151. Novel Leptomycins from a *Streptomyces* Strain A92-308902: Inhibitors of the Nucleo-cytoplasmic Translocation of the HIV-1 Regulatory Protein Rev

by **Ying Wang***, **Monique Ponelle**, and **Jean-Jacques Sanglier**

Core Technology Area, Research, *Novartis Pharma Inc.*, CH-4002 Basel

and **Barbara Wolff**

Novartis Research Institute, Brunner Strasse 59, A-1230 Vienna

(17.IV.97)

As one of the regulatory gene products in the HIV-1 genome, Rev protein must be translocated from the nucleus to the cytoplasm to exert its function. Therefore, inhibition of Rev protein translocation could be a useful target for HIV therapy. An extract from the *Streptomyces* strain A92-308902 with very potent inhibitory activity was found in the course of a high throughput screening with a Rev translocation assay (RTA). Bioassay-guided fractionation with gel filtration, normal-phase and reversed-phase chromatography yielded six RTA-active metabolites belonging to the leptomycin family, the known leptomycin A (**1**), leptomycin B (**2**), kazusamycin B (**3**), and kazusamycin A (**4**), and the hitherto unknown dilactonmycin (**5**) and delactonmycin (**6**), together with an inactive cyclic hexadepsipeptide L-156,620 (**7**). The structures were established mainly by spectroscopic methods (UV, FT-IR, FAB-MS, ^{1}H-NMR, ^{13}C-NMR(JMOD), DQ-COSY, ROESY, HSQC, and HMBC). The configuration of *all* C=C bonds of **1**–**6** was unambiguously established by analysis of coupling constants and ROESY spectra. All isolated leptomycins **1**–**6** inhibit Rev translocation at nanomolar concentrations. Six derivatives (**2a**–**c** and **4a**–**c**) of leptomycin B (**2**) and kazusamycin A (**4**) were also prepared and tested in the RTA for preliminary investigations on structure-activity relationships.

1. Introduction. – The acquired immunodeficiency syndrome (AIDS) is a life-threatening disease caused by the human immunodeficiency virus type 1 (HIV-1) [1]. Besides the genes encoding the structural proteins Gag, Pol, and Env, the HIV-1 genome contains several regulatory genes, such as Tat and Rev, which are essential for virus replication [2]. Rev protein is translated from completely spliced mRNA and then imported into the nucleus. After binding to the RRE (Rev response element), an HIV-specific RNA sequence, Rev mediates transport of unspliced and singly spliced HIV-1 mRNA from the nucleus to the cytoplasm. This allows the synthesis of Gag, Pol, and Env gene products and provides the complete proviral RNA for viral replication [3].

Recently, it has been found that Rev protein itself must be translocated from the nucleus to the cytoplasm to exert its function [4]. Therefore, inhibition of Rev-protein translocation could be a useful target for HIV-1 therapy. The cytoplasmic accumulation of Rev can be induced and amplified by inhibitors of ribosomal RNA synthesis, *e.g.*, actinomycin D, and monitored by immunofluorescence (Rev-translocation assay, RTA) [5].

In our high throughput screening based on the RTA, an extract with very potent inhibitory activity was identified from the *Streptomyces* strain A92-308902. Guided by

the RTA, we investigated this strain in an attempt to identify bioactive constituents which might be useful inhibitors of translocation of the HIV regulatory protein Rev.

Results. – The AcOEt extract of *Streptomyces* A92-308902 broth was defatted by partition between hexane and $MeOH/H_2O$ 9:1 and fractionated by gel filtration on *Sephadex LH-20*. From the fractions active in the Rev-translocation assay, compounds **1**–**6** were isolated by a combination of normal-phase and reversed-phase open column chromatography, or prep. reversed-phase HPLC (*RP-18*). All these six secondary metabolites showed quite potent inhibition of Rev translocation at nanomolar concentrations. As a major by-product in the fermentation broth, compound **7** was purified from an inactive fraction by chromatography on silica gel.

Compounds **1** and **2** were identified as leptomycins A and B, respectively, the first two members in this antibiotic family isolated from a *Streptomyces* strain in 1983 [6] [7]. The spectral data of compounds **3** and **4** revealed that they were identical with kazusamycins B and A, respectively, another two members in the same leptomycin family found in 1987 and 1984, respectively [8] [9]. All four compounds have a structure characteristic of an unsaturated, branched-chain fatty acid with a terminal δ-lactone ring. The only difference between the two groups is that Me–C(16)[1]) in leptomycins A (**1**) and B (**2**) has been oxygenated to $HOCH_2$–C(16)[1]) in kazusamycins B (**3**) and A (**4**).

To all C=C bonds in leptomycins A and B and kazusamycins B and A at C(6), C(12), C(14), and C(22)[1]) (*E*)-configuration was assigned based on the coupling constants *J*(6,7) (15.6 Hz) and *J*(12,13) (15.5 Hz), and the upfield chemical shift of C(29) (18.5 ppm) and C(33) (16.0 ppm) [7] [9b]. However, the configuration of the C=C bond at C(8) could not be determined although it was drawn in the (*E*)-configuration [7–9]. To establish the configuration of all C=C bonds, we carefully analysed nuclear *Overhauser* enhancement (NOE) in ROESY spectra of **1**–**4**. With the NOE signals for H–C(6)/H–C(25) and H–C(26), H–C(7)/H–C(10) and H–C(28), H–C(9)/H–C(26) and H–C(28), the C=C bond at C(8) in all four compounds was unambiguously assessed as having (*Z*)-configuration.

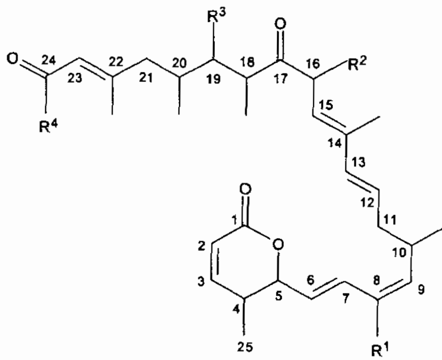
The strong absorption bands at 1779 and 1747 cm^{-1} in the IR spectrum of **5** reveal the presence of both γ- and δ-lactones. Its FAB-MS in positive mode shows quasimolecular ions at *m/z* 561 ($[M + Na]^+$) and 539 ($[M + H]^+$). The ^1H- and ^{13}C-NMR spectra of **5** resemble those of leptomycin B (**2**) and allowed the structure of compound **5** to be identified as dilactonmycin, a novel member in the leptomycin family.

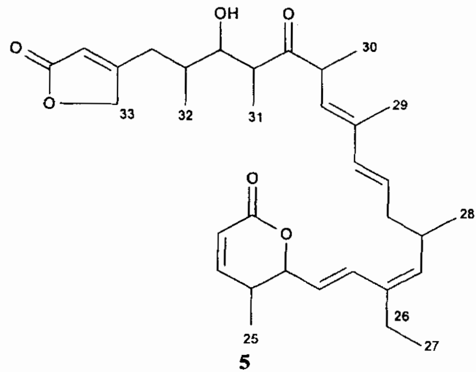
The major differences between **5** and leptomycin B (**2**) are new methylene signals at 4.77 and 4.68 ppm in the ^1H-NMR and at 73.17 ppm in the ^{13}C-NMR spectra of **5** in place of the signals of Me(33) at 2.13 ppm and 16.0 ppm in the NMR spectra of **2**. The signal of the C-atom adjacent C(22) (168.8 ppm) of **5** appears downfield by 7.9 ppm, whereas the signal of C(21) (33.30 ppm) is shifted upfield by 12.4 ppm. This clearly indicates that the COOH group and Me(33) of **2** form a new terminal γ-lactone in compound **5**. Detailed analysis of ^1H-, ^{13}C-NMR (JMOD), ROESY, HSQC, and HMBC spectra confirms this change, as well as the geometrical configurations of all C=C bonds (*Table 1*).

The spectroscopic data of **6** show that it is an analogue of the leptomycins but has significant differences. The positive FAB-MS spectrum of **5** exhibits quasimolecular ions at *m/z* 387 ($[M + Na]^+$) and 365 ($[M + H]^+$) much smaller than other leptomycins. The

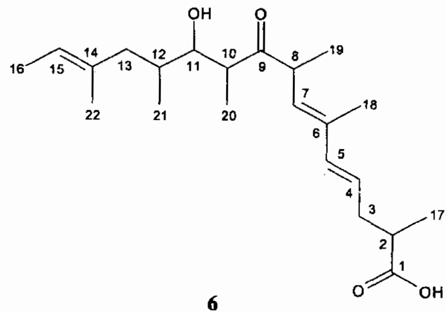
---

[1]) Arbitrary numbering; for systematic names, see. *Exper. Part.*





1	R^1 = Me, R^2 = Me, R^3 = R^4 = OH
2	R^1 = Et, R^2 = Me, R^3 = R^4 = OH
2a	R^1 = Et, R^2 = Me, R^3 = OAc, R^4 = OH
2b	R^1 = Et, R^2 = Me, R^3 = OH, R^4 = OMe
2c	R^1 = Et, R^2 = Me, R^3 = OAc, R^4 = OMe
3	R^1 = Me, R^2 = CH_2OH, R^3 = R^4 = OH
4	R^1 = Et, R^2 = CH_2OH, R^3 = R^4 = OH
4a	R^1 = Et, R^2 = CH_2OAc, R^3 = OAc, R^4 = OH
4b	R^1 = Et, R^2 = CH_2OH, R^3 = OH, R^4 = OMe
4c	R^1 = Et, R^2 = CH_2OAc, R^3 = OAc, R^4 = OMe



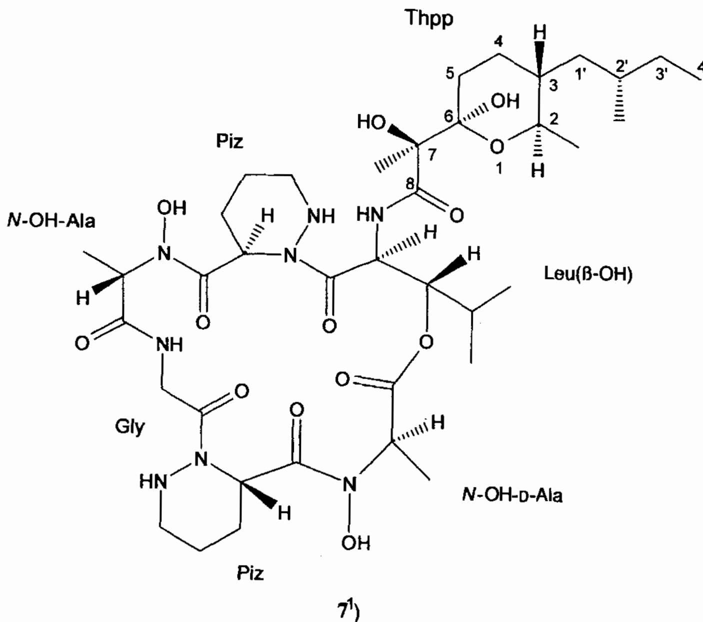


Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Dilactonmycin* (**5**)[1])

	^{1}H	ROESY	^{1}H,^{13}C Long-range correlations	^{13}C
C(1)				164.40
H-C(2)	5.99 (*dd*, *J* = 9.7, 0.8)	H-C(3)	C(1), C(4)	120.04
H-C(3)	6.95 (*dd*, *J* = 9.7, 5.5)	H-C(2), H-C(4), Me(25)	C(1), C(5), Me(25)	151.52
H-C(4)	2.56 (*m*)	H-C(3), H-C(5)	Me(25)	33.44
H-C(5)	4.98 (*ddd*, *J* = 6.9, 3.9, 1.1)	H-C(4), H-C(6), H-C(7)	C(3), C(4), C(6), C(7), Me(25)	81.46
H-C(6)	5.73 (*dd*, *J* = 15.9, 6.9)	H-C(5), Me(25), CH_2(26), Me(27)	C(5), C(8)	122.58
H-C(7)	6.65 (*d*, *J* = 15.9)	H-C(5), H-C(10), Me(28)	C(5), C(6), C(8), C(9), C(25)	130.34
H-C(8)				135.59
H-C(9)	5.25 (br. *d*, *J* = 9.7)	Me(28), H-C(11), CH_2(26), Me(27)	C(7), C(10), C(11), C(26), Me(28)	136.89
H-C(10)	2.67 (*m*)	H-C(7), H-C(11), Me(28)		32.31
H-C(11)	2.09 (*m*)	H-C(9), H-C(10), H-C(12), H-C(13)	C(9), C(10), C(12), C(13), Me(28)	40.78
H-C(12)	5.61 (*ddd*, *J* = 15.5, 7.2, 7.2)	H-C(11), Me(29)	C(11), C(14)	128.47
H-C(13)	6.02 (*d*, *J* = 15.5)	H-C(11), H-C(15)	C(11), C(14), C(15), Me(29)	134.84
C(14)				136.74
H-C(15)	5.04 (br. *d*, *J* = 10.2)	H-C(13), H-C(16), Me(30), Me(31)	C(13), C(14), C(16), Me(29), Me(30)	127.95
H-C(16)	3.67 (*m*)	Me(30)	C(15), C(17), Me(30)	45.52
C(17)				214.32
H-C(18)	2.81 (*dddd*, *J* = 7.0, 7.0, 7.0, 7.0)	H-C(19), H-C(20), Me(30), Me(32)	C(17), C(19), Me(31)	47.01
H-C(19)	3.67 (*dd*, *J* = 5.5, 5.5)	H-C(18), H-C(20), H_a-C(21), H_b-C(21), Me(31), Me(32)	C(17)	73.68
H-C(20)	1.76 (*m*)	H-C(18), H-C(19), H_a-C(21), H_b-C(21)		34.31
H_a-C(21)	2.53 (*dd*, *J* = 13.0, 9.1)	H-C(19), H-C(20), H-C(23), Me(32)	C(19), C(20), C(22), C(23), Me(32)	
H_b-C(21)	2.27 (*dd*, *J* = 13.0, 9.1)	H-C(19), H-C(20), H-C(23), Me(32)	C(19), C(20), C(22), C(23), Me(32)	33.30
C(22)				168.80
H-C(23)	5.88 (br. *s*)	H_a-C(21), H_b-C(21)	C(22)	116.70
C(24)				168.80
Me(25)	1.08 (*d*, *J* = 7.2)	H-C(3), H-C(6)	C(3), C(4), C(5)	12.35
CH_2(26)	2.22 (*dddd*, *J* = 7.2, 7.2, 7.2, 0.8)	H-C(6), H-C(9)	C(7), C(8), C(9), Me(27)	26.54
Me(27)	1.05 (*dd*, *J* = 7.4, 7.4)	H-C(6), H-C(9)	C(8), C(26)	13.56
Me(28)	0.98 (*d*, *J* = 6.6)	H-C(7), H-C(9), H-C(10)	C(9), C(10), C(11)	20.97
Me(29)	1.85 (*s*)	H-C(12), H-C(15)	C(13), C(14), C(15)	13.09
Me(30)	1.13 (*d*, *J* = 6.7)	H-C(15), H-C(16), H-C(18)	C(15), C(16), C(17)	15.79
Me(31)	1.18 (*d*, *J* = 7.0)	H-C(19)	C(17), C(18), C(19)	13.56
Me(32)	0.84 (*d*, *J* = 6.7)	H-C(18), H-C(19), H_a-C(21), H_b-C(21)	C(19), C(20), C(21)	13.35
H_a-C(33)	4.77 (*dd*, *J* = 16.1, 0.8)	H-C(20), H_a-C(21), H_b-C(21)	C(22), C(23), C(24)	
H_b-C(33)	4.68 (*dd*, *J* = 16.1, 0.8)	H-C(20), H_a-C(21), H_b-C(21)	C(22), C(23), C(24)	73.17

absence of a terminal δ-lactone in compound **5** is revealed by the comparison of both ^{1}H- and ^{13}C-NMR spectra with those of leptomycin B (**2**). By means of 2D homonuclear (DQ-COSY) and heteronuclear correlations (HSQC and HMBC), as well as detailed NOE analysis (ROESY) (*Table 2*), compound **6** was identified as delactonmycin, a novel natural product.

The four signals at 6.08, 5.60, 5.19, and 5.17 ppm in the ^{1}H-NMR of **6** are indicative of four vinyl groups, and this is confirmed by six olefinic C-atoms at 136.5, 135.8, 134.0, 129.2, 125.9, and 120.5 ppm. Seven signals between 12.0 to 16.4 ppm can be attributed to seven methyl groups.

The structure of the major by-product **7** was established by spectroscopic means and confirmed by comparison with the known ^{13}C-NMR of L-156,620 (*Table 3*), which belongs to the class of cyclic hexadepsipeptide antibiotics and has a 19-membered ring composed of six amino acids and a tetrahydropyranylpropanoic acid moiety (Thpp) [10]. L-156,602 was first reported to be a C5a antagonist [10] [11], and its complete structure and absolute configuration was accomplished by X-ray diffraction analysis and total synthesis [10] [12]. Recently, the *in vivo* selective anti-inflammatory effect of L-156,602 has also been investigated [13]. In our Rev-translocation assay, this compound did not show any inhibitory activity.

All six isolated leptomycins **1**–**6** inhibit Rev translocation at nanomolar concentrations, but also exert strong antiproliferative effects after incubation with cells for 72 h (*Table 4*). Leptomycin B (**2**) shows the most prominent inhibition activity in our test system. In an attempt to explore some preliminary structure-activity relationships, six derivatives were prepared from **2** and **4** by acetylation of OH group(s), methylation of the carboxylic group, or modification of both functional groups by acetylation and methylation. The ^{1}H-NMR data of **2a**–**c** and **4a**–**c**, together with their parent compounds **2** and **4**, are summarized in *Table 5*. Compounds **2b**, **2c**, and **4a** have been described before without giving physicochemical data [7] [8], whereas **2a**, **4b**, and **4d** are reported for the first time. From the results in the RTA and the proliferation assay (*Table 4*), it is apparent that any modification of the OH or COOH groups will decrease, to some extent, biological activity. However, these functional groups are not essential for the inhibition of Rev translocation and the antiproliferative effect.

Discussion. – As one of the regulatory gene products of the HIV-1 genome, Rev protein must be translocated from the nucleus to the cytoplasm to exert its function. Therefore, inhibition of Rev-protein translocation could provide a useful target for HIV therapy. Guided by the Rev-translocation assay, we isolated and characterized the highly active leptomycins **1**–**6** from the *Streptomyces* strain A92-308902.

The leptomycins had first been isolated because of their antifungal activities [6] [7], but they became more prominent as antitumor agents [8] [9] [14] [17]. Cytotoxic activity of leptomycins A (**1**) and B (**2**) against some human and mouse tumor cell lines has been demonstrated both *in vivo* and *in vitro* (*Table 4* and [15]). They cause reversible arrest of the cell cycle of rat 3Y1 fibroblasts in either G1 or G2 phase [16]. Besides leptomycins A (**1**) and B (**2**) and kazusamycins A (**4**) and B (**3**), other closely related compounds belonging to the leptomycin family are anguinomycins A–D [17], reductoleptomycin A [18], leptolstatin [19], and leptofuranins A–D [20]. Dilactonmycin (**5**) and delactonmycin (**6**) are two novel members in this family with unique structural features, namely with both a δ- and γ-lactone moiety (see **5**) or without any terminal unsaturated lactone (see **6**).

Table 2. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Delactonmycin* (**6**)[1])

	^{1}H	ROESY	^{1}H,^{13}C Long-range correlations	^{13}C
C(1)				180.8
H–C(2)	2.56 (*m*)	H_a–C(3), H_b–C(3), Me(17)	C(1), C(3), C(4), Me(17)	39.3
H_a–C(3)	2.26 (*dddd*, *J* = 15.0, 7.1, 7.1, 0.8)	H–C(2), H–C(4), H–C(5), H–C(6)	C(1), C(2), C(4), C(5), Me(17)	36.6
H_b–C(3)	2.50 (*dddd*, *J* = 15.0, 7.1, 7.1, 0.8)	H–C(2), H–C(4), H–C(5), H–C(6)	C(1), C(2), C(4), C(5), Me(17)	
H–C(4)	5.60 (*ddd*, *J* = 15.5, 7.2, 7.2)	H_a–C(3), H_b–C(3), Me(18)	C(2), C(3), C(6)	125.9
H–C(5)	6.08 (*d*, *J* = 15.5)	H_a–C(3), H_b–C(3), H–C(6), Me(17), Me(18)	C(3), C(6), C(7), Me(18)	136.5
C(6)				134.0
H–C(7)	5.17 (br. *d*, *J* = 10.2)	H–C(10), Me(19)	C(5), C(8), C(9), Me(18), Me(19)	129.2
H–C(8)	3.66 (*m*)	H–C(10), H–C(11), Me(18), Me(19)	C(6), C(7), C(9), Me(19)	45.7
C(9)				215.6
H–C(10)	2.86 (*dddd*, *J* = 7.0, 7.0, 7.0, 7.0)	H–C(7), H–C(8), H_a–C(13), H_b–C(13), Me(20), Me(21)	C(9), C(11), Me(20)	46.5
H–C(11)	3.56 (*dd*, *J* = 5.5, 5.5)	H–C(8), H–C(10), H_a–C(13), H_b–C(13), Me(20), Me(21)	C(9), C(10), C(12), C(13), Me(20), Me(21)	74.4
H–C(12)	1.65 (*m*)	H–C(10), H–C(11), H–C(12)	C(11), C(13), Me(21)	33.2
H_a–C(13)	1.70 (*dd*, *J* = 13.1, 9.1)	H–C(10), H–C(11), H–C(12), H–C(15)	C(11), C(12), C(14), C(15)	44.1
H_b–C(13)	1.98 (*dd*, *J* = 13.1, 9.1)	H–C(10), H–C(11), H–C(12), H–C(15)	C(11), C(12), C(14), C(15)	33.3
C(14)				135.8
H–C(15)	5.19 (*ddd*, *J* = 7.0, 7.0, 7.0)	H_a–C(13), H_b–C(13), Me(16)	C(13), Me(22)	120.5
Me(16)	1.58 (*d*, *J* = 6.8)	H–C(15)	C(14), C(15)	13.4
Me(17)	1.18 (*d*, *J* = 6.8)	H–C(2), H_a–C(3), H_b–C(3), H–C(5)	C(1), C(2), C(3)	16.4
Me(18)	1.83 (*s*)	H–C(4), H–C(5), H–C(8)	C(5), C(6), C(7)	13.1
Me(19)	1.15 (*d*, *J* = 6.8)	H–C(7), H–C(8)	C(7), C(8)	16.4
Me(20)	1.15 (*d*, *J* = 6.8)	H–C(10), H–C(11)	C(10), C(11)	12.0
Me(21)	0.78 (*d*, *J* = 6.8)	H–C(10), H–C(11), H_a–C(13), H_b–C(13)	C(11), C(12), C(13)	14.2
Me(22)	1.56 (*d*, *J* = 0.8)		C(13), C(14), C(15)	16.2

Table 3. *^{13}C-NMR Data of L-156,620* (**7**) [1])

	(D$_6$)DMSO	CD$_3$CN [a])		(D$_6$)DMSO	CD$_3$CN [a])		(D$_6$)DMSO	CD$_3$CN [a])
Leu (β-OH)			Piz′			Thpp		
C(α)	47.4	47.2	C(α)	48.5	50.1	C(2)	70.9	72.3
C(β)	78.3	79.5	C(β)	24.0	24.0	Me–C(2)	19.6	20.1
C(γ)	29.8	30.7	C(γ)	21.2	21.1	C(3)	39.7	40.5
C(δ)	19.0	18.5	C(δ)	47.2	47.2	C(4)	24.5	24.6
C(δ′)	19.4	20.0	*N*-OH-Ala′			C(5)	27.5	28.1
Gly			C(α)	53.7	54.4	C(6)	99.1	99.9
C(α)	42.1	42.6	C(β)	14.2	13.2	C(7)	75.9	77.2
Piz			Carbonyl			Me–C(7)	21.1	22.0
C(α)	48.8	49.4	COX	168.4	170.6	C(1′)	38.8	39.4
C(β)	25.3	25.8		169.4	170.6	C(2′)	31.0	31.8
C(γ)	21.4	22.3		169.6	170.7	Me–C(2′)	19.2	18.5
C(δ)	47.2	47.7		169.6	172.5	C(3′)	31.0	31.7
N-OH-Ala				172.0	173.0	C(4′)	11.9	11.9
C(α)	53.1	53.3		174.7	175.3			
C(β)	13.0	12.6		177.4	177.2			

[a]) From [10].

Table 4. *Inhibition of Rev Translocation by Leptomycins and Their Derivatives* [a])

	Rev translocation [b])		Cell proliferation [c])
	7 h	72 h	72 h
1	0.8	4	2.6
2	0.1	0.8	0.9
2a	10	10	6
2b	1	10	2
2c	1	10	1
3	10	20	7.4
4	1.2	3	2.9
4a	10	10	7
4b	0.5	10	2
4c	10	10	30
5	10	3	1.7
6	10	> 300	171

[a]) In average, the coefficient of error was 10%. [b]) Concentration [nM] at which Rev nucleo-cytoplasmic transport was fully inhibited. [c]) Concentration [nM] which caused a 50% reduction in cell number as compared to untreated controls.

The C=C bond at C(8) of **1**–**5** was assessed as having (*Z*)-configuration based on NOE analysis. This is identical with leptolstatin [19] and leptofuranins A–D [20]. Therefore, ist seems that almost all members of the leptomycin family have the same configuration, *i.e.*, (2*Z*,6*E*,8*Z*,12*E*,14*E*,22E) [1]).

All six identified leptomycins from *Streptomyces* A92-308902 inhibit Rev translocation at nanomolar concentrations. Their activity could not be improved by preliminary chemical modifications on the OH and COOH groups. Some other hydroxylated derivatives prepared in our biotransformation approach gave quite similar results [21]. In our

Table 5. *^{1}H-NMR Data* ($CDCl_3$) *of Leptomycin B* (**2**), *Kazusamycin A* (**4**), *and Their Derivatives* **2a–c** *and* **4a–c**[1])

	2	**2a**	**2b**	**2c**	**4**	**4a**	**4b**	**4c**
H–C(2)	5.99	5.99	5.99	5.99	5.99	5.99	5.99	5.99
H–C(3)	6.95	6.95	6.95	6.95	6.95	6.95	6.95	6.95
H–C(4)	2.53	2.53	2.53	2.53	2.53	2.53	2.53	2.53
H–C(5)	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99
H–C(6)	5.72	5.72	5.72	5.72	5.72	5.72	5.72	5.72
H–C(7)	6.65	6.65	6.65	6.65	6.65	6.65	6.65	6.65
H–C(9)	5.23	5.23	5.23	5.23	5.23	5.23	5.23	5.23
H–C(10)	2.68	2.68	2.68	2.68	2.68	2.68	2.68	2.68
CH_2(11)	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08
H–C(12)	5.60	5.60	5.60	5.60	5.65	5.66	5.65	5.65
H–C(13)	6.01	6.01	6.01	6.01	6.01	6.01	6.01	6.01
H–C(15)	5.08	5.08	5.08	5.08	5.06	4.98	5.06	5.06
H–C(16)	3.65	3.65	3.65	3.65	3.87	3.99	3.87	3.87
H–C(18)	2.83	2.95	2.83	2.93	2.79	2.88	2.79	2.88
H–C(19)	3.57	5.22	3.57	5.22	3.61	5.23	3.61	3.61
H–C(20)	1.75	1.65	1.75	1.82	1.77	1.85	1.77	1.80
H_a–C(21)	1.90	1.83	1.90	1.82	1.90	1.85	1.90	1.85
H_b–C(21)	2.20	2.18	2.20	2.20	2.22	2.20	2.22	2.20
H–C(23)	5.68	5.62	5.65	5.60	5.62	5.62	5.66	5.50
Me(25)	1.07	0.96	1.07	1.07	1.07	1.07	1.07	1.07
CH_2(26)	2.22	2.22	2.22	2.21	2.20	2.20	2.20	2.20
H–C(27)	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Me(28)	0.97	0.87	0.97	0.95	0.98	0.98	0.99	0.99
Me(29)	1.82	1.82	1.82	1.82	1.87	1.87	1.87	1.87
Me(30)	1.13	1.05	1.13	1.13				
H_a–C(30)					3.88	4.34	3.88	4.34
H_b–C(30)					3.63	4.07	3.63	4.07
Me(31)	1.16	1.14	1.16	1.13	1.20	1.09	1.20	1.20
Me(32)	0.81	0.71	0.70	0.70	0.78	0.70	0.78	0.70
Me(33)	2.13	2.08	2.13	2.09	2.12	2.09	2.12	2.12
MeCOO						1.99		1.99
MeCOO		2.07		2.08		2.08		2.08
MeO			3.690	3.67			3.67	3.67

further investigations, it was found that leptomycin B (**2**) inhibits Rev-dependent, but not Rev-independent gene expression in a short-term transfection assay. In primary human monocytes, it suppressed HIV-1 replication [5]. However, due to their antiproliferative activity, leptomycins cannot be used therapeutically, but could serve as a valuable tool for dissecting nuclear export pathways.

We appreciate the excellent technical assistance of *Henri Rey* and *Liliane Gschwind*. *Lukas Oberer* is acknowledged for his kind help in recording and analyzing NMR data of L-156,620 with a *Bruker-Advance-DMX-500* spectrometer. We would also thank *Hans Hofmann*, *Hans-Ulrich Gremlich*, and *Charles Quizquerez* for large-scale extraction, preliminary fractionation, and recording UV, FT-IR, and FAB mass spectra.

Experimental Part

General. TLC: silica gel *60 F254* precoated plates (*Merck*); *RP-18 W/UV254* precoated plates (*Merck*); detection at 254 nm. Open column chromatography (CC): *Sephadex LH-20* (*Pharmacia*), silica gel 40–63 µm (*Merck*), *LiChroprep RP-18* 40–63 µm (*Merck*). Anal. HPLC: *Hewlett-Packard-HP-1090* system with a photodi-

ode array UV detector and a *HP Chemstation*; *LiChrosphere-RP-18* column (5 μm, 5 × 100 mm i.d.; *Merck*), t_R in min. Prep. HPLC: *Labomatic-HD-200* pump coupled with a *Labomat-VS-200* gradient controller and a *Labocorod-700* UV detector; *LiChrosorb-RP-18* column, (7 μm, 25 × 250 mm i.d.; *Merck*). Buffer (pH 4.5) for anal. or prep. HPLC: H_3PO_4 (9.8 g) was dissolved in H_2O (1.9 l), the pH regulated to 4.5 with Et_3N, and the mixture diluted with H_2O (2 l) and MeCN (0.2 l). IR: FT-IR spectrometer *Bruker IFS 66*. ^1H- and ^{13}C-NMR: *Bruker-AMX-400* or *Bruker-Advance-DMX-500* spectrometer at 400 or 500 and 100.62 or 125.77 MHz, resp.; in (D_6)DMSO or $CDCl_3$; $SiMe_4$ as internal standard. MS (*m/z* (%)): *VG-7044SE* or *Finnigan-MAT-212* spectrometer, 8 keV Xe with nitrobenzyl alcohol or thioglycerin as matrix operating in the FAB positive-ion mode.

Micro-organism. By evaluating the RTA activity of MeOH extracts of many micro-organisms, we found strain A92-308902 that produced inhibitory compounds. A92-308902 was isolated from a soil sample collected at Dembo Bridge, Malawi. This strain has been deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, under the accession number DSM 7517.

Taxonomical Characterization of Producing Micro-organism. Morphology of the strain was ascertained by light microscopy (*Zeiss Axioplan*). Analysis of diaminopimelic acid was performed on the hydrolysate of cells grown on *Bennett*'s agar medium [22]. The analysis of fatty acids and whole-cells sugars was determined by gas chromatography [23]. On various agar media, the strain A92-308902 produced long spore chains of the *Rectus flexibilis* type. No fragmentation of the substrate hyphae was observed. Analysis of the cell wall hydrolysates of the strain revealed that the cell walls contained L,L-diaminopimelic acid = (2*S*,6*S*)-2,6-diaminoheptanedioic acid). The fatty acids are *iso*- and *anteiso*-branched, straight, and unsaturated. The sugar spectrum is non-distinctive. These taxonomic observations indicate that the strain A92-308902 belongs to the genus *Streptomyces*.

Fermentation. Cells from mature slants of the strain A92-308902 grown on a NZ-amine/yeast extract/starch medium were suspended in 10 ml of saline per slant, and 20 ml of this suspension were inoculated into each 2-l *Erlenmeyer* flask containing 1 l of seed medium composed of glycerol (0.75%), glucose (0.75%), malt-extract liquid (*Wander*; 0.75%), starch (0.75%), soya protein (*Siber Hegner*; 0.25%), NZ-amine type A (*Sheffield*; 0.25%). L-asparagine (0.1%), yeast extract (BBL; 0.135%), $CaCO_3$ (0.005%); KH_2PO_4 (0.025%), K_2HPO_4 (0.05%), $MgSO_4 \cdot 7\,H_2O$ (0.01%), NaCl (0.005%), trace-element soln. (Fe, Zn, Mn, Cu, Co, B, I), and agar (0.1%), in deionized H_2O (pH adjusted to pH 7.0 before sterilisation). The inoculated flasks were incubated on a rotary shaker (200 rpm) at 27° for 4 days. The seed cultures (2.5 l) were inoculated in 75 l bioreactors containing 50 l of the same medium. The cultures were fermented for 72 h at 27°. The bioreactors were rotated at 150 rpm, and air was introduced at a rate of 0.5 l/min per l of medium. Then, 50 l of these intermediate cultures were inoculated in 750-l bioreactors containing 500 l of the production medium composed of glucose (2.0%), malt-extract liquid (*Wander*; 0.2%), yeast extract (*Bacto*; 0.2%), soytone (*Bacto*; 0.2%), KH_2PO_4 (0.02%), K_2HPO_4 (0.04%), $MgSO_4 \cdot 7\,H_2O$ (0.02%), $CaCl_2 \cdot 2\,H_2O$ (0.005%), NaCl (0.005%), and trace-element solution (Fe, Zn, Mn, Cu, Co, B, I) in deionized H_2O (pH adjusted to 6.3 before sterilisation). The fermentation was carried out for 5 days at 24° with an agitation of 80 rpm and an aeration of 0.8 l/min per l of medium.

Rev-Translocation Assay (RTA). The assay is described in detail in [5]. Briefly, HeLa cells stably transfected with HIV-1 Rev cDNA (HeLa-Rev cells) were seeded into 96-well microtiter plates and synchronized by serum starvation for 2 days. Rev synthesis was then induced by the addition of serum. At this time, fermentation extracts or purified compounds were added in growth medium and incubated with the cells for a total of 7 or 72 h. During the last 2 h, actinomycin D (1 μg/ml) was added. The cells were then fixed, permeabilized, and immunostained for Rev protein, using a mouse monoclonal antibody against Rev and a dichlorotriazinyl amino fluorescein (DTAF)-labelled secondary antibody. Inhibition of Rev translocation was evaluated by microscopic examination of each individual well. Antiproliferative activity (IC_{50}) of the compounds was assessed after 72 h by staining cellular protein with sulforhodamine B [24]. Results were read in an SLT microtiter plate reader.

Extraction. The fermentation broth (1500 l) and AcOEt (1500 l) were homogenized with a *Dispax* reactor for 2 h and agitated for 3 h at r.t. The org. phase was separated from extracted broth with a *Westfalia* separator. The extraction was repeated once with the same procedure and afforded 809 g of AcOEt extract. The AcOEt extract was defatted three times by extraction with 10 l of MeOH/H_2O 9:1 and 10 l of hexane. The MeOH/H_2O phase was evaporated under reduced pressure to remove MeOH and extracted three times with AcOEt, giving 109 g of defatted AcOEt extract.

Isolation. The defatted AcOEt extract was subjected to gel filtration (10 kg of *Sephadex LH-20*, MeOH, 11 fractions). Compound **7** (9 g) was isolated from *Fr. 10* by CC (silica gel, AcOEt sat. by H_2O). The RTA-active *Fr. 7* was chromatographed CC (silica gel, AcOEt sat. by H_2O, 9 fractions). Compound **1** (14 g) and **2** (1.2 g), **3** (1.1 g) and **4** (0.25 g) were isolated from *Frs. 5* and *6*, resp., by CC (*RP-18*, MeCN/buffer (pH 4.5) 45:55 → 60:40). Compound **6** was obtained from *Fr. 3* by CC (silica gel, toluene/i-PrOH 98:2 → 80:20) and then prep. HPLC (*RP-18*, MeOH/H_2O (+ 0.01% AcOH) 70:30). *Fr. 2* was rechromatographed by CC (silica gel,

toluene/i-PrOH 99:1 → 90:10) and CC (silica gel, *t*-BuOEt/MeOH 97:3), giving compound **5** after a final purification by prep. HPLC (*RP-18*, MeOH/H_2O(+ 0.01% AcOH) 65:35).

Leptomycin A (= *(*2E,*10*E,*12*E,*16*Z,*18*E*)-19-(3,6-Dihydro-3-methyl-6-oxo-2*H*-pyran-2-yl)-6-hydroxy-3,5,7,9,11,15,17-heptamethyl-8-oxo-2,10,12,16,18-nonadecapentaenoic Acid*; **1**). Colourless viscous oil. TLC (SiO_2, *t*-BuOEt/MeOH 90:10): R_f 0.42. HPLC (*RP-18*, MeCN/buffer (pH 4.5) 50:50): t_R 7.2.

Leptomycin B (= *(*2E,*10*E,*12*E,*16*Z,*18*E*)-19-(3,6-Dihydro-3-methyl-6-oxo-2*H*-pyran-2-yl)-17-ethyl-6-hydroxy-3,5,7,9,11,15-hexamethyl-8-oxo-2,10,12,16,18-nonadecapentaenoic Acid*; **2**). Colourless viscous oil. TLC (SiO_2, *t*-BuOEt/MeOH 90:10): R_f 0.42. HPLC (*RP-18*, MeCN/buffer (pH 4.5) 50:50): t_R 5.3. ^{1}H-NMR: *Table 5*.

*19-*O*-Acetylleptomycin B* (**2a**). Colourless viscous oil. TLC (SiO_2, AcOEt/MeOH 95:5): R_f 0.47. ^{1}H-NMR: *Table 5*. FAB-MS (pos.-ion mode): 583 (5, $[M + H]^+$), 421 (14), 391 (31), 371 (27), 255 (19), 177 (48), 149 (100).

Leptomycin B Methyl Ester (**2b**). Colourless viscous oil. TLC (SiO_2, AcOEt/MeOH 90:10): R_f 0.69. ^{1}H-NMR: *Table 5*. FAB-MS (pos.-ion mode): 577 (34, $[M + Na]^+$), 555 (20, $[M + H]^+$), 537 (6, $[M + H - H_2O]^+$), 523 (12, $[M - MeO]^+$), 505 (8), 385 (74), 327 (10), 245 (14), 220 (100).

*19-*O*-Acetylleptomycin B Methyl Ester* (**2c**). Colourless viscous oil. TLC (SiO_2, AcOEt/acetone 95:5): R_f 0.57. ^{1}H-NMR: *Table 5*. FAB-MS (pos.-ion mode): 619 (78, $[M + Na]^+$), 597 (33, $[M + H]^+$), 565 (31, $[M - MeO]^+$), 537 (17), 487 (15), 391 (55), 371 (17), 327 (20), 269 (32), 247 (35), 219 (100).

Kazusamycin B (= *(*2E,*10*E,*12*E,*16*Z,*18*E*)-19-(3,6-Dihydro-3-methyl-6-oxo-2*H*-pyran-2-yl)-6-hydroxy-9-(hydroxymethyl)-3,5,7,11,15,17-hexamethyl-8-oxo-2,10,12,16,18-nonadecapentaenoic Acid*; **3**). Colourless viscous oil. TLC (SiO_2, *t*-BuOEt/MeOH 90:10): R_f 0.37. HPLC (*RP-18*, MeCN/buffer (pH 4.5) 45:55): t_R 4.0.

Kazusamycin A (= *(*2E,*10*E,*12*E,*16*Z,*18*E*)-19-(3,6-Dihydro-3-methyl-6-oxo-2*H*-pyran-2-yl)-17-ethyl-6-hydroxy-9-(hydroxymethyl)-3,5,7,11,15-pentamethyl-8-oxo-2,10,12,16,18-nonadecapentaenoic Acid*; **4**). Colourless viscous oil. TLC (SiO_2, *t*-BuOEt/MeOH 90:10): R_f 0.37. HPLC (*RP-18*, MeCN/buffer (pH 4.5) 45:55): t_R 5.1. ^{1}H-NMR: *Table 5*.

*19,30-Di-*O*-acetylkazusamycin A* (**4a**). Colourless viscous oil. TLC (SiO_2, AcOEt/MeOH 90:10): R_f 0.52. ^{1}H-NMR: *Table 5*. FAB-MS (pos.-ion mode): 663 (27, $[M + Na]^+$), 641 (5, $[M + H]^+$), 623 (9, $[M + H - H_2O]^+$), 563 (8), 485 (8), 391 (12), 283 (15), 219 (59), 177 (100).

Kazusamycin A Methyl Ester (**4b**). Colurless viscous oil. TLC (SiO_2, AcOEt/MeOH 90:10): R_f 0.57. ^{1}H-NMR: *Table 5*. FAB-MS (pos.-ion mode): 593 (32, $[M + Na]^+$), 571 (18, $[M + H]^+$), 553 (5, $[M + H - H_2O]^+$), 539 (10, $[M - MeO]^+$), 426 (10), 401 (28), 383 (15), 219 (100).

*19,30-Di-*O*-acetylkazusamycin A Methyl Ester* (**4c**). Colourless viscous oil. TLC (SiO_2, AcOEt/acetone 95:5): R_f 0.64. ^{1}H-NMR: *Table 5*. FAB-MS (pos.-ion mode): 677 (16, $[M + Na]^+$), 655 (5, $[M + H]^+$), 647 (4, $[M + H - H_2O]^+$), 623 (4, $[M - MeO]^+$), 391 (13), 351 (26), 295 (53), 247 (100), 219 (32).

Dilactonmycin (= *6-[(*1E,3Z,7E,9E*)-16-(2,5-Dihydro-5-oxofuran-3-yl)-3-ethyl-14-hydroxy-5,9,11,13,15-pentamethyl-12-oxohexadeca-1,3,7,9-tetraenyl]-5,6-dihydro-5-methyl-2*H*-pyran-2-one*; **5**). Colourless viscous oil. TLC (SiO_2, *t*-BuOEt/MeOH 97:3): R_f 0.33. HPLC (*RP-18*, MeOH/H_2O 75:25): t_R 4.7 min. UV (MeOH): 293 (1888), 242 (32946), 213 (35679), UV (MeOH + HCl): unchanged. UV(MeOH + NaOH): 296 (1870), 243 (24245). IR (liq. film): 3500*m* (OH), 2967*s*, 2931*s* (CH, aliph.), 1779*s* (C=O, γ-lactone), 1747*s* (C=O, δ-lactone), 1706*s* (C=O), 1620*w* (C=C), 1454*s*, 1374*m*, 1286*w*, 1247*s* (C–O), 1101*m*, 1045*w*, 1028*m*, 980*s*, 825*m*. ^{1}H-NMR, ROESY, HMBC, ^{13}C-NMR: *Table 1*. FAB-MS (pos.-ion mode): 561 (49, $[M + Na]^+$), 539 (39, $[M + H]^+$), 521 (17, $[M + H - H_2O]^+$), 419 (12), 385 (13), 351 (23), 303 (29), 295 (40), 247 (100), 219 (37), 191 (58).

Delactonmycin (= *(*4E,6E,14E*)-11-Hydroxy-2,6,8,10,12,14-hexamethyl-9-oxohexadeca-4,6,14-trienoic Acid*; **6**). Colourless viscous oil. TLC (SiO_2, toluene/i-PrOH 85:15): R_f 0.44. HPLC (*RP-18*, MeOH/H_2O 60:40): t_R 19.0 min. UV (MeOH): 296 (1386), 240 (20538). UV (MeOH + HCl): unchanged. UV (MeOH + NaOH): unchanged. IR (liq. film): 3480*m* (OH), 2972*s*, 2932*s* (CH, aliph.), 1709*s* (C=O, COOH), 1457*s*, 1381*m*, 1235*w*, 1126*w*, 974*s*, 874*w*. ^{1}H-NMR, ROESY, HMBC, ^{13}C-NMR: *Table 2*. FAB-MS (pos.-ion mode): 387 (15, $[M + Na]^+$), 365 (21, $[M + H]^+$), 347 (28, $[M + H - H_2O]^+$), 295 (8), 247 (17), 239 (100), 221 (37), 181 (87).

Cyclic Hexadepsipeptide L-156,602 (= *(*3S*)-3-Hydroxy-*N*-{2-hydroxy-1-oxo-2-[(*2R,5R,6R*)-tetrahydro-2-hydroxy-6-methyl-5-[(*2S*)-2-methylbutyl]-2*H*-pyran-2-yl]propyl}-*L*-leucyl-(*3R*)-hexahydropyridazine-3-carbonyl-*N*-hydroxy-*L*-alanylglycyl-(*3S*)-hexahydropyridazine-3-carbonyl-*N*-hydroxy-*D*-alanine (6.1 → 1.3)Lactone*, **7**). Colourless crystals. TLC (SiO_2, AcOEt sat. with H_2O): R_f 0.44. HPLC (*RP-18*, MeCN/H_2O(+ 0.27% H_3PO_4) 60:40): t_R 13.9. IR (KBr): 3418*m* (OH), 2945*s*, 2939*s* (CH, aliph.), 1752*m*, 1641*s* (N–C=O), 1529*w*, 1444*m*, 1377*w*, 1252*m*, 1199*m*, 1115*w*, 1081*w*, 1052*w*, 1005*w*, 960*w*, 925*w*. ^{13}C-NMR: *Table 3*.

Acetylation. The leptomycin (20 mg) in dry pyridine (0.5 ml) was treated at 4° for 15 h with freshly distilled Ac_2O (0.25 ml). The mixture was poured into ice-water and extracted with AcOEt. The AcOEt phase was washed twice with H_2O, dried (Na_2SO_4), and evaporated.

Methylation. The leptomycin (20 mg) or the acetylated leptomycin (18 mg) in $CHCl_3$ (1 ml) was treated with diazomethane (0.5 ml) for *ca.* 5 min. The soln. was evaporated and the methylated leptomycin purified by prep. TLC (silica gel, AcOEt/acetone 97:3, 95:5, or 90:10).

REFERENCES

[1] M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **1984**, *224*, 497.

[2] S. Arya, C. Guo, S. F. Josephs, F. Wong-Staal, *Science* **1985**, *229*, 69; E. Terwilliger, R. Burghoff, R. Sia, J. Sodroski, W. Haseltine, C. Rosen, *J. Virol.* **1988**, *62*, 655.

[3] B. Felber, M. Hadzopoulou-Cladaras, C. Cladaras, T. Copeland, G. N. Pavlaskis, *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1495; M. L. Hammarskjöld, J. Heumer, B. Hammarskjöld, I. Sangwan, L. Albert, D. Rekosch, *J. Virol.* **1989**, *63*, 1959; M. H. Malim, J. Haubner, S. Y. Le, J. V. Maizel, B. R. Cullen, *Nature (London)* **1989**, *338*, 254.

[4] B. Wolff, G. Cohen, J. Hauber, D. Meshcheryakova, C. Rabeck, *Exp. Cell Res.* **1995**, *217*, 31; B. E. Meyer, M. H. Malim, *Genes Dev.* **1994**, *8*, 1538; A. M. Szilvay, K. A. Brokstad, R. Kopperud, G. Haukenes, K. H. Kalland, *J. Virol.* **1995**, *69*, 3315.

[5] B. Wolff, J. J. Sanglier, Y. Wang, *Chem. Biol.* **1997**, *4*, 139.

[6] T. Hamamoto, S. Gunji, H. Tsuji, T. Beppu, *J. Antibiot.* **1983**, *36*, 639.

[7] T. Hamamoto, H. Seto, T. Beppu, *J. Antibiot.* **1983**, *36*, 646.

[8] K. Funaishi, K. Kawamura, Y. Sugiura, N. Nakahori, E. Yoshida, M. Okanishi, I. Umezawa, S. Funayama, K. Komiyama, *J. Antibiot.* **1987**, *40*, 778; K. Takamiya, E. Yoshida, T. Takahashi, A. Okura, M. Okanishi, K. Komiyama, I. Umezawa, *ibid.* **1988**, *41*, 1854.

[9] a) I. Umezawa, K. Komiyama, H. Oka, K. Okada, S. Tomisaka, T. Miyano, S. Takano, *J. Antibiot.* **1984**, *37*, 706; b) K. Komiyama, K. Okada, H. Oka, S. Tomisaka, T. Miyano, S. Funayama, I. Umezawa, *ibid.* **1985**, *38*, 220; c) K. Komiyama, K. Okada, Y. Hirokana, K. Masuda, S. Romisaka, I. Umezawa, *ibid.* **1985**, *38*, 224.

[10] O. D. Hensens, R. P. Borris, L. R. Koupal, C. G. Caldwell, S. A. Currie, A. A. Haidri, C. F. Homnick, S. S. Honeycutt, S. M. Lindenmayer, C. D. Schwartz, B. A. Weissberger, H. B. Woodruff, D. L. Zink, L. Zitano, J. M. Fieldhouse, T. Rollins, M. S. Springer, J. P. Springer, *J. Antibiot.* **1991**, *44*, 249.

[11] P. L. Durette, I. E. Kopka, T. J. Lanza, J. L. Goulet, J. F. Kinneary, C. G. Caldwell, M. L. Hammond, S. S. Bondy, R. A. Zambias, J. Boger, *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1033; R. F. Tsuji, M. Uramoto, H. Koshino, N. M. Tsuji, J. Magae, K. Nagai, M. Yamasaki, *Biosci. Biotechnol. Biochem.* **1992**, *56*, 1686.

[12] C. G. Caldwell, K. M. Rupprecht, S. S. Bondy, A. A. Davis, *J. Org. Chem.* **1990**, *55*, 2355; P. L. Durette, F. Baker, P. L. Barker, J. Boger, S. S. Bondy, M. L. Hammond, T. J. Lanza, A. A. Pessolano, C. G. Caldwell, *Tetrahedron Lett.* **1990**, *31*, 1237.

[13] R. F. Tsuji, J. Yamakoshi, M. Uramoto, H. Koshino, M. Saito, M. Kikuchi, T. Masuda, *Immunopharmacology* **1995**, *29*, 79.

[14] K. Komiyama, K. Okada, Y. Hirokawa, K. Masuda, S. Tomisaka, I. Umezawa, *J. Antibiot.* **1985**, *38*, 224.

[15] B. J. Roberts, K. L. Hamelehle, J. S. Sebolt, W. R. Leopold, *Cancer Chemother. Pharmacol.* ***1986***, *16*, 95.

[16] M. Yoshida, M. Nishikawa, K. Nishi, K. Abe, S. Horinouchi, T. Beppu, *Exp. Cell. Res.* **1990**, *187*, 150.

[17] Y. Hayakawa, K. Adachi, N. Komeshima, *J. Antibiot.* **1987**, *40*, 1349; Y. Hayakawa, K. Sohada, K. Shin-Ya, T. Hidaka, H. Seto, *ibid.* **1995**, *48*, 954.

[18] N. Hosokawa, H. Iinuma, H. Naganawa, M. Hamada, T. Takeuchi, *J. Antibiot.* **1993**, *46*, 676.

[19] K. Abe, M. Yoshida, S. Horinouchi, T. Beppu, *J. Antibiot.* **1993**, *46*, 728; K. Abe, M. Yoshida, H. Naoki, S. Horinouchi, T. Beppu, *ibid.* **1993**, *46*, 735.

[20] Y. Hayakawa, K. Sohda, K. Furihata, T. Kuzuyama, K. Shin-Ya, H. Seto, *J. Antibiot.* **1996**, *49*, 974; Y. Hayakawa, K. Sohda, H. Seto, *ibid.* **1996**, *49*, 980.

[21] M. Kuhnt, F. Bitsch, M. Ponelle, J. J. Sanglier, Y. Wang, B. Wolff, submitted.

[22] J. L. Staneck, G. D. Roberts, *Applied Microbiol.* **1974**, *28*, 226.

[23] A. G. O'Donnell, D. E. Minnikin, M. Goodfellow, in 'Chemical Methods in Bacterial Systematics', Eds. M. Goodfellow and D. E. Minnikin, Academic Press, London, 1985, pp. 131–144.

[24] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahan, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107.