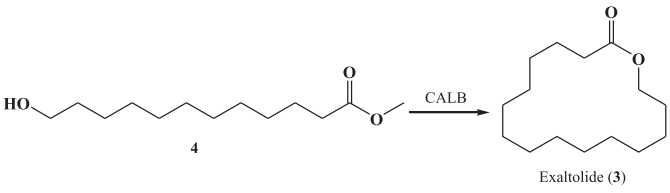
One of the advantages in using immobilized enzymes is their recovery from reaction environment. We evaluated this aspect by filtering the enzyme at the end of each reaction. Recovered enzyme was then introduced in the reaction flask in order to react with a new solution of substrate, under the same conditions. Recovery operations were performed for 4 times; after the fourth filtration we noted that the immobilized enzyme had lost the initial particle size. However, after the fourth recovery, CALB still showed sufficient activity for synthetize hexadecanolide giving a yield of 58% (Table 2).

Table 2
Enzyme reuse

Yield of hexadecanolide				
Initial reaction	1st recovery	2nd recovery	3rd recovery	4th recovery
79%	70%	65%	62%	58%

The synthetic protocol developed for obtaining hexadecanolide from 16-hydroxy hexadecanoic ester was applied to the lactonization of some other long chain ω-hydroxy esters. Two additional musky macrolactones of vegetable origin, exaltolide and ambrettolide, were prepared.

Exaltolide (**3**), isolated for the first time from the root of *Angelica archangelica*,[13,14] shows musk-like odour and fixative properties and is highly valued in the perfumery industry. Several syntheses of **3** have been reported in the literature, including also a biocatalytic preparation, which reports the acquisition of 77.8% of exaltolide using lipase from *Pseudomonas P* in benzene at 30 °C for over 24 h, starting from a solution of 15-hydroxy pentadecanoic acid.[15] In contrast, lactonization of methyl 15-hydroxy pentadecanoate (**4**), under our conditions, furnished exaltolide (**3**) in 88% yield after 2 h (Scheme 2).



Scheme 2. Synthesis of exaltolide (**3**).

Table 1
Screening of different lipases

		Yield of lactone after[a]				
		15 min	30 min	60 min	120 min	180 min
1	Amano Lipase PS from *Burkholderia cepacia* (*Pseudomonas cep.*)	n.d.	5%	13%	25%	30%
2	Lipase from Porcine Pancreas	n.d.	15%	36%	27%	23%
3	Amano Lipase A from *Aspergillus niger*	n.d.	n.d.	n.d.	n.d.	<5%
4	Amano Lipase from *Pseudomonas fluorescens*	n.d.	n.d.	n.d.	n.d.	<5%
5	Amano Lipase G from *Penicillium camemberti*	n.d.	n.d.	n.d.	n.d.	<5%
6	Lipase immobilized on acrylic resin from *Candida antarctica*	66%	69%	79%	76%	74%
7	Lipase immobilized from *Mucor miehei*	<5%	8%	15%	22%	30%
8	Lipase immobilized on Immobead 150 from *Pseudomonas cepacea*	7%	27%	53%	48%	40%

n.d.=undetectable.
[a] Isolated yields after chromatographic purification on silica.

(*Z*)-16-Hexadec-7-enolide (ambrettolide®) (**5**) is the principal odorous constituent of *Hibiscus abelmochus,* isolated from ambrette seed oil by Kerschbaum in 1927.[14] Owing to its importance in the perfume industry, several methods have been developed for the synthesis of this expensive naturally occurring macrolide.[16–18] Actually, the compound used in perfumery today is a synthetic isomer of the natural product: (*E*)-16-hexadec-9-enolide (isoambrettolide®). From a retro-synthetic point of view, the unsatured hydroxyester **6** needed for enzymatic lactonization was envisioned as a result of Wittig olefination of aldehyde **7** with the phosphorane derived from triphenylphosphonium salt **8**. The synthesis (Scheme 3) started with protection of alcohol group of **9** with *tert*-butyl dimethylsilylchloride (TBSCl); the next step was the reduction of the protected ester to aldehyde **7**. Ethyl 7-bromo heptanoate (**10**) was transformed into phosphonium salt **8** in 90% yield. The subsequent steps were olefination by Wittig reaction, followed by deprotection of the alcohol group. In the Wittig reaction, the phosphonium ion **8** was deprotonated by potassium of bis(trimethylsilyl)amide (TMSA-K) in dry THF. Subsequent removal of t-BDMSi protecting group was directly executed with aq fluoridric acid 48%. At the end, the olefin *Z*-**6** was exclusively formed and isolated in 62% yields (two steps). With hydroxyester **6** in our hand, we were ready for the cyclization step. Enzymatic lactonization of **6**, was conducted in the presence of CALB immobilized on acrylic resin under the established conditions of developed methodology. Again we found that the enzyme acts immediately on hydroxy ester, which begins to lactonize already in the first few minutes reaching the maximum yield of ambrettolide (83%) after 3 h.

The last target of our work was (15*R*)-15-hexadecanolide (**21**), a musk fragrance widely used in perfumery. This compound was isolated by Kaiser and Lamparsky as a trace component of the dried latex of *Ferula galbaniflua* and *F. rubicaulis*.[19] We prepared this macrocyclic lactone by direct enantioselective lactonization of the corresponding racemic hydroxy ester (±) −**18**. Once again the racemic hydroxy ester needed for enzymatic lactonization was envisioned as resulting from Wittig reaction of aldehyde **16** with the phosphorane derived from triphenylphosphonium salt **17**. The synthesis (Scheme 4) started with the preparation of TBS derivative of (±)—ethyl 2-hydroxybutanoate (**13**). Aldehyde **16** was then prepared by DIBAL-H reduction of **14**. The phosphonium salt **17** was obtained by treating methyl 12-bromododecanoate with triphenylphosphine. Wittig olefination of aldehyde **16** with phosphorane derived from **17** furnished compound (±) −**18**; then, removal of TBS protecting group gave hydroxyl ester (±) −**19** (55% yield, two steps). Enzymatic lactonization conducted in presence of CALB, under optimized conditions, gave, after 1 h, monolactone **20** with 80% of conversion (40% yield) with high enantiomeric purity (>99% ee). Finally, catalytic hydrogenation of **20** furnished (15*R*)-15-hexadecanolide (**21**) in 91% yield (>99% ee).

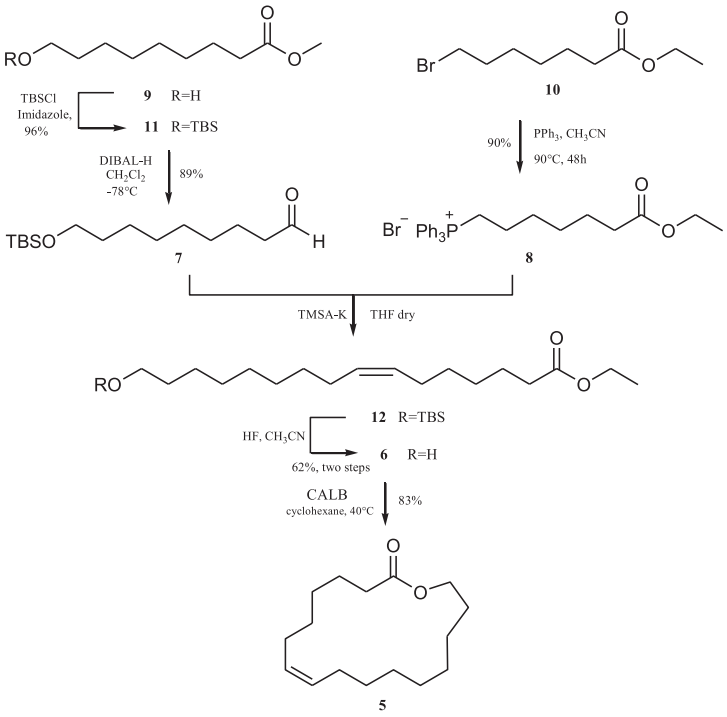
3. Conclusions

In the course of this investigation we have improved the enzymatic macrolactonization methodology by optimizing several parameters. The methodology was applied to the preparation of some musky macrolactones such as 16-hexadecanolide, exaltolide, ambrettolide and (15*R*)- (−)-15-hexadecanolide.

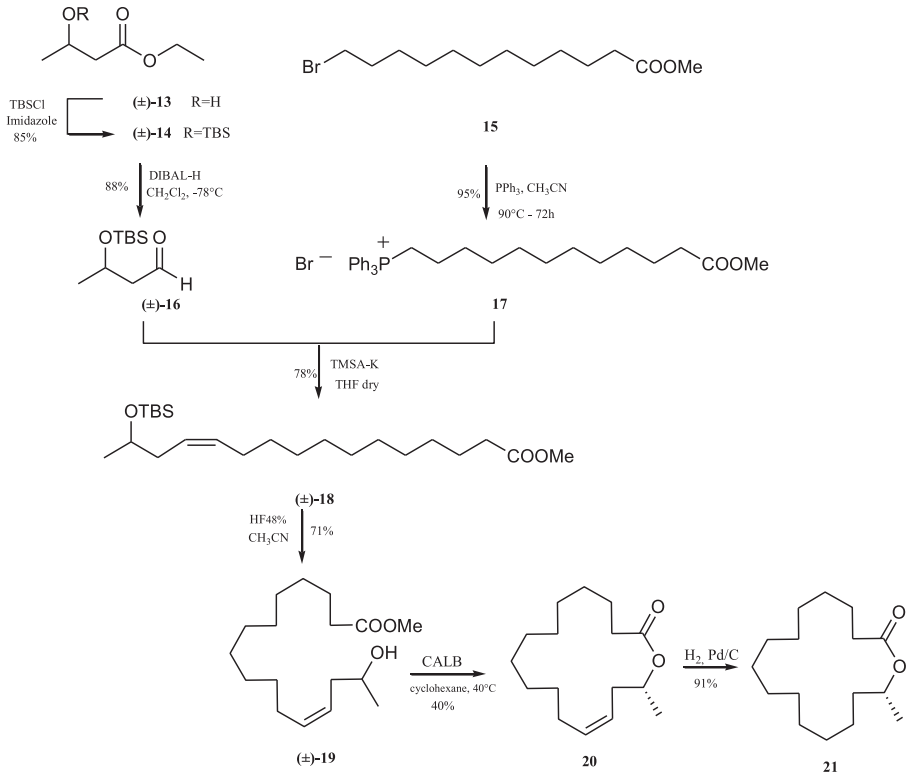
4. Experimental

4.1. General

All reactions were monitored by thin layer chromatography (TLC) and the products were visualized by UV light or by spraying of indicators, such as phosphomolybdic acid and cerium sulfate, or by exposure to iodine vapour. The products were purified using silica gel (Merck 70–230 mesh and Merck 230–400 mesh). All enzymes used in this work were purchased from Sigma -Aldrich. The NMR spectra were performed on Brucker AM 250 spectrometers (250.13 MHz for ^{1}H and 62.89 for ^{13}C), Bruker DRX 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) and Brucker DRX 400 (400.135 MHz for ^{1}H and 100.03 for ^{13}C) of the Department of Chemistry and Biology,



Scheme 3. Synthesis of ambrettolide (**5**).



Scheme 4. Synthesis of (15*R*)-(−)-15-hexadecanolide (**21**).

University of Salerno. The chemical shifts were reported in ppm: $CHCl_3$ signal (7.26 ppm for ^1H) and $CDCl_3$ (77.0 ppm for ^{13}C) were used as internal standard. ESIMS spectra were performed on a Micromass Quattro micro API™ mass spectrometer equipped with an electrospray ionization source operating in positive mode. IR spectra were obtained at a resolution of 2.0 cm^{-1} with a Vector 22 Bruker Spectrometer. Elemental analyses were performed on Flash EA 1112 (Thermo Electron Corporation) analyzer. Microwave experiments were conducted using a commercial available single-mode microwave apparatus equipped with a high sensitivity infrared sensor for temperature control and measurement (Discover LabMate, CEM Corporation). Enantiomeric excesses were determined by GC analyses carried out on a Agilent Technologies 6850 GC System FID detector using a Supelco Beta Dex ™ 20 Fused Silica Capillary column (30m×0.25 mm). The water activity in all the experiments was set by equilibrating the suspension containing the enzyme in organic solvent with aqueous saturated salt pairs (mixture of $Na_2HPO_4{\cdot}7H_20/2H_20$) solution in separated vessels overnight (at least 12 h).

4.2. Synthesis of 16-hexadecanolide (2)

The reaction vessel containing a suspension of 50 mg of enzyme CALB (lipase B acrylic resin from *C. antarctica* (EC 3.1.1.3, 10,000 U/g) in 40 mL of cyclohexane was placed in a closed chamber containing a saturated solution of salt hydrates ($Na_2HPO_4{\cdot}7H_20/2H_20$) and kept overnight in indirect contact, under vigorous agitation. Then, 16-hydroxyexadecanoic methyl ester (**1**) (11.5 mg, 0.04 mmol) was added and the reaction was vigorously stirred at 40 °C for 1 h. At the end, the enzyme was removed by filtration and the filtrate was concentrated under vacuum. Purification of the crude material on flash column chromatography, eluting with hexane/chloroform 6/4, gave **2** (8.1 mg, 79%) as colourless oil.

16-Hexadecanolide (**2**): R_f (50% $CHCl_3$/hexane) 0.67; IR ($CHCl_3$): 2923, 1732, 1164 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 4.10 (2H, t, J=5.5 Hz), 2.30 (2H, t, J=6.6 Hz), 1.69–1.54 (4H, m), 1.42–1.18 (22H, m). ^{13}C NMR (75 MHz $CDCl_3$): δ 174.0 (C), 64.2 (CH_2), 34.6 (CH_2), 28.6 (CH_2), 28.1 (CH_2), 27.8 (CH_2), 27.7 (CH_2), 27.6 (CH_2), 27.5 (CH_2), 26.9 (CH_2), 26.8 (CH_2), 26.78 (2 CH_2), 26.7 (CH_2), 25.5 (CH_2), 25.0 (CH_2). ES-MS (*m*/*z*): 255 $[M+H]^+$. Anal. Calcd for C16H30O2: C, 75.54; H, 11.89. Found: C, 75.29; H, 11.67.

4.3. Synthesis of exaltolide (3)

The reaction vessel containing a suspension of 50 mg of enzyme CALB (lipase B acrylic resin from *C. antarctica* (EC 3.1.1.3, 10,000 U/g) in 40 mL of cyclohexane was placed in a closed chamber containing a saturated solution of salt hydrates ($Na_2HPO_4{\cdot}7H_20/2H_20$) and kept overnight in indirectly contact, under vigorous agitation. Then, hydroxyester **4** (11.0 mg, 0.04 mmol) was added and the reaction was vigorously stirred at 40 °C for 2 h. At the end, the enzyme was removed by filtration and the filtrate was concentrated under vacuum. Purification of the crude material on flash column chromatography, eluting with hexane/chloroform 6/4, afforded exaltolide (**3**) (8.5 mg, 88%) as colourless oil.

Exaltolide (**3**): R_f (50% $CHCl_3$/hexane) 0.66; IR ($CHCl_3$): 2934, 1736, 1344 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 4.13 (2H, t, J=5.6 Hz), 2.33 (2H, t, J=6.6 Hz), 1.72–1.60 (4H, m), 1.43–1.29 (20H, m). ^{13}C NMR (100 MHz $CDCl_3$): δ 174.0 (C), 63.9 (CH_2), 34.4 (CH_2), 28.3 (CH_2), 27.7 (CH_2), 27.1 (2 CH_2), 26.8 (CH_2), 26.6 (CH_2), 26.3 (CH_2), 26.0 (CH_2), 25.9 (CH_2), 25.8 (CH_2), 25.1 (CH_2), 24.9 (CH_2). ES-MS (*m*/*z*): 263 $[M+Na]^+$, 241 $[M+H]^+$. Anal. Calcd for C15H28O2: C, 74.95; H, 11.74. Found: C, 74.81; H, 11.48.

4.4. Synthesis of ambrettolide

*4.4.1. TBS-protected alcohol **11**.* To a stirred solution of 9-hydroxy nonanoate methyl ester (1.0 g, 5.31 mmol) in dry dichloromethane (5.3 mL) was added imidazole (0.48 g, 7.0 mmol) and then TBSCl (1.1 g, 7.0 mmol). The resulting solution was stirred overnight

at room temperature. Then, the mixture was quenched with water and extracted with dichloromethane (3×20 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. Purification of the crude material on flash column chromatography, eluting with petroleum ether/diethyl ether 97/3, gave pure **11** (1.539 g, 96%) as colourless oil.

Compound (**11**): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.65 (3H, s, $-OCH_3$), 3.58 (2H, t, J=6.5 Hz), 2.29 (2H, t, J=7.7 Hz), 1.61 (2H, m), 1.49 (2H, m), 1.29 (8H, m), 0.88 (9H, s), −0.03 (6H, s). ^{13}C NMR (62.89 MHz $CDCl_3$): δ 174.3 (C), 63.2 (CH_2), 21.4 (CH_3), 34.1 (CH_2), 32.8 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 26.0 (3 CH_3), 25.7 (CH_2), 24.9 (CH_2), 18.3 (C), −5.3 (2 CH_3). ES-MS (m/z): 325 $[M+Na]^+$, 303 $[M+H]^+$.

*4.4.2. Aldehyde **7**.* To a stirred solution of **11** (1.40 g, 4.37 mmol) in anhydrous CH_2Cl_2 (10 mL), under N_2 atmosphere, DIBAL-H (10 mL, 10.5 mol/L in CH_2Cl_2) was added dropwise during 2 h, at −78 °C. Then, the resulted solution was warmed to 0 °C and quenched with a saturated solution of Na/K tartrate (10 mL) and extracted with dichloromethane (3×20 mL). The organic layer was dried over Na_2SO_4, and concentrated under vacuum. Purification of the crude material on flash column chromatography, eluting with pentane/diethyl ether 99/1, gave aldehyde **7** (1.060 g, 89%) as colourless oil.

Compound (**7**): ^{1}H NMR ($CDCl_3$, 250 MHz): δ 9.75 (1H, s), 3.58 (2H, t, J=6.5 Hz), 2.30 (2H, dt, J=7.4, 1.8 Hz), 1.62 (2H, m), 1.49 (2H, m), 1.29 (8H, m), 0.88 (9H, s), −0.03 (6H, s). ^{13}C NMR (62.89 MHz $CDCl_3$): δ 202.7 (CH), 63.1 (CH_2), 43.7 (CH_2), 32.6 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 25.8 (3 CH_3), 25.5 (CH_2), 21.9 (CH_2), 18.2 (C), −5.4 (2 CH_3). ES-MS (m/z): 295 $[M+Na]^+$, 273 $[M+H]^+$.

*4.4.3. Phosphonium salt (**8**).* A mixture containing ethyl 7-bromo heptanoate (1.35 g, 5.7 mmol), triphenilphosphine (1.5 g, 6.3 mmol) and 15 mL of CH_3CN was refluxed for 48 h. Upon cooling, the solution was concentrated under vacuum and subjected to flash column chromatography (petroleum ether/diethyl ether 97/3 and then with chloroform/methanol 95/5) to give phosphonium salt **8** (2.56 g, 90%).

Compound (**8**): ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.85–7.57 (15H, m), 3.98 (2H, q, J=7.1 Hz), 3.64 (2H, m), 2.14 (2H, t, J=7.3 Hz), 1.66–1.50 (4H, m), 1.44 (2H, m), 1.24 (2H, m), 1.12 (3H, t, J=7.1 Hz). ^{13}C NMR (75 MHz $CDCl_3$): δ 173.4 (C), 134.8 (CH, d, $^4J_{PC}$=3 Hz, aromatic carbon *para* to phosphorous), 133.4 (CH, d, $^3J_{PC}$=10 Hz, aromatic carbon *meta* to phosphorous), 130.3 (CH, d, $^2J_{PC}$=13 Hz, aromatic carbon *ortho* to phosphorous), 118.0 (C, d, $^1J_{PC}$=86 Hz, quaternary aromatic carbon attached to phosphorous), 59.9 (t), 33.8 (t), 29.7 (CH_2, d, $^2J_{PC}$=16 Hz), 28.2 (CH_2), 24.1 (CH_2), 22.4 (CH_2, d, $^1J_{PC}$=46 Hz), 22.1 (CH_2), 14.0 (CH_3).

*4.4.4. (Z)-ethyl 16-hydroxyhexadec-7-enoate (**6**).* Phosphonium salt **8** (2.55 g, 5.1 mmol) was dissolved in 10 mL of dry THF at 0 °C stirring for some minutes. Then, a solution of potassium bis(-trimethylsilyl)amide (10 mL 0.25 M in toluene) was added. The reaction mixture was warmed to rt and stirred for 30 min. After cooling at 0 °C, a solution of aldehyde **7** (1.054 g, 3.87 mmol) in dry THF (7 mL) was added dropwise. The resulting solution was stirred for 2 h before it was quenched by acidification with 10 ml of HCl 1.0 M and extracted with diethyl ether. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude material was dissolved in CH_3CN (100 mL), in a silicon vessel and 48% aqueous HF (30 mL) was added at room temperature. The resulting mixture was stirred for 3 h. Then, $NaHCO_3$ was carefully added until the mixture was neutralized. The organic solvent was removed in vacuo and the residual aqueous layer was extracted with ethyl acetate (3×20 mL). The combined organic extracts were dried over Na_2SO_4, concentrated in vacuo and purified on flash column chromatography, eluiting with petroleum ether/diethyl ether from 85/15 to 7/3, yielding pure **6** (714 mg, 62%) as colourless oil.

Compound (**6**): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.35 (1H, dt, J=11.2 and 5.8 Hz), 5.32 (1H, dt, J=11.2 and 5.8 Hz), 4.11 (2H, q, J=7.1 Hz), 3.63 (2H, t, J=6.6 Hz), 2.28 (2H, t, J=7.6 Hz), 2.00 (4H, m), 1.62 (2H, m), 1.55 (2H, m), 1.34–1.28 (14H, m), 1.24 (3H, t, J=7.1 Hz). ^{13}C NMR (100.03 MHz $CDCl_3$): δ 173.9 (C), 130.0 (CH), 129.5(CH), 62.9 (CH_2), 60.1 (CH_2), 34.3 (CH_2), 32.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (t), 28.7 (CH_2), 27.1 (CH_2), 26.9 (CH_2), 25.7 (CH_2), 24.8 (CH_2), 14.2 (CH_3). ES-MS (m/z): 321 $[M+Na]^+$, 299 $[M+H]^+$.

*4.4.5. Ambrettolide (**5**).* The reaction vessel containing a suspension of 50 mg of enzyme CALB (lipase B acrylic resin from *C. antarctica* (EC 3.1.1.3, 10,000 U/g) in 40 mL of cyclohexane was placed in a closed chamber containing a saturated solution of salt hydrates ($Na_2HPO_4{\cdot}7H_2O/2H_20$) and kept overnight in indirectly contact, under vigorous agitation. Then, hydroxy-ester **6** (12 mg, 0.04 mmol) was added and the reaction was vigorously stirred at 40 °C for 3 h. At the end, the enzyme was removed by filtration and the filtrate was concentrated under vacuum. Purification of the crude material on flash column chromatography, eluting with petroleum ether/chloroform 8/2, gave. ambrettolide **5** (8.4 mg, 83%) as colourless oil.

Ambrettolide (**5**): R_f (50% $CHCl_3$/hexane) 0.62; IR (cm^{-1}): 2927, 1738, 1468. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.32 (2H, m), 4.13 (2H, m), 2.32 (2H, t, J=6.6 Hz), 2.08–1.99 (4H, m), 1.66–1.57 (4H, m), 1.44–1.19 (14H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.9 (C), 130.2 (CH), 130.0 (CH), 63.7 (CH_2), 34.5 (CH_2), 29.4 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 28.3 (CH_2), 27.6 (CH_2), 26.9 (CH_2), 26.8 (CH_2), 25.3 (CH_2), 25.2 (CH_2). MS (m/z): 253 $[M+H]^+$. Anal. Calcd for C16H28O2: C, 76.14; H, 11.18. Found: C, 76.02; H, 11.27.

4.5. Synthesis of (15*R*)-15-hexadecanolide (21)

*4.5.1. TBS-protected alcohol (rac)-**14**.* To a stirred solution of (*rac*)-ethyl-3-hydroxybutanoate (1.00 g, 7.57 mmol) in dry dichloromethane (8.0 mL) was added imidazole (0.67 g, 9.84 mmol) and then TBSCl (1.5 g, 9.95 mmol). The resulting solution was stirred overnight at room temperature. Then, the mixture was quenched with water and extracted with dichloromethane (3×20 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. Purification of the crude material on flash column chromatography, eluting with petroleum ether/diethyl ether 95/5, gave pure (*rac*)-**14** (1.59 g, 85%) as colourless oil.

Compound (*rac*)-**14**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.26 (1H, m), 4.09 (2H, m), 2.45 (1H, dd, J=14.3, 7.5 Hz), 2.34 (1H, dd, J=14.3, 5.4 Hz), 1.24 (3H, t, J=7.5 Hz), 1.17 (3H, d, J=6.1 Hz), 0.84 (9H, s), 0.05 (3H, s), 0.03 (3H, s). ^{13}C NMR (62.89 MHz $CDCl_3$): δ 171.6 (C), 65.8 (CH), 60.1 (CH_2), 44.9 (CH_2), 25.7 (3 CH_3), 23.9 (CH_3), 17.9 (C), 14.2 (CH_3), −4.6 (CH_3), −5.1 (CH_3). ES-MS (m/z): 269 $[M+Na]^+$, 247 $[M+H]^+$.

*4.5.2. Aldehyde (rac)-**16**.* To a stirred solution of (*rac*)-**14** (1.5 g, 6.09 mmol) in anhydrous CH_2Cl_2 (6 mL), under N_2 atmosphere, DIBAL-H (12 mL, 0.5 mol/L in CH_2Cl_2) was added dropwise during 2 h, at −78 °C. Then, the resulted solution was warmed to 0 °C and quenched with a saturated solution of Na/K tartrate (10 mL) and extracted with dichloromethane (3×20 mL). The organic layer was dried over Na_2SO_4, and concentrated under vacuum. Purification of the crude material on flash column chromatography, eluting with pentane/diethyl ether 99/1, gave aldehyde (*rac*)-**16** (1.082 g, 88%) as colourless oil.

Compound (*rac*)-**16**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.72 (1H, br s), 4.29 (1H, m), 2.48 (1H, ddd, J=15.6, 6.8, 2.9 Hz), 2.39 (1H, ddd, J=15.6, 4.9, 2.0 Hz), 1.17 (3H, d, J=5.9 Hz), 0.80 (9H, s), 0.01 (3H, s),-0.01 (3H, s). ^{13}C NMR (75 MHz $CDCl_3$): δ 202.1 (CH), 64.4 (CH), 52.8

(CH_2), 25.6 (3 CH_3), 24.0 (CH_3), 17.8 (C), −4.5 (CH_3), −5.1 (CH_3). ES-MS (*m*/*z*): 241 $[M+K]^+$, 225 $[M+Na]^+$, 203 $[M+H]^+$.

*4.5.3. Phosphonium salt (**17**).* A mixture containing methyl 12-bromododecanoate (890 mg, 3.05 mmol), triphenilphosphine (800 mg, 3.05 mmol) and 10 mL of CH_3CN was refluxed for 5d. Upon cooling, the solution was concentrated under vacuum and subjected to flash column chromatography (dichloromethane/methanol 95/5) to give phosphonium salt **17** (1.61 g, 95%).

Compound **17**: ^1H NMR ($CDCl_3$, 400 MHz): δ 7.86–7.65 (15H, m), 3.74 (2H, m), 3.63 (3H, s), 2.26 (2H, t, *J*=7.5 Hz), 1.66–1.51 (6H, m), 1.29–1.12 (12H, m). ^{13}C NMR (100.03 MHz $CDCl_3$): δ 174.1 (C), 134.8 (CH, d, $^4J_{PC}$=3 Hz, aromatic carbon *para* to phosphorous), 133.2 (CH, d, $^3J_{PC}$=9.9 Hz, aromatic carbon *meta* to phosphorous), 130.1 (CH, d, $^2J_{PC}$=12.4 Hz, aromatic carbon *ortho* to phosphorous), 118.1 (C, d, $^1J_{PC}$=85 Hz, quaternary aromatic carbon attached to phosphorous), 51.0 (CH_3), 33.8 (CH_2), 30.1 (CH_2, d, $^2J_{PC}$=15 Hz), 29.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 24.6 (CH_2), 22.6 (CH_2, d, $^1J_{PC}$=44 Hz).

*4.5.4. Compound (rac)-**18**.* Phosphonium salt **17** (850 mg, 1.53 mmol) was dissolved in 5 mL of dry THF at 0 °C stirring for 10 min. Then, a solution of potassium bis(trimethylsilyl)amide (3,0 mL, 0.5 M in toluene) was added. The reaction mixture was warmed to rt and stirred for 30 min. After cooling at 0 °C, a solution of aldehyde (*rac*)-**16** (231 mg, 1.14 mmol) in dry THF (3 mL) was added dropwise. The resulting solution was stirred for 2 h before it was quenched by acidification with 10 mL of HCl 1.0 M and extracted with diethyl ether. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. Purification on flash column chromatography, eluiting with petroleum ether/diethyl ether from 99/1 to 95/5, afforded pure (*rac*)-**18** (356 mg, 78%) as colourless oil.

Compound (*rac*)-**18**: ^1H NMR ($CDCl_3$, 400 MHz): δ 5.42 (1H, dt, *J*=10.9, 7.5 Hz), 5.34 (1H, dt, *J*=10.9, 6.8 Hz), 3.78 (1H, m), 3.64 (3H, s), 2.28 (2H, t, *J*=7.6 Hz), 2.15 (4H, m), 2.00 (4H, m), 1.60 (2H, m), 1.34–1.28 (14H, m), 1.10 (3H, d, *J*=6.1 Hz), 0.87 (9H, s), 0.03 (6H, s). ^{13}C NMR (100.03 MHz $CDCl_3$): δ 174.2 (C), 131.4 (CH), 125.9(CH), 68.7 (CH), 51.3 (CH_3), 37.4 (CH_2), 34.0 (CH_2), 29.6 (CH_2), 29.5 (2 CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.8 (3 CH_3), 24.9 (CH_2), 23.4 (CH_3), 18.1 (C), −4.6 (CH_3), −4.8 (CH_3). ES-MS (*m*/*z*): 398 [M+].

*4.5.5. Hydroxyester (rac)-**19**.* To a stirred solution of (*rac*)-**18** (100 mg, 0.25 mmol) in CH_3CN (10 mL), in a silicon vessel, 48% aqueous HF (2.3 mL) was added at room temperature. The resulting mixture was stirred for 3 h. Then, $NaHCO_3$ was carefully added until the mixture was neutralized. The organic solvent was removed in vacuo and the residual aqueous layer was extracted with ethyl acetate (3×20 mL). The combined organic extracts were dried over Na_2SO_4,concentrated in vacuo and purified on flash column chromatography (petroleum ether/diethyl ether from 85/15 to 7/3) yielding pure (*rac*)-**19** (51 mg, 71%) as colourless oil.

Compound (*rac*)-**19**: ^1H NMR ($CDCl_3$, 400 MHz): δ 5.54 (1H, dt, *J*=10.3, 7.3 Hz), 5.38 (1H, dt, *J*=10.3, 7.3 Hz), 3.80 (1H, m), 3.65 (3H, s), 2.29 (2H, t, *J*=7.6 Hz), 2.21 (4H, m), 2.04 (4H, m), 1.60 (2H, m), 1.37–1.20 (14H, m), 1.19 (3H, d, *J*=6.0 Hz). ^{13}C NMR (100.03 MHz $CDCl_3$): δ 174.3 (C), 133.4 (CH), 125.0 (CH), 67.6 (CH), 51.4 (CH_3), 37.1 (CH_2), 34.0 (CH_2), 29.6 (CH_2), 29.5 (2 CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.3 (CH_2), 24.9 (CH_2), 22.7 (CH_3). ES-MS (*m*/*z*): 323 $[M+K]^+$, 307 $[M+Na]^+$.

*4.5.6. (12Z)-(15R)- 15-hexadeca-12-en-olide (**20**).* The reaction vessel containing a suspension of 50 mg of enzyme CALB (lipase B acrylic resin from *C. antarctica* (EC 3.1.1.3, 10,000 U/g) in 40 mL of cyclohexane was placed in a closed chamber containing a saturated solution of salt hydrates ($Na_2HPO_4 \cdot 7H_20/2H_20$) and kept overnight in indirectly contact, under vigorous agitation. Then, hydroxy-ester (±)**19** (11.5 mg, 0.04 mmol) was added and the reaction was vigorously stirred at 40 °C for 3 h. At the end, the enzyme was removed by filtration and the filtrate was concentrated under vacuum. Purification of the crude material on flash column chromatography, eluting with petroleum ether/chloroform 8/2, gave pure **20** (4.1 mg, 40%) as colourless oil.

Compound **20**: $[\alpha]_D^{26}$ +32 (*c*=0.4, $CHCl_3$), ee>99%. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.52 (1H, dt, *J*=10.3, 7.3 Hz), 5.35 (1H, dt, *J*=10.3, 7.3 Hz), 4.95 (1H, m), 2.47 (1H, m), 2.27 (2H, m), 2.23–1.94 (3H, m), 1.64 (2H, m), 1.40–1.28 (14H, m), 1.26 (3H, t, *J*=6.1 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.2 (C), 132.1 (CH), 124.1 (CH), 70.0 (CH), 34.2 (CH_2), 33.6 (CH_2), 27.3 (CH_2), 26.3 (CH_2), 26.2 (CH_2), 26.0 (CH_2), 25.9 (CH_2), 25.7 (CH_2), 25.6 (CH_2), 23.8 (CH_2), 19.1 (CH_3). ES-MS (*m*/*z*): 291$[M+K]^+$, 253 $[M+H]^+$.

*4.5.7. (15R)-15-hexadecanolide (**21**).* To a vigorously stirred suspension of **20** (4.0 mg, 0.016 mmol) and a catalytic amount of 10% Pd/C in EtOH (1 mL), in a conical flask, was introduced H_2 gas at room temperature. After 1 h the reaction mixture was filtered through a Celite bed and the filtrate was concentrated in vacuo. The residue was flash-chromatographed (5% diethyl ether in petroleum ether) affording pure **21** (3.7 mg, 91%) as colourless oil.

Compound **21**: $[\alpha]_D^{26}$ −13.9 (*c*=0.14, $CHCl_3$), ee>99%. $[\alpha]_D^{20}$ −16.5 (*c*=1.03), for natural (−)–**21**.[19] IR (cm^{-1}): 2927, 1738, 1468. ^1H NMR ($CDCl_3$, 400 MHz): δ 4.95 (1H, m), 2.29 (2H, m), 1.69 (1H, m), 1.64–1.47 (5H, m), 1.43–1.22 (18H, m), 1.21 (3H, d, *J*=6.3 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.7 (C), 70.6 (CH), 35.9 (CH_2), 34.8 (CH_2), 33.6 (CH_2), 29.7 (CH_2), 27.7 (CH_2), 27.5 (CH_2), 27.2 (CH_2), 26.6 (CH_2), 26.2 (CH_2), 25.7 (CH_2), 25.6 (CH_2), 25.0 (CH_2), 24.3 (CH_2), 20.4 (CH_3). ES-MS (*m*/*z*): 277 $[M+Na]^+$, 255 $[M+H]^+$.

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