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Synthesis and resolution of a key building block for epothilones: a comparison of asymmetric synthesis, chemical and enzymatic resolution☆

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Abstract—The asymmetric synthesis and kinetic resolution of a series of acyloins (α-hydroxy ketones) suitable as building blocks for the northern half of epothilones was studied. Three methods were applied to obtain nonracemic compounds at the eventual epothilone C15-position: asymmetric synthesis with Evans' auxiliary, chemical resolution and enzymatic resolution. The success rate in small scale applications increased in the order given, and the enzymatic resolution was studied in more detail. Out of a set of nine lipases and esterases, lipases from *Burkholderia cepacia*, *Pseudomonas* sp., lipase B from *Candida antarctica* and recombinant esterases from *Streptomyces diastatochromogenes* exhibited the highest enantioselectivities with *E*-values ranging from 60 to >200. Pig liver esterase exhibited inverse enantiopreference and only with recombinant enzyme could a moderate selectivity ($E = 50$, commercial PLE: $E = 8$) be observed.

1. Introduction

Enantiomerically pure chiral acyloins are valuable intermediates in the total synthesis of complex natural products.[1] Acyloin derivatives such as **6** are useful fragments for syntheses of epothilones B, D, and derivatives, which use a northern/southern half approach (Scheme 1).[2–5] However, installation of the remote C15 stereocentre of the epothilones **1**–**5** proved to be a major challenge. The epothilones are a family of closely related polyketides (isolated from *Sorangium cellulosum* by Höfle et al. in 1994) that display a strong in vitro cytotoxicity against mammalian cells.[6,7] The mode of action of these remarkable 16-membered macrolactones[8] was soon shown to be stabilization of microtubuli in a manner similar to paclitaxel (Taxol®).[9] The epothilones are competitive inhibitors of ^{3}H-labelled paclitaxel binding to tubulin, suggesting that the binding sites of both compounds at least overlap partially.[10] Moreover, the epothilones are far more active than paclitaxel against multidrug resistant cell lines. Several total syntheses of epothilones A–E have been published,[11,12] with many derivatives being synthesized within a short period of time after the first total syntheses.[13,14] The chemistry and biology of epothilones has been well described,[14–18] and several members of the epothilone family, as well as some synthetic derivatives, are currently in various stages of clinical development.

2. Results and discussion

In the course of our synthetic approach towards epothilone B (D) and derivatives, we envisioned the C7-17 northern half precursor **6**, in which the epothilone-C7–C14 moiety is derived from the natural diprenol nerol (cf. neryl bromide **8**, Scheme 2). Three distinct strategies to obtain **6** with the correct C3-stereocentre were investigated: (i) auxiliary-assisted asymmetric alkylation, (ii) chemical resolution of a racemic intermediate, and

☆ Part of this work was also conducted at the Dept. of Organic and Inorganic Chemistry, Vrije Universiteit Amsterdam, The Netherlands.

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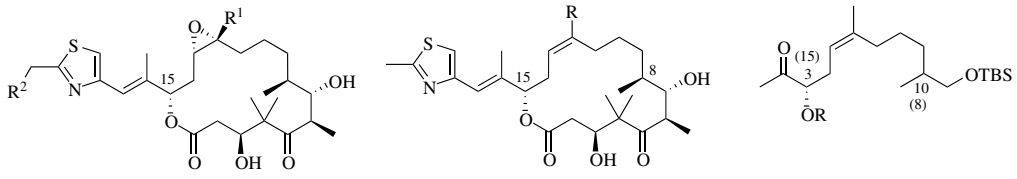


1 Epothilone A: R^1=R^2=H
2 Epothilone B: R^1=Me, R^2=H
5 Epothilone E: R^1=H, R^2=OH

3 Epothilone C: R=H
4 Epothilone D: R=Me

6 Northern Half Acyloin Building Block

Scheme 1. Epothilones A–E and northern half fragment **6** (in brackets: epothilone numbering).

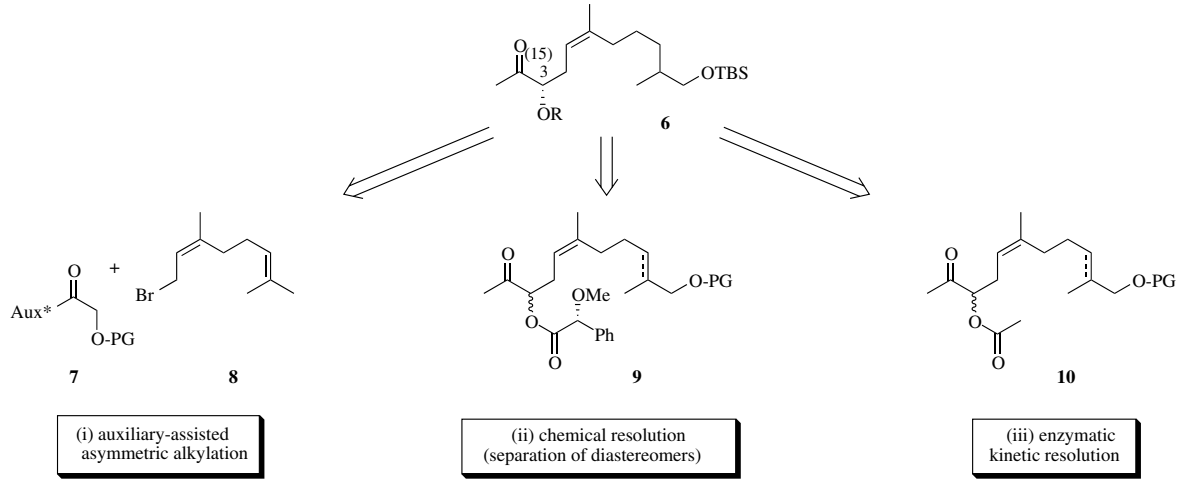
(iii) enzymatic kinetic resolution of a racemic intermediate (Scheme 2).

The first approach involved alkylation of chiral *O*-protected glycolic acid derivatives **7** (Scheme 3). Initially, we considered using Evans' oxazolidinones such as (*S*)-5-benzyl-1,3-oxazolidin-2-one **11** as chiral auxiliaries and TBS as the protecting group. Evans' amide **7a** was prepared by reaction of lithiated oxazolidinone **11** with (*tert*-butyldimethylsilyloxy)acetyl chloride.[19] However, deprotonation of this Evans' amide with LDA led to extensive cleavage of the TBS ether. Alkylation of the corresponding benzyloxy acetamide **7b** with LDA followed by neryl bromide **8** afforded the desired alkylation product, albeit in poor yield (⩽20%).

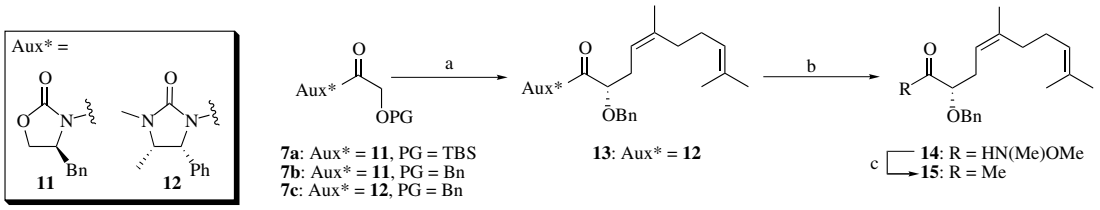
Cardillo et al. reported that the more basic L-(−)-ephedrine-derived imidazolidinone **12**[20] is an efficient chiral auxiliary in the alkylation of benzyloxyacetic acid derivatives.[21] Indeed, the alkylation of amide **7c** resulted in a significant increase in conversion with respect to the corresponding Evans' amide **7b**, but the isolated yield did not exceed 49%. The absolute configuration of alkylated imidazolinone **13** was assigned in analogy to the results of Cardillo et al.[21] Amide **13** was then treated with $AlMe_3$ and HN(Me)OMe·HCl to give the corresponding Weinreb amide **14**, which was smoothly converted to methyl ketone **15** (MeLi·LiBr, CH_2Cl_2).

After completion of these initial studies, Danishefsky et al. reported the alkylation of a TES-protected glycolic acid-derived Evans' amide using an allylic iodide and LiHMDS as base.[22] However, at that moment, our other two approaches towards **6** were more promising and we decided not to pursue this route further.

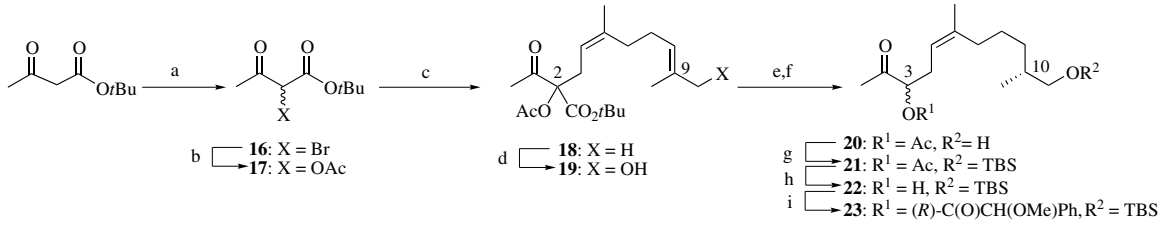
The second approach made use of our recently developed racemic acyloin synthesis[1] to obtain C3-racemic epothilone northern half precursors **9**.[2] Commercially available *tert*-butyl acetoacetate was subjected to bromination and subsequent displacement of the bromide by acetate to give *tert*-butyl 2-acetoxyacetoacetate **17**, which was subsequently alkylated (NaH, neryl bromide **8**, DMF) to give **18** (Scheme 4).[1] A SeO_2-oxidation can be used to introduce the hydroxy group at C7.[2] Catalytic regioselective hydrogenation, for example, with Ru[(*R*)-BINAP](OAc)$_2$, gives C9/C10-reduced intermediates with low (*R*)-preferential enantioselectivity at C10.[23] Selective de-alkoxycarbonylation of the *tert*-butyl ester gave C3-racemic, acetate mono-protected diol **20**; TBS protection afforded **21**, and mild hydrolysis (K_2CO_3, MeOH) resulted in the other mono-protected diol **22**. All the substrates for the enzymatic kinetic resolution (v.i.). For the chemical resolution, C3-alcohol **22** was reesterified with (*R*)-α-methoxyphenylacetic acid. The resulting diastereomers **23a** and **23b** were easily separable by normal flash chromatography on silica. In contrast to the acyloin acetates, the mandelates require much longer hydrolysis times. Under basic conditions, this leads to increased or even complete racemization of acyloins. Fortunately, mandelate can often serve as



Scheme 2. Strategies towards enantiomerically pure acyloin **6** derived from neryl derivatives as northern half precursor in the total synthesis of epothilones B/D (PG = Protective Group or H, TBS = *tert*-butyldimethylsilyl, Aux* = chiral auxiliary).



Scheme 3. (a) LDA, neryl bromide **8**, THF, −63 °C → rt: no yield with **7a**, 20% with **7b**, 49% with **7c**; (b) $AlMe_3$, HN(Me)OMe·HCl, CH_2Cl_2, 75%; (c) MeLi·LiBr, CH_2Cl_2, quant.



Scheme 4. (a) NBS, acetone, quant.; (b) NaOAc, DMF, 68%; (c) NaH, neryl bromide, DMF, 96%; (d) cat. SeO_2, *t*-BuOOH, CH_2Cl_2, 45%; (e) 100 bar H_2, cat. Ru[(*R*)-BINAP](OAc)$_2$, MeOH/H_2O, 89% (9*R*, ee = 21%); (f) TFA, CH_2Cl_2, 1 h, 75%; (g) TBSCl, Et_3N, cat. DMAP, CH_2Cl_2, 80%; (h) K_2CO_3, MeOH, 97%; or hydrolase (see Table 1) (i) (*R*)-α-methoxyphenylacetic acid, EDCI, cat. DMAP, CH_2Cl_2, 80%.

a protecting group instead of acetate in further steps. Substitution of the *O*-methyl-mandelic acid by THP-protected mandelate is possible, whereas (1*S*)-camphanic acid did not allow diastereomeric separation on silica.

The third approach was based on the enzyme-catalyzed resolution of **21** and alcohol **20**, protected and not protected at the primary hydroxy group, respectively. Such an approach would not be confronted with additional transesterification steps nor with basic racemization upon hydrolysis. We have already demonstrated[1] that related simple acyloin acetates can be efficiently resolved using lipase or esterase catalysis with high enantioselectivity to give (*S*)-acyloins as hydrolysis products (Schemes 3 and 4).

Here, we used a broader set of lipases and esterases and investigated their use for the resolution of these two acyloin acetates using an aqueous biphasic system composed of phosphate buffer/toluene (Table 1). Most importantly, a biocatalytic system for the selective hydrolysis with inverse stereopreference appeared desirable, that is, a preferential hydrolysis to (3*R*)-acyloins, because the remaining (3*S*)-acyloin acetates are the ideal protected building blocks for further synthetic steps towards the epothilones.[32]

Acyloin acetate **20** could be resolved with high selectivity using lipase from *Burkholderia cepacia* (Amano PS), E >300, lipase from *Pseudomonas* sp. (E >150) and a recombinant esterase from *Streptomyces diastatochromogenes* (E >100), affording the corresponding (3*S*)-diol in excellent ee (>99%). Interestingly, commercial and recombinant pig liver esterase exhibited the desired inverse enantiopreference compared to the other enzymes, hydrolyzing (3*R*)-**20** preferentially, thus leaving acetate protected (3*S*)-epothilone building block (3*S*)-**20** for further synthesis. Furthermore, the E-value increased from E = 8 (commercial PLE) to E = 50 for the recombinant enzyme. This may be due to the presence of a single isoenzyme for the recombinant enzyme, which has already been shown to have a strong effect on enantioselectivity and preference.[24,25] Similar enantioselectivities were determined in the resolution of the TBS-protected derivative **21** (E > 200 for BCL and PSL).

Table 1. Results of the hydrolase screening for the resolution of **20** (left columns) and **21** (right columns) via hydrolysis in a phosphate buffer/toluene biphasic system

Enzyme[a]	Time (h)	Ee_S (%) **20**	Ee_P (%)	cv (%)	E[b]	Time (h)	Ee_S (%) **21**	Ee_P (%) **22**	cv (%)	E[b]
BCL	24	>98	>99 (*S*)	50	≫300	24	37	>99 (*S*)	27	>200
PSL	2	>99	>99 (*S*)	50	150	24	87	>99 (*S*)	46	>200
PFL	4	>99	>99 (*S*)	50	>100	24	3	>99 (*S*)	3	>20
CAL-B	24	>99	>99 (*S*)	50	50					
PLE[c]	2	55	>99 (*R*)	35	8					
rPLE[c]	24	33	>99 (*R*)	25	50					
PFE I	2	15	48 (*S*)	24	15					
PFE II	24	<3	<1	75	>1					
SDE	4	>99	>99 (*S*)	50	>100					

[a] For abbreviations of enzymes see Experimental section; cv=conversion.
[b] Calculated according to Chen et al.[26]
[c] Inversed stereopreference.

3. Conclusions

We have demonstrated that the northern half of epothilones can be best obtained in a chemoenzymatic synthesis. Although the asymmetric synthesis using Evans' auxiliary or separation of diastereomers also affords the target compound, the lipase-catalyzed resolution allows the fastest access and highest overall yields. Asymmetric synthesis proved complicated, low yielding and demanded many expensive steps. Chemical resolution was easy to scale-up, but required additional transesterification, chromatographic separation, and if free acyloin was required, a hydrolysis step, which is prone to racemization. Enzymatic resolution emerged as the most straightforward method, and scale-up using pH-titration should be easy. Furthermore, enantioselectivities were outstanding, the biocatalysts are readily available, and each enantiomer can be obtained directly by choosing either an (*S*)- or (*R*)-selective hydrolase. An increase in the overall yield by a dynamic kinetic resolution with racemization of the nondesired enantiomer is currently under study.

4. Experimental

4.1. General

All commercial reagents were purchased from Fluka, Merck, Aldrich, Acros, Strem, Sigma and Lancaster, and used without further purification, unless otherwise stated. All oxygen- and water-sensitive reactions were carried out in oven-dried glassware under argon. THF was distilled from potassium/benzophenone ketyl, Et_2O was distilled from sodium/potassium/benzophenone ketyl, and CH_2Cl_2 was distilled from CaH_2. Other dry solvents were purchased from Fluka. Flash chromatography was performed using silica gel 60 (230–400 mesh, Merck). Thin-layer chromatography (TLC) was performed using silica plates (Merck, silica gel 60 F_{254}) and developed using Cer-MOP reagent [molybdatophosphoric acid (5.0 g), cerium (IV) sulfate (2.0 g), and concentrated H_2SO_4 (16 mL) in water (200 mL)]. Optical rotations were measured using a 1 mL cell with 1 dm path length on a Perkin–Elmer 241MC polarimeter. IR spectra were recorded as $CHCl_3$ solutions or as thin films between NaCl plates on a Bruker IFS 28. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on either Bruker ARX 200, ARX 250 and ARX 400 or Varian Mercury VX 300 and VX 400 spectrometers using TMS as the internal standard. Chemical shifts δ are reported in parts per million (ppm) and coupling constants J given in hertz (Hz). Mass spectrometry was performed on a Finnigan MAT-90 mass spectrometer operating at an ionization potential of 70 eV. High resolution mass spectra were obtained from a Finnigan MAT-90 mass spectrometer with isobutene as the ionization gas.

Derivatizations of alcohols for GC were done by exposing an ethyl ether solution of the analyte for 10 min with an excess of trifluoracetic anhydride, followed by removal of the reagent and solvent in a stream of dry nitrogen.

Please note that in this section IUPAC-numbering and not epothilone numbering is used.

4.1.1. (4*S*)-4-Benzyl-3-(*tert*-butyldimethylsilyloxyacetyl)-oxazolidin-2-one 7a. (*tert*-Butyldimethylsilyloxy)acetic acid (1.05 g, 5.0 mmol) was dissolved in benzene (20 mL) and 5 mL of benzene distilled off to remove water. The mixture was allowed to cool to room temperature and oxalyl chloride (0.73 mL, 1.065 g, 8.4 mmol) then added dropwise. After stirring for 30 min at room temperature, the mixture was heated to reflux for 30 min. Subsequently, excess oxalyl chloride was distilled off with half of the benzene. 0.50 mL of *n*-butyllithium (10 M in hexane, 5.0 mmol) was added to a solution of (4*S*)-4-benzyloxazolidin-2-one (886 mg, 5.0 mmol) in THF (25 mL) at −63 °C. After stirring for 5 min at −63 °C, the benzene solution of (*tert*-butyldimethylsilyloxy)acetyl chloride was slowly added and the mixture stirred for an additional 30 min. After the mixture was allowed to warm to room temperature, H_2O (10 mL) was added and the organic solvent removed in vacuo. The mixture was then extracted with CH_2Cl_2 (2 × 25 mL). The combined organic layers were subsequently washed with 0.5 M HCl (20 mL), saturated $NaHCO_3$ solution (20 mL) and brine (20 mL) and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo to yield (4*S*)-4-benzyl-3-(*tert*-butyldimethylsilyloxyacetyl)-oxazolidin-2-one **7a** as a yellow oil. Yield 1.60 g (4.58 mmol, 92%). ^1H NMR (200 MHz, $CDCl_3$, TMS): $\delta = 0.12$ (s, 6H), 0.93 (s, 9H), 2.76 (dd, 1H), 3.31 (dd, 1H), 4.21 (s, 2H), 4.65 (m, 1H), 4.81 (s, 2H), 7.16–7.35 (m, 5H) ppm.

4.1.2. (4*S*)-4-Benzyl-3-(benzyloxyacetyl)oxazolidin-2-one 7b. *n*-Butyllithium (2.71 mL, 10 M in hexane, 27.1 mmol) was added to a solution of (4*S*)-4-benzyloxazolidin-2-one (4.78 g, 27.1 mmol) in THF (100 mL) at −63 °C. After stirring the mixture at −78 °C for 10 min, benzyloxyacetyl chloride (3.0 g, 16.0 mmol) was added and the mixture stirred at −63 °C for an additional 1.5 h. The mixture was allowed to warm to room temperature and then poured into brine (500 mL) and extracted with CH_2Cl_2 (4 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo to yield a yellow oil. The crude product was purified by flash chromatography (column dimensions: 30 × 3 cm, EtOAc/petroleum ether = 1:1) to obtain (4*S*)-4-benzyl-3-(benzyloxyacetyl)oxazolidin-2-one as a colourless solid. Yield 4.93 g (15.1 mmol, 95%). ^1H NMR (200 MHz, $CDCl_3$, TMS): $\delta = 2.80$ (dd, 1H), 3.29 (dd, 1H), 4.19 (m, 2H), 4.64 (m, 1H), 4.68 (s, 2H), 4.70 (s, 2H), 7.17–7.43 (m, 10H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, TMS): $\delta = 37.46$, 54.52, 67.14, 69.59, 73.28, 127.26–129.33 (10C), 134.96, 137.25, 153.29, 169.97 ppm.

4.1.3. (4*S*,5*R*)-1-Benzyloxyacetyl-3,4-dimethyl-5-phenyl-imidazolidin-2-one 7c. Compound **7c** was synthesized according to Refs. 20 and 21.

4.1.4. *Z*-(4*S*,5*R*,2′*S*)-3,4-Dimethyl-5-phenyl-1-(2′-benzyloxy-5′,9′-dimethyldeca-4′,8′-dienoyl)-imidazolidin-2-one 13. A solution of diisopropylamine (2.65 mL, 1.90 g,

18.8 mmol) in THF (25 mL) was cooled to 0 °C and *n*-butyl-lithium (1.88 mL, 10 M in hexane, 18.8 mmol) added. The resulting LDA solution was slowly added at −63 °C to a solution of (4*S*,5*R*)-3,4-dimethyl-5-phenyl-1-(benzyloxyacetyl)-imidazolidin-2-one **7c** (5.30 g, 15.7 mmol) in THF (25 mL). After stirring the mixture at −63 °C for 1 h, neryl bromide (3.40 g, 15.7 mmol) was added. After stirring for an additional 3 h, the mixture was allowed to warm to room temperature and stirred for 15 min at room temperature. After addition of saturated NH_4Cl solution (25 mL), the organic solvent was removed in vacuo and H_2O (25 mL) added. The mixture was extracted with Et_2O (3 × 75 mL) and the combined organic layers dried over Na_2SO_4. After filtration, the solvent was removed in vacuo to give a yellow oil. The crude product was purified by flash chromatography (column dimensions: 30 × 3 cm, EtOAc/petroleum ether = 1:2) to obtain (4*S*,5*R*,2′*S*)-3,4-dimethyl-5-phenyl-1-(2′-benzyloxy-5′,9′-dimethyldeca-4′(*Z*),8′-dienoyl)-imidazolidin-2-one **13** as a yellow oil. Yield 3.64 g (7.67 mmol, 49%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ = 0.79 (d, 3H), 1.56 (s, 3H), 1.65 (s, 6H), 1.98 (s, 4H), 2.45 (m, 2H), 2.80 (s, 3H), 3.84 (m, 1H), 4.54 (q, 2H), 5.08–5.35 (m, 4H), 7.09–7.37 (m, 10H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ = 14.88, 17.48, 23.34, 25.56, 26.26, 28.00, 31.61, 31.94, 53.99, 58.76, 72.00, 77.43, 119.72, 124.30, 126.88–128.21 (10C), 131.07, 136.22, 137.60, 138.22, 155.08, 171.85 ppm.

4.1.5. *Z*-(2*S*)-2-Benzyloxy-*N*-methoxy-*N*,5,9-trimethyldeca-4,8-dienoylamide 14. Trimethyl aluminium (4.50 mL, 2 M in toluene, 9.00 mmol) was added at 0 °C to a suspension of *N*,*O*-dimethyl hydroxylamine hydrochloride (878 mg, 9.00 mmol) in CH_2Cl_2 (12 mL). The mixture was stirred at room temperature for 15 min, then recooled to −10 °C and a solution of *Z*-(4*S*,5*R*,2′*S*)-3,4-dimethyl-5-phenyl-1-(2′-benzyloxy-5′,9′-dimethyldeca-4′,8′-dienoyl)imidazolidin-2-one **13** (1.42 g, 3.00 mmol) in CH_2Cl_2 (12 mL) added. After the mixture was stirred at −10 °C for 1 h, at 0 °C for 2 h and at room temperature for 0.5 h, it was poured into a mixture of 0.5 N HCl (100 mL) and CH_2Cl_2 (50 mL). After shaking the mixture vigorously for 5 min, the organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (2 × 25 mL). The combined organic layers were subsequently washed with 0.5 M HCl (60 mL) and 1 M phosphate buffer (60 mL, pH = 7.0) and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo. The crude product was purified by flash chromatography (column dimensions: 30 × 2 cm, EtOAc/petroleum ether = 1:2) to obtain *Z*-(2*S*)-2-benzyloxy-*N*-methoxy-*N*,5,9-trimethyldeca-4,8-dienoylamide **14** as a colourless oil. Yield: 779 mg (2.25 mmol, 75%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ = 1.58 (s, 3H), 1.66 (s, 3H), 1.71 (s, 3H), 2.03 (s, 4H), 2.46 (t, 2H), 3.20 (s, 3H), 3.55 (s, 3H), 4.27 (t, 1H), 4.55 (q, 2H), 5.08 (s, 1H), 5.23 (t, 1H), 7.16–7.36 (m, 5H) ppm.

4.1.6. *Z*-(3*S*)-3-Benzyloxy-6,10-dimethylundeca-5,9-dien-2-one 15. A methyllithium lithium bromide complex (1.87 mL, 1.5 M in THF, 2.80 mmol) was added at −63 °C to a solution of *Z*-(2*S*)-2-benzyloxy-*N*-methoxy-*N*,5,9-trimethyldeca-4,8-dienoylamide **14** (345 mg, 1.00 mmol) in CH_2Cl_2 (15 mL). After the mixture was stirred for 40 min at −63 °C, the reaction was quenched by cannula transfer to a precooled (0 °C) and rapidly stirred mixture of saturated NH_4Cl solution (20 mL) and 3:1 hexane/CH_2Cl_2 (20 mL). The mixture was allowed to warm to room temperature and then diluted with brine (50 mL) and 3:1 hexane/CH_2Cl_2 (50 mL). After shaking the mixture vigorously, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (30 mL). The combined organic layers were washed with brine (150 mL) and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo to yield pure *Z*-(3*S*)-3-benzyloxy-6,10-dimethylundeca-5,9-dien-2-one **15** as a yellow oil. Yield: 308 mg (1.03 mmol, quantitative). ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ = 1.59 (s, 3H), 1.67 (s, 3H), 1.70 (s, 3H), 2.02 (s, 4H), 2.17 (s, 3H), 2.42 (t, 2H), 3.77 (t, 1H), 4.52 (q, 2H), 4.78–4.98 (m, 2H), 7.29–7.34 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ = 17.61, 23.44, 25.64, 25.70, 26.41, 30.55, 32.03, 72.21, 85.01, 119.03, 124.07, 127.75, 127.81, 128.38, 131.55, 137.60, 138.25. MS (CI, ammonia), *m*/*z* (%): 301 (13), $[M+H]^+$; 318 (100), $[M+NH_4]^+$. $[\alpha]_D^{25} = -18.0$ (*c* 1.0, $CHCl_3$).

4.1.7. *tert*-Butyl-2-bromoacetoacetate 16 and *tert*-butyl-2-acetoxyacetoacetate 17. Compounds **16** and **17** were synthesized according to Ref. 1.

4.1.8. *Z*-2-Acetoxy-2-acetyl-5,9-dimethyl-deca-4,8-dienoic-acid-*tert*-butylester 18. *tert*-Butyl-2-acetoxyacetoacetate **17** (19.5 g, 90 mmol) was added dropwise to a stirred suspension of NaH (2.59 g, 108 mmol) in DMF (180 mL) at 0 °C. After liberation of the hydrogen gas had stopped, neryl bromide (19.6 g, 90 mmol) was added dropwise at 0 °C. Afterwards, the ice bath was removed and the mixture stirred at room temperature for 16 h. The mixture was then diluted with ether (750 mL) and washed with water (3 × 200 mL) as well as with brine (1 × 200 mL). The solution was dried over Na_2SO_4, filtered and concentrated in vacuo, to give a slightly yellow oil. Yield 30.3 g (86 mmol, 96%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ = 1.46 (s, 9H), 1.60 (s, 3H), 1.68 (s, 3H), 1.70 (s, 3H), 2.00–2.05 (m, 4H), 2.16 (s, 3H), 2.31 (s, 3H), 2.82–2.87 (m, 2H), 5.00–5.09 (m, 2H) ppm.^{13} C NMR (50 MHz, $CDCl_3$, TMS): δ = 17.56, 20.60, 23.52, 25.61, 26.32, 26.95, 27.63, 31.90, 32.30, 83.08, 87.83, 116.22, 123.75, 131.84, 140.07, 165.96, 169.53, 201.06 ppm. IR (thin film): 845 (w), 1024 (w), 1072 (w), 1086 (w), 1115 (w), 1157 (s), 1236 (s), 1256 (s), 1314 (w), 1370 (s), 13895 (w), 1437 (w), 1452 (w), 1746 (s), 2861 (w), 2882 (w), 2932 (m), 2976 (m) cm^{-1}. MS (CI, isobut.): *m*/*z* (%) = 353 (13) $[M+H]^+$, 298 (21), 297 (100), 279 (14), 255 (10), 253 (13), 237 (27), 219 (20), 209 (65), 193 (10), 175 (6), 153 (7), 137 (16). HRMS: calculated for $C_{17}H_{31}O_5$ (MH^+): 353.23282; found: 353.23245.

4.1.9. (4*Z*,8*E*)-2-Acetoxy-2-acetyl-5,9-dimethyl-10-hydroxy-deca-4,8-dienoic-acid-*tert*-butylester 19. Powdered selenium dioxide (0.158 g, 1.42 mmol) was suspended in CH_2Cl_2 (50 mL), and a 70% aq *tert*-butyl hydroperoxide solution (10.2 g, 79.5 mmol) was added and stirred at room temperature for 30 min. Then 10.0 g (28.4 mmol)

tert-butyl (4*Z*)-*2*-acetoxy-2-acetyl-5,9-dimethyldeca-4,8-dienoate **18** were added and the reaction mixture stirred at room temperature for 48 h. After concentration in vacuo, 50 mL of toluene were added and removed in vacuo (removal of excess *tert*-butyl hydroperoxide). This was repeated three times and the obtained slightly yellow oil separated by flash chromatography (column dimensions: 5.0×19.5 cm, ethyl acetate/petroleum ether = 1:2). Yield: 4.65 g (12.6 mmol, 44%) colourless oil. ^{1}H NMR (250 MHz, $CDCl_3$, TMS): $\delta = 1.45$ (s, 9H), 1.66 (s, 3H), 1.71 (s, 3H), 1.90–2.15 (m, 4H), 2.15 (s, 3H), 2.31 (s, 3H), 2.85–2.88 (m, 2H), 3.99 (s, 2H), 5.02 (m, 1H), 5.38 (m, 1H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$, TMS): $\delta = 13.62$, 20.69, 23.47, 25.78, 27.03, 27.74, 31.62, 32.36, 68.71, 83.29, 87.97, 116.65, 124.95, 135.42, 139.69, 166.01, 169.60, 201.41 ppm. IR (thin film): 755 (w), 845 (w), 1018 (w), 1049 (w), 1072 (w), 1157 (m), 1236 (m), 1258 (m), 1371 (m), 1395 (w), 1435 (w), 1456 (w), 1742 (s), 2854 (w), 2934 (w), 2978 (w) cm^{-1}. MS (CI, isobut.): *m/z* (%) = 369 (6) $[M+H]^+$, 329 (6), 311 (26), 295 (100), 271 (11), 253 (24), 235 (14), 203 (10), 169 (9), 135 (10). HRMS: calculated for $C_{20}H_{33}O_6$ (MH^+): 369.22772; found: 369.22880.

4.1.10. *Z*-(9*R*)-2-Acetoxy-2-acetyl-5,9-dimethyl-10-hydroxy-deca-4-enoic acid *tert*-butyl ester 24. 7.94 g (21.6 mmol) (4*Z*)-2-acetoxy-2-acetyl-5,9-dimethyl-10-hydroxydeca-4,8-dienoic acid *tert*-butyl ester **19** was dissolved in 15.0 mL absolute methanol and 750 μL demineralized water added. The solution was degassed with three freeze-thaw-cycles before 185 mg Ru[(*R*)-BINAP](OAc)$_2$ were added and put in an autoclave under nitrogen atmosphere together with a magnetic stirring bar. After threefold purging with hydrogen (99.999%, water 5 vpm, oxygen 2 vpm), the autoclave was set under a pressure of 100 bar hydrogen and stirred at room temperature for 25 h. The hydrogen pressure was released and the solution concentrated in vacuo. The obtained brown oil was purified by flash chromatography (column dimensions: 4.5×25.0 cm, ethyl acetate/petroleum ether = 1:2). Yield: 7.08 g (19.1 mmol, 88%) colourless oil. The ee was determined after dealkoxycarbonylation to compound **20** (cf. next procedure) to be 21%. The racemic diastereomers behave like a single compound and show only one set of signals in NMR, that is, the stereocentres behave spectroscopically independent at the given resolution. ^{1}H NMR (250 MHz, $CDCl_3$, TMS): $\delta = 0.91$ (d, 3H, $J = 6.7$ Hz), 1.45 (s, 9H), 1.68 (s, 3H), 1.0–2.0 (m, 7H), 2.15 (s, 3H), 2.31 (s, 3H), 2.85 (m, 2H), 3.41 (dd, AB, $J_1 = 10.5$ Hz, J_2=6.3 Hz, 1H), 3.49 (dd, AB, $J_1 = 10.5$ Hz, $J_2 = 5.9$ Hz, 1H), 5.00 (m, 1H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$, TMS): $\delta = 16.59$, 20.70, 23.53, 25.20, 27.03, 27.73, 32.04, 32.29, 32.98, 35.71, 68.15, 83.23, 88.03, 116.21, 140.32, 165.99, 169.92, 201.36 ppm. IR (thin film): 756 (w), 845 (w), 1045 (m), 1065 (m), 1080 (m), 1157 (s), 1235 (s), 1258 (s), 1371 (s), 1395 (w), 1429 (w), 1456 (w), 1744 (s), 2874 (w), 2934 (m), 2972 (m) cm^1. MS (CI, isobut.): *m/z* (%) = 369 (6) $[M+H]^+$. HRMS: calculated for $C_{20}H_{33}O_6$ (MH^+): 371.2433; found: 371.242097. EA: calculated: C 64.84, H 9.25; found: C 64.46, H 9.36; $[\alpha]_D^{25} = +5.2$ (*c* 0.62, $CHCl_3$) at ee ≈21%.

4.1.11. (10*R*)-3-Acetoxy-11-hydroxy-6,10-dimethyl-5-undecen-2-one 20. 1.032 g (2.79 mmol) (9*R*)-(4*Z*)-*2*-acetoxy-2-acetyl-5,9-dimethyl-10-hydroxydeca-4-enoic acid *tert*-butyl ester **24** were dissolved in 28 mL CH_2Cl_2 and 2.80 mL TFA added. After stirring for 2 h at room temperature, all volatile matter was removed in vacuo and the remaining oil dissolved in 28 mL methanol. Then 5.6 mL saturated aqueous $NaHCO_3$ solution was added and the suspension stirred for 140 min at ambient temperature before dilution with 200 mL ether was performed. The organic layer was washed two times with 50 mL water as well as once with 50 mL brine. After drying over Na_2SO_4, filtration and removal of the solvents in vacuo, a slightly yellow oil was obtained. Purification was achieved by flash chromatography (column dimensions: 2.0×20.0 cm, ethyl acetate/petroleum ether = 2:3). Yield: 568 mg (2.10 mmol, 75%) colourless oil, ee = 21%. The enantiomeric excess at C10 was determined by chiral HPLC on a cyclobond I 2000 column (250×4.6 mm, 5 μL) with acetonitrile/0.2% triethylammonium acetate buffer. Diastereomers are not resolved in NMR at the given resolution. ^{1}H NMR (250 MHz, $CDCl_3$, TMS): $\delta = 0.92$ (d, 3H, $J = 6.6$ Hz), 1.00–1.20 (m, 1H), 1.30–1.50 (m, 3H), 1.60 (m, 1H), 1.70 (s, 3H), 1.73 (s, 1H, OH), 2.01 (m, 2H), 2.14 (s, 3H), 2.16 (s, 3H), 2.48 (m, 2H), 3.46 (m, 2H), 4.98 (m, 1H, C*H*OAc), 5.11 (m, 1H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$, TMS): $\delta = 16.57$, 20.69, 23.42, 25.20, 26.28, 29.06, 32.04, 33.02, 35.71, 68.19, 78.56, 117.90, 139.08, 170.59, 205.47 ppm. IR (thin film): 755 (w), 986 (w), 1047 (m), 1175 (w), 1242 (s), 1375 (m), 1435 (w), 1456 (w), 1730 (s), 1744 (s), 2872 (m), 2932 (s) cm^{-1}. $[\alpha]_D^{22} = +4.9$ (*c*, 1); MS (ESI–MS): *m/z* (%) = 563.3 (100) $[2M+Na]^+$, 293.0 (54) $[M+Na]^+$, 271.1 (7) $[M+H]^+$. EA: calculated, C 55.55, H 7.46; found: C 55.39, H 7.54.

4.1.12. *Z*-(10*R*)-3-Acetoxy-11-*tert*-butyldimethylsilyloxy-6,10-dimethyl-5-undecen-2-one 21. 528 mg (1.95 mmol) (10*R*)-3-Acetoxy-11-hydroxy-6,10-dimethyl-5-undecen-2-one **20** were dissolved in 10.0 mL absolute CH_2Cl_2. After addition of 541 μL (395 mg, 3.90 mmol), triethylamine and 12 mg (0.10 mmol) of DMAP, the solution was cooled with an ice bath. After stirring for 5 min 368 mg (2.44 mmol) of TBSCl were added at once and the resulting solution stirred at 0 °C for 2 h and additional 14 h at room temperature. The resulting colourless suspension was again cooled to 0 °C before 460 μL of methanol were added. After stirring for 30 min at 0 °C, all solvents were removed in vacuo. Ether (15 mL) as well as 15 mL saturated aqueous NH_4Cl solution were then added and after intensive stirring and separation, the aqueous phase extracted twice with 10 mL ether. The combined organic layers were washed with 15 mL brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The obtained oil was purified by means of flash chromatography (column dimensions: 2.0×20.0 cm, ethyl acetate/petroleum ether = 1:10). Yield: 597 mg (1.55 mmol, 80%) colourless oil. The diastereomers are not resolved in NMR at the given resolution. ^{1}H NMR (250 MHz, $CDCl_3$, TMS): $\delta = 0.04$ (s, 6H), 0.87 (d, $J = 6.7$ Hz, 3H), 0.89 (s, 9H), 1.00–1.20 (m, 1H), 1.30–1.50 (m, 3H), 1.52–1.60 (m, 1H), 1.70 (s, 3H), 1.97–2.03 (m, 2H), 2.13 (s, 3H), 2.15 (s, 3H),

2.44–2.50 (m, 2H), 3.37 (dd, $J = 9.7$ Hz, $J = 6.4$ Hz, 1H), 3.44 (dd, $J = 9.7$ Hz, $J = 6.0$ Hz, 1H), 4.98 (m, 1H), 5.09 (m, 1H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$, TMS): $\delta = -5.34$, 16.72, 18.36, 20.68, 23.45, 25.30, 25.97, 26.59, 29.02, 32.16, 33.11, 35.74, 68.28, 78.57, 117.81, 138.89, 170.52, 205.16 ppm. IR (thin film): 775 (m), 837 (m), 1053 (w), 1092 (m), 1248 (s), 1373 (w), 1458 (w), 1472 (w), 1734 (s), 1748 (s), 2857 (m), 2891 (w), 2905 (w), 2932 (m), 2955 (m) cm^{-1}. MS (CI, isobut.): *m/z* (%) = 385 (13) $[M+H]^+$, 327 (13), 267 (26), 253 (6), 193 (40), 175 (62), 117 (100). HRMS: calculated for $C_{21}H_{41}O_4Si$ (MH^+): 385.27740; found: 385.278524. $[\alpha]_D^{25} = +0.0$ (*c* 0.33, $CHCl_3$).

4.1.13. *Z*-(10*R*)-11-(*tert*-Butyldimethylsilyloxy)-3-hydroxy-6,10-dimethyl-undec-5-en-2-one 22. 1.943 g (5.05 mmol) (10*R*)-3-Acetoxy-11-(*tert*-butyl-dimethylsilyloxy)-6,10-dimethyl-5-undecen-2-one **21**, ee = 21%, were dissolved in 20.0 mL methanol and 400 mL of a saturated potassium carbonate solution then added. After stirring at ambient temperature for 5–14 min, 30 mL brine were added and fivefold extraction with 30 mL diethyl ether followed. The hydrolysis was already completed after 5 min according to TLC. A timely work-up is crucial, because the yield drops drastically if the reaction is not stopped in time. For example, stirring for 90 min resulted in dramatic drop of the yield to 43%. The combined organic layers were washed with 50 mL brine and dried over Na_2SO_4. After filtration and removal of the solvent in vacuo, the remaining oil was purified by flash chromatography (column dimensions: 2.0×20.0 cm, ethyl acetate/petroleum ether = 1:4). Yield: 1.686 g (4.92 mmol, 97%). The diastereomers were not resolved in NMR at the given resolution. ^{1}H NMR (250 MHz, $CDCl_3$, TMS): $\delta = 0.03$ (s, 6H, TBS), 0.83 (d, $J = 6.8$ Hz, 3H), 0.89 (s, 9H, TBS), 1.00–1.65 (m, 5H), 1.69 (s, 3H), 2.00 (m, 2H), 2.12 (s, 3H), 2.25–2.45 (m, 1H), 2.45–2.60 (m, 1H), 3.40 (m, 2H), 4.20 (m, 1H), 5.10 (m, 1H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$, TMS): $\delta = 5.43$, 16.63, 18.27, 23.41, 25.21, 25.38, 25.88, 32.02, 32.22, 33.04, 35.61, 68.18, 76.73, 118.07, 139.59, 209.55 ppm. IR (thin film): 667 (w), 775 (m), 837 (s), 1006 (w), 1093 (s), 1147 (w), 1163 (w), 1249 (s), 1362 (m), 1373 (m), 1387 (w), 1419 (w), 1436 (w), 1457 (m), 1464 (m), 1472 (m), 1653 (w), 1684 (w), 1700 (w), 1733 (s), 1747 (s), 2855 (m), 2881 (w), 2902 (m), 2907 (m), 2929 (m), 2950 (m), 2956 (m) cm^{-1}. $[\alpha]_D^{25} = +0.0$ (*c* 0.62, $CHCl_3$).

4.1.14. (*R*)-α-Methoxyphenylacetic acid *Z*-(1*R*,8*R*)-1-acetyl-9-(*tert*-butyldimethylsilyloxy)-4,8-dimethylnon-3-enyl ester 23a and (*R*)-α-methoxyphenylacetic acid *Z*-(1*S*,8*R*)-1-acetyl-9-(*tert*-butyldimethylsilyloxy)-4,8-dimethylnon-3-enyl ester 23b. To a solution of 2.413 g (7.04 mmol) (10*R*)-11-(*tert*-butyldimethylsilyloxy)-3-hydroxy-6,10-dimethyl-undec-5-en-2-one **22** of ee = 21%, 1.287 g (7.75 mmol) (*R*)-α-methoxyphenylacetic acid and 86 mg (0.70 mmol) of DMAP in 72.0 mL CH_2Cl_2, were added 2.701 g (14.09 mmol) of EDCI and the solution stirred 1 h 40 min at ambient temperature. Diethyl ether (250 mL) was then added and the resulting suspension extracted twice with 100 mL water as well as twice with 100 mL brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated in vacuo. The remaining yellow oil contained two diastereomeric esters*, which were separated by flash chromatography (column dimensions: 3.5×20.0 cm, diethyl ether/petroleum ether = 2:9). Total yield 2.796 g (5.697 mmol, 81%). The absolute configuration at C1 was assigned by NMR according to Raban and Mislow,[30] and in addition according to Riguera et al.,[31] with identical results.

Fraction one: (*R*)-α-methoxyphenylacetic acid 3*Z*-(1*R*,8*R**)-1-acetyl-9-(*tert*-butyldimethylsilyloxy)-4,8-dimethyl-non-3-enyl ester **23a**. Yield: 1.33 g (2.710 mmol, 38%). R_f value: 0.31 (diethyl ether/petroleum ether = 1:4). ^{1}H NMR (250 MHz, $CDCl_3$, TMS): $\delta = 0.05$ (s, 6H), 0.89 (d, 3H, $J = 6.6$ Hz), 0.91 (s, 9H), 1.00–1.65 (m, 5H), 1.68 (s, 3H), 1.82 (s, 3H), 1.99 (m, 2H), 2.50 (m, 2H), 3.35–3.46 (m, 2H), 3.47 (s, 3H), 4.84 (s, 1H), 5.00–5.09 (m, 2H), 7.37–7.51 (m, 5H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$, TMS): $\delta = -5.38$, 16.68, 18.33, 23.42, 25.26, 25.93, 26.07, 29.11, 32.13, 33.07, 35.68, 57.42, 68.26, 78.98, 82.44, 117.64, 127.32, 128.63, 128.93, 135.82, 139.91, 170.08, 204.99 ppm. IR (thin film): 665 (w), 697 (w), 732 (w), 757 (w), 776 (m), 814 (w), 837 (s), 916 (w), 939 (w), 1006 (w), 1033 (w), 1043 (w), 1097 (s), 1114 (s), 1170 (m), 1199 (m), 1254 (s), 1321 (w), 1360 (m), 1388 (w), 1459 (m), 1469 (m), 1729 (s), 1757 (s), 2855 (m), 2929 (s), 2952 (s) cm^{-1}.

Fraction two: (*R*)-α-methoxyphenylacetic acid 3*Z*-(1*S*,8*R**)-1-acetyl-9-(*tert*-butyldimethylsilyloxy)-4,8-dimethyl-non-3-enyl ester **23b**. Yield: 1.466 g (3.182 mmol, 42%). R_f value: 0.22 (diethyl ether/petroleum ether = 1:4). ^{1}H NMR (250 MHz, $CDCl_3$, TMS): $\delta = 0.04$ (s, 6H), 0.87 (d, 3H, $J = 6.7$ Hz), 0.91 (s, 9H), 0.95–1.75 (m, 5H), 1.57 (s, 3H), 1.75–2.05 (m, 2H), 2.11 (s, 3H), 2.30–2.50 (m, 2H), 3.37–3.51 (m, 2H), 3.47 (s, 3H), 4.84 (m, 1H), 4.90 (s, 1H), 5.00 (dd, 1H, $J = 7.6$ Hz, $J = 5.3$ Hz), 7.35–7.49 (m, 5H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$, TMS): $\delta = -5.38$, 16.67, 18.32, 23.31, 25.20, 25.93, 26.45, 28.83, 32.04, 33.05, 35.65, 57.47, 68.24, 79.04, 82.25, 117.42, 127.16, 128.58, 128.76, 135.98, 139.82, 170.31, 204.50 ppm. IR (thin film): 665 (w), 697 (w),733 (w), 756 (w), 776 (m), 814 (w), 837 (s), 916 (w), 939 (w), 1005 (w), 1032 (w), 1043 (w), 1098 (s), 1114 (s), 1171 (s), 1200 (m), 1254 (m), 1388 (m), 1459 (m), 1470 (m), 1731 (s), 1757 (s), 2855 (m), 2897 (m), 2929 (s), 2952 (s) cm^{-1}. $[\alpha]_D^{25} = -23.9$ (*c* 0.91, $CHCl_3$).

*C1/mandelic ester diastereomers. The additional stereocentre at C8 (de = 21%) had no influence on the chromatographic separation and on NMR at the given resolution.

4.2. Enzyme-catalyzed preparative scale resolutions of 21 and 20

All organic solvents were analytical grade or higher and dried overnight over activated molecular sieves (3 Å) before use. Commercial enzyme preparations were used as delivered. Lipases from the following organisms were used: *Candida antarctica* (Roche, Chirazyme L2, CAL-B),

Burkholderia cepacia (Amano PS, BCL). *Pseudomonas fluorescens* (Amano AK, PFL), *Pseudomonas* sp. (Chirazyme L6, PSL) and pig liver esterase (PLE, Sigma). Production of the recombinant esterases from *P. fluorescens* (PFE-I,[27] PFE-II[28]), *Streptomyces diastatochromogenes* (SDE[29]) by expression in *E. coli* has already been described. Recombinant pig liver esterase (rPLE) was produced by expression in the yeast *Pichia pastoris*.[24,25]

4.2.1. Method for the screening of suitable enzymes. Five milligrams hydrolase (lipase or esterase) were dissolved in 500 μL sodium phosphate buffer (pH 7.5, 50 mM) in 1.5 mL Eppendorf tubes and thermostated to 37 °C on a thermoshaker (Eppendorf, Hamburg, Germany). Reactions were initiated by the addition of 200 μL of a substrate solution (10 mg/mL acyloin acetate in toluene). Reaction mixtures were shaken at 900 rpm. For 24 h, samples of 20 μL from the organic phase were taken from each reaction and transferred to a new vial with 200 μL dichloromethane. The mixture was vortexed and centrifuged (2 min, 13,000 rpm) and the supernatant transferred to a new vial and dried over a small amount Na_2SO_4. After another centrifugation (2 min, 13,000 rpm), the organic phase was transferred to a new vial. Samples were analyzed by GC with a chiral column (Heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin, 25 m × 0.25 mm). For baseline separation of the enantiomers of alcohol **22**, analytical derivatization with trifluoro acetic acid anhydride was necessary (see general part).

4.2.2. Preparative enzymatic resolution of 20. Acyloin acetate **20** (479 mg, 1.248 mmol) was added to a biphasic mixture composed of 20 mL distilled water and 2 mL toluene at 37 °C. After addition of lipase CAL-B (98 mg), the reaction was stirred in a pH-stat (set to pH 7.5) and monitored by base consumption. The mixture was extracted three times with diethyl ether and dried over Na_2SO_4. After removal of excess solvent in vacuo, ester and alcohol were separated by column chromatography (hexane/diethyl ether = 10:1, 5:1). Yields: product (diol, >99% ee): 179 mg (42%), remaining substrate (acetate **20**, 46% ee): 186 mg (48%). Conv. 31%, E >200.

4.2.3. Preparative enzymatic resolution of 21. Acyloin acetate **21** (253 mg, 0.937 mmol) was added to a biphasic mixture composed of 30 mL phosphate buffer (pH 7.5, 50 mM) and 20 mL toluene at 37 °C. After addition of lipase Amano PS (270 mg), the reaction was stirred until GC analysis revealed that the desired optical purity and conversion was achieved. The enzyme was removed by centrifugation, filtrated and extracted twice with toluene (20 mL). The organic layer was dried over Na_2SO_4 and the excess solvent was removed in vacuum. Ester and alcohol were separated by column chromatography (hexane:ethyl acetate, 6:1). Yields: product (alcohol **22**, >99% ee): 59.7 mg (28%), remaining substrate (acetate **21**, 92% ee): 74.7 mg (27%). Conv. 48%, E >200.

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References

1. Scheid, G. O.; Kuit, W.; Ruijter, E.; Orru, R. V. A.; Henke, E.; Bornscheuer, U.; Wessjohann, L. A. *Eur. J. Org. Chem.* **2004**, 1063–1074.
2. Gabriel, T.; Wessjohann, L. A. *Tetrahedron Lett.* **1997**, *38*, 1363–1366; Gabriel, T.; Wessjohann, L. A. *Tetrahedron Lett.* **1997**, *38*, 4387–4388; Scheid, G. O.; Eichelberger, U.; Wessjohann, L. A. *Tetrahedron Lett.* submitted for publication.
3. Wessjohann, L. A.; Scheid, G. O. Deutsche Offenlegungsschrift DE0010051136A1 (18.04.2002), Germany, 16.10.2000, CA 136: 325358.
4. Wessjohann, L. A.; Scheid, G. O.; Bornscheuer, U.; Henke, E.; Kuit, W.; Orru, R. V. A. Deutsche Offenlegungsschrift DE0010134172A1 (23.01.2003), Germany, 13.07.2001.
5. Wessjohann, L. A.; Scheid, G. O.; Bornscheuer, U.; Henke, E.; Kuit, W.; Orru, R. V. A. Patent WO2002/032844A2 (25.04.2002), EP 200111992, International, 16.10.2001, CA 136:340534.
6. Gerth, K.; Bedorf, N.; Höfle, G.; Irschik, H.; Reichenbach, H. *J. Antibiotics* **1996**, *49*, 560–564.
7. Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed.* **1996**, *35*, 1567–1569.
8. Wessjohann, L. A.; Ruijter, E.; Garcia-Rivera, D.; Brandt, W. *Mol. Div.*, in press.
9. He, L.; Orr, G. A.; Horwitz, S. B. *Drug Discov. Today* **2001**, *6*, 1153–1164; Rudolf, E.; Červinka, M. *Curr. Med. Chem.—Anti-Cancer Agents* **2003**, *3*, 421–429.
10. Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325–2333.
11. Nicolaou, K. C.; Ninkovic, S.; Sarabia, F.; Vourloumis, D.; He, Y.; Vallberg, H.; Finlay, M. R. V.; Yang, Y. *J. Am. Chem. Soc.* **1997**, *119*, 7974–7991.
12. Balog, A.; Meng, D.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. *J. Angew. Chem.* **1996**, *108*, 2976–2978.
13. Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434–8456.
14. Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed.* **1998**, *37*, 2014–2045.
15. Mulzer, J. *Monatsh. Chem.* **2000**, *131*, 205–238.
16. Wessjohann, L. A. *Angew. Chem.* **1997**, *109*, 738–742; *Angew. Chem., Int. Ed.* **1997**, *36*, 715–718.
17. Wessjohann, L. A.; Scheid, G. O. In Schmalz, H.-G., Ed.; Organic Synthesis Highlights; Wiley-VCH: Weinheim, 2000; Vol. 4, pp 251–267.
18. Altmann, K.-H. *Curr. Opin. Chem. Biol.* **2001**, *5*, 424–431.
19. Bischofsberger, N.; Waldmann, H.; Saito, T.; Simon, E. S.; Lees, W.; Bednarski, M. D.; Whitesides, G. M. *J. Org. Chem.* **1988**, *53*, 3457–3465.
20. Close, W. J. *J. Org. Chem.* **1950**, *15*, 1131–1134.
21. Cardillo, G.; Orena, M.; Romero, M.; Sandri, S. *Tetrahedron* **1989**, *45*, 1501–1508.
22. Chapell, M. D.; Stachel, S. J.; Lee, C. B.; Danishefsky, S. *J. Org. Lett.* **2000**, *2*, 1633–1636.
23. Noyori, R. *Asymmetric Catalysis in Organic Synthesis*; John Wiley Sons: New York, 1993.
24. Musidlowska-Persson, A.; Bornscheuer, U. T. *Tetrahedron: Asymmetry* **2003**, *14*, 1341–1344.

25. Musidlowska, A.; Lange, S.; Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2001**, *40*, 2851–2853.
26. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
27. Krebsfänger, N.; Zocher, F.; Altenbuchner, J.; Bornscheuer, U. T. *Enzyme Microb. Technol.* **1998**, *21*, 641–646.
28. Khalameyzer, V.; Fischer, I.; Bornscheuer, U. T.; Altenbuchner, J. *Appl. Environm. Microbiol.* **1999**, *65*, 477–482.
29. Khalameyzer, V.; Bornscheuer, U. T. *Biotechnol. Lett.* **1999**, *21*, 101–104.
30. Raban, M.; Mislow, K. *Tetrahedron Lett.* **1965**, *48*, 4249–4253.
31. Latypov, S. K.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Am. Chem. Soc.* **1998**, *120*, 877–882.
32. For chemoenzymatic approaches to other epothilone fragments see: Shioji, K.; Kawaoka, H.; Miura, A.; Okuma, K. *Synth. Commun.* **2001**, *31*, 3569–3575; Zhu, B.; Panek, J. S. *Tetrahedron Lett.* **2000**, *41*, 1863–1866; Machajewski, T. D.; Wong, C.-H. *Synthesis* **1999**, 1469–1472; Bornscheuer, U. T.; Altenbuchner, J.; Meyer, H. H. *Biotechnol. Bioeng.* **1998**, *58*, 554–559.