Absolute stereostructures of novel cytotoxic metabolites, gymnastatins A–E, from a *Gymnascella* species separated from a *Halichondria* sponge

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Gymnastatins A–E have been isolated from a strain of *Gymnascella dankaliensis* originally separated from the sponge *Halichondria japonica*, and their absolute stereostructures have been established on the basis of spectroscopic analyses using 1D and 2D NMR techniques and some chemical transformations. Among them gymnastatins A–C exhibited significant cytotoxicity against cultured P388 cells.

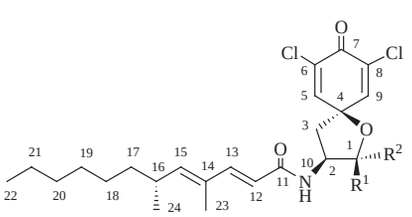
Introduction

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria,[1–4] we have focused our attention on new antitumour materials from microorganisms inhabiting the marine environment. As part of this program, we have found a number of antitumour and/or cytotoxic compounds from various fungi and an actinomycete originally isolated from various marine organisms, and elucidated their structures.[5–12] Our continuing search for cytotoxic compounds has led to the isolation of five metabolites designated gymnastatins A–E **1**–**5** from a strain of *Gymnascella dankaliensis* (Castellani) Currah OUPS-N134 which was separated from the sponge *Halichondria japonica.* We report herein the isolation and absolute stereostructure determination of **1**–**5**, of which the relative stereostructures for **1**–**3** except for the configuration of the 16-position have been briefly reported in a preliminary form,[13] together with their cytotoxic activities.

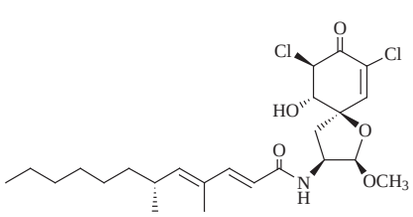
Results and discussion

The fungal strain was cultured at 27 °C for 4 weeks in a medium containing 1% malt extract, 1% glucose and 0.05% peptone in artificial seawater adjusted to pH 7.5. The MeOH extract of the mycelia was purified by bioassay-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatography and high-performance liquid chromatography (HPLC) to afford gymnastatins A–E **1**–**5** as colourless powders.

Gymnastatin A **1** had the molecular formula $C_{23}H_{31}Cl_2NO_4$ established by respective $[M - H_2O]^+$ and M^+ peaks of **1** and its acetate (**6a**) in high-resolution electron impact mass spectrometry (HREIMS), and the ratio of relative intensity of isotope peaks $(M^+ : [M + 2]^+ : [M + 4]^+ = ca.\ 9 : 6 : 1)$ in EIMS. Its IR spectrum exhibited bands at 3377, 3277, 1697, 1653 and 1606 cm^{-1}, characteristic of an alcohol, an amine, a conjugated ketone, an amide and a double bond. The ^1H and ^{13}C NMR spectra (Table 1) of **1** suggested that it existed in a 2 : 1 mixture of two stereoisomers (**1a** and **1b**), in which proton and carbon signals of two methines (δ_H 5.54, d, J 4.3 Hz, and δ_H 5.53, s; δ_C 96.50 and δ_C 103.58), assignable to a hemiacetal group, were observed. Two fractions separated from the mixture by HPLC each exhibited the NMR spectrum for a mixture of **1a** and **1b** in a 2 : 1 ratio. On the other hand, acetylation of **1** afforded two separable monoacetates (**6a** and **6b**), in which the ^1H and ^{13}C NMR signals for acetoxymethine groups appeared at δ_H 6.38 (d, J 4.6 Hz) and δ_C 95.24, and δ_H 6.38 (s) and δ_C 101.18, respectively (Table 2). Hydrolysis of **6a** by an aqueous ammonia solution gave compound **1** as a 2 : 1 mixture of two stereoisomers.



1a : R^1 = OH, R^2 = H
1b : R^1 = H, R^2 = OH
6a : R^1 = OAc, R^2 = H
6b : R^1 = H, R^2= OAc



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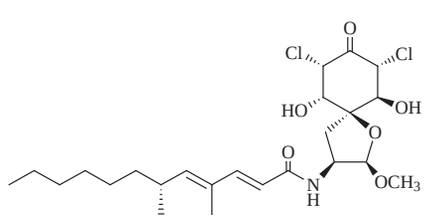

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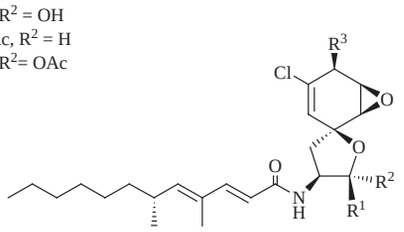
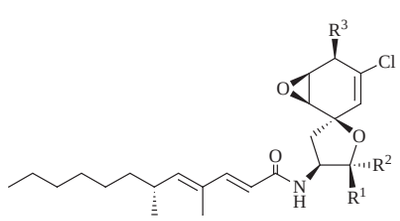


4a : R^1, R^3 = OH, R^2 = H
4b : R^1 = H, R^2, R^3 = OH
7a : R^1, R^3 = OAc, R^2 = H
7b : R^1 = H, R^2, R^3 = OAc



5a : R^1, R^3 = OH, R^2 = H
5b : R^1 = H, R^2, R^3 = OH
8a : R^1, R^3 = OAc, R^2 = H
8b : R^1 = H, R^2, R^3 = OAc

Table 1 ^{1}H and ^{13}C NMR data of gymnastatin A (**1**) in $CDCl_3$

	1a			**1b**		
Position	δ_H [a]	*J*/Hz	δ_C	δ_H [a]	*J*/Hz	δ_C
1	5.54d	4.3 (2)	96.50 (t) [b]	5.53s		103.58 (t)
2	4.79m		52.24 (t)	4.65m		58.15 (t)
3α	2.59dd	12.9 (3β), 8.3 (2)	38.35 (s)	2.82dd	12.9 (3β), 8.3 (2)	40.21 (s)
β	2.24t	12.9 (2,3α)		2.24t	12.9 (2,3α)	
4			78.98 (q)			80.30 (q)
5	7.03d	2.7 (9)	144.72 (t)	7.03d	2.7 (9)	144.72 (t)
6			130.61 (q)			130.61 (q)
7			172.78 (q)			172.78 (q)
8			130.69 (q)			130.69 (q)
9	7.14d	2.7 (5)	147.27 (t)	7.14d	2.7 (5)	147.27 (t)
10	6.18d	8.2 (2)		6.18d	8.2 (2)	
11			166.99 (q)			167.78 (q)
12	5.78d	15.1 (13)	116.64 (t)	5.78d	15.1 (13)	116.64 (t)
13	7.26d	15.1 (12)	147.84 (t)	7.26d	15.1 (12)	147.84 (t)
14			130.80 (q)			130.80 (q)
15	5.78d	9.8 (16)	148.98 (t)	5.78d	9.8 (16)	148.98 (t)
16	2.50m		33.24 (t)	2.50m		33.24 (t)
17	1.37m		37.21 (s)	1.37m		37.21 (s)
18	1.23m		27.48 (s)	1.23m		27.48 (s)
19	1.23m		29.39 (s)	1.23m		29.39 (s)
20	1.23m		31.83 (s)	1.23m		31.83 (s)
21	1.23m		22.64 (s)	1.23m		22.64 (s)
22	0.87t	6.7 (21)	14.11 (p)	0.87t	6.7 (21)	14.11 (p)
23	1.77s		12.52 (p)	1.77s		12.52 (p)
24	0.96d	6.6 (16)	20.51 (p)	0.96d	6.6 (16)	20.51 (p)
1-OH	5.11br s			5.11br s		

[a] ^{1}H Chemical-shift values (δ/ppm from TMS) followed by multiplicity and then the coupling constant (*J*/Hz). Figures in parentheses indicate the proton coupling with that position. [b] Letters, p, s, t and q, in parentheses indicate, respectively, primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

This evidence implied that **1** contains two stereoisomers (**1a** and **1b**) on a hemiacetal group, which coexist at equilibrium in a certain ratio. Acetate **6a** thus obtained was used for a structure analysis of **1**.

A close inspection of the ^{1}H and ^{13}C NMR spectral data (Table 2) of **6a** by DEPT and ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectroscopy (COSY) experiments revealed the presence of the following functionalities: three methyls (C-22 to C-24) including one primary, secondary and vinyl methyl each, six methylenes (C-3 and C-17 to C-21), one disubstituted and three trisubstituted double bonds (C-12, C-13, C-5, C-6, C-8, C-9, C-14 and C-15), a secondary amide (N-10 and C-11) and a quaternary sp^3-carbon (C-4) linked to an oxygen atom, two sp^3-hybridized methines (C-2 and C-16) including one methine linked to a nitrogen atom in addition to the acetoxymethine (C-1) of the hemiacetal group. The remaining functionality, corresponding to the carbon signal at δ_C 172.08, was shown to be a ketone in a cross-conjugated dienone system on the basis of HMBC correlations (5-H/C-7 and 9-H/C-7) shown in Fig. 1. The carbon signal of the conjugated ketone appeared shifted upfield by *ca*. 10 ppm, relative to a general cross-conjugated cyclohexadienone (δ_C 183–185),[14–16] suggesting that chlorine atoms exist at the α-position of the ketone.[16,17] In addition to the partial structure (C-5 to C-9) thus established, the ^{1}H–^{1}H COSY analysis of **6a** led to other partial structures shown by bold-faced lines in Fig. 1, which were supported by HMBC correlations (Table 2). The geometry of the diene in the side chain was deduced from a coupling constant ($J_{12,13}$ 15.1 Hz) of olefinic protons, a chemical shift (δ_C 12.49) of the ^{13}C NMR signal of a vinyl methyl,[18] and nuclear Overhauser enhancements (NOEs) for 12-H/23-H and 13-H/15-H. The connection of these partial structures and the remaining functional groups (C-4, C-11 and C-18–C-20) was determined on the basis of HMBC correlations shown in Fig. 1. Based on this evidence, the planar structure of acetate **6a** was elucidated. The side chain was supported by an EIMS fragment at *m*/*z* 179 ($[C_{13}H_{23}]^+$).

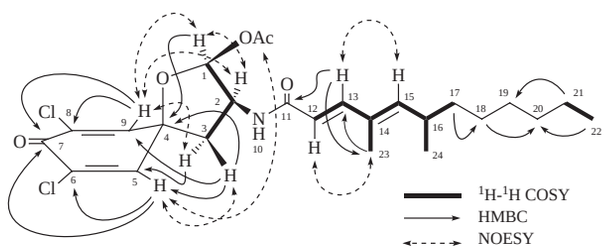


Fig. 1 Selected ^{1}H–^{1}H COSY, HMBC and NOESY correlations in gymnastatin A acetate **6a**.

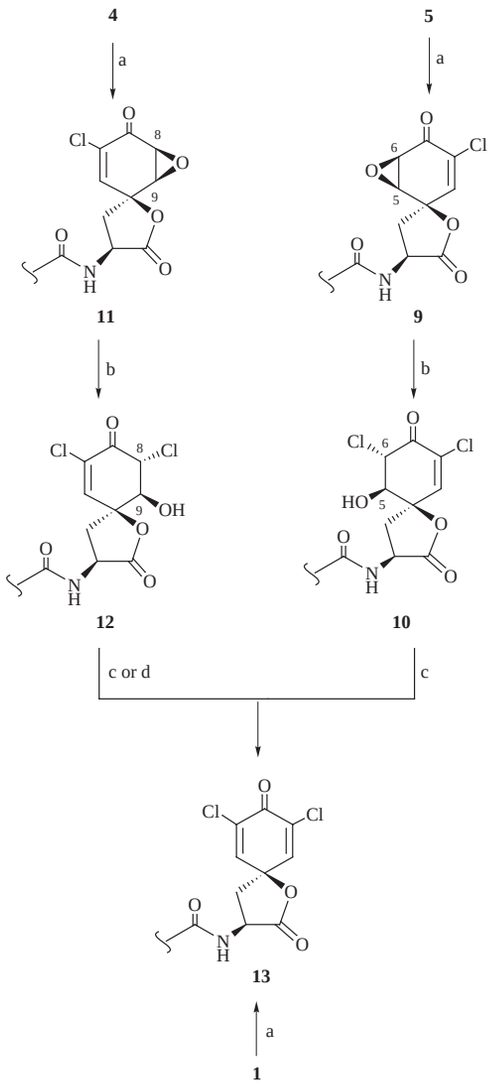
In an NOESY experiment, acetate **6a** showed NOEs for 1-H/9-H, 2-H/9-H and Ac/5-H (Fig. 1), whereas stereoisomeric acetate **6b** exhibited NOEs for 1-H/5-H, 2-H/9-H and Ac/9-H (Table 2). This finding indicated that 1-H and 2-H are in the same arrangement as 9-H in **6a**, and 1-OAc and 2-H are in the same arrangement as 9-H in **6b**, meaning that H-1 is *trans* to 2-H. Consequently, the relative stereostructures for gymnastatin A **1** as a mixture of diastereomers at the 1-position were established as **1a** and **1b** except for the configuration of the 16-position. The absolute stereostructure of **1** including the configuration of the 16-position was determined by a chemical transformation of gymnastatin E **5**, of which the absolute configuration was determined as described below, to the oxidation product **13** of **1** by a pyridine–CrO_3 complex (Scheme 1).

Gymnastatin E **5** was assigned the molecular formula $C_{23}H_{34}ClNO_5$ as deduced from a molecular ion peak in HREIMS. Its IR spectrum exhibited absorption bands for hydroxy and amino groups, an amide and a double bond, but did not show a band for a conjugated ketone. The ^{1}H and ^{13}C NMR spectra (Table 3) of **5** suggested that it exists in a 2:1 mixture of two stereoisomers (**5a** and **5b**) as in compound **1**. Acetylation of **5** by a standard method gave two diacetates **8a** and **8b**. Since these acetates could be separated by HPLC, acetate **8a** was used for a structure analysis of **5**.

Table 2 ^{1}H and ^{13}C NMR data of gymnastatin A acetates **6a** and **6b** in $CDCl_3$

	6a						**6b**			
Position	δ_H [a]	*J*/Hz	δ_C	^{1}H–^{1}H COSY	HMBC (C)	NOESY	δ_H [a]	*J*/Hz	δ_C	NOESY
1	6.38d	4.6 (2)	95.24 (t) [b]	2	1-O*C*OCH$_3$, 2, 3, 4	2, 9, 10	6.38s		101.18 (t)	2, 3β, 5, 10
2	5.06dddd	13.0 (3β), 8.7 (10), 7.8 (3α), 4.6 (1)	50.99 (t)	1, 3α, 3β, 10		1, 3α, 9, 10	4.74td	6.2 (3α, 10), 2.7 (3β)	56.78 (t)	1, 3α, 3β, 9, 10
3α	2.63dd	13.0 (3β), 7.8 (2)	38.42 (s)	3β	1, 2, 5	2, 3β, 9	2.75dd	14.4 (3β), 6.2 (2)	40.78 (s)	3β, 2, 9
β	2.20t	13.0 (2, 3α)		2, 3α	2, 4, 5, 9	3α, 5, 10	2.37dd	14.4 (3α), 2.7 (2)		2, 3α, 5, 10
4			79.87 (q)						81.74 (q)	
5	6.89d	2.7 (9)	145.03 (t)	9	6, 7, 9	3β, 1-OAc	6.99d	2.7 (9)	144.00 (t)	1, 3β
6			131.70 (q)						131.16 (q)	
7			172.08 (q)						172.11 (q)	
8			131.35 (q)						131.42 (q)	
9	7.05d	2.7 (5)	143.15 (t)	5	5, 7, 8	1, 2, 3α	7.01d	2.7 (5)	145.28 (t)	1-OAc, 2, 3α
10	5.64d	8.7 (2)		2	2, 11	1, 2, 3β	5.85d	6.2 (2)		1, 2, 3β
11			166.26 (q)						166.57 (q)	
12	5.73d	15.1 (13)	116.07 (t)	13	11, 13, 14	23	5.78d	15.2 (13)	116.08 (t)	23
13	7.30d	15.1 (12)	148.37 (t)	12	11, 12, 14, 15, 23	15	7.30d	15.2 (12)	148.34 (t)	15
14			130.65 (q)						130.69 (q)	
15	5.71d	9.6 (16)	149.20 (t)	16, 23	13, 14, 16, 17, 23, 24	13, 16, 24	5.70d	10.0 (16)	149.28 (t)	13, 24
16	2.52m		33.28 (t)	15, 17A, 17B, 24		15, 23, 24	2.52m		33.29 (t)	23, 24
17A	1.27m		37.20 (s)	16	18		1.27m		37.21 (s)	
B	1.35			16	18		1.35m			
18	1.22m		27.46 (s)		19		1.22m		27.47 (s)	
19	1.23m		29.37 (s)				1.24m		29.38 (s)	
20	1.27m		31.81 (s)		18		1.27m		31.81 (s)	
21	1.23m		22.60 (s)	22	19, 20		1.23m		22.62 (s)	
22	0.87t	6.6 (21)	14.06 (p)	21	20, 21		0.87t	6.9 (22)	14.09 (p)	
23	1.78s		12.49 (p)	15	13, 14, 15	12, 16	1.79s		12.51 (p)	12, 16
24	0.97d	6.7 (16)	20.48 (p)	16	15, 16, 17	15, 16	0.98d	6.6 (16)	20.49 (p)	15, 16
1-O*C*OCH$_3$			169.03 (q)						169.12 (q)	
1-OCO*CH*$_3$	2.21s		21.34 (p)		1-O*C*OCH$_3$	5	2.17s		21.18 (p)	9

[a,b] As in Table 1.



Scheme 1 *Reaction conditions*: (a) pyridine–CrO_3; (b) pyridinium chloride; (c) Ac_2O, pyridine; (d) MsCl, Et_3N.

The general features of the ^1H and ^{13}C NMR spectra (Table 4) of **8a** closely resembled those of **6a** except that the signals for the ketone and one of the trisubstituted double bonds in the cyclohexane ring of **6a** were respectively replaced by those of a secondary alcohol (δ_H 5.68; δ_C 66.66) and an epoxide [δ_H 3.34 and 3.58; δ_C 56.88 (J_{CH} 183.6 Hz) and 51.48 (J_{CH} 180.2 Hz)] in **8a**. The position of the three functional groups in the cyclohexane ring of **8a** was established by a combination of coupling relationships between vicinal protons and ^1H–^1H COSY correlations from 6-H to 5-H and 7-H, and from 9-H to 7-H and 5-H (long-range couplings). It is most likely that a cyclohexane ring with an epoxide and a double bond like **8a** exists in a boat conformation. The observation of NOEs from 9-H (a vinyl proton) to 1-H, 2-H and 3-H$^\alpha$, and from 5-H (an epoxymethine proton) to 3-H$^\beta$ in **8a** (Table 4) implied that the stereostructure of **8a** is either of two stereoisomers, in which the orientation of the epoxide is opposite each other. In order to choose between them for **8a**, gymnastatin E **5** was converted to keto-lactone **9**, which then was derivatized to chlorohydrin **10** by treatment with pyridinium chloride (Scheme 1). The large coupling constant ($J_{5,6}$ 10.4 Hz) between H-5 and H-6 in chlorohydrin **10** obtained indicated that the cyclohexane ring of **10** exists in a chair conformation, with 5-OH and 6-Cl in a coequatorial arrangement. In NOESY experiments of compound **10**, NOEs from 3-H$^\alpha$ to 2-H and 9-H (a vinyl proton), and from 3-H$^\beta$ to 5-H (a hydroxymethine proton) were observed, implying that 5-OH in chlorohydrin **10** is oriented *cis* to the carbonyloxy group, and consequently the epoxide bond in acetate **8a** is oriented *cis* to the ether bond (C-4–O). The summary of these considerations led to the relative stereostructures of acetate **8a** and, consequently, of **5** (**5a** and **5b**) for gymnastatin E, except for the 7- and 16-positions. In order to determine the relative configuration of the 7- and 16-positions in **5**, an X-ray crystal structure analysis was carried out for a single crystal of acetate **8a** (obtained by recrystallization from MeOH). The result obtained (Fig. 2) allowed assignment of the relative configuration of all the asymmetric centres and the conformation for **8a** and **5**. Though the chirality of **8a** was also investigated by using the abnormal dispersion factor of chlorine in the X-ray analysis, a conclusive result was not obtained. Therefore, the modified Mosher method[19] was applied for determination of the absolute configuration of compound **5**.

The '(*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)-acetic acid' (MTPA) esters **14a** and **14b** of compound **5** were prepared by a standard method. Though compound **5** is a mixture of two stereoisomers (**5a** and **5b**), this reaction gave only the 1,7-bis-MTPA esters with the 1-ester in a β-configuration, implying that these reaction conditions displaced the equilibrium of the hemiacetal group to one stereoisomer. The ^1H chemical-shift differences between the (*R*)- and (*S*)-1,7-bis-MTPA esters **14a** and **14b** are shown in Fig. 3, and the result suggested the *R* configuration for the asymmetric centre at C-7 and, consequently, led to absolute stereostructure **5** (**5a** and **5b**) for gymnastatin E. It is known that ^1H chemical-shift differences of MTPA esters with axial arrangements or with steric hindrances are irregularly distributed.[19] In this case, the 1-MTPA ester with steric hindrance also did not give data in accord with the rule.

As described above briefly, treatment of chlorohydrin **10** from **5** with acetic anhydride in pyridine afforded keto-lactone **13** which was identical with that derived from **1** by treatment with a pyridine–CrO_3 complex in all the respects including a specific rotation. This evidence led to the absolute configuration of all the asymmetric centres including the 16-position for gymnastatin A **1** (Scheme 1).

Gymnastatin D **4** is isomeric with compound **5**. The general features of ^1H and ^{13}C NMR spectra (Table 5) of **4** closely

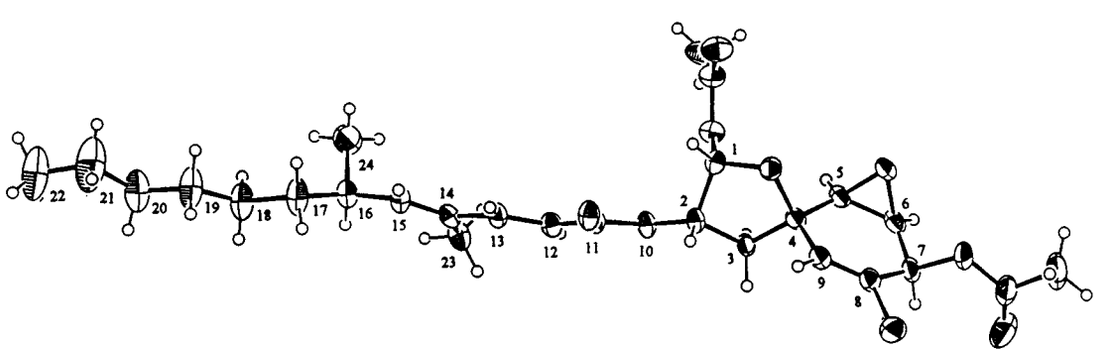
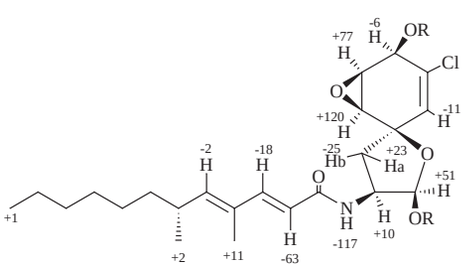


Fig. 2 X-Ray crystal structure for compound **8a**.

Table 3 ^{1}H and ^{13}C NMR data of gymnastatin E **5** in acetone-d_6

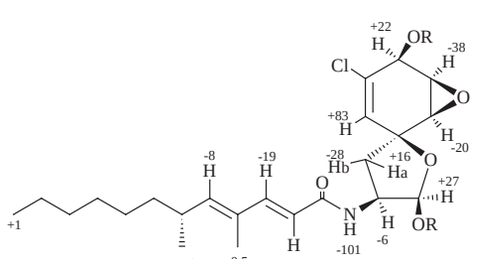
	5a			**5b**		
Position	δ_H [a]	*J*/Hz	δ_C	δ_H [a]	*J*/Hz	δ_C
1	5.42t	3.8 (2, 1-OH)	96.65 (t) [b]	5.48d	3.4 (1-OH)	103.13 (t)
2	4.60m		52.81 (t)	4.40m		58.09 (t)
3α	2.24dd	12.4 (3β), 8.2 (2)	39.38 (s)	2.44dd	14.0 (3β), 6.7 (2)	40.47 (s)
β	2.13t	12.4 (2,3α)		2.18t	14.0 (3α), 1.4 (2)	
4			81.23 (q)			82.99 (q)
5	3.43dd	4.1 (6), 2.4 (9)	59.00 (t)	3.45dd	4.1 (6), 2.4 (9)	57.74 (t)
6	3.53dd	4.1 (5), 2.8 (7)	55.03 (t)	3.52dd	4.1 (5), 2.8 (7)	54.74 (t)
7	4.41dd	2.8 (6), 1.6 (9)	66.65 (t)	4.43dd	2.8 (6), 1.6 (9)	66.52 (t)
8			132.58 (q)			132.15 (q)
9	5.80dd	2.4 (5), 1.6 (7)	129.08 (t)	5.86dd	2.4 (5), 1.6 (7)	131.19 (t)
10	7.13d	8.0 (2)		7.49d	6.6 (2)	
11			166.36 (q)			166.41 (q)
12	6.16d	15.4 (13)	119.99 (t)	6.11d	15.4 (13)	119.78 (t)
13	7.21d	15.4 (12)	145.74 (t)	7.23d	15.4 (12)	145.83 (t)
14			132.25 (q)			132.15 (q)
15	5.66d	9.8 (16)	146.66 (t)	5.66d	9.8 (16)	146.86 (t)
16	2.58m		33.68 (t)	2.58m		33.68 (t)
17A	1.29m		38.03 (s)	1.29m		38.03 (s)
B	1.38m			1.38m		
18	1.26m		28.18 (s)	1.26m		28.18 (s)
19	1.27m		30.05 (s)	1.27m		30.05 (s)
20	1.26m		32.54 (s)	1.26m		32.54 (s)
21	1.27m		23.24 (s)	1.27m		23.24 (s)
22	0.89t	6.9 (21)	14.29 (p)	0.89t	6.9 (21)	14.29 (p)
23	1.81s		12.70 (p)	1.79s		12.70 (p)
24	1.00d	6.6 (16)	20.91 (p)			20.91 (p)
1-OH	6.05d	4.1 (1)		5.88d	3.7 (1)	
7-OH	4.76d	8.5 (7)		4.79d	8.5 (7)	

[a,b] As in Table 1.



14a R= (*R*)-MTPA
14b R= (*S*)-MTPA

Fig. 3 ^{1}H Chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters **14a** and **14b** of gymnastatin E (**5**). $\Delta\delta$-Values are expressed in Hz (500 MHz).



15a R= (*R*)-MTPA
15b R= (*S*)-MTPA

Fig. 4 ^{1}H Chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters **15a** and **15b** of gymnastatin D (**4**). $\Delta\delta$-Values are expressed in Hz (500 MHz).

resembled those of its isomer **5** and also indicated it to be a mixture of two stereoisomers (**4a** and **4b**) on the hemiacetal group. Moreover, compound **4** gave two separable diacetates **7a** and **7b**, of which the NMR spectra (Table 6) also exhibited signals similar to diacetates **8a** and **8b** of compound **5**, respectively.

In NOESY experiments of diacetate **7a**, NOEs from 9-H (an epoxymethine proton) to 2-H and 3-H$^\alpha$, and from 5-H (a vinyl proton) to 3-H$^\beta$ were observed, whereas an NOE was not observed between 1-H and 9-H (Table 6). Though an NOE between 1-H and 2-H was observed in both diacetates **7a** and **7b**, there were obvious differences between the two acetates in selected difference NOE values between 1-H and 2-H [8.0% (**7a**) > 3.3% (**7b**)]. This finding suggested that 1-H and 2-H are oriented on the same side as the epoxymethine proton (9-H) in compound **7a**. However, the orientation of the epoxide in **7a** was not suggested by these observed NOEs. It was deduced from NOE data of chlorohydrin **12** derived by oxidation of compound **4** followed by treatment with pyridinium chloride as in the case of **5** (Scheme 1). The observation of NOEs from 5-H (a vinylic proton) to 3-H$^\beta$, and from 3-H$^\alpha$ to 2-H and 9-H (a hydroxymethine proton) in **12** implied that the epoxide bond in acetate **7a** is oriented *cis* to the ether bond (C-4–O). The coupling constant (2.8 Hz) between 7-H and 8-H in **7a** was almost the same value as that between 6-H and 7-H in **8a**, suggesting the pseudoequatorial arrangement of 7-OH in **7a** as in **8a**. The above-summarized evidence led to the relative stereostructure of **4** (**4a** and **4b**) for gymnastatin D.

Treatment of chlorohydrin **12** with acetic anhydride in pyridine or mesyl chloride (MsCl) and triethylamine (Et_3N) afforded keto-lactone **13** derived from gymnastatin E **5** (Scheme 1), allowing assignment of the absolute configuration to gymnastatin D **4**. This absolute configuration was identical with that assigned by application of the modified Mosher method for the (*R*)- and (*S*)-1,7-bis-MTPA esters **15a** and **15b** derived from **4** (Fig. 4). This result supported the pseudoequatorial arrangement of 7-OH in **4**, deduced from the observed coupling constant between 7-H and 8-H in acetate **7a**.

Gymnastatin B **2** had the molecular formula $C_{24}H_{35}Cl_2NO_5$ established by an M^+ peak of **2** in HREIMS. The general spectral features of compound **2** closely resembled those of **1a** except that one of the trisubstituted double bonds in the cyclohexane ring and the hydroxy group on the hemiacetal group in **1a** were replaced by two sp^3-methines linked to a hydroxy group

Table 4 ^{1}H and ^{13}C NMR data of gymnastatin E diacetates **8a** and **8b** in $CDCl_3$

	8a						**8b**		
Position	δ_H [a]	*J*/Hz	δ_C	^{1}H–^{1}H COSY	HMBC (C)	NOESY	δ_H [a]	*J*/Hz	δ_C
1	6.37d	4.4 (2)	95.34 (t) [b]	2	2, 3, 4	2, 9, 10	6.33s		100.55 (t)
2	4.94dddd	12.5 (3β), 9.4 (10), 8.3 (3α), 4.4 (1)	50.85 (t)	1, 3α, 3β, 10		1, 3α, 9, 10	4.63td	6.5 (3α, 10), 1,6 (3β)	56.46 (t)
3α	2.42dd	12.5 (3β), 8.3 (2)	38.89 (s)	2, 3β	2, 4, 5, 9	2, 3β, 9	2.48dd	14.3 (3β), 6.5 (2)	40.07 (s)
β	2.03t	12.5 (2, 3α)		2, 3α	1, 2, 5, 9	3α, 5	2.31dd	14.3 (3α), 1.6 (2)	
4			81.88 (q)						83.72 (q)
5	3.34dd	4.1 (6), 2.3 (9)	56.88 (t)	9	4, 9	3β, 6	3.42dd	4.1 (6), 2.5 (9)	56.19 (t)
6	3.58dd	4.1 (5), 2.7 (7)	51.48 (q)	5, 7	8	5, 7	3.57dd	4.1 (5), 2.7 (7)	51.58 (t)
7	5.68dd	2.7 (6), 1.8 (9)	66.66 (t)	6, 9	6, 9	6	5.68dd	2.7 (6), 1.8 (9)	66.52 (t)
8			127.97 (q)						127.73 (q)
9	5.88dd	2.3 (5), 1.8 (7)	128.68 (t)	5, 7	5, 7, 8	1, 2, 3α	5.81dd	2.5 (5), 1.8 (7)	129.94 (t)
10	5.69d	9.4 (2)		2		1, 2	5.94br d	6.5 (2)	
11			166.23 (q)						166.35 (q)
12	5.71d	15.2 (13)	116.36 (t)	13	11, 13, 14	23	5.73d	15.1 (13)	116.48 (t)
13	7.28d	15.2 (12)	147.98 (t)	12	11, 12, 14, 15, 23	15	7.26d	15.1 (12)	147.75 (t)
14			130.65 (q)						130.73 (q)
15	5.59d	9.4 (16)	148.85 (t)	16, 23	13, 14, 16, 17, 23	13, 24	5.67d	9.6 (16)	148.81 (t)
16	2.51m		33.26 (t)	15, 17A, 17B, 24		23, 24	2.51m		33.24 (t)
17A	1.26m		37.22 (s)	16	18		1.25m		37.22 (s)
B	1.35m			16	18		1.35m		
18	1.22m		27.47 (s)		19		1.20m		27.47 (s)
19	1.24m		29.38 (s)				1.23m		29.38 (s)
20	1.23m		31.82 (s)		18		1.27m		31.82 (s)
21	1.27m		22.62 (s)	22	19, 20		1.23m		22.62 (s)
22	0.87t	6.9 (21)	14.08 (p)	21	20, 21		0.87t	6.9 (21)	14.09 (p)
23	1.77s		12.50 (p)	15	13, 14, 15	12, 16	1.76s		12.48 (p)
24	0.98d	6.6 (16)	20.51 (p)	16	15, 16, 17	15, 16	0.97d	6.6 (16)	20.51 (p)
1-O*C*OCH$_3$			169.11 (q)						169.11 (q)
1-OCO*CH*$_3$	2.17s		21.45 (p)		1-O*C*OCH$_3$		2.11s		21.17 (p)
7-O*C*OCH$_3$			170.42 (q)						170.34 (q)
7-OCO*CH*$_3$	2.21s		20.71 (p)		7-O*C*OCH$_3$		2.21s		20.69 (p)

[a,b] As in Table 1.

Table 5 ^{1}H and ^{13}C NMR data of gymnastatin D (**4**) in acetone-d_6

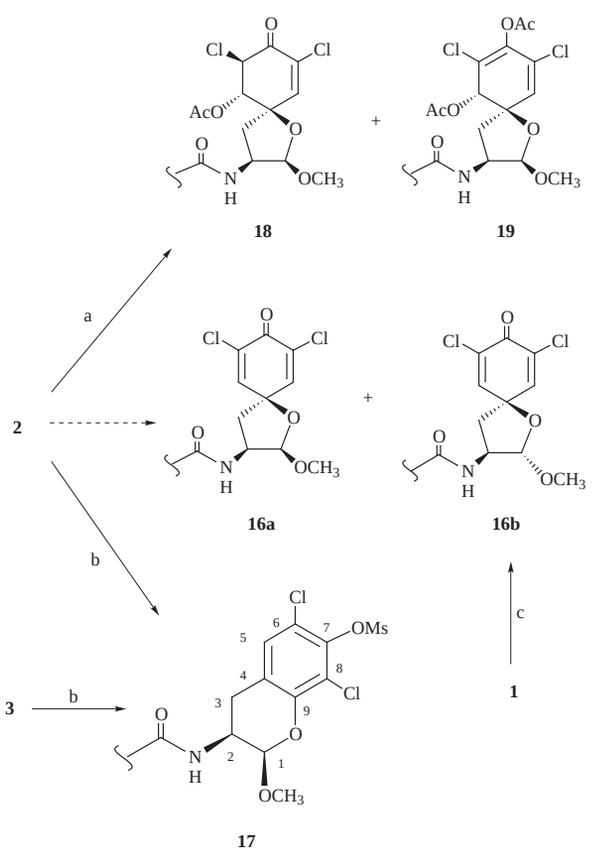
	4a			**4b**		
Position	δ_H [a]	*J*/Hz	δ_C	δ_H [a]	*J*/Hz	δ_C
1	5.40t	4.1 (2, 1-OH)	96.64 (t) [b]	5.46d	3.7 (1-OH)	103.13 (t)
2	4.62m		52.64 (t)	4.42m		57.73 (t)
3α	2.44dd	12.6 (3β), 8.0 (2)	38.33 (s)	2.63dd	14.0 (3β), 6.8 (2)	40.80 (s)
β	1.99dd	12.6 (3α), 11.4 (2)		1.93dd	14.0 (3α), 2.1 (2)	
4			80.86 (q)			82.83 (q)
5	5.76dd	2.3 (9), 1.6 (7)	130.47 (t)	5.79dd	2.3 (9), 1.6 (7)	129.54 (t)
6			132.18 (q)			132.08 (q)
7	4.40dd	2.9 (8), 1.6 (5)	66.47 (t)	4.42dd	2.5 (9), 1.6 (5)	66.53 (t)
8	3.55dd	4.1 (9), 2.9 (7)	54.79 (t)	3.53dd	4.1 (9), 2.9 (7)	55.13 (t)
9	3.43dd	4.1 (8), 2.3 (5)	57.46 (t)	3.48dd	4.1 (8), 2.3 (5)	59.15 (t)
10	7.17d	8.0 (2)		7.52d	6.6 (2)	
11			166.67 (q)			166.67 (q)
12	6.15d	15.3 (13)	119.77 (t)	6.05d	15.3 (13)	119.56 (t)
13	7.21d	15.3 (12)	145.98 (t)	7.21d	15.3 (12)	145.98 (t)
14			132.22 (q)			132.22 (q)
15	5.64d	9.8 (16)	146.95 (t)	5.64d	9.8 (16)	146.95 (t)
16	2.57m		33.65 (t)	2.57m		33.65 (t)
17A	1.29m		37.96 (s)	1.29m		37.96 (s)
B	1.38m			1.38m		
18	1.29m		28.14 (s)	1.29m		28.14 (s)
19	1.28m		30.05 (s)	1.28m		30.05 (s)
20	1.29m		32.49 (s)	1.29m		32.49 (s)
21	1.26m		23.20 (s)	1.26m		23.20 (s)
22	0.87t	6.9 (21)	14.28 (p)	0.87t	6.9 (21)	14.28 (p)
23	1.79s		12.69 (p)	1.76s		12.69 (p)
24	0.99d	6.7 (16)	20.88 (p)	0.99d	6.7 (16)	20.88 (p)
1-OH	6.05d	4.1 (1)		5.88d	3.7 (1)	
7-OH	4.76d	8.5 (7)		4.79d	8.5 (7)	

[a,b] As in Table 1.

and a chlorine atom [δ_H 4.29 (5-H), δ_C 75.46 (C-5); δ_H 5.40 (6-H), δ_C 60.85 (C-6)], and a methoxy group (δ_H 3.48; δ_C 55.10) in **2**, respectively, and the C-7 signal (δ_C 183.49) of the ketone in **2** appeared shifted downfield relative to that of **1a** in the NMR spectra (Table 7). The planar structure of **2** thus deduced from the NMR spectral analysis was confirmed by analysis of ^{1}H–^{1}H COSY and HMBC correlations (1-H/OMe, 5-H/C-4, 5-H/C-7, 5-H/C-9, 9-H/C-3, 9-H/C-7 *etc.*; Table 7).

The observations of NOEs from 9-H to 2-H and 3-H$^\alpha$, and from 5-H to 3-H$^\beta$ and OMe, and a W-type long-range coupling ($J_{5,9}$ 2.2 Hz) between 5-H and 9-H in **2** (Table 7) showed that 1-H and 2-H are on the same side as 9-H, and the 1-methoxy group is on the same side as 5-H with a pseudoequatorial arrangement on the cyclohexane ring. In addition, observation of NOEs from 6-H to 5-H and 5-OH, and the small coupling constant ($J_{5,6}$ 2.2 Hz) between 5-H and 6-H implied that the cyclohexane ring in **2** exists in a twist-chair conformation with 5-H and 6-H in a co-pseudoequatorial, consequently, *trans* arrangement.

An interconversion of compounds **2** and **1** was attempted for assignment of an absolute configuration for **2**. Treatment of **1** with trimethyl orthoformate under the presence of toluene-*p*-sulfonic acid afforded two separable methyl hemiacetals **16a** and **16b** (Scheme 2). In order to derive **16a** from **2**, compound **2** was treated with MsCl and Et_3N as for the derivatives (**12**) of gymnastatin D **4**. This reaction did not give the desired product, but compound **17** formed by rearrangement and aromatization (Scheme 2). The structure of **17** was established by analysis of ^{1}H–^{1}H COSY and HMBC correlations, and NOE data in **17**. Cross peaks were observed from 2-H to 1-H, 3-H$^\alpha$ and 3-H$^\beta$ in ^{1}H–^{1}H COSY, and from only one aromatic proton (5-H) to C-3 and two aromatic quaternary carbons (C-7 and C-9) bearing an oxygen atom, from 1-H to C-3 and C-9, and from 3-H$^\alpha$ and 3-H$^\beta$ to one (C-4) of three other aromatic quaternary carbons (C-4, C-6 and C-8) in HMBC. In additon to these correlations, the observation of NOEs from 5-H to 3-H$^\alpha$ and 3-H$^\beta$ suggested



Scheme 2 *Reaction conditions*: (a) Ac_2O, pyridine; (b) MsCl, Et_3N; (c) $CH(OCH_3)_3$, *p*-TsOH.

positional relationships of C-1–C-5, C-7 and C-9. Therefore, two chlorine atoms should be linked to the two remaining quaternary carbons (C-6 and C-8), and C-6 was assigned by an

Table 6 ^{1}H and ^{13}C NMR data of gymnastatin D diacetates **7a** and **7b** in $CDCl_3$

	7a					**7b**			
Position	δ_H [a]	*J*/Hz	δ_C	HMBC (C)	NOESY	δ_H [a]	*J*/Hz	δ_C	NOESY
1	6.36d	4.4 (2)	95.33 (t) [b]	2, 3, 4	2, 10	6.31s	4.4 (2)	100.85 (t)	2, 10
2	4.97dddd	13.0 (3β), 8.5 (10), 8.0 (3α), 4.4 (1)	50.56 (t)		1, 3α, 9, 10	4.69td	7.0 (3α, 10), 2.5 (3β)	55.92 (t)	1, 3α, 9, 10
3α	2.54dd	13.0 (3β), 8.0 (2)	37.93 (s)	4, 5, 9	2, 3β, 9	2.63dd	14.1 (3β), 7.0 (2)	40.87 (s)	2, 9
β	1.96t	13.0 (2, 3α)		1, 9	3α, 5	2.04dd	14.1 (3α), 2.5 (2)		5
4			81.65 (q)					83.52 (q)	
5	5.74dd	2.5 (9), 1.8 (7)	129.63 (t)	6, 7, 8	3β	5.82dd	2.5 (9), 1.8 (7)	129.11 (t)	3β
6			128.25 (q)					128.28 (q)	
7	5.69dd	2.8 (6), 1.8 (5)	66.54 (t)	6	8	5.68dd	2.7 (8), 1.8 (5)	66.64 (t)	8
8	3.59dd	4.1 (9), 2.8 (7)	51.35 (t)	6, 7, 9	7, 9	3.60dd	4.1 (9), 2.7 (7)	51.66 (t)	7, 9
9	3.43dd	4.1 (8), 2.5 (5)	55.57 (t)	4	2, 3α, 8	3.43dd	4.1 (8), 2.5 (5)	56.67 (t)	2, 3α, 8
10	5.61d	8.5 (2)		2, 11	1, 2	5.78d	7.0 (2)		1, 2, 3β
11			166.28 (q)					166.37 (q)	
12	5.71d	14.7 (13)	116.33 (t)	11, 13, 14	23	5.75d	15.1 (13)	116.44 (t)	23
13	7.28d	14.7 (12)	148.01 (t)	11, 12, 14, 15, 23	15	7.27d	15.1 (12)	147.86 (t)	15
14			130.66 (q)					130.74 (q)	
15	5.69d	9.4 (16)	148.88 (t)	13, 14, 16, 17, 23, 24	13, 24	5.68d	9.6 (16)	148.77 (t)	13, 24
16	2.51m		33.24 (t)		23, 24	2.50m		33.24 (t)	23, 24
17A	1.26m		37.20 (s)	18		1.26m		37.22 (s)	
B	1.35m			18		1.34m			
18	1.22m		27.45 (s)	19		1.21m		27.46 (s)	
19	1.22m		29.36 (s)			1.23m		29.39 (s)	
20	1.23m		31.80 (s)	18		1.23m		31.81 (s)	
21	1.26m		22.60 (s)	19, 20		1.26m		22.62 (s)	
22	0.87t	6.9 (21)	14.07 (p)	20, 21		0.87t	6.9 (21)	14.09 (p)	
23	1.77s		12.50 (p)	13, 14, 15	12, 16	1.77s		12.51 (p)	12, 16
24	0.98d	6.7 (16)	20.50 (p)	15, 16, 17	15, 16	0.97d	6.6 (16)	20.52 (p)	15, 16
1-$COCH_3$			169.13 (q)					169.12 (q)	
1-$CO\mathit{CH}_3$	2.18s		21.37 (p)			2.18s		21.24 (p)	
7-$COCH_3$			170.37 (q)					170.35 (q)	
7-$CO\mathit{CH}_3$	2.20s		20.68 (p)			2.20s		20.70 (p)	

[a,b] As in Table 1.

Table 7 ^{1}H and ^{13}C NMR data of gymnastatin B **2a** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	δ_C	^{1}H–^{1}H COSY	HMBC	NOESY
1	4.65d	3.7 (2)	96.62 (t) [b]	2	1-OMe, 3	1-OMe, 2, 10
2	4.08dddd	12.2 (3β), 8.7 (10), 4.7 (3α), 3.7 (1)	46.34 (t)	1, 3α, 10		1, 3α, 9
3α	2.23dd	12.2 (3β), 4.7 (2)	38.32 (s)	2, 3β	1, 2, 5, 9	2, 3β, 9
β	2.00t	12.2 (3α)		2, 3β	2, 3α	3α, 5
4			69.01 (q)			
5	4.29t	2.2 (6, 9)	75.46 (t)	6, 9	4, 6, 7, 9	1-OMe, 3β, 6
6	5.40d	2.2 (5)	60.85 (t)	5	5, 7	5, 5-OH
7			183.49 (q)			
8			132.98 (q)			
9	6.94d	2.2 (5)	143.01 (t)	5	3, 5, 7, 8	3α
10	5.85d	8.7 (2)		2	1, 2, 11	1, 12
11			166.59 (q)			
12	5.72d	15.3 (13)	116.66 (t)	13	11, 13, 14	10, 23
13	7.14d	15.3 (12)	147.65 (t)	12	11, 12, 14, 15, 23	15
14			130.69 (q)			
15	5.68d	9.6 (16)	148.91 (t)	16, 23	13, 14, 16, 17, 23, 24	13, 16, 24
16	2.51m		33.25 (t)	15, 17A, 17B, 24		15, 23, 24
17A	1.27m		37.14 (s)	16	18	
B	1.34m			16	18	
18	1.22m		27.45 (s)		19	
19	1.24m		29.35 (s)			
20	1.27m		22.60 (s)		18	
21	1.23m		31.79 (s)	22	19, 20	
22	0.87t	6.8 (21)	14.05 (p)	21	20, 21	
23	1.76s		12.48 (p)	15	13, 14, 15	12, 16
24	0.97d	6.6 (16)	20.43 (p)	16	15, 16, 17	15, 16
1-OMe	3.47s		55.10 (p)		1	1, 5
5-OH	5.12br s					6

[a,b] As in Table 1.

HMBC correlation from 5-H to C-6. The *cis* arrangement of 1-H and 2-H was deduced from NOEs for 10-H/3-H$^\beta$, 10-H/OMe, 1-H/2-H, 1-H/3-H$^\alpha$ and 2-H/3-H$^\alpha$.

Since the above-mentioned reaction did not give the desired compound **16a**, compound **2** was next treated with acetic anhydride in pyridine. This reaction also afforded undesirable compounds, 5-acetate **18** and enol acetate **19**.

The absolute configuration for gymnastatin B **2** has not been established by a chemical transformation as described above, but is assumed to be the same as for its co-metabolites, gymnastatins A **1**, D **4** and E **5**.

Gymnastatin C **3** was assigned the molecular formula $C_{24}H_{35}Cl_2NO_5$ as deduced from an M^+ peak of **3** in HREIMS. The general spectral features of compound **3** closely resembled those of **2** except that the trisubstituted double bond in the cyclohexane ring of **2** was replaced by two sp^3-methines linked to a hydroxy group and a chlorine atom [δ_H 4.83 (8-H), δ_C 66.58 (C-8); δ_H 4.14 (9-H), δ_C 73.80 (C-9)] in **3**, and the C-7 signal (δ_C 190.47) of the ketone in **3** appeared shifted downfield relative to that of **2** in the NMR spectra (Table 8). The planar structure for **3** established by analysis of the NMR spectra was confirmed by ^{1}H–^{1}H COSY and HMBC correlations (Table 8).

The observation of an NOE between 6-H and 8-H (Table 8) in **3** implied that the cyclohexane ring of **3** exists in a chair conformation with 6-H and 8-H in a coaxial arrangement. NOEs from 5-H to the 1-methoxy group, 6-H and 3-H$^\beta$, and the coupling constant ($J_{5,6}$ 3.6 Hz) indicated that the methoxy group and 3-H$^\beta$ are on the same side as 5-H with an equatorial arrangement on the cyclohexane ring. The large coupling constant ($J_{8,9}$ 10.1 Hz) between 8-H and 9-H suggested that these protons are arranged *trans*-diaxially. In addition, NOEs from 3-H$^\alpha$ to 2-H and 9-H showed that 2-H is on the same side as 9-H and thus oriented *trans* to the 1-methoxy group. The summary of these considerations allowed assignment of relative stereostructure **3** to gymnastatin C.

Transformation from **3** to **16a** for assignment of an absolute configuration to **3** was attempted by treatment with MsCl and Et_3N. The resulting product was not the desired **16a**, but the same compound **17** as obtained in the same reaction for **2**. This result implied that the absolute configuration of C-1, C-2 and C-16 in **3** is the same as those of **2**, and thus led to absolute stereostructure **3** for gymnastatin C.

Cytotoxic activities of compounds **1**–**5** were examined in the P388 lymphocytic leukemia test system in a cell culture, according to the method reported previously.[20] The results showed that three of the compounds (**1**–**3**) exhibited potent cytotoxic activity and two (**4** and **5**) exhibited weak cytotoxic activity (ED_{50} 0.018, 0.108, 0.106 and 10.5, 10.8 μg cm^{-3}, respectively). Gymnastatin A **1** of these compounds showed strongest cytotoxicity. This evidence suggested that conjugated ketones were important for enhancement of cytotoxicity in gymnastatin analogues, and hence the cytotoxic activity of compound **3** resulted from a conjugated ketone which might be derived from compound **3** in the test system.

Experimental

General procedures

UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin-Elmer FT-IR spectrometer 1720X. Optical rotations were obtained on a JASCO ORD/UV-5 spectropolarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. CD spectra were recorded on a JASCO J-500A spectrometer. 1D and 2D NMR spectra were recorded at 27 °C on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125.7 MHz for ^{1}H and ^{13}C, respectively, with TMS as an internal reference. EIMS was determined using a Hitachi M-4000H mass spectrometer. Liquid chromatography over silica gel (mesh 230–400) was performed under medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm × 20 mm i.d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent CH_2Cl_2–MeOH (19 : 1), and compounds were viewed under a UV lamp and sprayed with 10% H_2SO_4 followed by heating.

Table 8 ^{1}H and ^{13}C NMR data of gymnastatin C **3** in $CDCl_3$

Position	δ_H[a]	*J*/Hz	δ_C	^{1}H–^{1}H COSY	HMBC (C)	NOESY
1	4.67d	3.8 (2)	97.13 (t)[b]	2	1-OMe, 3	2, 10
2	4.22dddd	12.6 (3β), 8.7 (10), 5.2 (3α), 3.8 (1)	46.13 (t)	1, 3α, 3β, 10	1, 3, 11	1, 3α
3α	2.47dd	12.6 (3β), 5.2 (2)	34.66 (s)	3β	1, 2, 4, 9	2, 3β
β	1.73t	12.6 (2, 3α)		3α	1, 2, 4, 9	3α, 5
4			71.82 (q)			
5	4.34d	3.6 (6)	74.02 (t)	6	3, 4, 6, 7, 9	1-OMe, 3β, 6
6	5.19d	3.6 (5)	61.21 (t)	5, 8	5, 7	5, 8
7			190.47 (q)			
8	4.83d	10.1 (9)	66.58 (t)	6	3	6
9	4.14d	10.1 (8)	73.80 (t)	5, 8	7, 9	3α, 5, 8
10	5.86d	8.7 (2)			1, 2, 11, 12	1, 12
11			166.61 (q)			
12	5.71d	15.3 (13)	116.52 (t)	13	11, 13, 14	10, 23
13	7.20d	15.3 (12)	148.02 (t)	12	11, 12, 14, 15, 23	15
14			130.72 (q)			
15	5.68d	9.8 (16)	149.02 (t)	16, 23	13, 14, 16, 17, 23, 24	13, 16, 24
16	2.51m		33.26 (t)	15, 17A, 17B, 24		15, 23, 24
17A	1.26m		37.20 (s)	16	18	
B	1.35m			16	18	
18	1.22m		27.47 (s)		19	
19	1.23m		29.38 (s)			
20	1.27m		22.62 (s)		18	
21	1.23m		31.82 (s)	22	19, 20	
22	0.87t	6.9 (21)	14.07 (p)	21	20, 21	
23	1.76s		12.49 (p)	15	13, 14, 15	12, 16
24	0.97d	6.6 (16)	20.47 (p)	16	15, 16, 17	15, 16
1-OMe	3.47s		55.27 (p)		1	5
5-OH	4.14br s					
9-OH	2.76br s					

[a,b] As in Table 1.

Culturing and isolation of metabolites

A strain of *Gymnascella dankaliensis* (Castellani) Currah OUPS-N134 was initially isolated from the sponge *Halichondria japonica*, collected in the Osaka Bay of Japan in April, 1994. The sponge was washed with EtOH and its slices applied to the surface of nutrient agar layered in a Petri dish. Serial transfers of one of the resulting colonies provided a pure strain of *G. dankaliensis*. The fungal strain was grown in a liquid medium (90 dm^3) containing 1% malt extract, 1% glucose and 0.05% peptone in artificial seawater adjusted to pH 7.5 for four weeks at 27 °C. The culture was filtered under suction and the mycelia collected was extracted thrice with MeOH. The combined extracts were evaporated *in vacuo* to give a mixture of crude metabolites (11.0 g), the CH_2Cl_2–MeOH (1:1) soluble fraction of which exhibited cytotoxicity (ED_{50} 21.5 μg cm^{-3}). The culture filtrate was extracted thrice with AcOEt. The combined extracts were evaporated *in vacuo* to afford a mixture of crude metabolites (1.6 g), the CH_2Cl_2–MeOH (1:1) soluble fraction of which exhibited cytotoxicity (ED_{50} 7.0 μg cm^{-3}). The CH_2Cl_2–MeOH (1:1) soluble fraction of the MeOH extract was passed through Sephadex LH-20, using CH_2Cl_2–MeOH (1:1) as the eluent.

The second fraction (7.0 g), in which the activity was concentrated, was chromatographed on a silica gel column with a hexane–CH_2Cl_2–MeOH gradient as the eluent. The MeOH–CH_2Cl_2 (1:99) and (2:98) eluates were collected as 2 fractions [Fr. 1 (558.7 mg) and Fr. 2 (212.4 mg)] and 2 fractions [Fr. 3 (144 mg) and Fr. 4 (144.3 mg)], respectively. Silica gel column chromatography was repeated for Fr. 1 with a CH_2Cl_2–MeOH gradient as the eluent. The MeOH–CH_2Cl_2 (1:99) eluates were collected as 2 fractions [Fr. 5 (134.1 mg) and Fr. 6 (164.9 mg)], of which Fr. 6 was purified by HPLC using acetone–H_2O (4:1) to afford 3 fractions [Fr. 7 (6.7 mg), Fr. 8 (4.8 mg) and Fr. 9 (42.7 mg)]. Fr. 8 and Fr. 9 afforded **3** (1.5 mg), and **2** (11.1 mg) and **1** (19.6 mg), respectively, after purification by HPLC using acetone–H_2O (7:3) and (3:1) as the eluent, respectively. Fr. 3 and Fr. 4 were purified by HPLC using acetone–H_2O (7:3) as the eluent to afford **4** (13.1 mg) and **5** (15.3 mg), respectively.

Gymnastatin A 1. Obtained as an amorphous powder, mp 74.2–76.0 °C, $[\alpha]_D$ −3.8 (*c* 0.73, $CHCl_3$); λ_{max} (EtOH)/nm 266 (log ε/dm^3 mol^{-1} cm^{-1} 4.63); ν_{max} (KBr)/cm^{-1} 3377, 3277 (OH, NH), 1697 (C=C–CO), 1653 (CONH) and 1606 (C=C); *m*/*z* (EI) 437 ($M^+ - H_2O$, 1.4%), 277 ($[C_{10}H_{11}Cl_2NO_5]^+$, 53.8), 207 ($[C_{14}H_{23}O]^+$, 72.7) and 178 ($[C_{13}H_{22}]^+$, 100.0) [*m*/*z* (HREI) Found: $M^+ - H_2O$, 437.1507. $C_{23}H_{29}Cl_2NO_3$ requires $M - H_2O$, 437.1523]; CD λ (*c* 4.80 × 10^{-5} mol dm^{-3} in EtOH)/nm 79 (Δε 0), 270 (+2.22), 267 (+2.02), 254 (+2.55), 244 (+2.49), 234 (+2.66) and 220 (0). This compound is a mixture of stereoisomers **1a** and **1b** (2:1), and their ^{1}H and ^{13}C NMR data are listed in Table 1.

Gymnastatin B 2. Obtained as an amorphous powder, mp 73.5–77.5 °C, $[\alpha]_D$ −122.1 (*c* 0.18, $CHCl_3$); λ_{max} (EtOH)/nm 265 (log ε/dm^3 mol^{-1} cm^{-1} 4.50); ν_{max} (KBr)/cm^{-1} 3384, 3275 (OH, NH), 1725 (C=C–CO), 1650 (CONH) and 1610 (C=C); *m*/*z* (EI) 487 (M^+, 2.0%), 277 ($[C_{10}H_{11}Cl_2NO_5]^+$, 56.6), 207 ($[C_{14}H_{23}O]^+$, 81.9), 178 ($[C_{13}H_{22}]^+$, 100.0) and 95 ($[C_5H_5NO]^+$, 37.2) [*m*/*z* (HREI) Found: M^+, 487.1907. $C_{24}H_{35}Cl_2NO_5$ requires *M*, 487.1890]; CD λ (*c* 4.84 × 10^{-5} mol dm^{-3} in EtOH)/nm 286 (Δε 0), 258 (−5.63) and 234 (0). ^{1}H and ^{13}C NMR data are listed in Table 7.

Gymnastatin C 3. Obtained as an amorphous powder, mp 104.7–107.5 °C, $[\alpha]_D$ −101.2 (*c* 0.12, $CHCl_3$); λ_{max} (EtOH)/nm 265 (log ε/dm^3 mol^{-1} cm^{-1} 4.42); ν_{max} (KBr)/cm^{-1} 3430 (OH, NH), 1755 (CO), 1651 (CONH) and 1604 (C=C); *m*/*z* (EI) 505 (M^+, 1.0%), 487 ($[M - H_2O]^+$, 2.3), 455 ($[M - 2H_2O]^+$, 5.2), 277 ($[C_{10}H_{11}Cl_2NO_5]^+$, 20.4), 207 ($[C_{14}H_{23}O]^+$, 100.0), 179 ($[C_{13}H_{23}]^+$, 68.7) and 95 ($[C_5H_5NO]^+$, 80.6) [*m*/*z* (HREI) Found: M^+, 505.1993. $C_{24}H_{37}Cl_2NO_6$ requires *M*, 505.1996]; CD λ (*c* 5.26 × 10^{-5} mol dm^{-3} in EtOH)/nm 356 (Δε 0), 328 (+0.72), 282 (0), 272 (+0.52), 263 (0) and 238 (−2.79). ^{1}H and ^{13}C NMR data are listed in Table 8.

Gymnastatin D 4. Obtained as an amorphous powder, mp 86.4–88.2 °C, $[\alpha]_D$ −8.9 (*c* 0.45, $CHCl_3$); λ_{max} (EtOH)/nm 265

(log ε/dm^3 mol^{-1} cm^{-1} 4.44); ν_{max} (KBr)/cm^{-1} 3399, 3311 (OH, NH), 1652 (CONH) and 1612 (C=C); *m/z* (EI) 439 (M^+, 1.4%), 421 ([M − H_2O]$^+$, 14.0), 411 ([M − CO]$^+$, 7.0), 326 ([M − C_8H_{17}]$^+$, 18.0), 207 ([$C_{14}H_{23}O$]$^+$, 100.0), 179 ([$C_{13}H_{23}$]$^+$, 42.0) and 95 ([C_5H_5NO]$^+$, 31.0) [*m/z* (HREI) Found: M^+, 439.2127. $C_{23}H_{34}ClNO_5$ requires *M*, 439.2124]; CD λ (*c* 5.16 × 10^{-5} mol dm^{-3} in EtOH)/nm 295 ($\Delta\varepsilon$ 0), 267 (−0.88), 241 (0), 220 (+1.70) and 215 (0). This compound is a mixture of stereoisomers **4a** and **4b** (2 : 1), and their ^{1}H and ^{13}C NMR data are listed in Table 5.

Gymnastatin E 5. Obtained as an amorphous powder, mp 87.3–88.0 °C, $[\alpha]_D$ −8.5 (*c* 0.52, $CHCl_3$); λ_{max} (EtOH)/nm 266 (log ε/dm^3 mol^{-1} cm^{-1} 4.63); ν_{max} (KBr)/cm^{-1} 3334, 1652 (CONH) and 1612 (C=C); *m/z* (EI) 439 (M^+, 1.4%), 326 ([M − C_8H_{17}]$^+$, 16.0), 207 ([$C_{14}H_{23}O$]$^+$, 100.0), 179 ([$C_{13}H_{23}$]$^+$, 63.7) and 95 ([C_5H_5NO]$^+$, 97.4) [*m/z* (HREI) Found: M^+, 439.2122. $C_{23}H_{34}ClNO_5$ requires *M*, 439.2124]; CD λ (*c* 5.78 × 10^{-5} mol dm^{-3} in EtOH)/nm 288 ($\Delta\varepsilon$ 0), 272 (−0.39), 257 (0), 222 (+1.78) and 211 (0). This compound is a mixture of stereoisomers **5a** and **5b** (2 : 1), and their ^{1}H and ^{13}C NMR data are listed in Table 3.

Acetylation of gymnastatin A 1

Ac_2O (0.2 cm^3) was added to a pyridine solution (0.2 cm^3) of gymnastatin A **1** (23.3 mg), and the reaction mixture was left at room temperature overnight. The mixture was concentrated to dryness under reduced pressure, and the residue was purified by HPLC using acetone–H_2O (4 : 1) as the eluent to afford acetates **6a** (11.7 mg) and **6b** (5.3 mg).

Acetate 6a. Obtained as an amorphous powder, mp 175.5–177.8 °C, $[\alpha]_D$ −19.7 (*c* 0.91, $CHCl_3$); λ_{max} (EtOH)/nm 266 (log ε/dm^3 mol^{-1} cm^{-1} 4.61); ν_{max} (KBr)/cm^{-1} 3313 (NH), 1756 (OCO), 1702 (C=C–CO), 1647 (CONH) and 1624 (C=C); *m/z* (EI) 501 (M^+ + 4, 0.009%), 499 (M^+ + 2, 0.053%), 497 (M^+, 0.084%), 437 ([M − AcOH]$^+$, 8.8), 207 ([$C_{14}H_{23}O$]$^+$, 100.0), 179 ([$C_{13}H_{23}$]$^+$, 59.7) and 95 ([C_5H_5NO]$^+$, 61.2) [*m/z* (HREI) Found: M^+, 497.1742. $C_{25}H_{33}Cl_2NO_5$ requires *M*, 497.1734]; CD λ (*c* 1.03 × 10^{-4} mol dm^{-3} in EtOH)/nm 337 ($\Delta\varepsilon$ 0), 292 (+0.74), 279 (0), 269 (−0.88), 262 (0), 245 (+2.75), 233 (0) and 218 (−3.22). ^{1}H and ^{13}C NMR data are listed in Table 2.

Acetate 6b. Obtained as an amorphous powder, mp 68–69 °C, $[\alpha]_D$ −23.9 (*c* 0.53, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3269 (NH), 1751 (OCO), 1697 (C=C–CO), 1651 (CONH) and 1612 (C=C); *m/z* (EI) 497 (M^+, 0.1%), 437 ([M − AcOH]$^+$, 0.8), 207 ([$C_{14}H_{23}O$]$^+$, 50.4), 179 ([$C_{13}H_{23}$]$^+$, 56.1) and 95 ([C_5H_5NO]$^+$, 100.0) [*m/z* (HREI) Found: M^+, 497.1737. $C_{25}H_{33}Cl_2NO_5$ requires *M*, 497.1734]. ^{1}H and ^{13}C NMR data are listed in Table 2.

Alkaline hydrolysis of acetate 6a

A solution (1 drop) of 28% ammonia in water was added to a solution of acetate **6a** (3.5 mg) in MeOH (2 cm^3), and the reaction mixture was left at room temperature for 2 h. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography with hexane–AcOEt (1 : 1) to afford compound **1** as a mixture of **1a** and **1b** (2 : 1).

Formation of keto-lactone 13 from gymnastatin A 1

A solution of gymnastatin A **1** (16 mg) in pyridine (0.2 cm^3) was added to a pyridine–CrO_3 complex prepared from pyridine (0.2 cm^3) and CrO_3 (50 mg), and the reaction mixture was left at room temperature overnight. The mixture was diluted with water and extracted with CH_2Cl_2. The extract was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography [CH_2Cl_2–MeOH (99 : 1)] followed by HPLC [ODS; acetone–H_2O (4 : 1)] to afford keto-lactone **13** as an amorphous powder. Compound **13**: mp 62.8–64.2 °C, $[\alpha]_D$ −127.9 (*c* 0.18, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3387, 3290 (NH), 1796 (γ-lactone), 1699 (C=C–CO), 1652 (CONH) and 1613 (C=C); *m/z* (EI) 455 ([M + H_2]$^+$, 7.4%), 409 ([M − CO_2]$^+$, 65.0), 294 ([M − $C_{13}H_{23}$]$^+$, 53.3), 207 ([$C_{14}H_{23}O$]$^+$, 89.4), 179 ([$C_{13}H_{23}$]$^+$, 84.8) and 95 ([C_5H_5NO]$^+$, 100.0) [*m/z* (HREI) Found: M^+, 455.1619. $C_{23}H_{31}Cl_2NO_4$ requires *M*, 455.1630]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.98 (3H, d, *J* = 6.7 Hz, 24-H_3), 1.21 (2H, m, 18-H_2 or 19-H_2), 1.23 (4H, m, 19-H_2 or 18-H_2 and 21-H_2 or 20-H_2), 1.26 (3H, m, 20-H_2 or 21-H_2 and 17-H$^\beta$), 1.35 (1H, m, 17-H$^\alpha$), 1.77 (3H, s, 23-H_3), 2.51 (1H, m, 16-H), 2.61 (1H, dd, *J* = 13.5, 10.1 Hz, 3-H$^\beta$), 2.89 (1H, dd, *J* = 13.5, 10.1 Hz, 3-H$^\alpha$), 4.63 (1H, td, *J* = 10.1, 6.0 Hz, 2-H), 5.71 (1H, d, *J* = 9.6 Hz, 15-H), 5.78 (1H, d, *J* = 15.3 Hz, 12-H), 6.16 (1H, br d, *J* = 6.0 Hz, 10-H), 7.07 (1H, d, *J* = 2.7 Hz, 9-H), 7.28 (1H, d, *J* = 2.7 Hz, 5-H) and 7.31 (1H, d, *J* = 15.3 Hz, 13-H); δ_C ($CDCl_3$) 12.44 (C-23), 14.08 (C-22), 20.47 (C-24), 22.61 (C-20 or C-21), 27.45 (C-18 or C-19), 29.37 (C-19 or C-18), 31.80 (C-21 or C-20), 33.31 (C-16), 37.18 (C-17), 37.88 (C-3), 49.40 (C-2), 77.62 (C-4), 115.28 (C-12), 130.70 (C-14), 132.92 (C-8), 133.17 (C-6), 140.67 (C-9), 142.47 (C-5), 148.94 (C-13), 149.72 (C-15), 166.90 (C-11), 171.62 (C-7) and 172.27 (C-1); CD λ (*c* 5.23 × 10^{-5} mol dm^{-3} in EtOH)/nm 330 ($\Delta\varepsilon$ 0), 262 (−19.35), 242 (0) and 223 (+7.04).

Methylation of gymnastatin A 1 by trimethyl orthoformate

A solution of gymnastatin A **1** (28 mg), toluene-*p*-sulfonic acid (3 mg) and trimethyl orthoformate (0.02 cm^3) in anhydrous MeOH (3 cm^3) was refluxed under argon for 2 h and then diluted with diethyl ether. It was washed with a 1 : 1 mixture of 5% NaOH and saturated brine solutions, and then saturated brine solution, and evaporated under reduced pressure. The residue was purified by HPLC [acetone–H_2O (4 : 1)] to afford methyl acetals **16a** (6.9 mg) and **16b** (7.1 mg) as amorphous powders.

Methyl acetal 16a. Mp 119.5–120.5 °C, $[\alpha]_D$ −31.4 (*c* 0.57, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3305 (NH), 1696 (C=C–CO), 1650 (CONH) and 1615 (C=C); *m/z* (EI) 469 (M^+, 2.7%), 207 ([$C_{14}H_{23}O$]$^+$, 100.0), 179 ([$C_{13}H_{23}$]$^+$, 62.9) and 95 ([C_5H_5NO]$^+$, 55.8) [*m/z* (HREI) Found: M^+, 469.1778. $C_{24}H_{33}Cl_2NO_4$ requires *M*, 469.1786]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.98 (3H, d, *J* = 6.7 Hz, 24-H_3), 1.22 (2H, m, 18-H or 19-H), 1.23 (3H, m, 19-H or 18-H and 21-H or 20-H), 1.26 (1H, m, 17-H$^\beta$), 1.27 (2H, m, 20-H_2 or 21-H_2), 1.35 (1H, m, 17-H$^\alpha$), 1.77 (3H, s, 23-H_3), 2.11 (1H, dd, *J* = 13.0, 11.2 Hz, 3-H$^\beta$), 2.51 (1H, m, 16-H), 2.61 (1H, dd, *J* = 13.0, 8.2 Hz, 3-H$^\alpha$), 3.51 (3H, s, 1-OMe), 4.83 (1H, dddd, *J* = 11.2, 8.6, 8.2, 4.5 Hz, 2-H), 5.01 (1H, d, *J* = 4.5 Hz, 1-H), 5.69 (1H, d, *J* = 9.8 Hz, 15-H), 5.75 (1H, d, *J* = 15.3 Hz, 12-H), 5.91 (1H, d, *J* = 8.6 Hz, 10-H), 6.95 (1H, d, *J* = 2.6 Hz, 5-H), 7.03 (1H, d, *J* = 2.6 Hz, 9-H) and 7.27 (1H, d, *J* = 15.3 Hz, 13-H); δ_C ($CDCl_3$) 12.51 (C-23), 14.08 (C-22), 20.54 (C-24), 22.61 (C-20 or C-21), 27.46 (C-18 or C-19), 29.38 (C-19 or C-18), 31.82 (C-21 or C-20), 33.23 (C-16), 37.25 (C-17), 38.91 (C-3), 51.90 (C-2), 55.38 (1-OMe), 79.15 (C-4), 103.10 (C-1), 116.75 (C-12), 130.74 (C-14), 130.78 (C-8), 130.91 (C-6), 144.39 (C-9), 146.40 (C-5), 147.51 (C-13), 148.53 (C-15), 166.29 (C-11) and 172.39 (C-7).

Methyl acetal 16b. Mp 51.8–52.5 °C, $[\alpha]_D$ −28.8 (*c* 0.71, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3377, 3272 (NH), 1695 (C=C–CO), 1651 (CONH) and 1610 (C=C); *m/z* (EI) 469 (M^+, 1.2%), 207 ([$C_{14}H_{23}O$]$^+$, 100.0), 179 ([$C_{13}H_{23}$]$^+$, 73.1) and 95 ([C_5H_5NO]$^+$, 49.3) [*m/z* (HREI) Found: M^+, 469.1774. $C_{24}H_{33}Cl_2NO_4$ requires *M*, 469.1786]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.8 Hz, 22-H_3), 0.98 (3H, d, *J* = 6.7 Hz, 24-H_3), 1.22 (2H, m, 18-H_2 or 19-H_2), 1.23 (2H, m, 21-H_2 or 20-H_2), 1.24 (2H, m, 19-H_2 or 18-H_2), 1.26 (3H, m, 17-H$^\beta$), 1.27 (2H, m, 20-H_2 or 21-H_2), 1.35

(1H, m, 17-H$^{\alpha}$), 1.78 (3H, s, 23-H$_3$), 2.23 (1H, dd, J = 14.2, 1.6 Hz, 3-H$^{\beta}$), 2.51 (1H, m, 16-H), 2.72 (1H, dd, J = 14.2, 6.6 Hz, 3-H$^{\alpha}$), 3.45 (3H, s, 1-OMe), 4.83 (1H, td, J = 6.6, 1.6 Hz, 2-H), 5.09 (1H, s, 1-H), 5.69 (1H, d, J = 9.8 Hz, 15-H), 5.75 (1H, d, J = 6.6 Hz, 10-H), 5.78 (1H, d, J = 15.3 Hz, 12-H), 6.99 (1H, d, J = 2.6 Hz, 5-H), 7.03 (1H, d, J = 2.6 Hz, 9-H) and 7.30 (1H, d, J = 15.3 Hz, 13-H); δ_C ($CDCl_3$) 12.52 (C-23), 14.08 (C-22), 20.51 (C-24), 22.62 (C-20 or C-21), 27.46 (C-18 or C-19), 29.38 (C-19 or C-18), 31.81 (C-21 or C-20), 33.26 (C-16), 37.22 (C-17), 40.72 (C-3), 55.33 (1-OMe), 56.99 (C-2), 80.42 (C-4), 109.70 (C-1), 116.42 (C-12), 130.50 (C-8), 130.69 (C-14), 130.86 (C-6), 145.05 (C-5), 147.06 (C-9), 147.93 (C-13), 148.92 (C-15), 166.46 (C-11) and 172.45 (C-7).

Treatment of gymnastatin B 2 with MsCl and Et_3N

To a solution of gymnastatin B **2** (10.9 mg) in anhydrous CH_2Cl_2 were successively added Et_3N (0.05 cm^3) and MsCl (0.025 cm^3) under argon, while cooling the mixture in an ice bath. After stirring for 30 min, the reaction mixture was diluted with a 50% brine solution and extracted with CH_2Cl_2. The extract was washed with saturated aqueous $NaHCO_3$ and water. After evaporation of the solvent, the residue was purified by HPLC [acetone–H_2O (4:1)] to afford **17** (6.9 mg) as an amorphous powder. Compound **17**: mp 54.0–54.5 °C, $[\alpha]_D$ −25.9 (c 0.54, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3276 (NH), 1652 (CONH), 1615, 1538 (Ar C–C) and 1376, 1187 (OSO_2); *m/z* (EI) 547 (M^+, 100%), 516 ($[M - OCH_3]^+$, 11.3), 434 ($[M - C_8H_{17}]^+$, 33.9), 381 ($[M - C_{12}H_{22}]^+$, 28.3), 324 ($[M - C_{14}H_{25}NO]^+$, 63.7), 293 ($[M - C_{14}H_{25}NO - OCH_3]^+$, 41.8), 207 ($[C_{14}H_{23}O]^+$, 64.3), 179 ($[C_{13}H_{23}]^+$, 53.1) and 95 ($[C_5H_5NO]^+$, 44.9) [*m/z* (HREI) Found: M^+, 547.1564. $C_{25}H_{33}Cl_2NO_6S$ requires M, 457.1560]; δ_H ($CDCl_3$) 0.87 (3H, t, J = 6.6 Hz, 22-H$_3$), 0.98 (3H, d, J = 6.6 Hz, 24-H$_3$), 1.22 (2H, m, 18-H$_2$ or 19-H$_2$), 1.23 (2H, m, 21-H$_2$ or 20-H$_2$), 1.24 (2H, m, 19-H$_2$ or 18-H$_2$), 1.26 (1H, m, 17-H$^{\beta}$), 1.27 (2H, m, 20-H$_2$ or 21-H$_2$), 1.35 (1H, m, 17-H$^{\alpha}$), 1.78 (3H, s, 23-H$_3$), 2.51 (1H, m, 16-H), 2.82 (1H, ddd, J = 16.0, 12.0, 1.1 Hz, 3-H$^{\beta}$), 2.95 (1H, dd, J = 16.0, 6.1 Hz, 3-H$^{\alpha}$), 3.47 (3H, s, 7-OSO_2Me), 3.52 (3H, s, 1-OMe), 4.57 (1H, dddd, J = 12.0, 8.9, 6.1, 2.5 Hz, 2-H), 5.19 (1H, d, J = 2.5 Hz, 1-H), 5.67 (1H, d, J = 8.9 Hz, 10-H), 5.68 (1H, d, J = 9.4 Hz, 15-H), 5.76 (1H, d, J = 15.3 Hz, 12-H), 7.09 (1H, s, 5-H) and 7.29 (1H, d, J = 15.3 Hz, 13-H); δ_C ($CDCl_3$) 12.51 (C-23), 14.08 (C-22), 20.54 (C-24), 22.61 (C-20 or C-21), 27.00 (C-3), 27.46 (C-18 or C-19), 29.38 (C-19 or C-18), 31.82 (C-21 or C-20), 33.21 (C-16), 37.24 (C-17), 41.08 (7-OSO_2Me), 44.29 (C-2), 56.39 (1-OMe), 98.00 (C-1), 116.90 (C-12), 118.75 (C-6), 121.07 (C-8), 121.86 (C-4), 127.94 (C-5), 130.74 (C-14), 142.17 (C-7), 146.63 (C-9), 147.46 (C-13), 148.42 (C-15) and 166.17 (C-11); CD λ (c 4.94 × 10^{-5} mol dm^{-3} in EtOH)/nm 310 ($\Delta\varepsilon$ 0), 284 (−1.84), 268 (0), 245 (+0.92), 231 (0), 221 (+0.31), 217 (−0.25) and 206 (−3.31).

Treatment of gymnastatin C 3 with MsCl and Et_3N

Using the same procedure as above with compound **2**, gymnastatin C **3** (2.7 mg) was treated with Et_3N (0.05 cm^3) and MsCl (0.025 cm^3) in anhydrous CH_2Cl_2 and purified by HPLC [acetone–H_2O (4:1)] to afford compound **17** (0.8 mg) as an amorphous powder, identical with that derived from compound **2**.

Treatment of gymnastatin B 2 with Ac_2O

Using the same procedure as acetylation of comound **1**, gymnastatin B **2** (5.7 mg) was treated with Ac_2O (0.1 cm^3) in pyridine (0.1 cm^3) and purified by HPLC [acetone–H_2O (4:1)] to afford 5-acetate **18** (2.3 mg) and enol acetate **19** (2.2 mg).

5-Acetate 18. Obtained as an amorphous powder, mp 73.0–73.5 °C, $[\alpha]_D$ −83.5 (c 0.79, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3392, 3277 (NH), 1747 (OCO), 1731 (C=C–CO), 1650 (CONH) and 1611 (C=C); *m/z* (EI) 529 (M^+, 14.3%), 469 ($[M - Ac - OCH_3]^+$, 11.4), 277 ($[C_{10}H_{11}NO_5Cl_2]^+$, 28.1), 207 ($[C_{14}H_{23}O]^+$, 100.0), 179 ($[C_{13}H_{23}]^+$, 79.3) and 95 ($[C_5H_5NO]^+$, 71.3) [*m/z* (HREI) Found: M^+, 529.2000. $C_{26}H_{37}Cl_2NO_6$ requires M, 529.1998]; δ_H ($CDCl_3$) 0.87 (3H, t, J = 6.9 Hz, 22-H$_3$), 0.97 (3H, d, J = 6.6 Hz, 24-H$_3$), 1.22 (2H, m, 18-H$_2$ or 19-H$_2$), 1.23 (2H, m, 21-H$_2$ or 20-H$_2$), 1.24 (2H, m, 19-H$_2$ or 18-H$_2$), 1.27 (3H, m, 20-H$_2$ or 21-H$_2$ and 17-H$^{\beta}$), 1.35 (1H, m, 17-H$^{\alpha}$), 1.76 (3H, s, 23-H$_3$), 1.96 (1H, t, J = 12.4 Hz, 3-H$^{\beta}$), 2.07 (3H, s, 5-COMe), 2.50 (1H, m, 16-H), 3.03 (1H, dd, J = 12.4, 4.8 Hz, 3-H$^{\alpha}$), 3.48 (3H, s, 1-OMe), 4.24 (1H, dddd, J = 12.4, 9.1, 4.8, 3.4 Hz, 2-H), 4.39 (1H, t, J = 2.3 Hz, 5-H), 4.66 (1H, d, J = 3.4 Hz, 1-H), 5.15 (1H, d, J = 2.3 Hz, 6-H), 5.57 (1H, d, J = 9.1 Hz, 10-H), 5.67 (1H, d, J = 9.8 Hz, 15-H), 5.69 (1H, d, J = 15.3 Hz, 12-H), 7.23 (1H, d, J = 15.3 Hz, 13-H) and 7.48 (1H, d, J = 2.3 Hz, 9-H); δ_C ($CDCl_3$) 12.49 (C-23), 14.07 (C-22), 20.53 (C-24), 21.26 (5-CO*Me*), 22.61 (C-20 or C-21), 27.45 (C-3), 27.45 (C-18 or C-19), 29.38 (C-19 or C-18), 31.81 (C-21 or C-20), 33.21 (C-16), 34.53 (C-3), 37.23 (C-17), 45.30 (C-2), 55.34 (1-OMe), 60.07 (C-6), 73.50 (C-5), 76.14 (C-4), 97.07 (C-1), 116.88 (C-12), 130.70 (C-14), 134.96 (C-8), 139.58 (C-9), 147.39 (C-13), 148.35 (C-15), 165.76 (C-11), 168.59 (5-*C*OMe) and 182.11 (C-7).

Enol acetate 19. Obtained as an amorphous powder, mp 67.2–68.5 °C, $[\alpha]_D$ +85.4 (c 0.56, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3270 (NH), 1697 (C=C–CO), 1787, 1746 (OCO), 1651 (CONH) and 1613 (C=C); *m/z* (EI) 571 (M^+, 0.9%), 511 ($[M - OAc - H]^+$, 40.1), 246 ($[C_{10}H_9O_3Cl_2 - H]^+$, 100.0), 224 ($[C_{14}H_{24}NO + H]^+$, 59.9), 207 ($[C_{14}H_{23}O]^+$, 42.1), 179 ($[C_{13}H_{23}]^+$, 33.5) and 95 ($[C_5H_5NO]^+$, 31.7) [*m/z* (HREI) Found: M^+, 571.2086. $C_{28}H_{39}Cl_2NO_7$ requires M, 571.2103]; δ_H ($CDCl_3$) 0.87 (3H, t, J = 6.9 Hz, 22-H$_3$), 0.97 (3H, d, J = 6.6 Hz, 24-H$_3$), 1.22 (2H, m, 18-H$_2$ or 19-H$_2$), 1.23 (2H, m, 19-H$_2$ or 18-H$_2$), 1.24 (2H, m, 21-H$_2$ or 20-H$_2$), 1.26 (1H, m, 17-H$^{\beta}$), 1.27 (2H, m, 20-H$_2$ or 21-H$_2$), 1.34 (1H, m, 17-H$^{\alpha}$), 1.76 (3H, s, 23-H$_3$), 1.95 (1H, t, J = 12.4 Hz, 3-H$^{\beta}$), 1.98 (3H, s, 5-COMe), 2.29 (3H, s, 7-COMe), 2.50 (1H, m, 16-H), 2.97 (1H, dd, J = 12.4, 3.6 Hz, 3-H$^{\alpha}$), 3.51 (3H, s, 1-OMe), 4.21 (1H, d, J = 1.3 Hz, 5-H), 4.28 (1H, dddd, J = 12.4, 9.4, 3.6, 3.2 Hz, 2-H), 4.67 (1H, d, J = 3.2 Hz, 1-H), 5.51 (1H, d, J = 9.4 Hz, 10-H), 5.67 (1H, d, J = 9.9 Hz, 15-H), 5.69 (1H, d, J = 15.3 Hz, 12-H), 6.49 (1H, d, J = 1.3 Hz, 9-H) and 7.23 (1H, d, J = 15.3 Hz, 13-H); δ_C ($CDCl_3$) 12.49 (C-23), 14.08 (C-22), 20.08 (7-CO*Me*), 20.55 (C-24), 21.25 (5-CO*Me*), 22.61 (C-20 or C-21), 27.45 (C-18 or C-19), 29.38 (C-19 or C-18), 31.81 (C-21 or C-20), 33.19 (C-16), 33.55 (C-3), 37.24 (C-17), 45.05 (C-2), 55.32 (1-O*C*H$_3$), 71.34 (C-5), 75.99 (C-4), 96.39 (C-1), 117.11 (C-12), 126.32 (C-9), 129.73 (C-6 and C-8), 130.73 (C-14), 140.75 (C-7), 147.15 (C-13), 148.10 (C-15), 165.75 (C-11), 166.76 (7-*C*OMe) and 169.12 (5-*C*OMe).

Acetylation of gymnastatin D 4

Using the same procedure as acetylation of compound **1**, gymnastatin D **4** (19.7 mg) was treated with Ac_2O (0.2 cm^3) in pyridine (0.2 cm^3) and purified by HPLC using acetone–H_2O (7:3) as the eluent to afford diacetates **7a** (12.3 mg) and **7b** (2.9 mg) as an amorphous powder.

Diacetate 7a. Mp 74.5–75.0 °C, $[\alpha]_D$ −22.9 (c 1.22, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3284 (NH), 1750 (OCO), 1655 (CONH) and 1615 (C=C); *m/z* (EI) 522 (M^+, 1.7%), 463 ($[M - OAc]^+$, 55.2), 404 ($[M - 2OAc]^+$, 6.6), 350 ($[M - 2OAc - C_4H_9]^+$, 29.6), 207 ($[C_{14}H_{23}O]^+$, 100.0), 179 ($[C_{13}H_{23}]^+$, 64.0) and 95 ($[C_5H_5NO]^+$, 73.4) [*m/z* (HREI) Found: M^+, 522.2241. $C_{27}H_{37}ClNO_7$ requires M, 522.2259]. ^{1}H and ^{13}C NMR data are listed in Table 6.

Diacetate 7b. Mp 78.0–79.0 °C, $[\alpha]_D$ −18.7 (c 0.30, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3274 (NH), 1749 (OCO), 1652 (CONH) and 1614 (C=C); *m/z* (EI) 522 (M^+, 0.5%), 463 ($[M - OAc]^+$, 100.0), 404 ($[M - 2OAc]^+$, 12.8), 350 ($[M - 2OAc - C_4H_9]^+$, 62.4),

206 ($[C_{14}H_{22}O]^+$, 100.0) and 177 ($[C_{13}H_{21}]^+$, 74.5) [*m*/*z* (HREI) Found: M^+, 522.2247. $C_{27}H_{37}ClNO_7$ requires *M*, 522.2259]. ^1H and ^{13}C NMR data are listed in Table 6.

Oxidation of gymnastatin D 4 by a pyridine–CrO_3 complex

Using the same procedure as above with compound **1**, gymnastatin D **4** (29.1 mg) in pyridine (0.2 cm^3) was treated with a pyridine–CrO_3 complex prepared from pyridine (0.2 cm^3) and CrO_3 (50 mg) and purified by silica gel column chromatography [hexane–AcOEt (1 : 1)] followed by HPLC [ODS; acetone–H_2O (4 : 1)] to afford keto-lactone **11** (7.3 mg) as an amorphous powder. Keto-lactone **11**: mp 80.5–81.5 °C, ν_{max} (KBr)/cm^{-1} 3365 (NH), 1795 (γ-lactone), 1713 (C=C–CO), 1652 (CONH) and 1615 (C=C); *m*/*z* (EI) 435 (M^+) [*m*/*z* (HREI) Found: M^+, 435.1818. $C_{23}H_{30}ClNO_5$ requires *M*, 435.1812]; δ_H (acetone-d_6) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.99 (3H, d, *J* = 6.6 Hz, 24-H_3), 1.20–1.42 (10H, m, 17–21-H_2), 1.79 (3H, s, 23-H_3), 2.58 (1H, m, 16-H), 2.60 (1H, dd, *J* = 14.0, 8.9 Hz, 3-H^β), 3.10 (1H, dd, *J* = 14.0, 10.3 Hz, 3-H^α), 3.78 (1H, d, *J* = 4.1 Hz, 8-H), 4.24 (1H, dd, *J* = 4.1, 2.8 Hz, 9-H), 4.87 (1H, ddd, *J* = 10.3, 8.9, 7.1 Hz, 2-H), 5.70 (1H, d, *J* = 9.9 Hz, 15-H), 6.01 (1H, d, *J* = 15.3 Hz, 12-H), 7.14 (1H, d, *J* = 2.8 Hz, 6-H), 7.22 (1H, d, *J* = 15.3 Hz, 13-H) and 7.99 (1H, d, *J* = 7.1 Hz, 10-H).

Formation of chlorohydrin 12 from keto-lactone 11

A solution of keto-lactone **11** (3.2 mg) and pyridinium chloride (3.5 mg) in pyridine (0.2 cm^3) was left at room temperature overnight, and then diluted with water and extracted with CH_2Cl_2. The extract was washed with water and concentrated to dryness. The residue was purified by HPLC [acetone–H_2O (4 : 1)] to afford chlorohydrin **12** (5.9 mg) as an amorphous powder. Chlorohydrin **12**: mp 81.5–82.5 °C, $[\alpha]_D$ −17.9 (*c* 0.22, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3368, 3316 (NH, OH), 1790 (γ-lactone), 1723 (C=C–CO), 1651 (CONH) and 1615 (C=C); *m*/*z* (EI) 471 (M^+, 2.0%), 453 ($[M - H_2O]^+$, 32.5), 409 ($[M - CO_2]^+$, 22.4), 224 ($[C_{14}H_{26}NO]^+$, 100.0), 207 ($[C_{14}H_{23}O]^+$, 87.5), 179 ($[C_{13}H_{23}]^+$, 87.3) and 138 ($[C_{10}H_{18}]^+$, 83.7) [*m*/*z* (HREI) Found: M^+, 471.1562. $C_{23}H_{31}Cl_2NO_5$ requires *M*, 471.1579]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.8 Hz, 22-H_3), 0.97 (3H, d, *J* = 6.6 Hz, 24-H_3), 1.20 (2H, m, 18-H_2 or 19-H_2), 1.23 (2H, m, 21-H_2 or 20-H_2), 1.24 (2H, m, 19-H_2 or 18-H_2), 1.26 (1H, m, 17-H^β), 1.27 (2H, m, 20-H_2 or 21-H_2), 1.34 (1H, m, 17-H^α), 1.76 (3H, s, 23-H_3), 2.51 (1H, m, 16-H), 2.53 (1H, dd, *J* = 13.1, 10.1 Hz, 3-H^β), 3.05 (1H, dd, *J* = 13.1, 10.1 Hz, 3-H^α), 4.05 (1H, d, *J* = 11.0 Hz, 9-H), 4.73 (1H, td, *J* = 10.1, 6.1 Hz, 2-H), 4.90 (1H, d, *J* = 11.0 Hz, 8-H), 5.69 (1H, d, *J* = 9.9 Hz, 15-H), 5.78 (1H, d, *J* = 15.3 Hz, 12-H), 6.39 (1H, d, *J* = 6.1 Hz, 10-H), 7.13 (1H, s, 5-H) and 7.29 (1H, d, *J* = 15.3 Hz, 13-H); δ_C ($CDCl_3$) 12.50 (C-23), 14.09 (C-22), 20.45 (C-24), 22.62 (C-20 or C-21), 27.46 (C-18 or C-19), 29.37 (C-19 or C-18), 31.81 (C-21 or C20), 33.29 (C-16), 37.16 (C-17), 39.10 (C-3), 50.54 (C-2), 64.27 (C-8), 75.49 (C-9), 80.52 (C-4), 115.53 (C-12), 130.71 (C-14), 136.62 (C-6), 141.33 (C-5), 148.69 (C-13), 149.59 (C-15), 167.34 (C-11), 173.92 (C-1) and 182.64 (C-7).

Dehydration of chlorohydrin 12

(a). Using the same procedure as acetylation of gymnastatin A **1**, chlorohydrin **12** (1.9 mg) was treated with Ac_2O (0.1 cm^3) in pyridine (0.1 cm^3) and purified by HPLC [acetone–H_2O (7 : 3)] to afford keto-lactone **13** (1.2 mg) as an amorphous powder, identical with that derived from gymnastatin A **1**.

(b). Using the same procedure as above with gymnastatin B **2**, chlorohydrin **12** (3.6 mg) was treated with Et_3N (0.05 cm^3) and MsCl (0.05 cm^3) in anhydrous CH_2Cl_2 (1 cm^3) and purified by HPLC [acetone–H_2O (4 : 1)] to afford **13** (1.0 mg) as an amorphous powder.

Formation of the (*R*)- and (*S*)-MTPA esters 15a and 15b from gymnastatin D 4

(*R*)-MTPA (15 mg), dicyclohexylcarbodiimide (DCC) (20 mg) and 4-(dimethylamino)pyridine (DMAP) (10 mg) were added to a CH_2Cl_2 solution (1 cm^3) of gymnastatin D **4** (7.9 mg), and the reaction mixture was left at room temperature for 3 h. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography with hexane–AcOEt (1 : 1) followed by HPLC (ODS) using acetone–H_2O (4 : 1) as the eluent to afford ester **15a** (6.7 mg). The same reaction with **4** (5.5 mg) using (*S*)-MTPA (10 mg) gave ester **15b** (3.6 mg).

Ester 15a. Obtained as an amorphous powder; *m*/*z* (EI) 871 (M^+) [*m*/*z* (HREI) Found: M^+, 871.2923. $C_{43}H_{48}ClF_6NO_9$ requires *M*, 871.2922]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.99 (3H, d, *J* = 6.6 Hz, 24-H_3), 1.18–1.40 (10H, m, 17–21-H_2), 1.75 (3H, s, 23-H_3), 1.85 (1H, t, *J* = 13.0 Hz, 3-H^β), 2.43 (1H, dd, *J* = 13.0, 8.0 Hz, 3-H^α), 2.52 (1H, m, 16-H), 3.43 (1H, dd, *J* = 4.1, 2.7 Hz, 9-H), 3.53 (3H, s, OMe), 3.62 (3H, s, OMe), 3.72 (1H, dd, *J* = 4.1, 2.8 Hz, 6-H), 5.07 (1H, dddd, *J* = 13.0, 8.9, 8.0, 4.3 Hz, 2-H), 5.38 (1H, dd, *J* = 2.8, 1.4 Hz, 5-H), 5.40 (1H, d, *J* = 8.9 Hz, 10-H), 5.53 (1H, d, *J* = 15.3 Hz, 12-H), 5.68 (1H, d, *J* = 9.8 Hz, 15-H), 5.73 (1H, dd, *J* = 2.7, 1.4 Hz, 7-H), 6.54 (1H, d, *J* = 4.3 Hz, 1-H), 7.26 (1H, d, *J* = 15.3 Hz, 13-H), 7.42 (6H, m, ArH), 7.49 (2H, m, ArH) and 7.60 (2H, m, ArH).

Ester 15b. Obtained as an amorphous powder; *m*/*z* (EI) 871 (M^+) [*m*/*z* (HREI) Found: M^+, 871.2926. $C_{43}H_{48}ClF_6NO_9$ requires *M*, 871.2922]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.99 (3H, d, *J* = 6.6 Hz, 24-H_3), 1.18–1.40 (10H, m, 17–21-H_2), 1.77 (3H, s, 23-H_3), 1.80 (1H, t, *J* = 13.1 Hz, 3-H^β), 2.46 (1H, dd, *J* = 13.1, 8.0 Hz, 3-H^α), 2.52 (1H, m, 16-H), 3.39 (1H, dd, *J* = 4.1, 2.5 Hz, 9-H), 3.53 (3H, s, OMe), 3.61 (3H, s, OMe), 3.64 (1H, dd, *J* = 4.1, 3.0 Hz, 8-H), 5.06 (1H, dddd, *J* = 13.1, 8.9, 8.0, 4.1 Hz, 2-H), 5.20 (1H, d, *J* = 8.9 Hz, 10-H), 5.42 (1H, d, *J* = 15.3 Hz, 12-H), 5.54 (1H, dd, *J* = 2.5, 1.6 Hz, 5-H), 5.68 (1H, d, *J* = 10.3 Hz, 15-H), 5.78 (1H, dd, *J* = 3.0, 1.6 Hz, 7-H), 6.59 (1H, d, *J* = 4.1 Hz, 1-H), 7.26 (1H, d, *J* = 15.3 Hz, 13-H), 7.42 (3H, m, ArH), 7.47 (3H, m, ArH), 7.52 (2H, m, ArH) and 7.58 (2H, m, ArH).

Acetylation of gymnastatin E 5

Using the same procedure as above with compound **1**, gymnastatin E **5** (15.8 mg) was treated with Ac_2O (0.2 cm^3) in pyridine (0.2 cm^3) and purified by HPLC using acetone–H_2O (7 : 3) as the eluent to afford diacetates **8a** (12.7 mg) and **8b** (2.7 mg).

Diacetate 8a. Obtained as colourless plates, mp 100.5–103.0 °C, $[\alpha]_D$ −36.4 (*c* 0.31, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3283 (NH), 1748 (OCO), 1657 (CONH) and 1614 (C=C); *m*/*z* (EI) 522 (M^+, 0.5%), 463 ($[M - OAc]^+$, 27.5), 404 ($[M - 2OAc]^+$, 11.1), 350 ($[M - 2OAc - C_4H_9]^+$, 13.6), 207 ($[C_{14}H_{23}O]^+$, 100.0) and 179 ($[C_{13}H_{23}]^+$, 74.5) [*m*/*z* (HREI) Found: M^+, 522.2244. $C_{27}H_{37}ClNO_7$ requires *M*, 522.2259]. ^1H and ^{13}C NMR data are listed in Table 4.

Diacetate 8b. Obtained as an amorphous powder, mp 67.0–68.0 °C, $[\alpha]_D$ −15.4 (*c* 0.27, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3267 (NH), 1751 (OCO), 1651 (CONH) and 1613 (C=C); *m*/*z* (EI) 522 (M^+, 1.1%), 463 ($[M - OAc]^+$, 100.0), 404 ($[M - 2OAc]^+$, 80.7), 350 ($[M - 2OAc - C_4H_9]^+$, 66.1), 207 ($[C_{14}H_{23}O]^+$, 97.6) and 177 ($[C_{13}H_{21}]^+$, 74.5) [*m*/*z* (HREI) Found: M^+, 522.2223. $C_{27}H_{37}ClNO_7$ requires *M*, 522.2259]. ^1H and ^{13}C NMR data are listed in Table 4.

Oxidation of gymnastatin E 5 by a pyridine–CrO_3 complex

Using the same procedure as above with compound **1**,

gymnastatin E **5** (25.5 mg) in pyridine (0.2 cm^3) was treated with a pyridine–CrO_3 complex prepared from pyridine (0.2 cm^3) and CrO_3 (50 mg) and purified by silica gel column chromatography [hexane–AcOEt (1:1)] followed by HPLC [acetone–H_2O (4:1)] to afford keto-lactone **9** (3.8 mg) as an amorphous powder. Keto-lactone **9**: mp 81.0–82.0 °C, $[\alpha]_D$ −52.4 (*c* 0.37, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3294 (NH), 1795 (γ-lactone), 1713 (C=C–CO), 1653 (CONH) and 1614 (C=C); *m/z* (EI) 435 (M^+, 5.1%), 322 ($[M - C_8H_{17}]^+$, 8.2), 224 ($[C_{14}H_{26}NO]^+$, 100.0), 207 ($[C_{14}H_{23}O]^+$, 31.6), 179 ($[C_{13}H_{23}]^+$, 50.6) and 95 ($[C_5H_5NO]^+$, 76.2) [*m/z* (HREI) Found: M^+, 435.1812. $C_{23}H_{30}ClNO_5$ requires *M*, 435.1812]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.97 (3H, d, *J* = 6.6 Hz, 24-H_3), 1.20 (2H, m, 18-H_2 or 19-H_2), 1.23 (4H, m, 19-H_2 or 18-H_2 and 21-H_2 or 20-H_2), 1.26 (1H, m, 17-H^β), 1.27 (2H, m, 20-H_2 or 21-H_2), 1.35 (1H, m, 18-H^α), 1.76 (3H, s, 23-H_3), 2.51 (1H, m, 16-H), 2.64 (1H, dd, *J* = 13.5, 10.0 Hz, 3-H^β), 2.77 (1H, dd, *J* = 13.5, 10.0 Hz, 3-H^α), 3.70 (1H, d, *J* = 3.9 Hz, 6-H), 4.12 (1H, dd, *J* = 3.9, 2.8 Hz, 5-H), 4.62 (1H, td, *J* = 10.0, 6.3 Hz, 2-H), 5.69 (1H, d, *J* = 9.8 Hz, 15-H), 5.78 (1H, d, *J* = 15.2 Hz, 12-H), 6.31 (1H, d, *J* = 6.3 Hz, 10-H), 6.72 (1H, d, *J* = 2.8 Hz, 9-H) and 7.29 (1H, d, *J* = 15.2 Hz, 13-H); δ_C ($CDCl_3$) 12.44 (C-23), 14.08 (C-22), 20.46 (C-24), 22.61 (C-20 or C-21), 27.45 (C-18 or C-19), 29.37 (C-19 or C-18), 31.80 (C-21 or C-20), 33.30 (C-16), 37.17 (C-17), 37.92 (C-3), 49.38 (C-2), 51.90 (C-6), 55.66 (C-5), 79.79 (C-4), 115.38 (C-12), 129.66 (C-8), 130.72 (C-14), 139.48 (C-9), 148.82 (C-13), 149.63 (C-15), 166.96 (C-11), 172.70 (C-1) and 185.52 (C-7).

Formation of chlorohydrin 10 from keto-lactone 9

Using the same procedure as above with compound **11**, keto-lactone **9** (3.2 mg) was treated with pyridinium chloride (3.5 mg) in pyridine (0.2 cm^3) and purified by HPLC [acetone–H_2O (3:1)] to afford chlorohydrin **10** (1.9 mg) as an amorphous powder. Chlorohydrin **10**: mp 70.0–71.0 °C, $[\alpha]_D$ −42.1 (*c* 0.19, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3359, 3302 (NH, OH), 1790 (γ-lactone), 1723 (C=C–CO), 1652 (CONH) and 1614 (C=C); *m/z* (EI) 471 (M^+, 0.8%), 453 ($[M - H_2O]^+$, 14.7), 409 ($[M - CO_2]^+$, 35.1), 224 ($[C_{14}H_{26}NO]^+$, 100.0), 207 ($[C_{14}H_{23}O]^+$, 94.4), 179 ($[C_{13}H_{23}]^+$, 90.1), 138 ($[C_{10}H_{18}]^+$, 53.8) and 95 ($[C_5H_5NO]^+$, 32.5) [*m/z* (HREI) Found: M^+, 471.1571. $C_{23}H_{31}Cl_2NO_5$ requires *M*, 471.1579]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.98 (3H, d, *J* = 6.6 Hz, 24-H_3), 1.22 (2H, m, 18-H_2 or 19-H_2), 1.23 (2H, m, 21-H_2 or 20-H_2), 1.24 (2H, m, 19-H_2 or 18-H_2), 1.27 (3H, m, 17-H^β and 20-H_2 or 21-H_2), 1.36 (1H, m, 18-H^α), 1.76 (3H, s, 23-H_3), 2.51 (1H, m, 16-H), 2.68 (1H, dd, *J* = 13.9, 8.2 Hz, 3-H^β), 2.84 (1H, dd, *J* = 13.9, 10.1 Hz, 3-H^α), 3.62 (1H, br s, 5-OH), 4.05 (1H, d, *J* = 10.4 Hz, 5-H), 4.92 (1H, d, *J* = 10.4 Hz, 6-H), 5.05 (1H, ddd, *J* = 10.1, 8.9, 8.2 Hz, 2-H), 5.69 (1H, d, *J* = 9.9 Hz, 15-H), 5.77 (1H, d, *J* = 15.3 Hz, 12-H), 6.34 (1H, d, *J* = 8.9 Hz, 10-H), 7.03 (1H, s, 9-H) and 7.30 (1H, d, *J* = 15.3 Hz, 13-H); δ_C ($CDCl_3$) 12.45 (C-23), 14.09 (C-22), 20.49 (C-24), 22.62 (C-20 or C-21), 27.46 (C-18 or C-19), 29.38 (C-19 or C-18), 31.82 (C-21 or C-20), 33.28 (C-16), 37.20 (C-17), 37.46 (C-3), 48.27 (C-2), 63.76 (C-6), 74.53 (C-5), 80.61 (C-4), 115.85 (C-12), 130.76 (C-14), 136.35 (C-8), 139.50 (C-9), 148.56 (C-13), 149.29 (C-15), 166.81 (C-11), 172.88 (C-1) and 182.13 (C-7).

Dehydration of chlorohydrin 10 by Ac_2O

Using the same procedure as acetylation of gymnastatin A **1**, chlorohydrin **10** (1.1 mg) was treated with Ac_2O (0.1 cm^3) in pyridine (0.1 cm^3) and purified by HPLC [acetone–H_2O (4:1)] to afford keto-lactone **13** (0.8 mg) as an amorphous powder, identical with that derived from gymnastatin A **1**.

Formation of the (*R*)- and (*S*)-MTPA esters 14a and 14b from gymnastatin E 5

Using the same procedure as above with gymnastatin D **4**, gymnastatin E **5** (5.3 and 3.7 mg) was treated with (*R*)-MTPA (10 mg) and (*S*)-MTPA (10 mg) to afford esters **14a** (4.1 mg) and **14b** (4.3 mg), respectively.

Ester 14a. Obtained as an amorphous powder; *m/z* (EI) 871 (M^+) [*m/z* (HREI) Found: M^+, 871.2919. $C_{43}H_{48}ClF_6NO_9$ requires *M*, 871.2922]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.99 (3H, d, *J* = 6.7 Hz, 24-H_3), 1.18–1.38 (10H, m, 17–21-H_2), 1.74 (3H, s, 23-H_3), 1.94 (1H, t, *J* = 12.6 Hz, 3-H^β), 2.34 (1H, dd, *J* = 12.6, 8.2 Hz, 3-H^α), 2.52 (1H, m, 16-H), 2.94 (1H, dd, *J* = 3.9, 2.4 Hz, 5-H), 3.51 (1H, dd, *J* = 3.9, 2.8 Hz, 6-H), 3.51 (3H, s, OMe), 3.60 (3H, s, OMe), 5.03 (1H, dddd, *J* = 12.6, 9.0, 8.2, 4.3 Hz, 2-H), 5.42 (1H, d, *J* = 9.0 Hz, 10-H), 5.55 (1H, d, *J* = 15.2 Hz, 12-H), 5.68 (1H, d, *J* = 9.3 Hz, 15-H), 5.76 (1H, dd, *J* = 2.8, 1.6 Hz, 7-H), 5.90 (1H, dd, *J* = 2.4, 1.6 Hz, 9-H), 6.53 (1H, d, *J* = 4.3 Hz, 1-H), 7.26 (1H, d, *J* = 15.2 Hz, 13-H), 7.39 (3H, m, ArH), 7.43 (3H, m, ArH), 7.48 (2H, m, ArH) and 7.59 (2H, m, ArH).

Ester 14b. Obtained as an amorphous powder; *m/z* (EI) 871 (M^+) [*m/z* (HREI) Found: M^+, 871.2920. $C_{43}H_{48}ClF_6NO_9$ requires *M*, 871.2922]; δ_H ($CDCl_3$) 0.87 (3H, t, *J* = 6.9 Hz, 22-H_3), 0.99 (3H, d, *J* = 6.6 Hz, 24-H_3), 1.19–1.41 (10H, m, 17–21-H_2), 1.77 (3H, s, 23-H_3), 1.89 (1H, t, *J* = 12.8 Hz, 3-H^β), 2.39 (1H, dd, *J* = 12.8, 8.5 Hz, 3-H^α), 2.52 (1H, m, 16-H), 3.18 (1H, dd, *J* = 4.0, 2.6 Hz, 5-H), 3.55 (3H, s, OMe), 3.63 (3H, s, OMe), 3.67 (1H, dd, *J* = 4.0, 3.0 Hz, 6-H), 5.05 (1H, dddd, *J* = 12.8, 8.9, 8.5, 4.4 Hz, 2-H), 5.19 (1H, d, *J* = 8.9 Hz, 10-H), 5.42 (1H, d, *J* = 15.1 Hz, 12-H), 5.68 (1H, d, *J* = 9.3 Hz, 15-H), 5.75 (1H, dd, *J* = 3.0, 1.8 Hz, 7-H), 5.88 (1H, dd, *J* = 2.6, 1.8 Hz, 9-H), 6.63 (1H, d, *J* = 4.4 Hz, 1-H), 7.22 (1H, d, *J* = 15.1 Hz, 13-H), 7.42 (3H, m, ArH), 7.45 (3H, m, ArH), 7.54 (2H, m, ArH) and 7.60 (2H, m, ArH).

X-Ray crystallography of diacetate 8a from gymnastatin E 5

Diacetate **8a** from gymnastatin E **5** was crystallized from methanol solution by the vapour diffusion method. Crystal data: $C_{27}H_{37}ClNO_7$, *M* = 523.03, monoclinic, *C*2, *a* = 19.175(7) Å, *b* = 9.322(3) Å, *c* = 17.751(4) Å, β = 103.00(2)°, *V* = 3091.8(15) $Å^3$, *Z* = 4, d_x = 1.124 Mg m^{-3}, *F*(000) = 1116, μ(Cu-K*α*) = 1.422 cm^{-1}. Data collection was performed by a Rigaku AFC5R using graphite-monochromated radiation (λ = 1.5418 Å). Total 2743 reflections were collected until θ = 63.29°, in which 2557 reflections were observed [$I > 2\sigma(I)$]. The crystal structure was solved by the direct method using SHELXS-86.[21] The structure was refined by the full matrix least-squares method on *F* using SHELXL-93.[22] In the structure refinements, non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were calculated on the geometrically ideal positions by the 'ride on' method, and were included in the calculation of structure factors with isotropic temperature factors. In the final stage, *R* = 0.0882, *Rw* = 0.2259 and *S* = 1.061 were obtained.

Full crystallographic details, excluding structure factor tables, have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc.*, *Perkin Trans. 1*, available *via* the RSC Web page (http://www.rsc.org/authors). Any request to the CCDC for this material should quote the full literature citation and the reference number 207/262.

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References

1 M. Yotsu, T. Yamazaki, Y. Meguro, A. Endo, M. Murata, H. Naoki and T. Yasumoto, *Toxicon*, 1987, **25**, 225; T. Yasumoto,

D. Yasumura, M. Yotsu, T. Michishita, A. Endo and Y. Kotaki, *Agric. Biol. Chem.*, 1986, **50**, 793; T. Noguchi, J. K. Jeon, O. Arakawa, H. Sugita, Y. Deguchi, Y. Shida and K. Hashimoto, *J. Biochem.* (*Tokyo*), 1986, **99**, 311.
2 T. Kosuge, K. TsujiI, K. Hirai and T. Fukuyama, *Chem. Pharm. Bull.*, 1985, **33**, 3059.
3 M. Kodama, T. Ogata and S. Sato, *Agric. Biol. Chem.*, 1988, **52**, 1075.
4 R. E. Moore, P. Helfrich and G. M. L. Patterson, *Oceanus*, 1989, **25**, 54.
5 A. Numata, C. Takahashi, T. Matsushita, T. Miyamoto, K. Kawai, Y. Usami, M. Inoue, H. Ohishi and T. Shingu, *Tetrahedron Lett.*, 1992, **33**, 1621.
6 C. Takahashi, A. Numata, Y. Ito, E. Matsumura, H. Araki, H. Iwaki and K. Kushida, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 1859.
7 C. Takahashi, A. Numata, E. Matsumura, K. Minoura, H. Eto, T. Shingu, T. Ito and T. Hasegawa, *J. Antibiot.*, 1994, **47**, 1242.
8 C. Takahashi, Y. Takai, Y. Kimura, A. Numata, N. Shigematsu and H. Tanaka, *Phytochemistry*, 1995, **38**, 155.
9 A. Numata, C. Takahashi, Y. Ito, K. Minoura, T. Yamada, C. Matsuda and K. Nomoto, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 239.
10 A. Numata, M. Iritani, T. Yamada, K. Minoura, E. Matsumura, T. Yamori and T. Tsuruo, *Tetrahedron Lett.*, 1997, **38**, 8215.
11 T. Amagata, Y. Usami, K. Minoura, T. Ito and A. Numata, *J. Antibiot.*, 1998, **51**, 33.
12 C. Iwamoto, K. Minoura, S. Hagishita, K. Nomoto and A. Numata, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 449, and references cited therein.
13 A. Numata, T. Amagata, K. Minoura and T. Ito, *Tetrahedron Lett.*, 1997, **38**, 5675.
14 T. Higa, *Tetrahedron Lett.*, 1985, **26**, 2335.
15 A. Rieker and S. Berger, *Org. Magn. Reson.*, 1972, **4**, 857.
16 C. J. Pouchert and J. Behnke, *The Aldrich Library of 13C and 1H FTNMR Spectra*, Edition 1, vol. 1, pp. 696, 713–716, Aldrich Chemical Company, 1993.
17 M. F. Grenier-Loustalot, P. Iratcabal, A. Forchioni and F. Metras, *Org. Magn. Reson.*, 1976, **8**, 544.
18 G. Englert, *Helv. Chim. Acta*, 1975, **58**, 2367.
19 I. Ohtani, T. Kusumi, Y. Kashman and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.
20 A. Numata, P. Yang, C. Takahashi, R. Fujiki, M. Nabae and E. Fujita, *Chem. Pharm. Bull.*, 1989, **37**, 648.
21 G. M. Sheldrick, SHELXS-86, Program for the Solution of Crystal Structures, University of Gottingen, Gottingen, 1986.
22 G. M. Sheldrick, SHELXS-93, Program for the Refinement of Crystal Structures from Diffraction Data, University of Gottingen, Gottingen, 1993.

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