Stereochemistry of enacyloxins. Part 3.[1] (12′*S*,17′*R*,18′*S*,19′*R*)-Absolute configuration of enacyloxins, a series of antibiotics from *Frateuria* sp. W-315

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The absolute configuration of the 12′,17′,18′,19′-positions of enacyloxins (ENXs), a series of polyhydroxy-polyenic antibiotics from *Frateuria* sp. W-315, was determined. As degradation of decarbamoyl (dec) ENX IIa gave (5*R*,6*S*,1′*E*)-6-(but-1′-enyl)-5-chloro-5,6-dihydro-2*H*-pyran-2-one, which corresponded to the 15′–23′ skeleton of dec ENX IIa, its enantiomers were synthesized from tri-*O*-acetyl-D-glucal. Comparison of the HPLC retention time of these naturally derived and synthetic compounds revealed the 17′*R*,18′*S*,19′*R*-configuration of ENXs. Hydrogenation and oxidation of ENX IIa gave methyl 13-hydroxy-6,12-dimethyltridecanoate, which was converted to the 13-MTPA ester. Comparison of the ^{1}H NMR chemical shifts and the coupling constants with the model compounds revealed the 12′*S*-configuration. This absolute stereochemistry is necessarily applicable to other enacyloxins.

Introduction

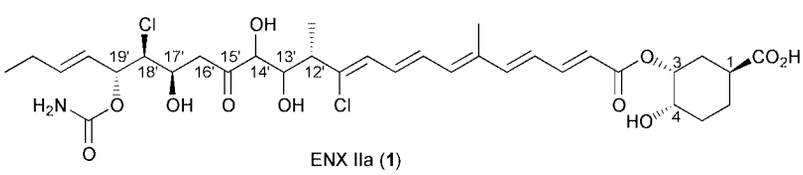
Enacyloxins (ENXs) are polyhydroxy-polyenic and yellow-colored antibiotics produced by *Frateuria* sp. W-315 in a Czapek-Dox medium spent by *Neurospora crassa*.[2] ENX IIa **1** is the main product and has antibiotic activity against Gram-positive and Gram-negative bacteria.[3] Its mode of action was considered to be the inhibition of peptide biosynthesis,[4] and its inhibition mechanism on ribosomes was recently reported.[5] Furthermore, ENXs have attracted considerable attention because they show antibiotic inhibitory activity toward organelle protein synthesis in *Plasmodium falciparum*.[6] ENX IIa **1** is an enzymatic oxidation product of ENX IVa **2**[3d] and both are also converted enzymatically to their decarbamoylated products, dec ENX IIa (cyclic form **3** and linear form **3′**) and dec ENX IVa **4**, respectively (Scheme 1).[1,3d] As shown in Scheme 1, the relative 17′*S**,18′*R**,19′*S**-configuration of **1** was elucidated from the 6-membered hemiacetal form of **3**. To determine the absolute configuration of this fragment of **1**, a degradation study was performed. In this paper we describe the determination of the absolute configuration of this fragment.

Results and discussion

1. Determination of the absolute configuration of the 17′,18′,19′-positions

Degradation study. Treatment of dec ENX IIa **3** with $NaIO_4$ in MeOH gave hemiacetal **5** (excess $NaIO_4$) or ester **6** (1–2 eq. $NaIO_4$), while lactone **7** was formed using $Pb(OAc)_4$ in MeCN at 0 °C. As **7** could be dehydrated to give the simpler lactone **8** under neutral conditions (DCC, CuCl),[7] we aimed to synthesize both enantiomers of **8** to determine the absolute configuration (Scheme 2).

Synthesis of both enantiomers of the lactone 8′. As shown in Scheme 3, our synthesis started from tri-*O*-acetyl-D-glucal **9**, which was converted to the methoxyphenyl (MP) ether **10** by our reported procedure.[8] The hydroxy group was oxidized under Swern conditions followed by a Wittig–Horner reaction to give ester **11**. The ethoxycarbonyl group of **11** was reduced to give **12** and the resulting hydroxy group was acetylated to afford **13**. Copper catalyzed Grignard substitution of this allylic acetate gave **14**. The methoxyphenyl group was removed using silver dipicolinate $[Ag(DPAH)_2]$[9] and the resulting hemiacetal was oxidized to give lactone **15**. Deprotection of the THP group gave alcohol **16**, and Mitsunobu conditions using *p*-nitrobenzoic acid successfully inverted the 5-position to give **17**. However, isomerization to the more stable γ-lactone **18** occurred during removal of the PNB group. To prevent this isomerization, we chose acetic acid as the Mitsunobu donor. The acetyl group of **19** was successfully removed by enzymatic hydrolysis without isomerization to give (−)-**20**. Finally, the hydroxy group of (−)-**20** was converted to the chloride with inversion of configuration to afford the desired (+)-**8′**. Next we prepared the enantiomer. Alkaline treatment of alcohol **15** gave the corresponding hydroxy acid, which was submitted to Mitsunobu conditions to give lactone **21**. Compound **21** was converted to (−)-**8′** *via* (+)-**20** as described above.



ENX IIa (**1**)

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Determination of the absolute configuration. Chiral HPLC analysis of the naturally derived and the synthetic **8′** was performed (Column, Shiseido Ceramosphere Chiral RU-2, 4.6 × 150 mm; MeOH–H_2O = 1 : 1 at 1.0 ml min^{-1}; 245 nm). The retention time of the naturally derived **8** coincided with that of (+)-**8′** (4.8 min), whilst that of (−)-**8′** was 2.9 min, so the absolute configuration of naturally derived **8** was confirmed to be 5*S*,6*R*. Consequently, the absolute configuration of ENX IIa **1** was determined to be 17′*R*,18′*S*,19′*R*.

2. Determination of the absolute configuration of the 12′-position

Degradation study. We adopted the MTPA ester method which is used in the determination of the absolute configuration of α-methyl fatty acids.[10] The polyene fragment of ENX IIa methyl ester **22** was hydrogenated over PtO_2 to give **23** (Scheme 4). The 13′,14′-vicinal diol was cleaved with $NaIO_4$–$NaBH_4$ to afford a mixture of ester **24** and free acid **25**, which were treated with CH_2N_2 followed by alkaline methanol to give alcohol **26**. This compound was converted to the corresponding (*S*)-MTPA ester **27**.

The model compound, (*S*)-2-methyltetradecanol **30**, was synthesized from methyl (*S*)-3-hydroxy-2-methylbutanoate **28** *via* the known iodide **29**.[11] The alcohol **30** was then converted to its (*S*)-MTPA ester **31**.[10a] The corresponding 2*RS* compound **32** was also prepared.[12] The ^{1}H NMR spectrum of **27** was compared with those of the model compounds **31** and **32**.[10b] As shown in Fig. 1, the chemical shifts and the splitting pattern of **27** closely match those of **31**. Consequently, the absolute configuration of the 12′-position was determined to be *S*.

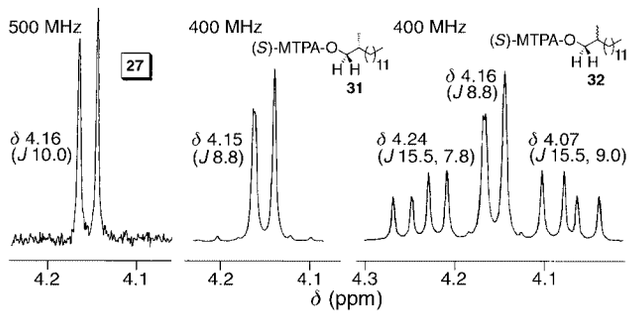


Fig. 1 Parts of the ^{1}H NMR spectra of the natural and the model compounds.

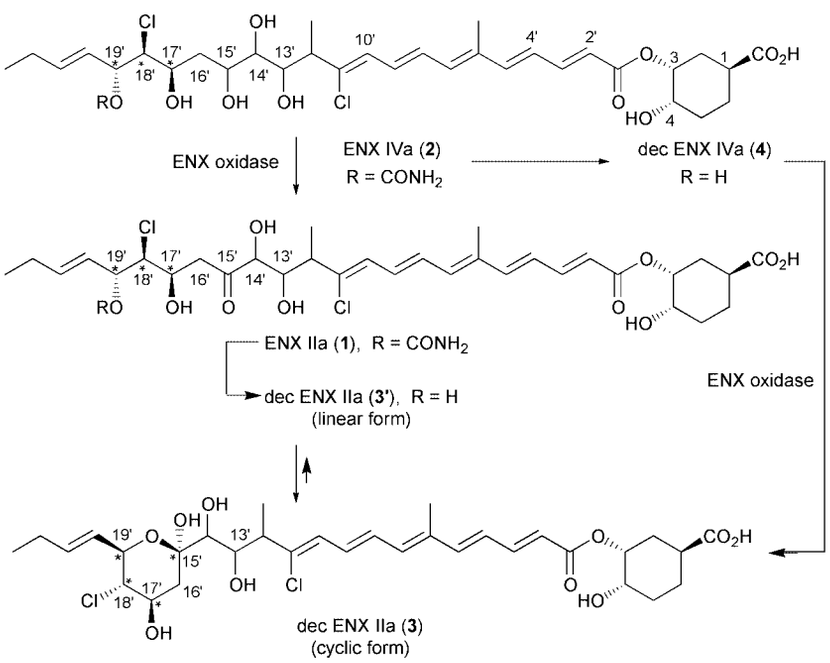
Conclusion

The 12′*S*,17′*R*,18′*S*,19′*R*-absolute configuration of ENX IIa, a polyhydroxy-polyenic antibiotic from *Frateuria* sp. W-315, was determined by comparison of degradation products of the natural compounds and the synthetic model compounds. This absolute stereochemistry is necessarily applicable to all ENXs.

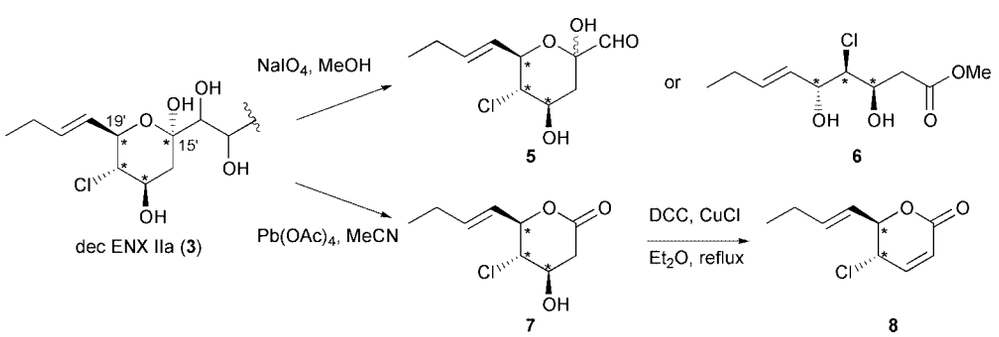
Experimental

General

NMR spectra were obtained on Varian Unity Inova 500



Scheme 1 Enacyloxins and decarbamoyl enacyloxins.



Scheme 2 Degradation of dec ENX IIa **3**.

(500 MHz for ^{1}H) and GEMINI 3000 (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometers in $CDCl_3$ using Me_4Si as an internal standard, mass spectra were recorded on a JEOL DX-303 HF, and IR spectra were measured on a JASCO IR-810. HPLC were measured on a Shimadzu LC-10. Optical rotations are given in 10^{-1} deg cm^2 g^{-1}.

Determination of the 17,18,19-positions

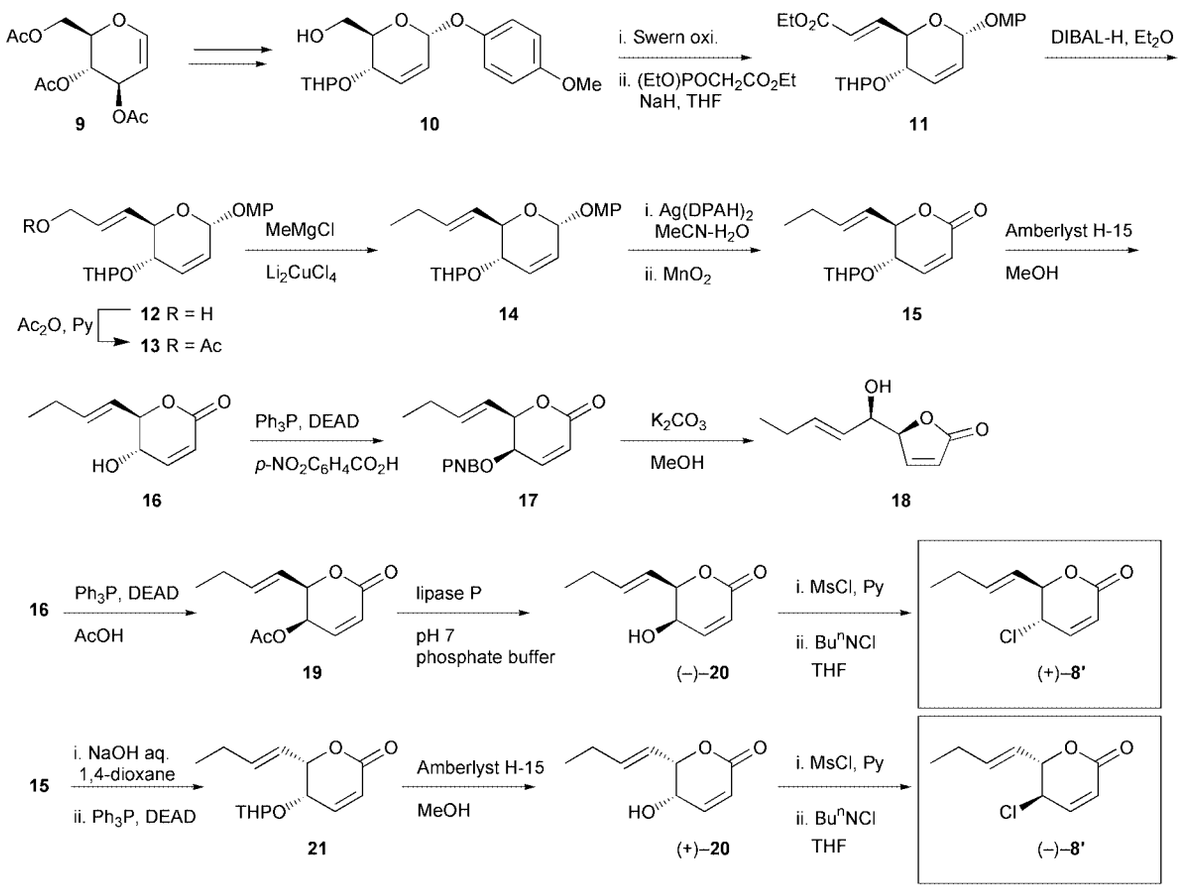
Natural (4*R*,5*S*,6*R*,1′*E*)-6-(but-1′-enyl)-5-chloro-2-formyl-3,4,5,6-tetrahydro-2*H*-pyran-2,4-diol 5 and methyl (3*R*,4*S*,5*R*,6*E*)-4-chloro-3,5-dihydroxynon-6-enoate 6. To a solution of dec ENX IIa (1.0 mg, 1.5 μmol) in MeOH (1 cm^3) was added a solution of $NaIO_4$ (1 mg, 5 mmol) in H_2O (1 cm^3) and the mixture was stirred for 1 h at 20 °C. Then the mixture was diluted with EtOAc, washed with aq. $Na_2S_2O_3$, H_2O and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 3 : 1) to give **5** and **6** in trace amounts. **5**; δ_H (500 MHz) 1.16 (3 H, t, *J* 7.4 Hz, 4′-H), 1.82 (2 H, pseudo dd, *J* 12.0, 3.8 Hz, 3-H), 2.12 (2 H, m, 3′-H), 2.34 (1 H, ddd, *J* 12.0, 4.9, 0.8 Hz, 3-H), 2.65 (1 H, m, 4′-H), 3.36 (1 H, dt, *J* 10.2, 11.8 Hz, 5-H), 4.49 (1 H, dd, *J* 7.4, 10.2 Hz, 6-H), 5.50 (1 H, ddt, *J* 15.4, 9.1, 1.6 Hz, 1′-H), 5.94 (1 H, dd, *J* 7.4, 10.2 Hz, 2′-H), 8.11 (1 H, s, CHO); *m*/*z* (EI) 234 (M$^+$). **6**; δ_H (500 MHz) 1.04 (3 H, t, *J* 7.4 Hz, 9-H), 2.12 (2 H, m, 8-H), 2.58 (1 H, pseudo d, *J* 16.2 Hz, 2-H), 2.78 (1 H, dd, *J* 16.2, 8.6 Hz, 2-H), 3.90 (1 H, dt, *J* 1.9, 5.8 Hz), 4.44 (1 H, m), 4.63 (1 H, m, 5-H), 5.57 (1 H, dd, *J* 15.1, 6.3 Hz, 6-H), 5.91 (1 H, dt, *J* 15.4, 6.3, 7-H).

Natural (4*R*,5*S*,6*R*,1′*E*)-6-(but-1′-enyl)-5-chloro-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-one 7. To a solution of dec ENX IIa (1.2 mg, 1.8 μmol) in MeCN (0.6 cm^3) was added slowly a suspension of $Pb(OAc)_4$ (10 mg) in MeCN (1 cm^3) at 0 °C. After the mixture was stirred for 30 min, ethylene glycol (0.5 cm^3) was added. The mixture was stirred for a further 10 min, diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 2 : 1) to give **7** (0.20 μg, 54%); δ_H (500 MHz) 1.04 (3 H, t, *J* 7.4 Hz, 4′-H), 1.56 (2 H, dq, *J* 6.4, 7.4 Hz, 3′-H), 2.60 (1 H, d, *J* 2.9 Hz, OH), 2.65 (1 H, dd, *J* 9.5, 17.6 Hz, 3-H), 3.14 (1 H, dd, *J* 6.2, 17.6 Hz, 3-H), 3.75 (1 H, dd, *J* 10.5, 9.4 Hz, 5-H), 4.15 (1 H, dddd, *J* 2.9, 6.2, 9.4, 9.5 Hz, 4-H), 4.60 (1 H, dd, *J* 7.8, 10.5 Hz, 6-H), 5.50 (1 H, dd, *J* 7.8, 15.4 Hz, 1′-H), 6.00 (1 H, dt, *J* 6.4, 15.4 Hz, 2′-H); *m*/*z* (EI) 204 (*M*$^+$). This compound was used in the next step without further purification.

Natural (5*S*,6*R*,1′*E*)-6-(but-1′-enyl)-5-chloro-5,6-dihydro-2*H*-pyran-2-one (5*S*,6*R*)-8. A solution of **7** (0.20 μg, 0.98 μmol), dicyclohexylcarbodiimide (DCC, 1 mg, 5 μmol) and CuCl (cat.) in ether (5 cm^3) was stirred at reflux for 1 h under Ar. After being cooled to 20 °C, the mixture was concentrated *in vacuo* and the residue was purified by HPLC (hexane–Pr^{i}OH = 20 : 1, ERC-Si-1811, 20 °C, 1.0 cm^3 min^{-1}, 222 nm, t_R = 7.5 min) to give (5*S*,6*R*)-**8** (0.10 μg, 55%) as a colourless oil. The spectral data were identical with those of synthetic (5*S*,6*R*)-**8**.

(2*R*,5*S*,6*R*,1′*E*,2″*RS*)-5,6-Dihydro-6-(2′-ethoxycarbonyleth-1′-enyl)-2-(*p*-methoxyphenoxy)-5-(tetrahydro-2″*H*-pyran-2″-yloxy)-2*H*-pyran 11. To a solution of oxalyl chloride (1.2 cm^3, 14 mmol) in THF (60 cm^3) was added DMSO (2.5 cm^3, 32 mmol) at −78 °C under Ar and the mixture was stirred for 0.5 h. A solution of **10** (4.00 g, 11.1 mmol) in THF (20 cm^3) was added dropwise and the mixture was stirred as the temperature gradually rose. At −30 °C, to this was added Et_3N (4.0 cm^3, 28 mmol) and the resulting mixture was stirred for 3 h until the temperature increased to 0 °C. The mixture was poured into H_2O and extracted with ether. The extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. This crude aldehyde was used in the next step without further purification.

To a suspension of NaH (*ca.* 60% oil dispersion, 0.50 g, *ca.* 13 mmol) in dry THF (60 cm^3) was added triethyl phosphono-

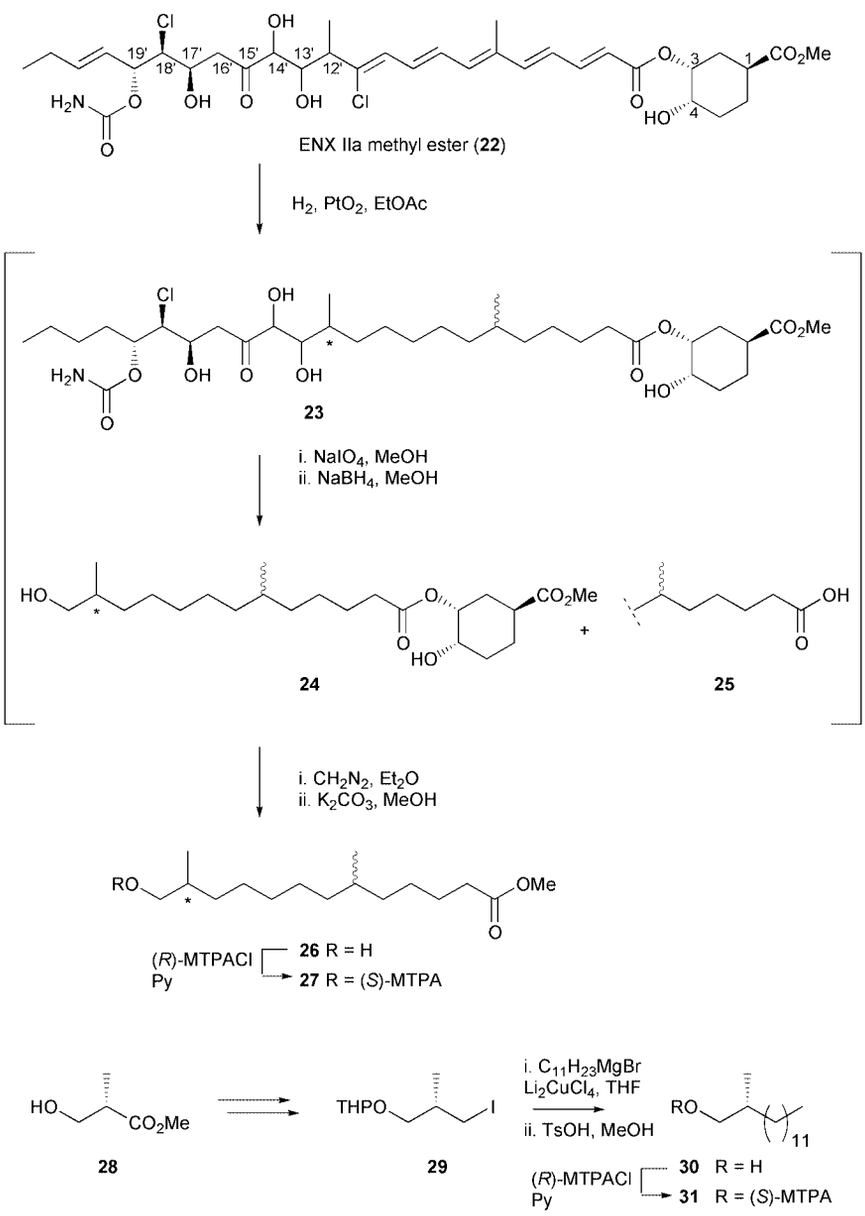


Scheme 3 Synthesis of both enantiomers of the lactone **8′**.

acetate (2.5 cm^3, 13 mmol) at 0 °C. After the mixture was cooled to −20 °C, a solution of the above mentioned oil in dry THF (20 cm^3) was added. The resulting mixture was stirred until the temperature reached 0 °C. The reaction mixture was quenched with aq. NH_4Cl and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 3 : 1) to give **11** (4.50 g, 80.7%) as a colourless oil; ν_{max}(film)/cm^{-1} 1720, 1650 and 1500; δ_H (300 MHz) 1.28 and 1.29 (total 3 H, each t, *J* 7.1 Hz, CH_3CH_2), 1.55–2.01 [6 H, m, $(CH_2)_3$], 3.53 (1 H, m, THP), 3.78 (3 H, s, MeO), 3.97 (1 H, m, 6-H), 4.01 and 4.02 (total 1 H, dd, *J* 1.6, 9.6 Hz, 2″-H), 4.20 (total 2 H, each d, *J* 7.1 Hz, CH_3CH_2), 4.57 (0.5 H, dd, *J* 1.4, 1.8 Hz, THP), 4.59 (0.5 H, dd, *J* 1.8, 9.6 Hz, THP), 4.68 (0.5 H, dd, *J* 2.7, 4.4 Hz, 6-H), 4.91 (0.5 H, dd, *J* 2.7, 3.0 Hz, 6-H), 5.57 and 5.59 (total 1 H, pseudo d, *J* 2.5 Hz, 5-H), 5.90 (0.5 H, ddd, *J* 2.2, 2.7, 10.4 Hz, 4-H), 5.96 (0.5 H, ddd, *J* 1.9, 2.7, 10.4 Hz, 4-H), 6.14 and 6.17 (total 1 H, dd, *J* 1.9, 15.7 Hz, 2′-H), 6.18 (0.5 H, m, 3-H), 6.23 (0.5 H, pseudo d, *J* 10.2 Hz, 3-H), 6.8–5.05 (4 H, m, Ar), 7.04 (0.5 H, dd, *J* 4.4, 15.7 Hz, 1′-H), 7.23 (0.5 H, dd, *J* 4.1, 15.7 Hz, 1′-H). δ_C 14.3, 19.0, 19.6, 25.4, 30.8, 55.8, 60.5, 62.3, 63.0, 69.1, 69.5, 74.6, 94.2, 95.2, 101.4, 116,7, 121.9, 126.0, 132.5, 144.6, 153.5, 166.7; *m*/*z* (EI) 404.1835 (M^+. $C_{22}H_{28}O_7$ requires 404.1833).

(2*R*,5*S*,6*R*,1′*E*)-6-(3′-Hydroxyprop-1′-enyl)-5,6-dihydro-2-(*p*-methoxyphenoxy)-5-(tetrahydro-2″*H*-pyran-2″-yloxy)-2*H*-pyran 12. To a solution of **11** (3.7 g, 9.5 mmol) in dry Et_2O (100 cm^3) was added DIBAL-H (1 M in hexane, 21.0 cm^3, 21 mmol) at −78 °C under Ar and the mixture was stirred for 1 h at this temperature. To this was added MeOH, and the mixture was stirred until the whole solution became a gel. The resulting gel was filtered through a Celite pad and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel (elution with hexane–EtOAc = 1 : 1) to give **12** (2.9 g, 84%) as a colourless oil (Found: C, 66.04; H, 7.31. $C_{20}H_{26}O_6$ requires C, 66.28; H, 7.23%); ν_{max}(film)/cm^{-1} 3430 and 1500; δ_H (300 MHz) 1.5–1.9 [6 H, m, $(CH_2)_3$], 3.4–3.6 (1.5 H, m), 3.76 (3 H, s, OMe), 3.8–4.1 (2.5 H, m), 4.16 (2 H, m), 4.41 (1 H, m), 4.69 (0.5 H, dd, *J* 2.7, 4.4 Hz, 5-H), 4.86 (0.5 H, dd, *J* 3.0, 3.3 Hz, 5-H), 5.54 (1 H, m, 2-H), 5.7–6.1 (3 H, m), 6.15 (0.5 H, pseudo d, *J* 10.2 Hz, 3-H), 6.21 (0.5 H, pseudo d, *J* 10.2 Hz, 3-H), 6.75–6.85 (2 H, m, Ar), 6.95–7.1 (2 H, m, Ar). δ_C 19.3, 19.5, 25.5, 30.9, 55.8, 62.7, 63.2, 63.3, 70.1, 70.6, 74.6, 94.3, 95.6, 101.1, 116.8, 126.1, 128.0, 131.5, 133.5, 152.7.

(2*R*,5*S*,6*R*,1′*E*)-6-(3′-Acetoxyprop-1′-enyl)-5,6-dihydro-2-(*p*-methoxyphenoxy)-5-(tetrahydro-2″*H*-pyran-2″-yloxy)-2*H*-pyran 13. A solution of **12** (5.00 g, 13.8 mmol) and acetic



Scheme 4 Degradation of ENX IIa methyl ester **22**.

anhydride (10 cm^3) in pyridine (25 cm^3) was stirred for 1 h at 20 °C. The reaction mixture was diluted with EtOAc, successively washed with 1 M HCl aq. and aq. $NaHCO_3$, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane–EtOAc (3 : 1) gave **13** (5.57 g, quant) as a colourless oil; ν_{max}(film)/cm^{-1} 1735 and 1500; δ_H (300 MHz) 1.5–1.8 [6 H, m, $(CH_2)_3$], 2.06 (3 H, s, Ac), 3.50 (1 H, m), 3.76 (3 H, s, OMe), 3.9 (1 H, m), 3.98 and 4.14 (total 1 H, each dd, *J* 1.5, 9.5 Hz, 6-H), 4.41 (1 H, m), 4.58 (2 H, dd, *J* 4.1, 8.5 Hz, 3′-H), 4.66 and 4.92 (total 1 H, each m, 5-H), 5.54 (1 H, m, 2-H), 5.8–6.0 (3 H, m), 6.20 (1 H, dd, *J* 1.4, 10.2 Hz, 3-H), 6.8–7.3 (4 H, m, Ar). δ_C 18.7, 19.4, 20.8, 25.3, 30.6, 55.6, 61.8, 62.7, 64.3, 69.6, 70.3, 74.3, 94.2, 94.7, 100.9, 116.5, 125.8, 126.7, 131.2, 132.8, 153.3, 170.8; *m*/*z* (EI) 404.1832 (M^+. $C_{22}H_{28}O_7$ requires 404.1833).

(2*R*,5*S*,6*R*,1′*E*)-6-(But-1′-enyl)-5,6-dihydro-2-(*p*-methoxyphenoxy)-5-(tetrahydro-2″*H*-pyran-2″-yloxy)-2*H*-pyran 14. To a solution of **13** (5.50 g, 13.0 mmol) and Li_2CuCl_4 (0.1 M in THF, 1.3 cm^3, 0.13 mmol) in THF (40 cm^3) was added MeMgCl (3 M in THF, 6.0 cm^3, 18 mmol) in THF (30 cm^3) at −8 °C and the mixture was stirred for 1 h at −8 °C. The reaction mixture was poured into water, extracted with ether, washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane–EtOAc (8 : 1) gave **14** (4.00 g, 85.4%) as a colourless oil; ν_{max}(film)/cm^{-1} 1500; δ_H (300 MHz) 0.99 and 1.00 (total 3 H, each t, *J* 7.7 Hz, 4′-H), 1.5–1.8 [6 H, m, $(CH_2)_3$], 2.08 (2 H, m, 3′-H), 3.52 (1 H, m), 3.77 (3 H, s, OMe), 3.90 (1 H, m), 4.02 and 4.15 (total 1 H, each m, 5-H), 4.35 (1 H, m), 4.70 and 4.95 (total 1 H, each m, 6-H), 5.48 and 5.59 (total 1 H, each, ddt, *J* 15.5, 6.8, 1.5 Hz, 1′-H), 5.51 (1 H, m, 2-H), 5.85 (1 H, m, 2′-H), 5.87 and 5.92 (total 1 H, ddd, *J* 0.8, 1.9, 10.2 Hz, 3-H), 6.15 and 6.18 (total 1 H, ddd, *J* 1.1, 1.6, 10.2 Hz, 4-H), 6.8–7.1 (4 H, m, Ar). δ_C 13.1, 18.4, 19.3, 25.4, 30.5, 55.6, 61.3, 62.5, 69.6, 71.0, 71.7, 74.0, 94.3, 94.5, 100.5, 112.6, 125.8, 126.5, 126.7, 136.7, 153.4; *m*/*z* (EI) 360.1943 (M^+. $C_{21}H_{28}O_5$ requires 360.1935).

(5*S*,6*R*,1′*E*)-6-(But-1′-enyl)-5,6-dihydro-5-(tetrahydro-2″*H*-pyran-2″-yloxy)-2*H*-pyran-2-one 15. A solution of **14** (0.900 g, 2.48 mmol), silver dipicolinate [$Ag(DPAH)_2$, 2.3 g, 5.0 mmol] in MeCN–H_2O (4 : 1, 10 cm^3) was stirred at 20 °C for 1 h. The mixture was filtered, the filtrate was poured into aq. $Na_2S_2O_3$ and extracted with ether. The extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (elution with hexane–EtOAc = 2 : 1) to give a colourless oil, which was used in the next step without further purification.

A suspension of the above mentioned oil and active MnO_2 (3.0 g) in toluene (10 cm^3) was shaken at 20 °C for 2 h. The mixture was filtered through a Celite pad and concentrated *in vacuo*. The residue was chromatographed on silica gel (elution with hexane–EtOAc = 4 : 1) to **15** (0.500 g, 79.9%) as a colourless oil; ν_{max}(film)/cm^{-1} 1725 and 1670; δ_H (300 MHz) 1.00 (3 H, t, *J* 7.4 Hz, 4′-H), 1.5–1.9 [6 H, m, $(CH_2)_3$], 2.10 (2 H, dq, *J* 0.8, 7.4 Hz, 3′-H), 3.54 and 3.88 (total 1 H, each m, OCH_2 of THP), 4.26 (1 H, ddd, *J* 1.9, 3.0, 7.9 Hz, 5-H), 4.70 (1 H, m, OCHO of THP), 4.76 (1 H, dd, *J* 7.9, 0.8 Hz, 6-H), 5.50 (1 H, ddt, *J* 15.4, 7.4, 1.6 Hz, 2′-H), 5.94 (1 H, ddt, *J* 15.4, 0.8, 6.4 Hz, 1′-H), 5.99 (1 H, dd, *J* 1.9, 10.2 Hz, 3-H), 6.91 (1 H, dd, *J* 2.7, 10.2 Hz, 4-H). δ_C 13.1, 19.4, 25.3, 25.4, 30.4, 63.0, 71.6, 81.9, 100.5, 121.0, 124.0, 139.2, 147.8, 163.1; *m*/*z* (EI) 252.1345 (M^+. $C_{14}H_{20}O_4$ requires 252.1360).

(5*S*,6*R*,1′*E*)-6-(But-1′-enyl)-5,6-dihydro-5-hydroxy-2*H*-pyran-2-one 16. A suspension of **15** (0.329 g, 1.30 mmol) and Amberlyst H-15 in MeOH (2 cm^3) was stirred at 20 °C for 3 h. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by PTLC (hexane–EtOAc = 3 : 2) to give **16** (0.210 g, 96.2%) as a colourless oil, $[\alpha]_D^{25}$ −310 (*c* 0.080, $CHCl_3$); ν_{max}(film)/cm^{-1} 3400 and 1720; δ_H (300 MHz) 1.04 (3 H, t, *J* 7.4 Hz, 4′-H), 2.14 (2 H, m, 3′-H), 2.20 (1 H, m, OH), 4.33 (1 H, pseudo d, *J* 2.5 Hz, 5-H), 4.66 (1 H, ddd, *J* 8.5, 8.0, 0.8 Hz, 6-H), 5.53 (1 H, ddt, *J* 15.7, 8.0, 1.5 Hz, 1′-H), 6.20 (1 H, ddt, *J* 15.7, 6.0, 0.8 Hz, 2′-H), 6.00 (1 H, dd, *J* 9.9, 1.9 Hz, 3-H), 6.87 (1 H, dd, *J* 2.5, 9.9 Hz, 4-H). δ_C 13.0, 25.4, 66.3, 83.7, 121.1, 123.6, 141.0, 148.0, 163.1; *m*/*z* (EI) 169.0871 (M^+ + H. $C_9H_{13}O_3$ requires 169.0864).

(5*R*,6*R*,1′*E*)-6-(But-1′-enyl)-5,6-dihydro-5-(*p*-nitrobenzoyloxy)-2*H*-pyran-2-one 17. To a solution of **16** (0.40 g, 2.4 mmol), *p*-nitrobenzoic acid (0.44 g, 2.6 mmol) and Ph_3P (0.70 g, 2.6 mmol) in dry THF (10 ml) was added DEAD (0.4 cm^3, 2.5 mmol) at 0 °C and the mixture was stirred for 2 h at 20 °C. Then the solution was concentrated *in vacuo*, and the residue was chromatographed on silica gel (hexane–EtOAc = 3 : 1) to give **17** (0.50 g, 1.7 mmol, 72.1%) as yellow plates, mp 152 °C; ν_{max}(KBr)/cm^{-1} 3100, 3060, 1730, 1720 and 1600; δ_H (300 MHz) 0.95 (3 H, t, *J* 7.4 Hz, 4′-H), 2.02 (2 H, qd, *J* 6.3, 7.4 Hz, 3′-H), 5.10 (1 H, pseudo ddd, *J* 0.8, 2.7, 6.9 Hz, 6-H), 5.52 (1 H, dd, *J* 2.7, 5.5 Hz, 5-H), 5.62 (1 H, ddt, *J* 15.7, 6.9, 1.6 Hz, 1′-H), 6.06 (1 H, ddt, *J* 15.7, 6.3, 1.1 Hz, 2′-H), 6.32 (1 H, d, *J* 9.6 Hz, 3-H), 7.10 (1 H, dd, *J* 5.5, 9.6 Hz, 4-H), 8.13–8.34 (4 H, m, Ar).

(2*R*,1′*R*,2′*E*)-2,5-Dihydro-2-(1′-hydroxypent-2′-enyl)furan-5-one 18. A solution of **17** (0.10 g, 0.34 mmol) and K_2CO_3 (5 mg) in MeOH–H_2O (3 : 1, 4 cm^3) was stirred at 20 °C for 30 min. The reaction gave a complex mixture monitored by TLC. The mixture was diluted with EtOAc and washed with water and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 2 : 1) to give **18** as the main product; ν_{max}(film)/cm^{-1} 3450, 3010, 1770 and 760.

(5*S*,6*R*,1′*E*)-5-Acetoxy-6-(but-1′-enyl)-5,6-dihydro-2*H*-pyran-2-one 19. To a solution of **16** (0.210 g, 1.25 mmol), Ph_3P (1.36 g, 5.20 mmol) and acetic acid (0.36 cm^3, 4.8 mmol) in dry THF (10 cm^3) was added a solution of DEAD (0.80 cm^3, 5.0 mmol) in THF (3 cm^3) at −45 °C under N_2. After being stirred at 20 °C for 12 h, the mixture was poured into water and extracted with EtOAc. The extract was washed with 1 M HCl, aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (elution with hexane–EtOAc = 2 : 1) to give **19** (184 mg, 69.9%) as colourless needles, mp 48.5–49.5 °C, $[\alpha]_D^{25}$ −65 (*c* 0.013, $CHCl_3$); ν_{max}(film)/cm^{-1} 1740 and 1720; δ_H (300 MHz) 1.04 (3 H, t, *J* 7.4 Hz, 4′-H), 2.10 (3 H, s, Ac), 2.11 (2 H, m, 3′-H), 4.96 (1 H, ddd, *J* 0.8, 3.0, 7.1 Hz, 6-H), 5.25 (1 H, dd, *J* 3.0, 5.5 Hz, 5-H), 5.57 (1 H, ddt, *J* 15.6, 7.1, 1.6 Hz, 1′-H), 5.99 (1 H, ddt, *J* 15.6, 6.3, 0.8 Hz, 2′-H), 6.12 (1 H, d, *J* 9.6 Hz, 3-H), 6.96 (1 H, dd, *J* 5.5, 9.6 Hz, 4-H). δ_C 13.0, 20.5, 25.3, 64.1, 79.5, 121.4, 125.0, 139.7, 140.8, 162.8, 170.2; *m*/*z* (EI) 211.0973 (M^+ + H. $C_{11}H_{15}O_4$ requires 211.0969).

(5*R*,6*R*,1′*E*)-6-(But-1′-enyl)-5,6-dihydro-5-hydroxy-2*H*-pyran-2-one (−)-20. A solution of **19** (46.3 mg, 0.220 mmol) and lipase P (Amano) in Pr^i_2O (4 cm^3) and phosphate buffer (pH 7.0, 0.1 M, 2 cm^3) was stirred at 20 °C for 2 d. The mixture was diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by PTLC (hexane–EtOAc = 1 : 1) to give (−)-**20** (42.5 mg, 96.2%) as a colourless oil, $[\alpha]_D^{26}$ −250 (*c* 0.060, $CHCl_3$); ν_{max}(film)/cm^{-1} 3400 and 1720; δ_H (300 MHz) 1.05 (3 H, t, *J* 7.4 Hz, 4′-H), 2.17 (2 H, dq, *J* 7.4, 6.3 Hz, 3′-H), 2.10 (1 H, br, OH), 4.20 (1 H, m, 5-H), 4.84 (1 H, m), 5.70 (1 H, ddt, *J* 15.5, 7.0, 1.5 Hz, 1′-H), 6.05 (1 H, ddt, *J* 15.5, 6.3, 0.8 Hz, 2′-H), 6.13 (1 H, d, *J* 9.6 Hz, 3-H), 6.99 (1 H, dd, *J* 5.5, 9.6 Hz, 4-H). δ_C 13.0, 25.5, 63.2, 81.3, 121.7, 123.0, 139.9, 144.8, 163.5; *m*/*z* (EI) 169.0867 (M^+ + H. $C_9H_{13}O_3$ requires 169.0864).

(5*S*,6*R*,1′*E*)-6-(But-1′-enyl)-5-chloro-5,6-dihydro-2*H*-pyran-2-one (+)-8′. To a solution of (−)-**20** (19 mg, 0.11 mmol) in THF (0.5 cm^3) was added successively pyridine (0.2 cm^3) and MsCl (0.05 cm^3) at 0 °C and the mixture was stirred for 1 h. Then to this was added a solution of tetrabutylammonium chloride (0.34 g, 6.0 mmol) in THF (2 cm^3) and the mixture was stirred at 20 °C for 1 d. The reaction mixture was diluted with EtOAc, washed with 1 M HCl, aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by PTLC (hexane–EtOAc = 3 : 1) to give (+)-**8′** (14 mg, 71%) as a colourless oil, $[\alpha]_D^{25}$ +220 (*c* 0.030, $CDCl_3$); ν_{max}(film)/cm^{-1} 1730; δ_H (300 MHz) 1.02 (3 H, t, *J* 7.4 Hz, 4′-H), 2.12 (2 H, dq, *J* 7.4, 6.3 Hz, 3′-H), 4.50 (1 H, ddd, *J* 1.5, 3.3, 6.9 Hz, 5-H), 4.93 (1 H, dd, *J* 7.1, 6.9 Hz, 6-H), 5.52 (1 H, ddt, *J* 15.4, 7.1, 1.6 Hz, 1′-H), 6.01 (1 H, ddt, *J* 15.4, 6.3, 1.1 Hz, 2′-H), 6.09 (1 H, dd, *J* 9.9, 1.5 Hz, 3-H), 6.48 (1 H, dd, *J* 3.6, 9.9 Hz, 4-H); *m*/*z* (EI) 186.0433 (M^+. $C_9H_{11}O_2{}^{35}Cl$ requires 186.0447).

(5*S*,6*S*,1′*E*)-6-(But-1′-enyl)-5,6-dihydro-5-(tetrahydro-2″*H*-pyran-2″-yloxy)-2*H*-pyran-2-one 21. To a solution of **16** (0.24 g, 0.94 mmol) in 1,4-dioxane–H_2O (1 : 1, 3 cm^3) was added 1 M NaOH (1.5 cm^3) at 0 °C and the mixture was stirred at 0 °C for 0.5 h. The solution was acidified with 1 M HCl and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residual oil was used in the following step without further purification.

To a solution of PPh_3 (0.25 g, 0.95 mmol) in THF (20 cm^3) was added DEAD (0.16 cm^3, 0.10 mmol) and the above mentioned oil in THF (10 cm^3) at −40 °C and the mixture was stirred for 12 h as the temperature of this gradually rose to 20 °C. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel (elution with hexane–EtOAc = 3 : 1) to give **21** (62 mg, 26%) as a colourless oil; ν_{max}(film)/cm^{-1} 1725 and 1670; δ_H (300 MHz) 1.02 (3 H, t, *J* 7.4 Hz, 4′-H), 1.4–1.8 [6 H, m, $(CH_2)_3$], 2.10 (2 H, dq, *J* 0.8, 7.4 Hz, 3′-H), 3.54 and 3.88 (total 1 H, each m, OCH_2 of THP), 4.26 (1 H, ddd, *J* 1.9, 3.0, 7.9 Hz, 5-H), 4.70 (1 H, m, OCHO of THP), 4.76 (1 H, dd, *J* 7.9, 0.8 Hz, 6-H), 5.50 (1 H, ddt, *J* 15.4, 7.4, 1.6 Hz, 2′-H), 5.94 (1 H, ddt, *J* 15.4, 0.8, 6.4 Hz, 1′-H), 5.99 (1 H, dd, *J* 1.9, 10.2 Hz, 3-H), 6.91 (1 H, dd, *J* 2.7, 10.2 Hz, 4-H). δ_C 13.1, 19.4, 25.3, 25.4, 30.4, 63.0, 71.6, 81.9, 100.5, 121.0, 124.0, 139.2, 147.8, 163.1; *m*/*z* (EI) 252.1345 (M^+. $C_{14}H_{20}O_4$ requires 252.1360).

(5*S*,6*S*,1′*E*)-6-(But-1′-enyl)-5,6-dihydro-5-hydroxy-2*H*-pyran-2-one (+)-20. In a similar manner as described for **16**, **21** (56 mg, 0.22 mmol) gave (+)-**20** (35 mg, 0.21 mmol, 90%).

(5*R*,6*S*,1′*E*)-6-(But-1′-enyl)-5-chloro-5,6-dihydro-2*H*-pyran-2-one (−)-8′. In a similar manner as described for (+)-**8′**, (+)-**20** (35 mg, 0.21 mmol) gave (−)-**8′** (20 mg, 0.11 mmol, 55%); *m*/*z* (FAB) 187.0535 (M + H^+. $C_9H_{12}O_2{}^{35}Cl$ requires 187.0525).

HPLC analysis of the natural derived 8 and the synthetic 8′. Column, Shiseido Ceramosphere Chiral RU-2, 4.6 × 150 mm; temperature, 20 °C; eluent, MeOH–H_2O = 1 : 1 at 1.0 ml min^{-1}; t_R, 2.9 min [(−)-**8′**] and 4.8 min [(+)-**8′** and **8**]; detection at 245 nm.

Determination of the 12′-position

Methyl (6*RS*,12*R*)-13-hydroxy-6,12-dimethyltridecanoate 26. A suspension of ENX IIa methyl ester (**22**, 11.7 mg, 16.7 μmol) and PtO_2 (cat.) in MeOH (1 cm^3) was stirred under H_2 for 2 h. The mixture was filtered through a silica gel pad and concentrated *in vacuo* to give crude compound **23**. This residue (14.9 mg) and $NaIO_4$ (18.0 mg, 84.0 μmol) in MeOH (1 cm^3) was stirred at 20 °C for 1.5 h. The mixture was diluted with EtOAc, washed with aq. $Na_2S_2O_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. A solution of the residue and $NaBH_4$ (10.0 mg, 30.0 μmol) was stirred at 20 °C for 12 h. The reaction was quenched with 1 M HCl, diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give a mixture of **24** and **25**. This mixture was treated with CH_2N_2 in ether to give a pale yellow oil. This oil was stirred with K_2CO_3 (1.0 mg) in MeOH (1 cm^3) for 12 h. The mixture was diluted with EtOAc, washed with H_2O and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 4 : 1) to give **26** (2.0 mg, 6.5 μmol, 39%) as a colourless oil; δ_H 0.84 (3 H, d, *J* 6.3 Hz, Me), 0.92 (3 H d, *J* 6.3 Hz, Me), 1.0–1.5 (16 H, m, CH_2), 1.5–1.7 (2 H, m, CH), 2.31 (2 H, dd, *J* 7.4, 7.7 Hz, 2-H), 3.4–3.53 (2 H, m, 13-H), 3.67 (3 H, s, OMe); *m*/*z* (FAB) 273.2422 (M^+ + H. $C_{16}H_{33}O_3$ requires 273.2428).

Methyl (6*RS*,12*R*)-13-[(*S*)-α-methoxy-α-trifluoromethylphenylacetoxy]-6,12-dimethyltridecanonate 27. To a solution of **26** (0.90 mg, 2.9 μmol) in pyridine (0.4 cm^3) was added (+)-(*R*)-MTPACl (13 mg, 53 μmol) and the mixture was stirred at 20 °C for 12 h. The reaction mixture was diluted with water and EtOAc, washed with 1 M HCl, aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 10 : 1) to give **27** (1.0 mg, 1.8 μmol, 62%) as a colourless oil. δ_H 0.83 (3 H, d, *J* 6.3 Hz, Me), 0.92 (3 H, d, *J* 6.3 Hz, Me), 1.0–2.0 (18 H, m, CH_2), 2.31 (2 H, t, *J* 7.7 Hz, 2-H), 3.67 (3 H, s, OMe), 4.15 (1 H, m, 13-H), 4.17 (1 H, m, 13-H), 7.4–7.55 (5 H, m, Ar).

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