

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

Ryo Takeuchi,^a Hiromasa Kiyota,^{*a} Manabu Yaosaka,^a Toshihiko Watanabe,^b Keiji Enari,^b
Takeyoshi Sugiyama^a and Takayuki Oritani^a

^a Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiya,
Aoba-ku, Sendai 981-8555, Japan

^b Faculty of Engineering, Tohoku Institute of Technology, 35-1, Yagiyama-kasumicho,
Taihaku-ku, Sendai 982-8577, Japan

Received (in Cambridge, UK) 17th May 2001, Accepted 17th August 2001

First published as an Advance Article on the web 1st October 2001

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 ¹H 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.

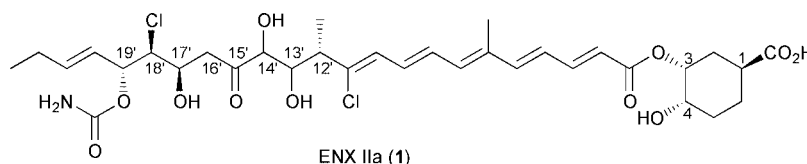
Results and discussion

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

Degradation study. Treatment of dec ENX IIa **3** with NaIO₄ in MeOH gave hemiacetal **5** (excess NaIO₄) or ester **6** (1–2 eq. NaIO₄), 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.



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–H₂O = 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 PtO₂ to give **23** (Scheme 4). The 13',14'-vicinal diol was cleaved with NaIO₄–NaBH₄ to afford a mixture of ester **24** and free acid **25**, which were treated with CH₂N₂ 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 ¹H 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

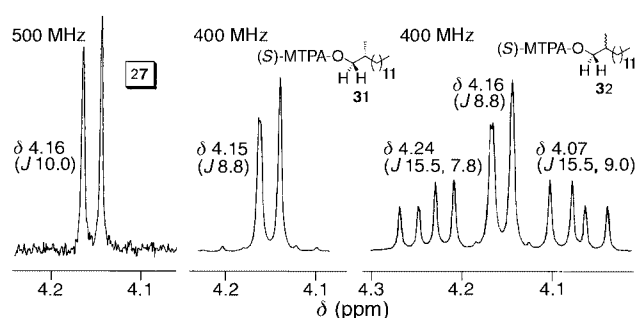


Fig. 1 Parts of the ¹H NMR spectra of the natural and the model compounds.

absolute configuration of the 12'-position was determined to be *S*.

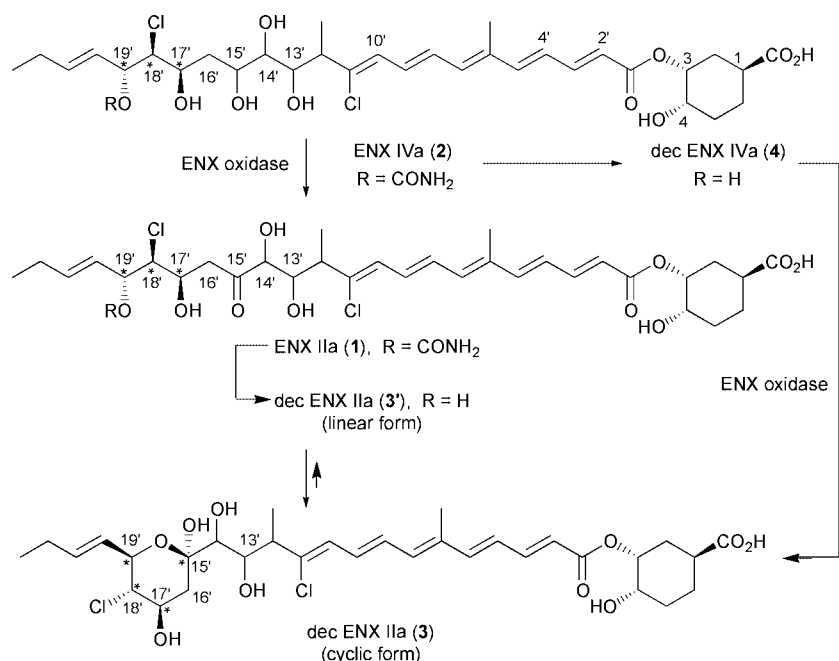
Conclusion

The 12'*S*,17'*R*,18'*S*,19'*R*-absolute configuration of ENX IIa, a polyhydroxy-polyenic antibiotic from *Frateruia* 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.

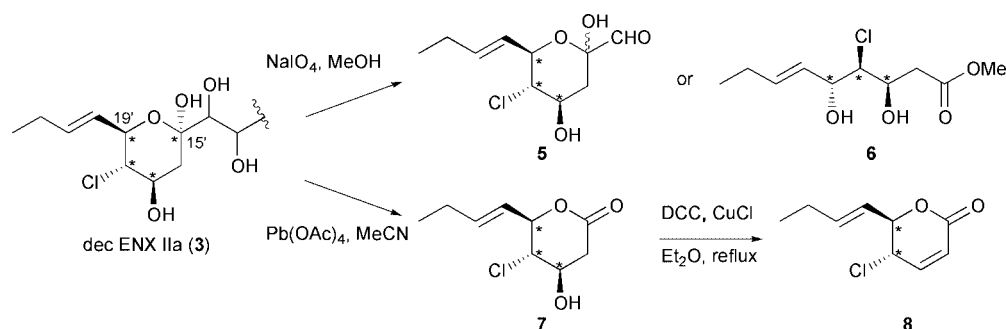
Experimental

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 ^1H) and GEMINI 3000 (300 MHz for ^1H 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 $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$.

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. $\text{Na}_2\text{S}_2\text{O}_3$, 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).

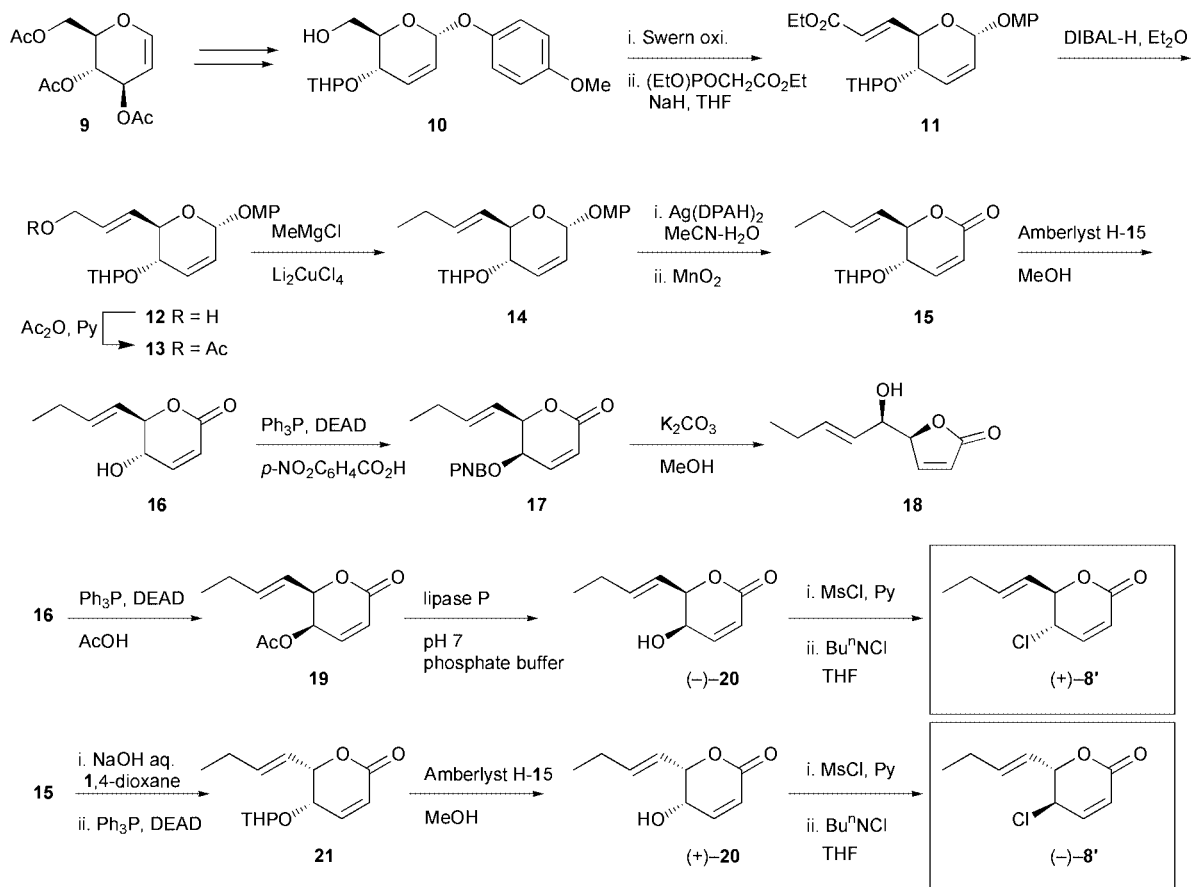
Natural (4*R*,5*S*,6*R*,1'*E*)-6-(but-1'-enyl)-5-chloro-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-one 7. To a solution of dec ENX IIa (1.2 mg, 1.8 μmol) in MeCN (0.6 cm^3) was added slowly a suspension of $\text{Pb}(\text{OAc})_4$ (10 mg) in MeCN (1 cm^3) at 0 °C. After the mixture was stirred for 30 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^iOH = 20 : 1, ERC-Si-1811, 20 °C, 1.0 $\text{cm}^3 \text{ min}^{-1}$, 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'-ethoxycarbonyl-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'**.

anhydride (10 cm³) in pyridine (25 cm³) was stirred for 1 h at 20 °C. The reaction mixture was diluted with EtOAc, successively washed with 1 M HCl aq. and aq. NaHCO₃, dried (MgSO₄) 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; $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 1735 and 1500; δ_{H} (300 MHz) 1.5–1.8 [6 H, m, (CH₂)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*⁺. C₂₂H₂₈O₇ 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 Li₂CuCl₄ (0.1 M in THF, 1.3 cm³, 0.13 mmol) in THF (40 cm³) was added MeMgCl (3 M in THF, 6.0 cm³, 18 mmol) in THF (30 cm³) 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 (MgSO₄) 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; $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 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, (CH₂)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*⁺. C₂₁H₂₈O₅ 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–H₂O (4 : 1, 10 cm³) was stirred at 20 °C for 1 h. The mixture was filtered, the filtrate was poured into aq. Na₂S₂O₃ and extracted with ether. The extract was washed with brine, dried (MgSO₄) 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 MnO₂ (3.0 g) in toluene (10 cm³) 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; $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 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, (CH₂)₃], 2.10 (2 H, dq, *J* 0.8, 7.4 Hz, 3'-H), 3.54 and 3.88 (total 1 H, each m, OCH₂ 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*⁺. C₁₄H₂₀O₄ 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 cm³) 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, $[\alpha]_{\text{D}}^{25}$ –310

(*c* 0.080, CHCl₃); $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 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. C₉H₁₃O₃ 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 Ph₃P (0.70 g, 2.6 mmol) in dry THF (10 ml) was added DEAD (0.4 cm³, 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; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 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 K₂CO₃ (5 mg) in MeOH–H₂O (3 : 1, 4 cm³) 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 MgSO₄ and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 2 : 1) to give **18** as the main product; $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 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), Ph₃P (1.36 g, 5.20 mmol) and acetic acid (0.36 cm³, 4.8 mmol) in dry THF (10 cm³) was added a solution of DEAD (0.80 cm³, 5.0 mmol) in THF (3 cm³) at –45 °C under N₂. 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. NaHCO₃ and brine, dried (MgSO₄) 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, $[\alpha]_{\text{D}}^{25}$ –65 (*c* 0.013, CHCl₃); $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 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. C₁₁H₁₅O₄ 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 Pr₂O (4 cm³) and phosphate buffer (pH 7.0, 0.1 M, 2 cm³) was stirred at 20 °C for 2 d. The mixture was diluted with EtOAc, washed with aq. NaHCO₃ and brine, dried (MgSO₄) 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, $[\alpha]_{\text{D}}^{26}$ –250 (*c* 0.060, CHCl₃); $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 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. C₉H₁₃O₃ requires 169.0864).

(5S,6R,1'E)-6-(But-1'-enyl)-5-chloro-5,6-dihydro-2H-pyran-2-one (+)-8'. To a solution of (–)-**20** (19 mg, 0.11 mmol) in THF (0.5 cm³) was added successively pyridine (0.2 cm³) and MsCl (0.05 cm³) 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 cm³) and the mixture was stirred at 20 °C for 1 d. The reaction mixture was diluted with EtOAc, washed with 1 M HCl, aq. NaHCO₃ and brine, dried (MgSO₄) 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, CDCl₃); $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 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*⁺. C₉H₁₁O₂³⁵Cl requires 186.0447).

(5S,6S,1'E)-6-(But-1'-enyl)-5,6-dihydro-5-(tetrahydro-2'H-pyran-2'-yloxy)-2H-pyran-2-one 21. To a solution of **16** (0.24 g, 0.94 mmol) in 1,4-dioxane–H₂O (1 : 1, 3 cm³) was added 1 M NaOH (1.5 cm³) 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 (MgSO₄) and concentrated *in vacuo*. The residual oil was used in the following step without further purification.

To a solution of PPh₃ (0.25 g, 0.95 mmol) in THF (20 cm³) was added DEAD (0.16 cm³, 0.10 mmol) and the above mentioned oil in THF (10 cm³) 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; $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 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, (CH₂)₃], 2.10 (2 H, dq, *J* 0.8, 7.4 Hz, 3'-H), 3.54 and 3.88 (total 1 H, each m, OCH₂ 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*⁺. C₁₄H₂₀O₄ requires 252.1360).

(5S,6S,1'E)-6-(But-1'-enyl)-5,6-dihydro-5-hydroxy-2H-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%).

(5R,6S,1'E)-6-(But-1'-enyl)-5-chloro-5,6-dihydro-2H-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⁺. C₉H₁₂O₂³⁵Cl 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–H₂O = 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 (6RS,12R)-13-hydroxy-6,12-dimethyltridecanoate 26. A suspension of ENX IIa methyl ester (**22**, 11.7 mg, 16.7 μmol)

and PtO₂ (cat.) in MeOH (1 cm³) was stirred under H₂ 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 NaIO₄ (18.0 mg, 84.0 μmol) in MeOH (1 cm³) was stirred at 20 °C for 1.5 h. The mixture was diluted with EtOAc, washed with aq. Na₂S₂O₃ and brine, dried (MgSO₄) and concentrated *in vacuo*. A solution of the residue and NaBH₄ (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. NaHCO₃ and brine, dried (MgSO₄) and concentrated *in vacuo* to give a mixture of **24** and **25**. This mixture was treated with CH₂N₂ in ether to give a pale yellow oil. This oil was stirred with K₂CO₃ (1.0 mg) in MeOH (1 cm³) for 12 h. The mixture was diluted with EtOAc, washed with H₂O and brine, dried (MgSO₄) 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, CH₂), 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. C₁₆H₃₃O₃ requires 273.2428).

Methyl (6RS,12R)-13-[(S)-α-methoxy-α-trifluoromethylphenylacetoxyl]-6,12-dimethyltridecanoate 27. To a solution of **26** (0.90 mg, 2.9 μmol) in pyridine (0.4 cm³) was added (+)-(R)-MTPACI (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. NaHCO₃ and brine, dried (MgSO₄) 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, CH₂), 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).

References

- Part 2, T. Watanabe, H. Kiyota, R. Takeuchi, K. Enari and T. Oritani, *Heterocycl. Commun.*, in the press.
- (a) T. Watanabe, T. Sugiyama, M. Takahashi, J. Shima, K. Yamashita, K. Izaki, K. Furihata and H. Seto, *Agric. Biol. Chem.*, 1990, **54**, 259; (b) T. Watanabe, T. Sugiyama, M. Takahashi, J. Shima, K. Yamashita, K. Furihata and H. Seto, *J. Antibiot.*, 1992, **45**, 470.
- (a) T. Watanabe, J. Shima, K. Izaki and T. Sugiyama, *J. Antibiot.*, 1992, **45**, 575; (b) T. Watanabe, T. Sugiyama, K. Chino, T. Suzuki, S. Wakabayashi, H. Hayashi, R. Itami, J. Shima and K. Izaki, *J. Antibiot.*, 1992, **45**, 476; (c) T. Watanabe, T. Sugiyama and K. Izaki, *J. Antibiot.*, 1994, **58**, 496; (d) R. Oyama, T. Watanabe, H. Hanzawa, T. Sano and T. Sugiyama, *Biosci. Biotechnol. Biochem.*, 1994, **58**, 1914.
- R. Cetin, I. M. Krab, P. H. Anborgh, R. H. Cool, T. Watanabe, T. Sugiyama, K. Izaki and A. Parmeggiani, *EMBO J.*, 1996, **15**, 2606.
- A. M. Zuurmond, *J. Mol. Biol.*, 1999, **294**, 627.
- B. Clough, *Protistologica*, 1999, **150**, 189.
- C. Alexandre and F. Rouessac, *Bull. Soc. Chim. Fr.*, 1971, 1837.
- A. Hiratate, H. Kiyota, T. Noshita, R. Takeuchi and T. Oritani, *J. Pestic. Sci.*, 2001, **26**, in the press.
- T. Noshita, T. Sugiyama, Y. Kitazumi and T. Oritani, *Biosci. Biotechnol. Biochem.*, 1995, **59**, 2052.
- (a) F. Yasuhara, S. Yamaguchi, R. Kasai and O. Tanaka, *Tetrahedron Lett.*, 1986, **27**, 4033; (b) K. Kikuchi, Bachelor Thesis, Tohoku University, 1989.
- K. Mori, H. Harada, P. Zagatti, A. Cork and D. R. Hall, *Liebigs Ann. Chem.*, 1991, 259.
- O. Nordin, E. Hedenström and H.-E. Högbert, *Tetrahedron: Asymmetry*, 1994, **5**, 785.