Synthesis and Conformational Analysis of Macrocycles Related to 10-Oxa-epothilone

Delphine Quintard,[a] Philippe Bertrand,[a] Christian Bachmann,[b] and Jean-Pierre Gesson*[a]

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A short and convergent synthesis of macrocyclic lactones related to 10-oxa-epothilone is based on aldolisation of a 3-(2′-methylallyloxy) aldehyde derived from methyl (2*S*)-3-hydroxy-2-methylpropionate followed by ring-closing metathesis.

Introduction

Epothilones A (EpoA, **1**) and B (EpoB, **2**), isolated by Höfle from the myxobacterium *Sorangium cellulosum*,[1] are potent antitumour agents (Figure 1).[2−4] These compounds act by microtubule stabilization, being able to displace paclitaxel from its tubulin complex. They are also characterised by improved properties (high potency, activity against MDR cell lines, better water solubility) with respect to this widely used clinical drug. Their 16-membered macrocyclic structure and side chain are reminiscent of other microtubule stabilisers such as sarcodictyn A,[5] eleutherobin,[6] laulimalide[7] and peloruside A.[8] The ongoing interest in epothilones has prompted numerous synthetic studies,[9−12] a large number of analogues having already been prepared by several groups and interesting structure-activity relationships (SARs) pointed out: desoxy EpoB (EpoD, **3**), for example, shows a better therapeutic index and is now in phase I clinical trials,[13] the presence of a hydroxy group at C21 (EpoF) affords better water solubility associated with improved efficacy,[14] different side chains are allowed at C15,[15] and a lactam function may be present instead of the lactone.[16,17] At the beginning of these SAR studies, few attempts to modify the unfunctionalised C9-C11 part were made. Recently, though, (*E*)-10,11-dehydro EpoB (Epo490, **4**) (as well as its analogue in the EpoA series) has been isolated and shown to be highly cytotoxic,[18,19] together with synthetic (*E*)-9,10-dehydro-12,13-desoxy EpoB (**5a**).[20] On the other hand, (*Z*)-9,10-dehydro EpoB (**5b**), independently synthesized by White[21−23] and by Ermolenko and Potier,[24] appears to be much less active. Few attempts to modify the C1−C3 part have been made. Initial synthetic studies by Nicolaou on 2,3-dehydro epoA (**6a**) have shown that this compound retains tubulin affinity.[9] Recently, Vite[25] has demonstrated that 2,3-dehydro epoB (**6b**), prepared by dehydration of epoB (**2**),[26] is almost as active (IC_{50} 3.4 nM) as epoB (0.8 nM) against HCT-116 human colon carcinoma cells [**6a** being 10 times less potent than epoA (**1**) against the same cell line]. Moreover, the (3*R*)-cyano derivative **7** obtained by cyanide addition to **6b** is also very active against tubulin polymerisation and cancer cell lines in vitro, in contrast to the (3*S*) diastereoisomer.[23]

These results, together with those obtained by ring enlargement or ring contraction of the macrocycle in the epoA[27] or epoB series,[28] can be interpreted in terms of the different conformations, the crucial importance of which − particularly for the aldol moiety (vide infra) − has been elegantly demonstrated by Taylor.[29,30] The more stable conformer, in solution, for EpoA, EpoB and EpoD, deduced from computational studies (MM2 force field) and NOE measurements, is similar to the solid-state structure found by X-ray for EpoB.[1] Conformational flexibility of these macrocycles is important. however, and numerous 3D models of tubulin-bound epoA or B were proposed[31−38] before the very recent high-resolution NMR structure of epoA bound to tubulin.[39] This shows two main conformational switches between the 3D structures of free and tubulin-bound epoA: the C16−C17−C18−C19 dihedral angle changes from *anti*-periplanar to *syn*-periplanar, probably to allow hydrogen bonding of the nitrogen atom of the thiazole ring with the protein, and the C2−C3−C4−C5 dihedral angle changes from a *gauche*+ to a *gauche*− conformation to allow the C3 hydroxy group to point outwards from the macrocycle ring. No change occurs in the import-

[a] UMR 6514, Université de Poitiers et CNRS,
40, Avenue du Recteur Pineau, 86022 Poitiers, France
Fax: (internat.) + 33-5-49453501
E-mail: jean-pierre.gesson@univ-poitiers.fr
[b] UMR 6503, Université de Poitiers et CNRS,
40, Avenue du Recteur Pineau, 86022 Poitiers, France

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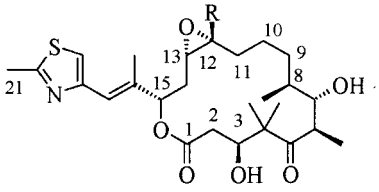
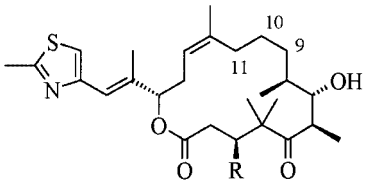
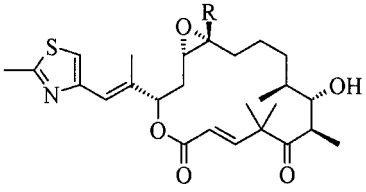


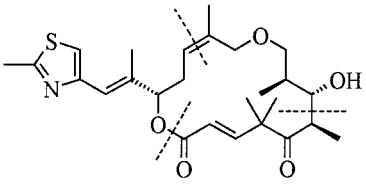
1: epothilone A, R = H
2: epothilone B, R = Me



3: R = OH, epothilone D
4: R = OH, epothilone 490, (*E*)-10,11-dehydro
5: R = OH, (*E*)- or (*Z*)-9,10-dehydro
7: R = CN



6a: R = H
6b: R = Me



8

Figure 1. Epothilone structures and target analogue **8**

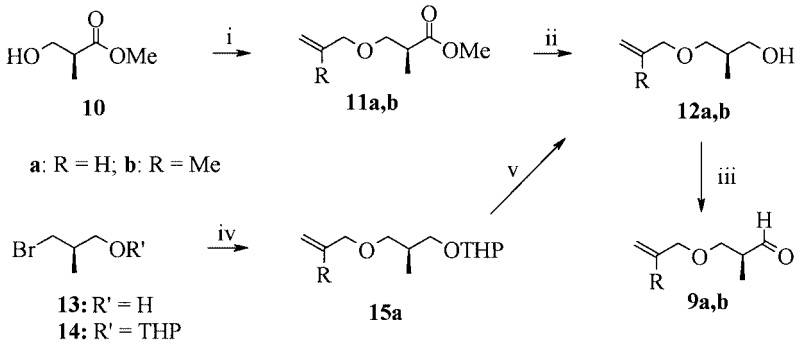
ant aldol part (C5−C8), in agreement with previous observations made by Taylor.

In a search for new epothilone analogues, it seems promising to replace one of the methylene groups at C9 or C10 by a heteroatom such as oxygen or sulfur (thereby further enhancing water solubility). Furthermore, a short route should be achievable if a C2−C3 double bond is present instead of the C3 hydroxy group, and this double bond should be amenable for further modifications to prepare a new set of analogues, as shown by Vite. Indeed, multistep syntheses of 9-oxa-[40] and 10-oxa-epothilones,[41] using ring-closing metathesis (RCM) as the final step, have recently been patented by the Schering group. In our case the retrosynthetic strategy toward **8** is based on the preparation of the 3-(2′-methylallyloxy) aldehyde **9b**, followed by aldolisation and RCM. To test the feasibility of such a strategy in the presence of an unsaturated ester, an analogue of epoA bearing a phenyl group in place of the thiazole moiety was first prepared by use of β-allyloxy aldehyde **9a** and a racemic homoallylic alcohol.

Results and Discussion

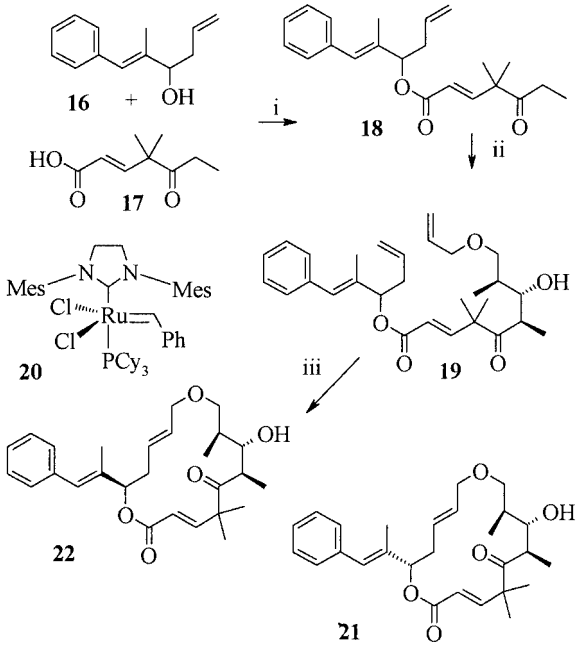
Preparation of the key aldehydes **9a** and **9b** started from the commercially available methyl (2*S*)-3-hydroxy-2-methylpropionate (**10**). Etherification with allyl or 2-methylallyl bromide afforded **11a** (70%) or **11b** (85%), which were then reduced with LAH to give **12a** and **12b** (54 and 58%). An alternative route starting from the commercially available (2*R*)-3-bromo-2-methylpropan-1-ol (**13**) was less efficient; protection as a THP ether **14** (or alternatively as a TBDMS ether) was carried out first, but all conditions used to prepare allyl ether **15** were limited due to competitive elimination of HBr from **14**. The best conditions turned out to be under phase-transfer catalysis, but only a limited (31%) isolated yield of **15a** was obtained, together with a 49% yield of the THP ether of 2-methyl-prop-2-en-1-ol. Deprotection afforded alcohol **12a** (91%), which was converted into aldehyde **9a** with PCC (70%) or under Swern conditions (73%). Swern oxidation of **12b** afforded **9b** (76%) (Scheme 1).

Esterification of racemic alcohol **16** with the known acid **17**[42] (DCC, DMAP, CH_2Cl_2) afforded **18** in only 25% isolated yield with use of 1 equiv. of **17** and DCC with respect to **16**, but the yield was increased to 75% with use of 2 equiv. of acid and DCC (Scheme 2). Ester **18** was converted into the corresponding (*Z*) enolate (LDA, THF).[13] Upon addition of **9a** (−78 °C, 30 min), a 5:1 mixture of two aldols was obtained in 58% yield. Only the major aldol **19** could be isolated in pure form (23%) after flash chromatography, together with mixed fractions that could be recycled. The *syn-anti* configuration is consistent with similar aldolisation reactions in the epothilone series, as deduced from comparison of NMR spectroscopic data ($J_{H7,H8}$ = 8.3 Hz and $J_{H6,H7}$ = 2.5 Hz) with literature data.[40,43,44] It may be noted that, as would be expected, the ratio in favour of the *anti* isomer is increased from ca. 2:1 to 5:1 upon replacement of a methylene by an oxygen atom at the β position of the aldehyde. In the former case the *anti* selectivity is best explained by the Roush model[45] (due to the presence of the alkene moiety) while in our case chelation (affording the same isomer) may be more important. The final RCM of **19** (3×10^{-3} M) was carried out at room temp. in CH_2Cl_2 in the presence of 0.1 equiv. of the second generation Grubbs catalyst **20**.[46] After chromatography, the macrocycles **21** (30%) and **22** (18%), differing only in their side chain configurations, were isolated. The (*E*) configurations of the newly created alkenes were confirmed by NMR (J = 14.9 and 15.2 Hz, respectively). Comparison of the H3 chemical shifts in **21/22** and compounds **23/24**[40] (as well as with the methyl analogue **28**, vide infra) was used to determine the C15 configuration, since this proton is more shielded in the natural (15*S*) isomer (Table 1).

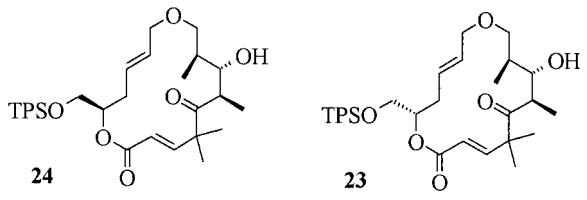


i) Br-CH_2-CH(R)=CH_2, Ag_2O, 70-85%. ii) LAH, 54-58%.
iii) PCC or Swern 70-76%.
iv) DHP, H^+ then HO-CH_2-CH(R)=CH_2, PTC, 31%. v) MeOH, H^+, 91%

Scheme 1



i) DCC, DMAP, CH_2Cl_2, 75%. ii) LDA, THF, -78 °C, 58%
iii) Grubbs I, 10%, CH_2Cl_2, 30% (**21**) and 28% (**22**)



Scheme 2

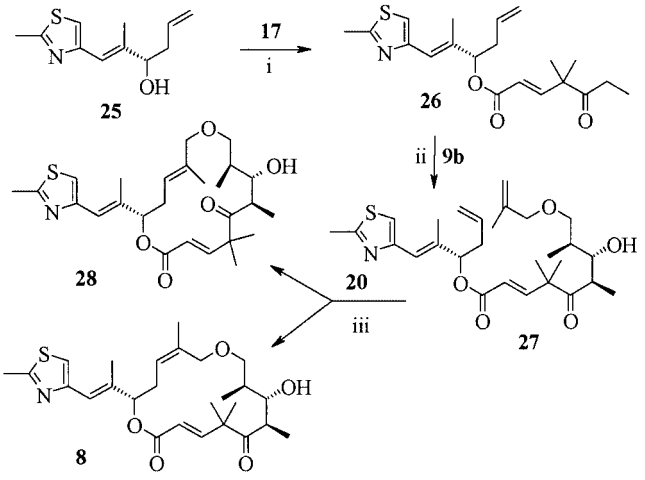
Alcohol **25** was prepared from the corresponding aldehyde by a slight modification of the reported procedure:[47,48] (−)-Ipc_2Ballyl was generated from allylmagnesium bromide and DIP-Cl[49] (under these conditions no filtration of magnesium salts is needed). Compound **25** {$[\alpha]_D = -18.6$ ($c = 0.6$, $CHCl_3$) (ref.: $[\alpha]_D = -15.9$ ($c = 4.9$, $CHCl_3$)[40] and $[\alpha]_D = -20.2$ ($c = 1$, $CHCl_3$)[41]} was obtained in 70% yield and 96% *ee* (chiral HPLC). As before, esterification of **17**[40] with **25** (DCC, DMAP, CH_2Cl_2) afforded **26** (78%). Aldolisation with **9b** afforded a 5:1 mixture of two aldols (49%), the major of which could be isolated in pure form after flash chromatography (**27**, 19%). The observed NMR spectroscopic data ($J_{H7,H8} = 8.5$ Hz and $J_{H6,H7} = 2.8$ Hz) are similar to those of **19**. The final RCM was carried out as before with **20** (0.15 equiv., CH_2Cl_2, room temp., 72 h), to give a 46% yield of **28** and **8** (1.3:1 ratio), which were readily separated by flash chromatography. It is interesting to point out that such RCMs have previously been reported with the Schrock Mo catalyst[50,51] while the use of the Grubbs ruthenium carbene [$RuCl_2(PCy_3)_2CHPh$] was unsuccessful, the formation of a lactone by metathesis between the C13−C14 and the C2−C3 alkenes not having been observed under such conditions. The (*E*) configuration in **28** is in agreement with ^{13}C chemical shifts and with NOE experiments.

Table 1. Comparison of ^1H chemical shifts (ppm) of protons H3 and H15 in **21/22/28** and **23/24**

Compound	**21**	**22**	**23**	**24**	**28**
δ H3	6.82	7.17	6.78	7.02	6.77
δ H15	5.65	5.51	5.16−5.22	5.21−5.26	5.62

Conformational Analysis

As mentioned earlier, the epothilone macrocycle is fairly flexible but the configuration of the aldol moiety (C5−C8) is critical for bioactivity. Conformation I exists in equilibrium with conformation II and their relative contributions in solution may be deduced from $J_{H6,H7}$. According to Taylor, in the case of EpoA, the computationally predicted



Scheme 3

value of $J_{H6,H7}$ is 10.5 Hz for the more stable conformer I and 1 Hz for conformer II (Figure 2).

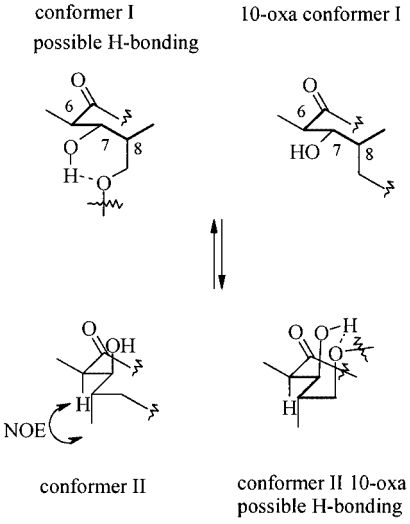


Figure 2. Taylor models of the C5−C8 region (left) and oxa compounds (right) showing possible hydrogen bonding

Examination of the ^1H NMR spectra reveals that the H6,H7 coupling constants for the two isomers are different: 9.5 Hz for **28** and 2.7 Hz for **8**. The observed coupling constant for **28** is thus similar to the value reported for EpoA (J = 8.5 Hz, CD_2Cl_2), in agreement with a 9:1 ratio for conformers I and II (ratio 4:1 for EpoA). A reversal of stability between the two conformers is found for **8**, the observed coupling constant being in agreement with a 1:4 ratio between conformers I and II. In addition, in the case of **8**, a NOE is clearly observed between H6 and Me8, and this is only possible for conformer II. Similarly, it was found in the phenyl series that the (15*S*) isomer **21** exhibits a larger $J_{H6,H7}$ value (9.1 Hz) than the (15*R*) isomer **22**. The conformation of the epothilone macrocycle is thus modified by inversion of configuration at C15.

Furthermore, for epothilones C and D, Taylor[52] has found two stable conformers for the C11−C15 part, conformer III being the more stable. For the compound *trans*-**28**, NOEs are observed between H15 and H13, and the coupling constant between H15 and H14b is 10.3 Hz while that with H14a is <1 Hz. Those data fit better with model III. For the *cis* compound **8**, NOEs cannot be observed between H13 and H15, since their NMR signals are collapsed. The coupling constants between H15 and H14a/H14b are 6.8 and 6.9 Hz, respectively. This indicates that conformer IV is predominant in this case (Figure 3).

Molecular modelling at the RHF/3.21G level (Gaussian 98[53]) was then carried out and, as expected, large sets of possible conformations were found both for **8** and for **28**. As indicated above, important aspects are the position of the C12 methyl group, which may be found either "up" or "down" with respect to the median plane of macrocycle, and the C5−C6−C7−C8 torsion angle. The most interesting result is the presence, in the more stable conformers, of a hydrogen bond between the OH aldol and O10, thus producing conformations of type I (Figure 2). Hence, for the more stable conformers of **28** (E_{rel} = −12.8 kcal/mol, total energy = −1822.42555 au), the H bonding is characterised by a 1.78 Å distance and by a 144° bond angle, while values of 1.78 Å and 148.5° are observed for **8** (E_{rel} = −8.2 kcal/mol). The C5−C6−C7−C8 torsion angles in these conformers are −59.3° and −63.1°, respectively (Figure 4). For (*E*) isomer **28**, these calculations are in agreement with NMR spectroscopic data corresponding to conformer I. The more stable conformer II was found at a much higher energy (E_{rel} = 0 kcal/mol) with an *s-cis* ester configuration.

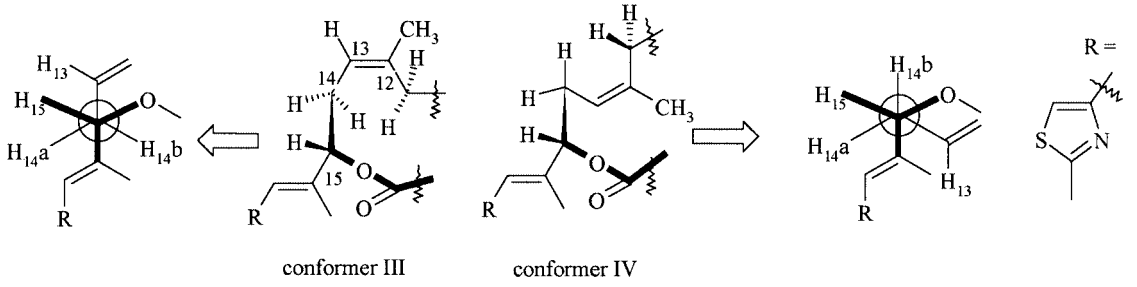
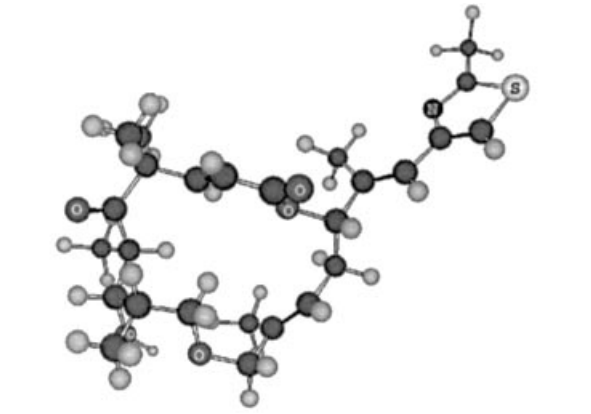
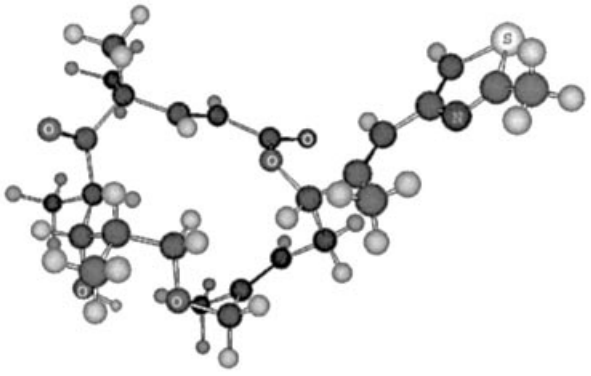


Figure 3. Taylor models of the C1−C13 region showing possible NOEs



28 (*E*, more stable conformer)



8 (*Z*, more stable conformer)

Figure 4. Conformation of **28** and **8** obtained from calculations (C atoms black, H atoms light grey, other atoms as indicated)

For the (*Z*) isomer, the more stable conformer does not fit with NMR spectroscopic data, but conformer II is closer in energy ($E_{rel} = -2.6$ kcal/mol). It is characterised by an *s-cis* ester configuration and an intermolecular hydrogen bond between the OH aldol and the ester carbonyl.

Conclusion

In conclusion, 10-oxa-epothilone analogues have been prepared in only 14 operations (7 steps in the longer linear sequence) by aldolisation of a chiral 3-(2′-methylallyloxy)-propanal, followed by ring-closing metathesis. These macrocycles are amenable to further transformations such as epoxidation at C12, C13 or Michael addition at C3. No significant cytotoxic activity against L1210 leukemia was observed for **8**, **21**, **22** and **28** ($IC_{50} > 10$ μM). Whether the observed lack of activity is or is not due to the introduction of the oxygen atom at C10 remains to be studied.

Experimental Section

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker 300 MHz spectrometer, in $CDCl_3$ as solvent with TMS as internal standard. Assignment of ^{1}H NMR and ^{13}C NMR spectra was achieved by COSY ^{1}H-^{1}H, NOE and DEPT (Multiplicity by DEPT: s = C, d = CH, t = CH_2, q = CH_3). High-resolution MS were performed by the "Service Central de Microanalyse" (CNRS, Lyon). All reactions were run under an inert atmosphere. THF was dried with and distilled from sodium/benzophenone, and CH_2Cl_2 was distilled from $CaCl_2$. Organic extract mixtures were dried with anhydrous Na_2SO_4 and filtered, and the solvent was then removed under reduced pressure. All separations were performed under flash chromatography (MPLC) conditions on silica gel (25−40 mμ), completed, if necessary, by preparative thin-layer chromatography (TLC) performed on silica gel plates (60 GF_{254}).

Methyl (2*R*)-3-Allyloxy-2-methylpropanoate (11a): In a flask wrapped with aluminium foil and under N_2, methyl (+)-L-β-hydroxyisobutyrate (2 mL, 18.05 mmol) was added to a suspension of $MgSO_4$ (435 mg, 18 mmol) and Ag_2O (5.4 g, 23.5 mmol) in petroleum ether (80 mL). After the system had been stirred for 1 h at room temp., the flask was cooled in an ice bath, and allyl bromide (2.34 mL, 27.07 mmol) was slowly added. After 30 min, a second fraction of Ag_2O was added (5.9 g, 25.3 mmol), and the mixture was stirred at room temp. overnight. Filtration through Celite and magnesium sulfate, concentration in vacuo and chromatography on silica gel (eluent: petroleum ether/EtOAc, 90:10) afforded 1.8 g of **11a** (70%) as a colourless oil. $[\alpha]_D^{20} = +13$ ($c = 1.7$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 1.17$ (d, $^3J_{1H} = 7.1$ Hz, 3 H, 2-CH_3), 2.75 (qt, $^3J_{3H} = 7.1$, $^3J_{2H} = 6.9$ Hz, 1 H, 2-H), 3.63 (dd, $^2J_{1H} = 9.2$, $^3J_{1H} = 7.3$ Hz, 2 H, 3-H_2), 3.70 (s, 3 H, CH_3 ester), 3.98 (dt, $^3J_{1H} = 5.5$, $^4J_{2H} = 1.4$ Hz, 2 H, 1′-H_2), 5.16 (dd, $^3J_{1Hcis} = 10.3$, $^2J_{1H} = 1.4$ Hz, 1 H, 3′-H*cis*), 5.25 (ddd, $^3J_{1Htrans} = 17.2$, $^4J_{1H} = 1.4$, $^2J_{1H} = 0.6$ Hz, 1 H, 3′-H_{trans}), 5.90 (ddt, $^3J_{1Htrans} = 17.2$, $^3J_{1Hcis} = 10.3$, $^3J_{2H} = 5.5$ Hz, 1 H, 2′-H) ppm.^{13}C NMR ($CDCl_3$): $\delta = 15.2$, 41.4, 53.0, 73.1, 73.3, 118.3, 135.8, 176.5 ppm. IR (cm^{-1}): $\tilde{\nu} =$ 3082, 1736, 1647, 1459, 1394, 992, 927.

Methyl (*R*)-2-Methyl-3-(2′-methylallyloxy)propanoate (11b): Use of the same procedure as above with **10** (1 mL, 9 mmol) and 3-bromo-2-methylpropene (1.36 mL, 13.54 mmol) (except for stirring at room temp. for 3 d) gave **11b** (1.33 g, 85%) as a colourless oil. $[\alpha]_D^{20} = +14$ ($c = 0.5$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 1.17$ (d, $^3J_{1H} = 8.5$ Hz, 3 H, 2-CH_3), 1.71 (br. s, 3 H, 2′-CH_3), 2.75 (m, 1 H, 2-H), 3.43 (dd, $^2J_{1H} = 9.2$, $^3J_{1H} = 5.9$ Hz, 1 H, 3-Ha), 3.59 (dd, $^2J_{1H} = 9.2$, $^3J_{1H} = 7.3$ Hz, 1 H, 3-Hb), 3.70 (s, 3 H, CH_3 ester), 3.88 (t, $^4J_{2H} = 0.7$ Hz, 2 H, 1′-H_2), 4.88 (t, $^4J_{2H} = 0.6$ Hz, 1 H, 3′-Ha), 4.93 (t, $^4J_{2H} = 0.9$ Hz, 1 H, 3′-Hb) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 13.2$, 19.3, 40.1, 50.2, 71.7, 75.1, 112.3, 142.1, 175.4 ppm. IR (cm^{-1}): $\tilde{\nu} =$ 3077, 1736, 1657, 1455, 1375, 1251, 1199, 1095, 991, 901.

(2*R*)-3-Allyloxy-2-methylpropanol (12a): A solution of **11a** (2.76 g, 19.3 mmol) in anhydrous diethyl ether (30 mL) was added dropwise under N_2 to a suspension of lithium aluminium hydride (293 mg, 7.7 mmol) in diethyl ether (50 mL). The mixture was heated at reflux for 1 h, stirred at room temp. overnight and cooled to 0 °C, and ethyl acetate (1.32 mL, 13.5 mmol) followed by ethanol (5.1 mL, 86.8 mmol) were then slowly added to quench excess $LiAlH_4$. The salts were precipitated by acidification with HCl (1 N, 6 mL). Water was added, and the aqueous phase was extracted with diethyl ether (3 × 20 mL). The organic extracts were then washed with brine, dried ($MgSO_4$) and concentrated under vacuo to provide a yellow oil (1.95 g), which was chromatographed on silica gel (petroleum ether/EtOAc, 80:20 to 60:40 as eluent) to afford **12a** (1.1 g, 44%, 54% based on recovered starting material). $[\alpha]_D^{20} = +16$ ($c = 0.46$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 0.87$ (d, $^3J_{1H} = 6.9$ Hz,

3 H, 2-CH_3), 2.07 (m, 1 H, 2-H), 2.64 (s, 1 H, OH), 3.45 (dd, $^2J_{1H}$ = 9.5, $^3J_{1H}$ = 6.5 Hz, 2 H, 1-H_2), 3.62 (dd, $^2J_{1H}$ = 9.5, $^3J_{1H}$ = 6.2 Hz, 2 H, 3-H_2), 3.99 (dd, $^3J_{1H}$ = 5.5, $^4J_{1H}$ = 1.2 Hz, 2 H, 1′-H_2), 5.22 (dq, $^3J_{1Hcis}$ = 10.0, $^2J_{1H}$ = $^4J_{2H}$ = 1.5 Hz, 1 H, 3′-H_{cis}), 5.29 (dq, $^3J_{1Htrans}$ = 17.2, $^4J_{2H}$ = $^2J_{1H}$ = 1.6 Hz, 1 H, 3′-H_{trans}), 5.90 (ddt, $^3J_{1Htrans}$ = 17.2, $^3J_{1Hcis}$ = 10.0, $^3J_{2H}$ = 5.5 Hz, 1 H, 2′-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.4, 35.4, 67.9, 72.2, 75.6, 117.1, 134.4) ppm. IR (cm^{-1}): $\tilde{\nu}$ = 3391, 3081, 1647, 1455, 1266, 1197, 1093, 993, 926.

(2*R*)-2-Methyl-3-(2′-methylallyloxy)propanol (12b): This compound was produced by the same procedure as used for **12a**, starting from **11b** (1.3 g, 7.5 mmol) and giving **12b** (0.41 g, 38%). $[\alpha]_D^{20}$ = +15 (c = 0.23, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.17 (d, $^3J_{1H}$ = 8.5 Hz, 3 H, 2-CH_3), 1.71 (br. s, 3 H, 2′-CH_3), 2.75 (m, 1 H, 2-H), 3.42 (dd, $^2J_{1H}$ = 9.4, $^3J_{1H}$ = 3.5 Hz, 1 H, 3-Ha), 3.46 (dd, $^2J_{1H}$ = 9.4, $^3J_{1H}$ = 4.9 Hz, 1 H, 3-Hb), 3.69 (d, $^3J_{1H}$ = 7.0 Hz, 2 H, 1-H_2), 3.88 (t, $^4J_{2H}$ = 0.7 Hz, 2 H, 1′-H_2), 4.89 (t, $^4J_{2H}$ = 0.5 Hz, 1 H, 3′-Ha), 4.94 (t, $^4J_{2H}$ = 0.7 Hz, 1 H, 3′-Hb) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.2, 19.3, 40.1, 67.7, 71.7, 75.1, 112.3, 142.1 ppm. IR (cm^{-1}): $\tilde{\nu}$ = 3436, 3077, 1258, 1200, 1095, 984, 899.

(2*R*)-3-Allyloxy-2-methylpropanal (9a): DMSO (1.20 mL, 16.9 mmol) in solution in CH_2Cl_2 was slowly added under N_2 to a cooled (−50 °C) solution of oxalyl chloride (0.74 mL, 8.46 mmol) in freshly distilled CH_2Cl_2 (25 mL). After the mixture had been stirred at − 60 °C for 15 min, **12a** (1 g, 7.69 mmol) in solution in CH_2Cl_2 (7.5 mL) was slowly added (1 mL/min). The reaction was stirred at − 60 °C for 1 h 30, Et_3N (5.34 mL, 38.4 mmol) was added, and the reaction flask was allowed to warm to room temp. The mixture was then washed with water and with satd. NH_4Cl solution and the combined aqueous phases were extracted with CH_2Cl_2 (3 × 15 mL), dried ($MgSO_4$) and concentrated in vacuo to afford **9a** as a yellow oil (719 mg, 76%) after chromatography on silica gel (eluent: petroleum ether/EtOAc, 90:10). $[\alpha]_D^{20}$ = +3.6 (c = 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.13 (d, $^3J_{1H}$ = 7.1 Hz, 3 H, 2-CH_3), 2.63 (m, 1 H, 2-H), 3.60 (dd, $^2J_{1H}$ = 9.5, $^3J_{1H}$ = 5.3 Hz, 1 H, 3-Ha), 3.66 (dd, $^2J_{1H}$ = 9.5, $^3J_{1H}$ = 6.7 Hz, 1 H, 3-Hb), 3.99 (dt, $^3J_{1H}$ = 5.6, $^4J_{1H}$ = $^2J_{1H}$ = 1.4 Hz, 2 H, 1′-H_2), 5.19 (dt, $^3J_{1Hcis}$ = 10.3, $^2J_{1H}$ = $^4J_{1H}$ = 1.4 Hz, 1 H, 3′-H_{cis}), 5.27 (dt, $^3J_{1Htrans}$ = 17.2, $^4J_{1H}$ = $^2J_{1H}$ = 1.4 Hz, 1 H, 3′-H_{trans}), 5.80 (ddt, $^3J_{1Htrans}$ = 17.2, $^3J_{1Hcis}$ = 10.3, $^3J_{2H}$ = 5.6 Hz, 1 H, 2′-H), 9.7 (d, $^3J_{1H}$ = 1.5 Hz, 1 H, CHO) ppm.^{13}C NMR ($CDCl_3$): δ = 10.7, 29.7, 70.1, 72.2, 117.2, 134.4, 203.9. IR (cm^{-1}) = 3080, 2851, 1731, 1645, 1189, 1096, 926, 798 ppm.

(2*R*)-2-Methyl-3-[2′-methylallyloxy]propanal (9b): This compound was produced by the same procedure as used for **12a**, starting from **12b** (400 mg, 2.77 mmol) and affording **9b** (299 mg, 76%) as a yellow oil. $[\alpha]_D^{20}$ = −0.2 (c = 1.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.17 (d, $^3J_{1H}$ = 8.5 Hz, 3 H, 2-CH_3), 1.71 (br. s, 3 H, 2′-CH_3), 2.75 (m, 1 H, 2-H), 3.42 (dd, $^2J_{1H}$ = 9.4, $^3J_{1H}$ = 3.5 Hz, 1 H, 3-Ha), 3.48 (dd, $^2J_{1H}$ = 9.4, $^3J_{1H}$ = 4.9 Hz, 1 H, 3-Hb), 3.88 (t, $^4J_{2H}$ = 0.7 Hz, 2 H, 1′-H_2), 4.91 (t, $^4J_{2H}$ = 0.5 Hz, 1 H, 3′-Ha), 4.94 (t, $^4J_{2H}$ = 0.7 Hz, 1 H, 3′-Hb), 9.74 (br. s, 1 H, 1-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.9, 19.3, 46.7, 69.8, 75.2, 112.2, 142.1, 203 ppm. IR (cm^{-1}): $\tilde{\nu}$ = 3077, 2856, 2726, 1725, 1656, 1254, 1097, 1057, 982, 902.

(±)-3-Hydroxy-2-methyl-1-phenylhexa-1,5-diene (16): 2-(Methyl)-cinnamaldehyde (10 mL, 71.6 mmol) was dissolved in THF (70 mL), and zinc in powder form (9.36 g, 143 mmol) was then added portionwise and allyl bromide dropwise (13 mL, 143 mmol). The reaction mixture was stirred overnight at room temperature and quenched with saturated NH_4Cl solution (50 mL). After filtration and washing with diethyl ether (200 mL), the aqueous phase of the filtrate was extracted with diethyl ether (3 × 40 mL). The organic extracts were combined, dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel (eluent:petroleum ether/EtOAc, 90:10) afforded **16** in quantitative yield. ^{1}H NMR (CDCl3): δ = 1.88 (br. s, 3 H, 16-CH_3), 2.42 (m, 2 H, 14-H_2), 4.24 (t, $^3J_{2H}$ = 6.0 Hz, 15-H), 5.14 (d, $^3J_{1Hcis}$ = 8.2 Hz, 1 H, 12-H_{cis}), 5.17 (d, $^3J_{1Htrans}$ = 15.3 Hz, 1 H, 12-H_{trans}), 5.83 (m, 1 H, 13-H), 6.52 (br. s, 1 H, 17-H), 7.3 (m, 5 H, Ph) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0, 40.2, 77.1, 118.1, 126.2, 126.8, 128.5, 128.6, 129.0, 129.4, 135.0, 137.5, 140.0 ppm. MS: m/z = 188 (12), 147 (100), 129 (94), 115 (47), 91 (91), 77 (39), 69 (37), 65 (23), 55 (15), 51 (28). IR (cm^{-1}): $\tilde{\nu}$ = 3432, 3064, 3028, 1659, 1642, 1600, 1584, 1495.

Keto Ester 18: 4-(Dimethylamino)pyridine (1.435 g, 11.75 mmol) and dicyclohexylcarbodiimide (2.42 g, 11.75 mmol) were added under N_2 to a solution of 2-methyl-1-phenylhexa-1,5-dien-1-ol (2.21 g, 11.75 mmol) and 4,4-dimethyl-5-oxohept-2-enoic acid (2.0 g, 11.75 mmol) in dichloromethane (80 mL). After stirring overnight at room temp., the mixture was washed with water (50 mL). The aqueous phases were combined and extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated on a rotavapor. Chromatography on silica gel (petroleum ether/EtOAc, 95:5) afforded **18** (1.0 g, 25%). ^{1}H NMR ($CDCl_3$): δ = 1.01 (t, $^3J_{2H}$ = 7.0 Hz, 3 H, 6-CH_3), 1.32 (s, 6 H, $CH_{3,gem}$), 1.89 (d, $^4J_{1H}$ = 1.4 Hz, 3 H, 16-CH_3), 2.50 (q, $^3J_{3H}$ = 7.0 Hz, 2 H, 6-H_2), 2.59 (m, 2 H, 14-H_2), 5.07 (dd, $^3J_{1Hcis}$ = 10.1, $^2J_{1H}$ = 1.8 Hz, 1 H, 12-H_{cis}), 5.13 (dd, $^3J_{1Htrans}$ = 15.6, $^2J_{1H}$ = 1.8 Hz, 1 H, 12-H_{trans}), 5.42 (t, $^3J_{2H}$ = 7.6 Hz, 1 H, 15-H), 5.76 (ddt, $^3J_{1Htrans}$ = 17.1, $^3J_{1Hcis}$ = 10.1, $^3J_{2H}$ = 7.0 Hz, 1 H, 13-H), 5.90 (d, $^3J_{1Htrans}$ = 15.8 Hz, 1 H, 2-H], 6.54 (s, 1 H, 17-H), 7.07 (d, $^3J_{1Htrans}$ = 15.8 Hz, 1 H, 3-H), 7.25−7.30 (m, 5 H, Ph) ppm. ^{13}C NMR ($CDCl_3$): δ = 8.4, 13.8, 23.6, 31.5, 37.5, 50.5, 78.7, 117.7, 120.7, 126.7, 128.1, 129.0, 133.4, 135.3, 137.0, 151.2, 165.5, 211.7 ppm.

Aldol 19: A solution of freshly distilled diisopropylamine (0.380 mL, 2.71 mmol) was added dropwise at −30 °C under N_2 to a solution of *n*BuLi in hexanes (1.70 mL, 2.71 mmol) in THF (7 mL). After stirring for 15 min at −30 °C, the resulting LDA was diluted with THF (7 mL) and was then cooled to −78 °C. Keto ester **18** (840 mg, 2.47 mmol) in solution in THF (3.5 mL) was then added. After the mixture had been stirred for one hour at −78 °C, aldehyde **9a** (316.2 mg, 2.47 mmol) in THF (3.5 mL) was added dropwise over 5 min. After 30 minutes at −78 °C, the reaction mixture was allowed to warm to room temperature and was then quenched by addition of a saturated NH_4Cl solution (10 mL). After extraction of the aqueous phase with diethyl ether (3 × 15 mL), the combined organic extracts were washed with brine (25 mL), dried ($MgSO_4$) and concentrated. Separation by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) afforded pure aldol **19** (23%). ^{1}H NMR ($CDCl_3$): δ = 0.9 (d, $^3J_{1H}$ = 6.9 Hz, 3 H, 8-CH_3), 1.06 (d, $^3J_{1H}$ = 6.9 Hz, 3 H, 6-CH_3), 1.32 (s, 6 H, $CH_{3,gem}$), 1.89 (3 H, s, 16-CH_3), 2.55 (m, 2 H, 14-H_2), 3.10 (qd, $^3J_{3H}$ = 6.9, $^3J_{1H}$ = 2.8 Hz, 1 H, 6-H), 3.41 (tqd, $^3J_{2H}$ = 7.8, $^3J_{3H}$ = 5.5, $^3J_{1H}$ = 4.2 Hz, 1 H, 8-H), 3.49 (dd, $^2J_{1H}$ = 9.1, $^3J_{1H}$ = 4.2 Hz, 1 H, 9-Ha), 3.50 (dd, $^2J_{1H}$ = 9.1, $^3J_{1H}$ = 4.7 Hz, 1 H, 9-Hb), 3.56 (dt, $^3J_{1H}$ = 8.3, $^3J_{2H}$ = 2.5 Hz, 1 H, 7-H), 3.94 (dt, $^3J_{1H}$ = 5.4, $^4J_{2H}$ = 1.5 Hz, 2 H, 11-H_2), 5.06−5.27 (m, 4 H, C=CH_2), 5.43 (t, $^3J_{2H}$ = 6.7 Hz, 1 H, 15-H), 5.81 (m, 2 H, C=CH_2), 5.95 (d, $^3J_{1Htrans}$ = 15.9 Hz, 1 H, 2-H), 6.54 (s, 1 H, 17-H), 7.10 (d, $^3J_{1Htrans}$ = 15.9 Hz, 1 H, 3-H), 7.24−7.30 (m, 5 H, Ph) ppm. ^{13}C NMR ($CDCl_3$): δ = 9.8, 12.9, 13.3, 22.51, 22.55, 35.1, 36.6, 41.7, 50.5, 71.2, 72.1, 72.6, 77.8, 115.9, 116.9, 120.5, 125.0, 125.8,

127.2, 128.1, 132.3, 132.4, 133.7, 134.2, 134.3, 136.0, 149.9, 164.3, 214.9 ppm. IR (cm^{-1}): $\tilde{\nu}$ = 3507,3079, 1715, 1644, 1600, 1492, 1417, 1366, 1294, 1021. HRMS: calcd. 469.2954; found 469.2960.

Macrocycles 21 and 22: Grubbs catalyst **20** (0.1 equiv.) was added to a solution of diene **19** (as a mixture of (6*R*,7*S*,8*S*) and (6*S*,7*R*,8*R*) diastereoisomers, 39 mg, 0.083 mmol) in CH_2Cl_2 and the resulting mixture was stirred at room temp. under N_2 for 3 days. Removal of solvent in vacuo afforded a crude mixture, which was directly purified by preparative TLC on silica (petroleum ether/EtOAc, 85:15, as eluent) to give **21** (10.8 mg, 30%) and then **22** (6.8 mg, 18%).

Compound 21: ^{1}H NMR ($CDCl_3$): δ = 0.93 (d, $^3J_{1H}$ = 7.1 Hz, 3 H, 8-H_3), 1.07 (d, $^3J_{1H}$ = 6.8 Hz, 3 H, 6-CH_3), 1.27 (s, 3 H, $CH_{3,gem}$), 1.36 (s, 3 H, $CH_{3,gem}$), 1.89 (m, 1 H, 8-H), 1.95 (s, 3 H, 16-CH_3), 2.52 (m, 2 H, 14-H_2), 2.90 (br. s, 1 H, OH), 3.11 (dq, $^3J_{3H}$ = 6.7, $^3J_{1H}$ = 3.3 Hz, 1 H, 6-H), 3.28 (dd, $^3J_{1H}$ = 9.3, $^3J_{1H}$ = 4.6 Hz, 1 H, 7-H), 3.72 (br. m, 1 H, 9-H), 3.83 (dd, $^2J_{1H}$ = 12.7, $^3J_{1H}$ = 5.5 Hz, 1 H, 11-Ha), 3.97 (dd, $^2J_{1H}$ = 12.7, $^3J_{1H}$ = 4.5 Hz, 1 H, 11-Hb), 5.53 (dd, $^3J_{1H}$ = 9.4, $^3J_{1H}$ = 4.0 Hz, 1 H, 15-H), 5.63 (dt, $^3J_{1Htrans}$ = 15.3, $^3J_{2H}$ = 4.8 Hz, 1 H, 12-H), 5.71 (dt, $^3J_{1Htrans}$ = 15.3, $^3J_{2H}$ = 6.3 Hz, 1 H, 13-H), 6.09 (d, $^3J_{1Htrans}$ = 15.9 Hz, 1 H, 2-H), 6.57 (br. s, 1 H, 17-H), 7.17 (d, $^3J_{1Htrans}$ = 15.9 Hz, 1 H, 3-H), 7.20−7.35 (m, 5 H, Ph) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.7, 14.6, 14.8, 23.3, 24.7, 36.9, 37.2, 44.3, 51.5, 71.4, 72.9, 73.2, 76.6, 78.1, 122.1, 127.1, 127.2, 128.5, 128.6, 129.0, 129.4, 129.7, 136.3, 137.4, 151.5, 165.6, 214.8 ppm. HRMS: calcd. 463.2460; found 463.2464.

Compound 22: ^{1}H NMR ($CDCl_3$): δ = 1.13 (d, $^3J_{1H}$ = 7.0 Hz, 3 H, 8-H_3), 1.20 (s, 3 H, $CH_{3,gem}$), 1.26 (d, $^3J_{1H}$ = 6.8 Hz, 3 H, 6-CH_3), 1.41 (s, 3 H, $CH_{3,gem}$), 1.89 (m, 1 H, 8-H), 1.95 (d, $^4J_{1H}$ = 1.4 Hz, 3 H, 16-CH_3), 2.52 (m, 2 H, 14-H_2), 2.93 (dd, $^2J_{1H}$ = 9.5, $^3J_{1H}$ = 3.1 Hz, 9-Ha), 3.04 (dq, $^3J_{1H}$ = 9.1, $^3J_{3H}$ = 6.7 Hz, 1 H, 6-H), 3.28 (br. s, 1 H, OH), 3.55 (dd, $^2J_{1H}$ = 12.4, $^3J_{1H}$ = 9.6 Hz, 1 H, 11-Ha), 3.65 (dd, $^3J_{1H}$ = 9.1, $^3J_{1H}$ = 4.0 Hz, 1 H, 7-H), 3.79 (dd, $^2J_{1H}$ = 9.5, $^3J_{1H}$ = 2.8 Hz, 9-Hb), 4.26 (dd, $^2J_{1H}$ = 12.4, $^3J_{1H}$ = 3.7 Hz, 1 H, 11-Hb), 5.53−5.77 (m, 3 H, 12-H, 13-H, 15-H), 6.09 (d, $^3J_{1Htrans}$ = 15.8 Hz, 1 H, 2-H), 6.61 (s, 1 H, 17-H), 6.83 (d, $^3J_{1Htrans}$ = 15.8 Hz, 1 H, 3-H), 7.23−7.37 (m, 5 H, Ph) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.4, 16.4, 16.7, 23.0, 23.3, 35.2, 37.7, 46.1, 52.4, 71.2, 72.7, 76.9, 78.0, 122.2, 126.8, 127.4, 128.2, 128.98, 129.0, 129.6, 132.1, 135.7, 136.9, 151.4, 165.0, 213.7 ppm. HRMS: calcd. 463.2460; found 463.2462.

(3*S*)-3-Hydroxy-2-methyl-1-[2′-methyl-4′-thiazolyl]hexa-1,5-diene (25): (−)-Diisopinocampheylborane chloride (1.44 g, 4.5 mmol) was dissolved in anhydrous diethyl ether (5 mL) under nitrogen in a Schlenk flask. Then, after the mixture had been cooled to −40 °C, a solution of allylmagnesium bromide in diethyl ether (1 M, 3.75 mL, 3.75 mmol) was added dropwise and the mixture was warmed to room temperature over about one hour. The resulting solution of (allyl)diisopinocampheylborane was then cooled to −78 °C, and 2-methyl-3-[2′-methylthiazol-4′-yl]propenal (502 mg, 6.66 mmol) in diethyl ether (2 mL) was very slowly added. After stirring for 3.5 hours at −78 °C, the reaction mixture was allowed to warm to 0 °C and was then quenched by addition of acetaldehyde (1.01 mL, 18 mmol). The mixture was then oxidised with sodium acetate solution (3 M, 3 mL, 9 mmol) and hydrogen peroxide in water (35%, 1.5 mL) followed by a conventional workup to provide a yellow oil (1.70 g). Chromatography over silica gel (eluent: petroleum ether/EtOAc, 70:30) afforded **25** (441.3 mg, 70%). $[\alpha]_D^{20}$ = −18.6 (*c* = 0.58, $CHCl_3$), *ee*: 96.1%, {ref.: $[\alpha]_D$ = −15.9}, (Chiralcel OD-H, 0.46 × 25 cm, eluent: hexane/2-propanol, 90:10, 1 mL/min, UV detection 256 nm). ^{1}H NMR ($CDCl_3$): δ = 1.96 (s, 3 H, 16-CH_3), 2.30−2.40 (m, 2 H and 1 H, 14-H_2, OH), 2.63 (s, 3 H, 21-H_3), 4.13 (dd, $^3J_{1H}$ = 6.0, $^3J_{1H}$ = 6.1 Hz, 1 H, 15-H), 5.03 (dd, $^3J_{1Hcis}$ = 10.0, $^2J_{1H}$ = 1.5 Hz, 1 H, 12-H_{cis}), 5.08 (dd, $^3J_{1Htrans}$ = 17.0, $^2J_{1H}$ = 1.5 Hz, 1 H, 12-H_{trans}), 5.75 (ddt, $^3J_{1Htrans}$ = 17.0, $^3J_{1Hcis}$ = 10.0, $^3J_{2H}$ = 7.1 Hz, 1 H, 13-H), 6.48 (br. s, 1 H, 17-H), 6.85 (s, 1 H, 20-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.2, 18.9, 39.8, 76.4, 115.6, 117.5, 118.8, 134.6, 141.6, 152.5, 164.5) ppm.

Keto Ester 26: 4-(Dimethylamino)pyridine (1.22 g, 10 mmol) and dicyclohexylcarbodiimide (2.06 g, 10 mmol) were added under nitrogen to a solution of **25** (1.05 g, 5 mmol) and **17** (1.7 g, 10 mmol) in dichloromethane (40 mL). After stirring for 7 days at room temp., the mixture was washed with water (4 × 15 mL). The aqueous phases were combined and extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded **26** (1.40 g, 77.5%). $[\alpha]_D^{20}$ = −15.8 (*c* = 0.12, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.02 (t, $^3J_{2H}$ = 7.2 Hz, 3 H, 6-CH_3), 1.29 (s, 6 H, $CH_{3,gem}$), 2.09 (s, 3 H, 16-CH_3), 2.48 (q, $^3J_{3H}$ = 7.2 Hz, 2 H, 6-H_2), 2.54 (m, 2 H, 14-H_2), 2.70 (s, 3 H, 21-H_3), 5.06 (dd, $^3J_{1Hcis}$ = 10.0, $^2J_{1H}$ = 1.7 Hz, 1 H, 12-H_{cis}), 5.10 (dd, $^3J_{1Htrans}$ = 17.2, $^2J_{1H}$ = 1.7 Hz, 1 H, 12-H_{trans}), 5.38 (t, $^3J_{2H}$ = 6.5 Hz, 1 H, 15-H), 5.74 (ddt, $^3J_{1Htrans}$ = 17.2, $^3J_{1Hcis}$ = 10.0, $^3J_{2H}$ = 6.9 Hz, 1 H, 13-H), 5.90 (d, $^3J_{1Htrans}$ = 15.9 Hz, 1 H, 2-H), 6.54 (s, 1 H, 17-H), 6.95 (s, 1 H, 20-H), 7.05 (d, $^3J_{1Htrans}$ = 15.9 Hz, 1 H, 3-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 8.3, 10.8, 16.2, 20.5, 20.5, 29.0, 35.4, 50.4, 82.6, 115.1, 116.6, 119.0, 120.2, 137.3, 141.9, 142.0, 149.0, 165.9, 166.1, 212.0 ppm. HRMS: calcd. 384.1609; found 384.1607. MS: *m*/*z* = 361 (2), 320 (9), 208 (53), 192 (65), 176 (11), 168 (55), 151 (61), 134 (8), 125 (72), 112 (27), 96 (48), 81 (37), 67 (42), 57 (100). IR (cm^{-1}): $\tilde{\nu}$ = 3106, 3077, 1714, 1644, 1505, 1099, 1040, 990.

Aldol 27: A solution of *n*BuLi in hexanes (0.98 mL, 1.57 mmol) was added at −30 °C to a solution of freshly distilled diisopropylamine (0.219 mL, 1.57 mmol) in THF (1.4 mL). After stirring at −30 °C for 15 min, the resulting LDA was cooled to −78 °C, and **26** (524 mg, 1.445 mmol) dissolved in THF (1.4 mL) was then added. After the system had been stirred at −78 °C for one hour, **9b** (11.5 mg, 0.784 mmol) was added dropwise, and after 30 min at the same temperature the reaction mixture was quenched with glacial acetic acid (0.215 mL, 3.76 mmol). The reaction mixture was then allowed to warm to room temp., and saturated NH_4Cl was added. After extraction of the aqueous phase with diethyl ether (3 × 15 mL), the combined organic extracts were washed with brine (25 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (petroleum ether/EtOAc, 85:15) afforded **27** (74.3 mg, 19%) as the major product. $[\alpha]_D^{20}$ = −1.3 (*c* = 1.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.9 (d, $^3J_{1H}$ = 6.9 Hz, 3 H, 8-CH_3), 1.06 (d, $^3J_{1H}$ = 7.15 Hz, 3 H, 6-CH_3), 1.32 (s, 6 H, $CH_{3,gem}$), 1.70 (br. s, 3 H, 12′-CH_3), 1.75 (m, 1 H, 8-H), 2.08 (3 H, s, 16-CH_3), 2.54 (m, 2 H, 14-H_2), 2.71 (s, 3 H, 21-H_3), 3.1 (qd, $^3J_{3H}$ = 7.15, $^3J_{1H}$ = 2.9 Hz, 1 H, 6-H), 3.47 (m, 2 H and 1 H, 9-H_2, OH), 3.57 (dd, $^3J_{1H}$ = 8.2, $^3J_{1H}$ = 2.9 Hz, 1 H, 7-H), 3.81 (s, 2 H, 11-H_2), 4.86 (s, 1 H, C=CH), 4.92 (q, $^4J_{3H}$ = 0.9 Hz, 1 H, C=CH), 5.06 (dd, $^3J_{1Hcis}$ = 10.1, $^2J_{1H}$ = 1.2 Hz, 1 H, C=CH), 5.11 (dd, $^3J_{1Htrans}$ = 17.2, $^2J_{1H}$ = 1.2 Hz, 1 H, C=CH), 5.40 (t, $^3J_{2H}$ = 6.9 Hz, 1 H, 15-H), 5.74 (ddt, $^3J_{1Htrans}$ = 17.2, $^3J_{1Hcis}$ = 10.1, $^3J_{2H}$ = 6.9 Hz, 1 H, C=CH), 5.95 (d, $^3J_{1Htrans}$ = 15.7 Hz, 1 H, 2-H), 6.54 (s, 1 H, 17-H), 6.95 (s, 1 H, 20-H), 7.05 (d, $^3J_{1Htrans}$ = 15.7 Hz, 1 H, 3-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.7, 14.2, 14.7, 19.1, 29.7, 29.7, 36.1, 37.6, 42.7, 51.4, 72.8, 73.6, 75.2, 78.5, 116.3, 116.4, 117.8, 120.9, 121.0, 133.3, 136.8, 137.4, 142.1, 152.4, 164.6,

165.1, 215.9 ppm. HRMS: calcd. 526.2603; found 526.2601. IR (cm^{-1}): $\tilde{\nu}$ = 3490, 3078, 1714, 1643, 1503.

2,3-Dehydro-10-oxa-epothilone B (8) and Macrocycle 28: Grubbs catalyst **20** (14.2 mg, 0.0165 mmol) was added under N_2 to a solution of **27** (56 mg, 0.111 mmol) in dichloromethane (37 mL). After the mixture had been stirred for 5 days at room temp., conversion of **27** was complete and the reaction mixture was concentrated in vacuo and purified by preparative TLC (petroleum ether/EtOAc, 75:25). Two compounds were isolated: **8** (R_f: 0.50, 26%) and **26** (R_f: 0.35, 20%).

Compound 28: Oil; $[\alpha]_D^{20}$ = +82 (*c* = 0.2; $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.00 (s, 1 H), 6.77 (d, *J* = 15.8 Hz, 1 H), 6.64 (s, 1 H), 6.09 (d, *J* = 15.8 Hz, 1 H), 5.62 (d, *J* = 10.5 Hz, 1 H), 5.48 (dd, *J* = 10.7, 5.0 Hz, 1 H), 4.18 (d, *J* = 12.2 Hz, 1 H), 3.70 (ddd, *J* = 9.5, 2.4, 1.9 Hz, 1 H), 3.70 (m, 1 H), 3.56 (d, *J* = 12.2 Hz, 1 H), 3.48 (m, 1 H, OH), 2.97 (qd, *J* = 6.7, 9.5 Hz, 1 H), 2.90 (dd, *J* = 9.8, 2.4 Hz, 1 H), 2.72 (s, 3 H), 2.70 (dd, *J* = 10.5, 11.6 Hz, 1 H), 2.40 (dd, *J* = 3.4, 11.6 Hz, 1 H), 2.16 (s, 3 H), 1.70 (s, 3 H), 1.40 (s, 3 H), 1.28 (d, *J* = 6.7 Hz, 3 H), 1.28 (s, 3 H) and 1.18 (d, *J* = 7.2 Hz, 3 H) ppm. ^{13}C NMR (75 M Hz, $CDCl_3$): δ = 13.2, 15.5, 16.7, 17.5, 19.2, 22.8, 23.2, 29.6, 34.9, 46.3, 52.5, 70.1, 76.3, 77.4, 78.4, 116.3, 120.0, 122.4, 125.6, 135.1, 137.5, 151.4, 152.3, 164.8, 164.9, 213.7 ppm. HRMS: $[M + Na]^+$ calcd. 476.2471; found 476.2470.

Compound 8: Oil; $[\alpha]_D^{20}$ = −44 (*c* = 0.2; $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.12 (d, *J* = 15.8 Hz, 1 H), 6.98 (s, 1 H), 6.58 (s, 1 H), 6.00 (d, *J* = 15.8 Hz, 1 H), 5.47 (m, 2 H), 3.82 (d, *J* = 12.1 Hz, 1 H), 3.78 (d, *J* = 12.1 Hz, 1 H), 3.74 (dd, *J* = 8.9, 2.8 Hz, 1 H), 3.72 (dd, *J* = 7.1, 2.7 Hz, 1 H), 3.29 (dd, *J* = 9.1, 4.1 Hz, 1 H), 3.10 (qd, *J* = 6.9, 2.7 Hz, 1 H), 2.95 (br. s, 1 H), 2.71 (s, 3 H), 2.51 (dd, *J* = 6.9, 6.8 Hz, 2 H), 2.16 (s, 3 H), 1.74 (m, 1 H), 1.60 (s, 3 H), 1.37 (s, 3 H), 1.27 (s, 3 H), 1.08 (d, *J* = 6.9 Hz, 3 H) and 0.94 (d, *J* = 7.2 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.6, 14.3 (2 ×), 15.4, 19.2, 22.5, 24.0, 31.6, 36.9, 43.2, 51.2, 72.0, 72.1, 75.3, 78.0, 116.1, 119.6 120.1, 122.1, 134.1, 137.8, 150.7, 152.4, 164.7, 165.2, 214.8 ppm. HRMS: $[M + Na]^+$ calcd. 476.2471; found 476.2474.

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