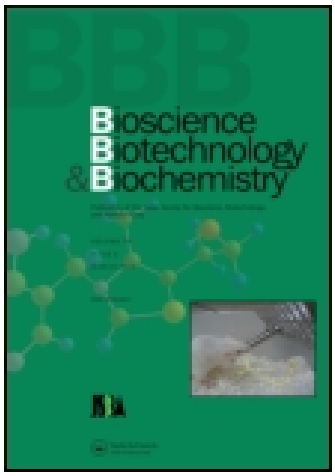

Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

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Published online: 12 Jun 2014.

To cite this article: Yoshihisa Tsukamoto, Hisaki Kajino, Kazuo Sato, Keiji Tanaka & Toshiaki Yanai (1997) Synthesis of 24a-Substituted Milbemycin A_4 Derivatives and Their Acaricidal Activity against Tetranychus uvticae, Bioscience, Biotechnology, and Biochemistry, 61:5, 806-812, DOI: 10.1271/bbb.61.806

To link to this article: http://dx.doi.org/10.1271/bbb.61.806

Biosci. Biotech. Biochem., **61** (5), 806-812, 1997

Synthesis of 24a-Substituted Milbemycin A_4 Derivatives and Their Acaricidal Activity against *Tetranychus urticae*

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Received October 17, 1996

A series of 24a-substituted milbemycin A_4 derivatives were synthesized from 24a-hydroxymilbemycin A_4, which had been obtained by the microbial oxidation of milbemycin A_4. The acaricidal activity of each synthesized derivative against *Tetranychus urticae* was tested and some of the derivatives showed higher activity than parent milbemycin A_4. Among them, 24a-methylmilbemycin A_4 (22) was the most active derivative, with 100% mortality of the mite at a concentration of 1 ppm and 50% mortality at a concentration of 0.1 ppm.

Key words: milbemycin; 24a-substituted milbemycin A_4 derivatives; *Tetranychus urticae*; two-spotted spider mite

Milbemycins[1,2] are sixteen-membered ring macrolides that have been isolated from *Streptomyces hygroscopicus*. They demonstrate potent and diverse activities as anthelmintics, acaricides and insecticides. Among them, a mixture of milbemycin A_3 (**1a**) and milbemycin A_4 (**1b**) has recently been developed and commercialized as an agricultural acaricide under the name Milbeknock®, while milbemycin D (**1c**) has been put on the market as a parasiticide for dogs. In the wake of this, a mixture of 5-deoxy-5-oxomilbemycin A_3 5-oxime (**1d**) and 5-deoxy-5-oxomilbemycin A_4 5-oxime (**1e**) has also been launched as a parasiticide for dogs.[3] Avermectins **2**, which have a similar structure to milbemycins, have also shown potent and diverse activities as anthelmintics, acaricides, and insecticides[4] (Scheme 1).

The remarkable biological features of milbemycins and avermectins has stimulated the interest of many research groups, and enormous effort has been made to produce semi-synthetic analogs of milbemycins and avermectins with improved biological activities.[2] Among the many reports on semi-synthetic milbemycins, avermectins, and related macrolactonic compounds, preparations of novel C-24-substituted milbemycin and avermectin derivatives have been reported by two groups.[5] Their preparations of the derivatives involved degradation of the spiroketal portions of milbemycins and avermectins, which have a hydroxy group at the 22- or 23-position, and resynthesis of the modified spiroketal parts to afford novel C-24- and C-25-substituted milbemycins and avermectins. However, this method is not applicable to the synthesis of C-24-substituted analogs from milbemycin A_4, which has no hydroxy group at the 22- or 23-position.

Nakagawa and co-workers have recently reported the microbial oxidation of milbemycins and related compounds to give 24a-hydroxymilbemycins, which are difficult to obtain by chemical transformation of milbemycins.[6] In this paper, the synthesis of 24a-substituted milbemycin A_4 derivatives from 24a-hydroxymilbemycin A_4 and their acaricidal activities are described.

Acylation of 24a-hydroxymilbemycin A_4 (**3**) with pivaloyl chloride in the presence of pyridine afforded 24a-pivaloyloxymilbemycin A_4 (**4**) in a 65% yield, but a diacylated product, 5-*O*-pivaloyl-24a-pivaloyloxymilbemycin A_4 (**5**), was also obtained in a 9% yield (Scheme 2).

To selectively modify the 24a-hydroxy group, we used 5-deoxy-24a-hydroxy-5-oxomilbemycin A_4 (**6**) and 5-*O*-*t*-butyldimethylsilyl-24a-hydroxymilbemycin A_4 (**7**) as intermediates, in which the original 5-hydroxy group of milbemycin A_4 was converted to a masking group, oxo group and *t*-butyldimethylsilyloxy group, respectively. Oxidation of 24a-hydroxymilbemycin A_4 (**3**) with active manganese dioxide enabled the allylic hydroxy group at the 5-position to be selectively oxidized, giving a promising intermediate, 5-deoxy-24a-hydroxy-5-oxomilbemycin A_4 (**6**), in an 81% yield. Another intermediate **7** was synthesized by converting 24a-hydroxy group of the ketone **6** to a 24a-allyloxycarbonyloxy derivative, and then reducing the 5-oxo group and protecting the resulting 5-hydroxy group by *t*-butyldimethylsilylation to afford 24a-allyloxycarbonyloxy-5-*O*-*t*-butyldimethylsilylmilbemycin A_4 (**8**) in a 72% yield from **6**. Palladium-catalyzed cleavage of the allyloxycarbonyl group of **8** gave key intermediate **7** in an 89% yield (Scheme 3).

Starting from **7**, 24a-(2,6-difluorobenzoyloxy)milbemycin A_4 (**14**), 24a-(2-methyl-2-phenylpropionyloxy)milbemycin A_4(**15**), 24a-ethoxycarbonyloxymilbemycin A_4 (**16**), 24a-(*p*-toluenesulfonyloxy)milbemycin A_4 (**17**) and 24a-methanesulfonyloxymilbemycin A_4 (**18**) were synthesized by acylation or sulfonylation of the 24a-hydroxy group and succeeding desilylation of the 5-hydroxy group (Scheme 4).

Next, 24a-fluoro and 24a-methy derivatives were synthesized by substitution reactions. Treatment of **6** with diethylaminosulfur trifluoride (DAST) afforded 5-deoxy-24a-fluoro-5-oxomilbemycin A_4 (**19**), the reduction of which with sodium borohydride gave 24a-fluoromilbemycin A_4 (**20**) in a 15% yield from **6**. 24a-Methylmilbemycin A_4 (**22**) was prepared by reacting 5-*O*-*t*-butyldimethylsilyl-24a-(*p*-toluenesulfonyloxy)milbemycin A_4 (**12**) with lithium dimethylcuprate and then deprotecting the 5-hydroxy group (Scheme 5).

The acaricidal activities of the 24a-substituted milbe-

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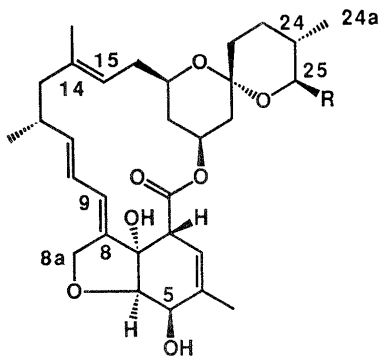
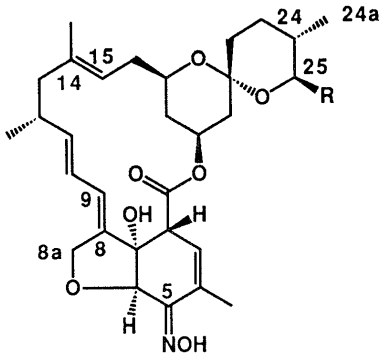
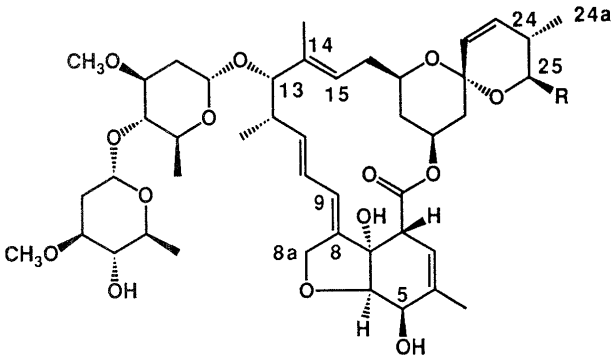




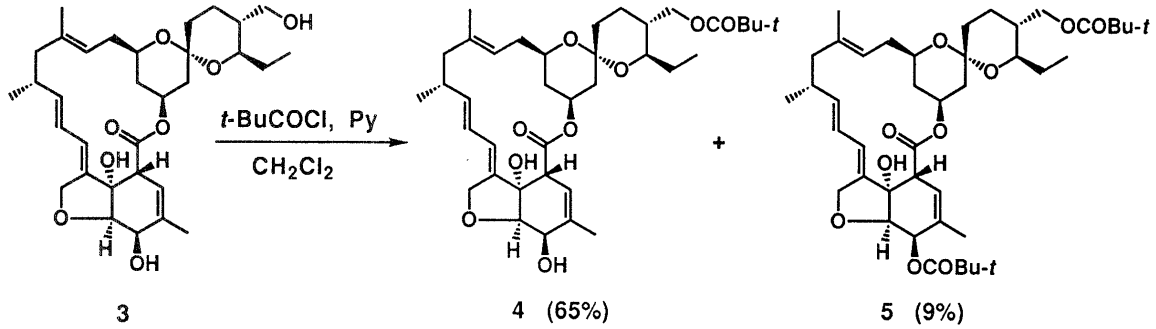
1a Milbemycin A_3 (R=Me)
1b Milbemycin A_4 (R=Et)
1c Milbemycin D (R=*i*-Pr)

1d 5-Deoxy-5-oxomilbemycin A_3 5-oxime (R=Me)
1e 5-Deoxy-5-oxomilbemycin A_4 5-oxime (R=Et)



2a Avermectin B_{1a} (R= *s*-Bu)
2b Avermectin B_{1b} (R= *i*-Pr)

Scheme 1. Structures of Milbemycins, 5-Deoxy-5-oxomilbemycin 5-Oximes, and Avermectins.

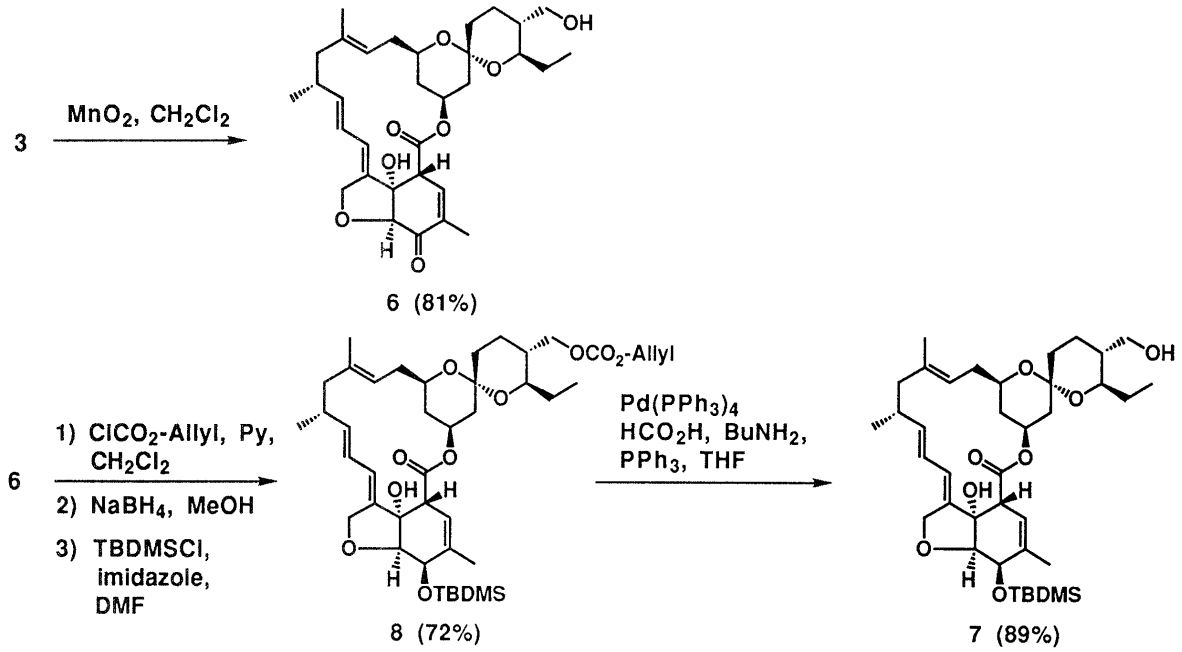


3 **4** (65%) **5** (9%)

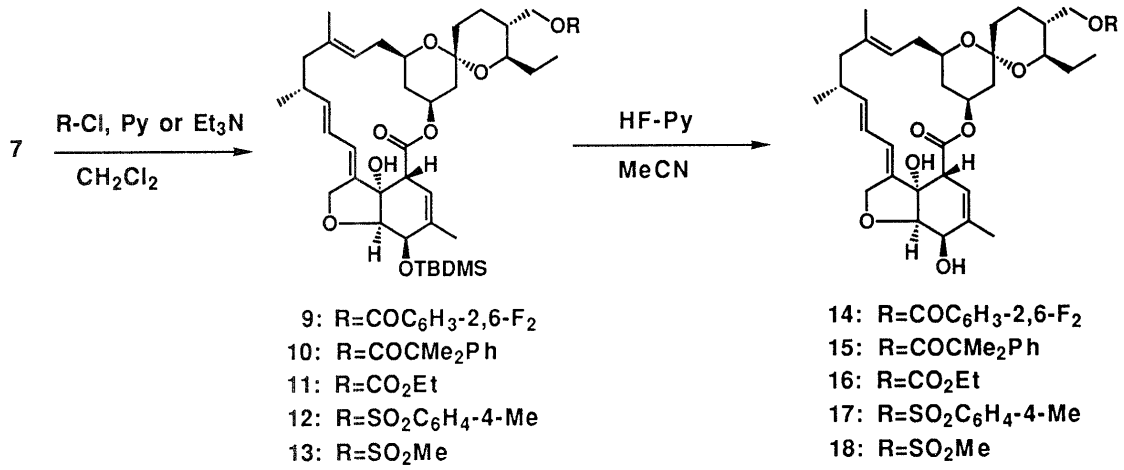
Scheme 2. Acylation of 24a-Hydroxymilbemycin A_4 (**3**) with Pivaloyl Chloride.

mycin A_4 derivatives were studied on the primary leaves of plants of the *Vigna sinensis Savi* species infested with organophosphate-sensitive mites (*Tetranychus urticae*). The results are summarized in the Table. The parent milbemycin A_4 (**1b**) exhibited moderate acaricidal activity at a concentration of 3 ppm. Introduction of a hydroxy, pivaloyloxy, 2,6-difluorobenzoyloxy, or methanesulfonyloxy group to the 24a-position of milbemycin A_4 resulted in decreased acaricidal activity (compounds **3**, **4**, **14**, and **18**). On the other hand, 24a-(2-methyl-2-phenylpropionyloxy)milbemycin A_4 (**15**), 24a-ethoxycarbonyloxymilbemycin A_4 (**16**), 24a-(*p*-toluenesulfonyloxy)milbemycin A_4 (**17**), 24a-fluoromilbemycin A_4 (**20**), and 24a-methylmilbemycin A_4 (**22**) each showed higher miticidal activity than that of milbemycin A_4 at 3 ppm. Among them, **22** was the most active derivative, with 100% mortality of the mite at a concentration of 1 ppm and 50% mortality at a concentration of 0.1 ppm.

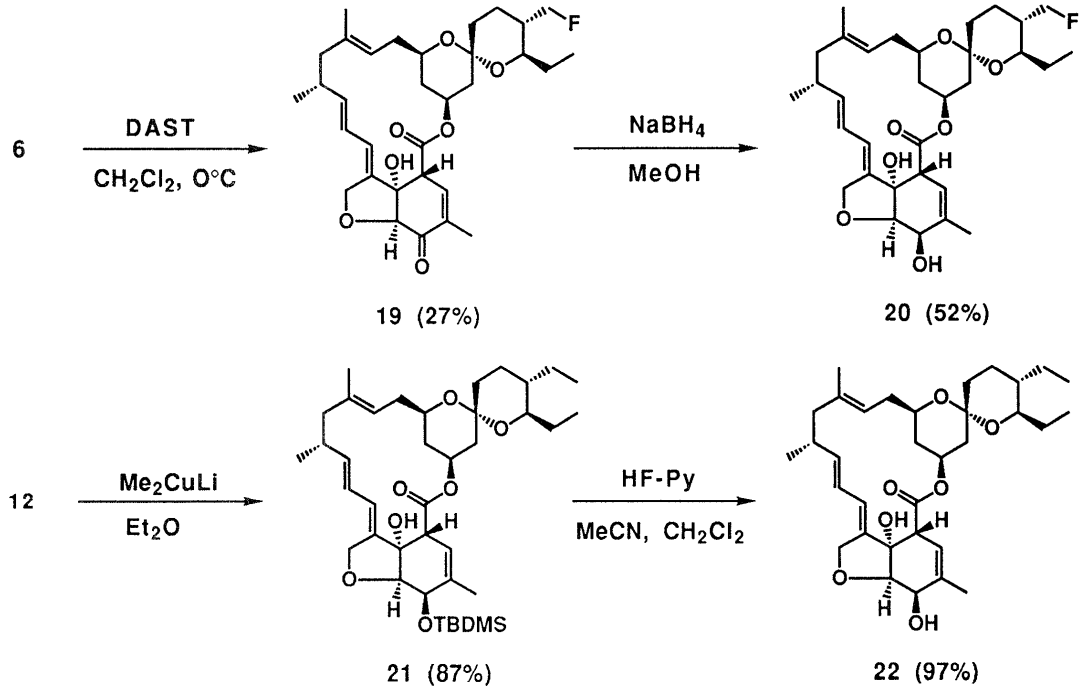
In conclusion, novel 24a-substituted derivatives of milbemycin A_4 were synthesized from 24a-hydroxymilbemycin A_4 (**3**), which had been obtained by the microbial oxidation of milbemycin A_4. Among the synthesized derivatives, 24a-



Scheme 3. Syntheses of 5-Deoxy-24a-hydroxy-5-oxomilbemycin A_4 (**6**) and 5-*O*-*t*-Butyldimethylsilyl-24a-hydroxymilbemycin A_4 (**7**).



Scheme 4. Syntheses of the 24a-Ester and Sulfonic Ester Derivatives of Milbemycin A_4.



Scheme 5. Syntheses of 24a-Fluoro- and 24a-Methylmilbemycin A_4.

Table Acaricidal Activity of 24a-Substituted Milbemycin A_4 Derivatives against *Tetranychus urticae*

Compound	Substituent at the 24-position	Mortality (%)			
		3 ppm	1 ppm	0.3 ppm	0.1 ppm
1b	Me	40	32	8	
3	CH_2OH	7			
4	CH_2OCOBu-*t*	7		6	
14	$CH_2OCOC_6H_3$-2,6-F_2	14		0	
15	$CH_2OCOC(Me)_2Ph$	75		0	
16	CH_2OCO_2Et	100		40	
17	$CH_2OSO_2C_6H_4$-4-Me	53		11	
18	CH_2OSO_2Me	13		0	
20	CH_2F	100		8	
22	Et	100	100		50

methylmilbemycin A_4 (**22**) exhibited the highest activity against *Tetranychus urticae*, indicating a potency of more than ten times that of the parent milbemycin A_4 (**1b**).

Experimental

IR spectra were recorded with a Perkin Elmer 1600 Series FT IR spectrometer. ^{1}H-NMR spectra were measured with a JEOL JNM-GX 270 FT NMR spectrometer with tetramethylsilane as an internal standard. Mass spectra were measured with a JEOL JMS-D 300 or Fisons Instruments AutoSpec-Ultima spectrometer. Column chromatography was performed on silica gel (Merck silica gel 60, 230–400 mesh or Wakogel C-100, 40–100 mesh). Preparative thin-layer chromatography was performed on silica gel (Merck Kieselgel 60 PF_{254}) of 0.5 mm thickness. All new compounds were characterized by their NMR, IR, and mass spectral data and by high-resolution mass spectra.

Reaction of 24a-hydroxymilbemycin A_4 (**3**) *with pivaloyl chloride to give 24a-pivaloyloxymilbemycin A_4* (**4**) *and 5-O-pivaloyl-24a-pivaloyloxymilbemycin A_4* (**5**). To a solution of **3** (112 mg) in dichloromethane (5 ml) in an ice bath were added pyridine (32 µl) and pivaloyl chloride (49 µl). The reaction mixture was stirred in an ice bath for 1 h and then at room temperature for 1 h. Brine was added to the reaction mixture, and the resulting mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by silica-gel chromatography (hexane–ethyl acetate gradient) to give 81 mg (65% yield) of **4** and 12 mg (9% yield) of **5**.

24a-Pivaloyloxymilbemycin A_4 (**4**). IR ν_{max} (KBr) cm^{-1}: 3477 (OH), 1730 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (1H, q, J=12.1 Hz, H–C (18)), 1.01 (3H, d, J=6.5 Hz, CH_3–C (12)), 1.01 (3H, t, J=7.3 Hz, CH_3–CH_2–C (25)), 1.20 (9H, s, $(CH_3)_3C$–CO_2–CH_2–C (24)), 1.23–1.95 (9H, m, H–C (13), H–C (18), H_2–C (22), H_2–C (23), H–C (24), CH_3–CH_2–C (25)), 1.37 (1H, t, J=11.7 Hz, H–C (20)), 1.53 (3H, s, CH_3–C (14)), 1.87 (3H, s, CH_3–C (4)), 1.99–2.06 (1H, m, H–C (20)), 2.17–2.28 (3H, m, H–C (13), H_2–C (16)), 2.35–2.51 (2H, m, HO–C (5), H–C (12)), 3.26–3.28 (1H, m, H–C (2)), 3.37 (1H, td, J_t=8.9 Hz, J_d=2.4 Hz, H–C (25)), 3.50–3.62 (1H, m, H–C (17)), 3.91–4.08 (2H, m, $(CH_3)_3C$–CO_2–CH_2–C (24)), 3.96 (1H, d, J=6.0 Hz, H–C (6)), 4.04 (1H, s, HO–C (7)), 4.29 (1H, t, J=6.0 Hz, H–C (5)), 4.62–4.74 (2H, m, CH_2–C (8)), 4.94–4.99 (1H, m, H–C (15)), 5.31–5.43 (3H, m, H–C (3), H–C (11), H–C (19)), 5.69–5.83 (2H, m, H–C (9), H–C (10)). MS *m/z*: 642 (M^+, $C_{37}H_{54}O_9$), 624, 606, 514, 496, 364, 345, 314, 295, 267, 248, 193, 165, 151. HR-MS *m/z* (M^+): calcd. for $C_{37}H_{54}O_9$, 642.3768; found, 642.3756.

5-O-Pivaloyl-24a-pivaloyloxymilbemycin A_4 (**5**). IR ν_{max} (KBr) cm^{-1}: 3480 (OH), 1732 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (1H, q, J=11.7 Hz, H–C (18)), 0.99–1.04 (6H, m, CH_3–C (12), CH_3–CH_2–C (25)), 1.18–1.91 (9H, m, H–C (13), H–C (18), H_2–C (22), H_2–C (23), H–C (24), CH_3–CH_2–C (25)), 1.20 (9H, s, $(CH_3)_3C$–CO_2–CH_2–C (24)), 1.24 (9H, s, $(CH_3)_3C$–CO_2–C (5)), 1.36 (1H, t, J=11.7 Hz, H–C (20)), 1.53 (3H, s, CH_3–C (14)), 1.75 (3H, d, J=1.6 Hz, CH_3–C (4)), 1.99–2.08 (1H, m, H–C (20)), 2.17–2.28 (3H, m, H–C (13), H_2–C (16)), 2.35–2.51 (1H, m, H–C (12)), 3.34–3.40 (2H, m, H–C (2), H–C (25)), 3.50–3.62 (1H, m, H–C (17)), 3.90–4.02 (3H, m, HO–C (7), $(CH_3)_3C$–CO_2–CH_2–C (24)), 4.06 (1H, d, J=6.1 Hz, H–C (6)), 4.56 (1H, dd, J=14.5, 2.0 Hz, CH–C (8)), 4.62 (1H, dd, J=14.5, 2.0 Hz, CH–C (8)), 4.93–4.99 (1H, m, H–C (15)), 5.29–5.41 (2H, m, H–C (11), H–C (19)), 5.46 (1H, dt, J_d=6.1 Hz, J_t=1.2 Hz, H–C (5)), 5.52 (1H, q, J=1.6 Hz, H–C (3)), 5.66–5.82 (2H, m, H–C (9), H–C (10)). MS *m/z*: 726 (M^+, $C_{42}H_{62}O_{10}$), 606, 514, 364, 345, 295, 279, 267, 248, 193, 165, 151, 149. HR-MS *m/z* (M^+): calcd. for $C_{42}H_{62}O_{10}$, 726.4343; found, 726.4351.

Synthesis of 5-deoxy-24a-hydroxy-5-oxomilbemycin A_4 (**6**). To a solution of **3** (2.06 g) in dichloromethane (30 ml) was added active manganese dioxide (14.2 g), and the mixture was stirred at room temperature for 3 h. The reaction mixture was filtered through Celite®, and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel, using hexane–ethyl acetate as the eluent, to afford 1.65 g (81% yield) of **6**.

5-Deoxy-24a-hydroxy-5-oxomilbemycin A_4 (**6**). IR ν_{max} (KBr) cm^{-1}: 3470 (OH), 1732 (C=O), 1680 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.84–1.00 (1H, m, H–C (18)), 1.00–1.05 (6H, m, CH_3–C (12), CH_3–CH_2–C (25)), 1.20–1.95 (11H, m, H–C (13), H–C (18), H–C (20), H_2–C (22), H_2–C (23), H–C (24), HO–CH_2–C (24), CH_3–CH_2–C (25)), 1.53 (3H, s, CH_3–C (14)), 1.89–1.90 (3H, m, CH_3–C (4)), 2.02–2.09 (1H, m, H–C (20)), 2.15–2.30 (3H, m, H–C (13), H_2–C (16)), 2.35–2.50 (1H, m, H–C (12)), 3.35 (1H, td, J_t=9.3 Hz, J_d=2.8 Hz, H–C (25)), 3.47–3.67 (4H, m, H–C (2), H–C (17), HO–CH_2–C (24)), 3.85 (1H, s, H–C (6)), 4.01 (1H, s, HO–C (7)), 4.68–4.80 (2H, m, CH_2–C (8)), 4.93–4.99 (1H, m, H–C (15)), 5.38–5.50 (2H, m, H–C (11), H–C (19)), 5.74 (1H, dd, J=14.5, 11.3 Hz, H–C (10)), 5.87 (1H, dt, J_d=11.3 Hz, J_t=2.4 Hz, H–C (9)), 6.55–6.57 (1H, m, H–C (3)). MS *m/z*: 556 (M^+, $C_{32}H_{44}O_8$), 538, 430, 211, 183, 151. MR-MS *m/z* (M^+): calcd. for $C_{32}H_{44}O_8$, 556.3036; found, 556.3037.

Synthesis of 24a-allyloxycarbonyloxy-5-O-t-butyldimethylsilylmilbemycin A_4 (**8**). To a solution of **6** (59.0 mg) in dichloromethane (1 ml) were added pyridine (25 µl) and allyl chloroformate (75 µl), and the mixture was stirred at room temperature for 2 h. Saturated, aqueous sodium bicarbonate was then added, and the mixture was extracted with dichloromethane. The extract was dried ($MgSO_4$) and evaporated under reduced pressure to leave a residue. This residue was dissolved in methanol (2 ml), to which was added sodium borohydride (5.8 mg) at 0°C. The resulting mixture was stirred for 20 min, before aqueous ammonium chloride was added. The resulting mixture was extracted with ethyl acetate, and the extract was dried over anhydrous magnesium sulfate. Removal of the solvent left a white powder which was dissolved in *N*,*N*-dimethylformamide (2 ml). To this solution were added imidazole (90 mg) and *t*-butyldimethylsilyl chloride (90 mg), and the mixture was stirred at room temperature for 2 h. Brine was then added, before the mixture was extracted with 40% ethyl acetate in hexane. The extract was evaporated, and the resulting residue was purified by preparative thin-layer chromatography (hexane–ethyl acetate = 4 : 1) to give 57.3 mg (72% yield) of **8**.

24a-Allyloxycarbonyloxy-5-O-t-butyldimethylsilylmilbemycin A_4 (**8**). IR ν_{max} (KBr) cm^{-1}: 3478 (OH), 1748 (C=O), 1713 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, $(CH_3)_2$–Si–O–C (5)), 0.82–1.00 (1H, m, H–C (18)), 0.93 (9H, s, $(CH_3)_3C$–Si–O–C (5)), 0.98–1.03 (6H, m, CH_3–C (12), CH_3–CH_2–C (25)), 1.26–1.90 (9H, m, H–C (13), H–C (18), H_2–C (22), H_2–C (23), H–C (24), CH_3–CH_2–C (25)), 1.36 (1H, t, J=11.7 Hz, H–C (20)), 1.54 (3H, s, CH_3–C (14)), 1.79 (3H, s, CH_3–C (4)), 2.00–2.06 (1H, m, H–C (20)), 2.16–2.27 (3H, m, H–C (13), H_2–C (16)), 2.35–2.51 (1H, m, H–C (12)), 3.29–3.40 (2H, m, H–C (2), H–C (25)), 3.50–3.60 (1H, m, H–C (17)), 3.81 (1H, d, J=5.6 Hz, H–C (6)), 3.96 (1H, dd, J=10.9, 5.6 Hz, CH_2=$CHCH_2$–OCO_2–CH–C (24)), 4.06 (1H, s, HO–C (7)), 4.12 (1H, dd, J=10.9, 4.0 Hz, CH_2=$CHCH_2$–OCO_2–CH–C (24)), 4.42–4.46 (1H, m, H–C (5)), 4.55–4.70 (4H, m, CH_2–C (8), CH_2=$CHCH_2$–OCO_2–CH_2–C (24)), 4.95 (1H, t, J=7.7 Hz, H–C (15)), 5.25–5.43 (5H, m, H–C (3), H–C (11), H–C (19), CH_2=$CHCH_2$–OCO_2–CH_2–C (24)), 5.68–5.80 (2H, m, H–C (9), H–C (10)), 5.86–6.01 (1H, m, CH_2=$CHCH_2$–OCO_2–CH_2–C (24)). MS *m/z*: 756 (M^+, $C_{42}H_{64}O_{10}Si$), 738, 699, 681, 606, 514, 364, 295, 267, 243, 225, 193, 165, 151. HR-MS *m/z* (M^+): calcd. for $C_{42}H_{64}O_{10}Si$, 756.4269; found, 756.4269.

Synthesis of 5-O-t-butyldimethylsilyl-24a-hydroxymilbemycin A_4 (**7**). To a suspension of **8** (55.0 mg), *n*-butylamine (10 µl), formic acid (26 µl) and triphenylphosphine (7.2 mg) in tetrahydrofuran (2 ml) was added tetrakis-

(triphenylphosphine)palladium(0) (7.2 mg) at room temperature. The mixture was stirred for 40 min, before aqueous ammonium chloride was added. The resulting mixture was extracted with dichloromethane, and the extract was dried over anhydrous magnesium sulfate. Removal of the solvent and purification of the residue by preparative thin-layer chromatography (hexane–ethyl acetate = 1 : 1) gave 43.5 mg (89% yield) of **7**.

5-O-t-Butyldimethylsilyl-24a-hydroxymilbemycin A$_4$ (**7**). IR ν_{max} (KBr) cm^{-1}: 3483 (OH), 1713 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, $(CH_3)_2$–Si–O–C (5)), 0.82–1.00 (1H, m, H–C (18)), 0.93 (9H, s, $(CH_3)_3$C–Si–O–C (5)), 0.98–1.04 (6H, m, CH_3–C (12), CH_3–CH_2–C (25)), 1.26–1.90 (10H, m, H–C (13), H–C (18), H_2–C (22), H_2–C (23), H–C (24), HO–CH_2–C (24), CH_3–CH_2–C (25)), 1.36 (1H, t, J = 11.7 Hz, H–C (20)), 1.54 (3H, s, CH_3–C (14)), 1.79 (3H, s, CH_3–C (4)), 2.00–2.07 (1H, m, H–C (20)), 2.16–2.27 (3H, m, H–C (13), H_2–C (16)), 2.34–2.50 (1H, m, H–C (12)), 3.29–3.40 (2H, m, H–C (2), H–C (25)), 3.45–3.65 (1H, m, H–C (17)), 3.48 (1H, dd, J = 10.9, 6.4 Hz, HO–CH–C (24)), 3.62 (1H, dd, J = 10.9, 4.4 Hz, HO–CH–C (24)), 3.81 (1H, d, J = 5.6 Hz, H–C (6)), 4.11 (1H, s, HO–C (7)), 4.41–4.46 (1H, m, H–C (5)), 4.58 (1H, d, J = 14.5 Hz, CH–C (8)), 4.67 (1H, d, J = 14.5 Hz, CH–C (8)), 4.91–4.97 (1H, m, H–C (15)), 5.27–5.43 (3H, m, H–C (3), H–C (11), H–C (19)), 5.68–5.79 (2H, m, H–C (9), H–C (10)). MS *m/z*: 672 (M^+, $C_{38}H_{60}O_8Si$), 654, 597, 522, 430, 280, 261, 243, 225, 211, 183, 165, 151. HR-MS *m/z* (M^+): calcd. for $C_{38}H_{60}O_8Si$: 672.4057; found, 672.4060.

Synthesis of 5-O-t-butyldimethylsilyl-24a-(2,6-difluorobenzoyloxy)milbemycin A$_4$ (**9**). To a solution of **7** (38.4 mg) in dichloromethane (1 ml) were added pyridine (10 μl) and 2,6-difluorobenzoyl chloride (15 μl) at room temperature. The reaction mixture was stirred for 1 h and poured into ice-cold water. The resulting mixture was extracted with ethyl acetate, and the extract was successively washed with water and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The resulting residue was purified by preparative thin-layer chromatography (hexane–ethyl acetate = 4 : 1) to afford 30.5 mg (66% yield) of **9**.

5-O-t-Butyldimethylsilyl-24a-(2,6-difluorobenzoyloxy)milbemycin A$_4$ (**9**). IR ν_{max} (KBr) cm^{-1}: 3467 (OH), 1737 (C=O), 1714 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, $(CH_3)_2$–Si–O–C (5)), 0.83–1.00 (1H, m, H–C (18)), 0.93 (9H, s, $(CH_3)_3$C–Si–O–C (5)), 0.99–1.05 (6H, m, CH_3–C (12), CH_3–CH_2–C (25)), 1.35–1.91 (9H, m, H–C (13), H–C (18), H_2–C (22), H_2–C (23), H–C (24), CH_3–CH_2–C (25)), 1.37 (1H, t, J = 11.7 Hz, H–C (20)), 1.54 (3H, s, CH_3–C (14)), 1.79 (3H, s, CH_3–C (4)), 2.01–2.08 (1H, m, H–C (20)), 2.17–2.28 (3H, m, H–C (13), H_2–C (16)), 2.35–2.51 (1H, m, H–C (12)), 3.35–3.38 (1H, m, H–C (2)), 3.44 (1H, td, J_t = 9.3 Hz, J_d = 2.4 Hz, H–C (25)), 3.52–3.62 (1H, m, H–C (17)), 3.82 (1H, d, J = 5.2 Hz, H–C (6)), 4.07 (1H, s, HO–C (7)), 4.21–4.33 (2H, m, $C_6H_3F_2$–CO_2–CH_2–C (24)), 4.42–4.45 (1H, m, H–C (5)), 4.59 (1H, d, J = 14.5 Hz, CH–C (8)), 4.67 (1H, d, J = 14.5 Hz, CH–C (8)), 4.97 (1H, t, J = 7.7 Hz, H–C (15)), 5.27–5.44 (3H, m, H–C (3), H–C (11), H–C (19)), 5.69–5.79 (2H, m, H–C (9), H–C (10)), 6.91–6.99 (2H, m, 2H of $C_6H_3F_2$–CO_2–CH_2–C (24)), 7.36–7.47 (1H, m, 1H of $C_6H_3F_2$–CO_2–CH_2–C (24)). MS *m/z*: 812 (M^+, $C_{45}H_{62}O_9F_2Si$), 794, 756, 377, 351, 346, 333, 318, 295, 254, 225, 196, 167, 149, 141. HR-MS *m/z* (M^+): calcd. for $C_{45}H_{62}O_9F_2Si$, 812.4131; found, 812.4131.

According to the procedure for the synthesis of **9**, compounds **10** and **11** were synthesized in 54% and 67% yields, respectively.

5-O-t-Butyldimethylsilyl-24a-(2-methyl-2-phenylpropionyloxy)milbemycin A$_4$ (**10**). IR ν_{max} (KBr) cm^{-1}: 3467 (OH), 1732 (C=O), 1715 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, $(CH_3)_2$–Si–O–C (5)), 0.79–1.00 (1H, m, H–C (18)), 0.88 (3H, t, J = 7.3 Hz, CH_3–CH_2–C (25)), 0.93 (9H, s, $(CH_3)_3$C–Si–O–C (5)), 1.00 (3H, d, J = 6.9 Hz, CH_3–C (12)), 1.16–1.35 (1H, m, CH_3–CH–C (25)), 1.33 (1H, t, J = 11.7 Hz, H–C (20)), 1.36–1.70 (6H, m, H_2–C (22), H_2–C (23), H–C (24), CH_3–CH–C (25)), 1.54 (3H, s, CH_3–C (14)), 1.57 (3H, s, $PhC(CH_3)_2$–CO_2–CH_2–C (24)), 1.59 (3H, s, $PhC(CH_3)_2$–CO_2–CH_2–C (24)), 1.77–1.83 (1H, m, H–C (18)), 1.79 (3H, s, CH_3–C (4)), 1.87 (1H, t, J = 12.5 Hz, H–C (13)), 1.96–2.02 (1H, m, H–C (20)), 2.15–2.27 (3H, m, H–C (13), H_2–C (16)), 2.35–2.51 (1H, m, H–C (12)), 3.18 (1H, td, J_t = 9.3 Hz, J_d = 2.4 Hz, H–C (25)), 3.34–3.37 (1H, m, H–C (2)), 3.40–3.53 (1H, m, H–C (17)), 3.81 (1H, d, J = 5.6 Hz, H–C (6)), 3.90 (1H, dd, J = 11.3, 4.0 Hz, $PhC(CH_3)_2$–CO_2–CH–C (24)), 3.95 (1H, dd, J = 11.3, 4.8 Hz, $PhC(CH_3)_2$–CO_2–CH–C (24)), 4.07 (1H, s, HO–C (7)), 4.42–4.45 (1H, m, H–C (5)), 4.58 (1H, d, J = 14.5 Hz, CH–C (8)), 4.66 (1H, d, J = 14.5 Hz, CH–C (8)), 4.95 (1H, dd, J = 9.7, 4.8 Hz, H–C (15)), 5.22–5.43 (3H, m, H–C (3), H–C (11), H–C (19)), 5.68–5.78 (2H, m, H–C (9), H–C (10)), 7.19–7.34 (5H, m, $C_6H_5C(CH_3)_2$–CO_2–CH_2–C (24)). MS *m/z*: 818 (M^+, $C_{48}H_{70}O_9Si$), 800, 761, 743, 668, 576, 426, 407, 357, 329, 242, 225, 193, 165, 151, 119. HR-MS *m/z* (M^+): calcd. for $C_{48}H_{70}O_9Si$, 818.4789; found, 818.4791.

5-O-t-Butyldimethylsilyl-24a-ethoxycarbonyloxymilbemycin A$_4$ (**11**). IR ν_{max} (KBr) cm^{-1}: 3467 (OH), 1747 (C=O), 1713 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, $(CH_3)_2$–Si–O–C (5)), 0.82–1.00 (1H, m, H–C (18)), 0.93 (9H, s, $(CH_3)_3$C–Si–O–C (5)), 0.98–1.03 (6H, m, CH_3–C (12), CH_3–CH_2–C (25)), 1.23–1.90 (10H, m, H–C (13), H–C (18), H–C (20), H_2–C (22), H_2–C (23), H–C (24), CH_3–CH_2–C (25)), 1.31 (3H, t, J = 7.3 Hz, CH_3–CH_2–OCO_2–CH_2–C (24)), 1.54 (3H, s, CH_3–C (14)), 1.79 (3H, s, CH_3–C (4)), 2.00–2.06 (1H, m, H–C (20)), 2.17–2.27 (3H, m, H–C(13), H_2–C(16)), 2.35–2.51 (1H, m, H–C (12)), 3.30–3.38 (2H, m, H–C(2), H–C (25)), 3.49–3.61 (1H, m, H–C (17)), 3.81 (1H, d, J = 5.6 Hz, H–C (6)), 3.94 (1H, dd, J = 10.5, 3.6 Hz, CH_3–CH_2–OCO_2–CH–C (24)), 4.07 (1H, s, HO–C (7)), 4.10 (1H, dd, J = 10.5, 4.4 Hz, CH_3–CH_2–O–CO_2–CH–C (24)), 4.19 (2H, q, J = 7.3 Hz, CH_3–CH_2–OCO_2–CH_2–C (24)), 4.42–4.45 (1H, m, H–C (5)), 4.57 (1H, d, J = 14.5 Hz, CH–C (8)), 4.67 (1H, d, J = 14.5 Hz, CH–C (8)), 4.92–4.98 (1H, m, H–C (15)), 5.26–5.43 (3H, m, H–C (3), H–C (11), H–C (19)), 5.68–5.79 (2H, m, H–C (9), H–C (10)). MS *m/z*: 744 (M^+, $C_{41}H_{64}O_{10}Si$), 726, 687, 669, 595, 503, 485, 445, 440, 416, 353, 333, 283, 255, 225, 193, 165, 151. HR-MS *m/z* (M^+): calcd. for $C_{41}H_{64}O_{10}Si$, 744.4269; found, 744.4271.

Synthesis of 5-O-t-butyldimethylsilyl-24a-(p-toluenesulfonyloxy)milbemycin A$_4$ (**12**). To a solution of **7** (394 mg) in dichloromethane (20 ml) were added *p*-toluenesulfonyl chloride (500 mg) and triethylamine (0.380 ml) at room temperature. The reaction mixture was stirred for 12 h and aqueous sodium bicarbonate was then added. The mixture was extracted with dichloromethane, and the resulting extract was dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was purified by silica-gel chromatography (hexane–ethyl acetate gradient) to give 417 mg (86% yield) of **12**.

5-O-t-Butyldimethylsilyl-24a-(p-toluenesulfonyloxy)milbemycin A$_4$ (**12**). IR ν_{max} (KBr) cm^{-1}: 3476 (OH), 1711 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, $(CH_3)_2$–Si–O–C (5)), 0.81–1.00 (1H, m, H–C (18)), 0.93 (9H, s, $(CH_3)_3$C–Si–O–C (5)), 0.94–1.01 (6H, m, CH_3–C (12), CH_3–CH_2–C (25)), 1.23–1.90 (9H, m, H–C (13), H–C (18), H_2–C (22), H_2–C (23), H–C (24), CH_3–CH_2–C (25)), 1.33 (1H, t, J = 11.7 Hz, H–C (20)), 1.53 (3H, s, CH_3–C (14)), 1.79 (3H, s, CH_3–C (4)), 1.97–2.03 (1H, m, H–C (20)), 2.14–2.25 (3H, m, H–C (13), H_2–C (16)), 2.35–2.51 (1H, m, H–C (12)), 2.45 (3H, s, CH_3–C_6H_4–SO_3–CH_2–C (24)), 3.24–3.31 (1H, m, H–C (25)), 3.34–3.37 (1H, m, H–C (2)), 3.43–3.55 (1H, m, H–C (17)), 3.81 (1H, d, J = 5.2 Hz, H–C (6)), 3.87 (1H, dd, J = 10.1, 5.2 Hz, CH_3–C_6H_4–SO_3–CH–C (24)), 3.94 (1H, dd, J = 10.1, 3.6 Hz, CH_3–C_6H_4–SO_3–CH–C (24)), 4.01 (1H, s, HO–C (7)), 4.41–4.45 (1H, m, H–C (5)), 4.58 (1H, d, J = 14.5 Hz, CH–C(8)), 4.66 (1H, d, J = 14.5 Hz, CH–C (8)), 4.91–4.97 (1H, m, H–C (15)), 5.22–5.42 (3H, m, H–C (3), H–C (11), H–C (19)), 5.68–5.80 (2H, m, H–C (9), H–C (10)), 7.35 (2H, d, J = 8.1 Hz, 2H of CH_3–C_6H_4–SO_3–CH_2–C (24)), 7.78 (2H, d, J = 8.1 Hz, 2H of CH_3–C_6H_4–SO_3–CH_2–C (24)). MS *m/z*: 826 (M^+, $C_{45}H_{66}O_{10}SSi$), 808, 676, 504, 365, 337, 243, 225, 193, 165, 149. HR-MS *m/z* (M^+): calcd. for $C_{45}H_{66}O_{10}SSi$, 826.4146; found, 826.4150.

In accordance with the procedure for the synthesis of **12**, compound **7** was converted to **13** (68% yield).

5-O-t-Butyldimethylsilyl-24a-methanesulfonyloxymilbemycin A$_4$ (**13**). IR ν_{max} (KBr) cm^{-1}: 3473 (OH), 1712 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, $(CH_3)_2$–Si–O–C (5)), 0.83–1.00 (1H, m, H–C (18)), 0.93 (9H, s, $(CH_3)_3$C–Si–O–C (5)), 0.98–1.04 (6H, m, CH_3–C (12), CH_3–CH_2–C (25)), 1.25–1.91 (9H, m, H–C (13), H–C (18), H_2–C (22), H_2–C (23), H–C (24), CH_3–CH_2–C (25)), 1.37 (1H, t, J = 11.7 Hz, H–C (20)), 1.54 (3H, s, CH_3–C (14)), 1.79 (3H, s, CH_3–C (4)), 2.01–2.07 (1H, m, H–C (20)), 2.15–2.27 (3H, m, H–C (13), H_2–C (16)), 2.35–2.51 (1H, m, H–C (12)), 3.00 (3H, s, CH_3–SO_3–CH_2–C (24)), 3.32–3.39 (2H, m, H–C (2), H–C (25)), 3.51–3.61 (1H, m, H–C (17)), 3.81 (1H, d, J = 5.2 Hz, H–C (6)), 4.01 (1H, s, HO–C (7)), 4.06 (1H, dd, J = 10.1, 5.2 Hz, CH_3–SO_3–CH–C (24)), 4.16 (1H, dd, J = 10.1, 3.6 Hz, CH_3–SO_3–CH–C (24)), 4.42–4.45 (1H, m, H–C (5)), 4.58 (1H, d, J = 14.5 Hz, CH–C (8)), 4.67 (1H, d, J = 14.5 Hz, CH–C (8)), 4.92–4.98 (1H, m, H–C (15)), 5.24–5.43 (3H, m, H–C (3), H–C (11), H–C (19)), 5.68–5.79 (2H, m, H–C (9), H–C (10)). MS *m/z*: 750 (M^+, $C_{39}H_{62}O_{10}SSi$), 732, 675, 654, 600, 579, 508, 415, 358, 339, 317, 302, 289, 261, 257, 243, 225, 193, 165, 151. HR-MS *m/z* (M^+): calcd. for

$C_{39}H_{62}O_{10}SSi$, 750.3833; found, 750.3831.

Synthesis of 24a-(2,6-difluorobenzoyloxy)milbemycin A_4 (**14**). To a solution of **9** (28 mg) in acetonitrile (1 ml) was added hydrogen fluoride–pyridine (HF = 68%, 0.15 ml) at room temperature. The reaction mixture was stirred for 2 h and then poured into saturated, aqueous sodium bicarbonate. The resulting mixture was extracted with ethyl acetate, before the extract was successively washed with water and brine, dried (Na_2SO_4), and concentrated. Preparative thin-layer chromatography of the residue on silica gel, eluting with 50% ethyl acetate in hexane, yielded 23.6 mg (98% yield) of **14**.

24a-(2,6-Difluorobenzoyloxy)milbemycin A_4 (**14**). IR ν_{max} (KBr) cm^{-1}: 3469 (OH), 1736 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.89 (1H, q, J = 12.1 Hz, H-C (18)), 0.99–1.05 (6H, m, CH_3-C (12), CH_3-CH_2-C (25)), 1.35–1.95 (9H, m, H-C (13), H-C (18), H_2-C (22), H_2-C (23), H-C (24), CH_3-CH_2-C (25)), 1.38 (1H, t, J = 11.7 Hz, H-C (20)), 1.54 (3H, s, CH_3-C (14)), 1.87 (3H, s, CH_3-C (4)), 2.00–2.07 (1H, m, H-C (20)), 2.17–2.28 (3H, m, H-C (13), H_2-C (16)), 2.35–2.51 (2H, m, HO-C (5), H-C (12)), 3.26–3.29 (1H, m, H-C (2)), 3.44 (1H, td, J_t = 9.3 Hz, J_d = 2.4 Hz, H-C (25)), 3.52–3.62 (1H, m, H-C (17)), 3.96 (1H, d, J = 6.1 Hz, H-C (6)), 4.05 (1H, s, HO-C (7)), 4.21–4.33 (3H, m, H-C (5), $C_6H_3F_2$-CO_2-CH_2-C (24)), 4.62–4.74 (2H, m, CH_2-C (8)), 4.94–5.00 (1H, m, H-C (15)), 5.30–5.43 (3H, m, H-C (3), H-C (11), H-C (19)), 5.69–5.84 (2H, m, H-C (9), H-C (10)), 6.91–6.99 (2H, m, 2H of $C_6H_3F_2$-CO_2-CH_2-C (24)), 7.36–7.47 (1H, m, 1H of $C_6H_3F_2$-CO_2-CH_2-C (24)). MS *m/z*: 698 (M^+, $C_{39}H_{48}O_9F_2$), 680, 662, 570, 552, 420, 401, 351, 323, 249, 243, 193, 165, 151, 141. HR-MS *m/z* (M^+): calcd. for $C_{39}H_{48}O_9F_2$, 698.3266; found, 698.3263.

According to the procedure for the synthesis of **14**, compounds **15**, **16**, **17**, and **18** were synthesized in 87%, 96%, 93%, and 97% yields, respectively.

24a-(2-Methyl-2-phenylpropionyloxy)milbemycin A_4 (**15**). IR ν_{max} (KBr) cm^{-1}: 3478 (OH), 1730 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.78–0.91 (1H, m, H-C (18)), 0.88 (3H, t, J = 7.3 Hz, CH_3-CH_2-C(25)), 1.01 (3H, d, J = 6.5 Hz, CH_3-C (12)), 1.17–1.35 (1H, m, CH_3-CH-C (25)), 1.33 (1H, t, J = 11.7 Hz, H-C (20)), 1.36–1.70 (6H, m, H_2-C (22), H_2-C (23), H-C (24), CH_3-CH-C (25)), 1.53 (3H, s, CH_3-C (14)), 1.57 (3H, s, $PhC(CH_3)_2$-CO_2-CH_2-C (24)), 1.59 (3H, s, $PhC(CH_3)_2$-CO_2-CH_2-C (24)), 1.77–1.84 (1H, m, H-C (18)), 1.83–1.93 (1H, m, H-C (13)), 1.87 (3H, s, CH_3-C (4)), 1.94–2.01 (1H, m, H-C (20)), 2.15–2.28 (3H, m, H-C (13), H_2-C (16)), 2.35–2.51 (2H, m, HO-C (5), H-C (12)), 3.18 (1H, td, J_t = 8.9 Hz, J_d = 2.4 Hz, H-C (25)), 3.24–3.28 (1H, m, H-C (2)), 3.42–3.52 (1H, m, H-C (17)), 3.87–3.99 (2H, m, $PhC(CH_3)_2$-CO_2-CH_2-C (24)), 3.96 (1H, d, J = 6.5 Hz, H-C (6)), 4.05 (1H, s, HO-C (7)), 4.29 (1H, t, J = 6.5 Hz, H-C (5)), 4.61–4.73 (2H, m, CH_2-C (8)), 4.92–4.98 (1H, m, H-C (15)), 5.27–5.43 (3H, m, H-C (3), H-C (11), H-C (19)), 5.69–5.83 (2H, m, H-C (9), H-C (10)), 7.19–7.34 (5H, m, $C_6H_5C(CH_3)_2$-CO_2-CH_2-C (24)). MS *m/z*: 704 (M^+, $C_{42}H_{56}O_9$), 668, 576, 558, 426, 407, 357, 329, 242, 193, 165, 151, 119. HR-MS *m/z* (M^+): calcd. for $C_{42}H_{56}O_9$, 704.3944; found, 704.3927.

24a-Ethoxycarbonyloxymilbemycin A_4 (**16**). IR ν_{max} (KBr) cm^{-1}: 3476 (OH), 1745 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (1H, q, J = 12.1 Hz, H-C (18)), 1.00 (3H, d, J = 6.5 Hz, CH_3-C (12)), 1.01 (3H, t, J = 7.3 Hz, CH_3-CH_2-C (25)), 1.23–1.95 (10H, m, H-C (13), H-C (18), H-C (20), H_2-C (22), H_2-C (23), H-C (24), CH_3-CH_2-C (25)), 1.31 (3H, t, J = 7.3 Hz, CH_3-CH_2-OCO_2-CH_2-C (24)), 1.53 (3H, s, CH_3-C (14)), 1.87 (3H, s, CH_3-C (4)), 1.99–2.05 (1H, m, H-C (20)), 2.17–2.27 (3H, m, H-C (13), H_2-C (16)), 2.35–2.51 (2H, m, HO-C (5), H-C (12)), 3.26–3.36 (2H, m, H-C (2), H-C (25)), 3.50–3.61 (1H, m, H-C (17)), 3.94 (1H, dd, J = 10.9, 6.0 Hz, CH_3-CH_2-OCO_2-CH-C (24)), 3.96 (1H, d, J = 6.4 Hz, H-C (6)), 4.04 ((1H, s, HO-C (7)), 4.11 (1H, dd, J = 10.9, 4.0 Hz, CH_3-CH_2-OCO_2-CH-C (24)), 4.19 (2H, q, J = 7.3 Hz, CH_3-CH_2-OCO_2-CH_2-C (24)), 4.29 (1H, t, J = 6.4 Hz, H-C (5)), 4.62–4.74 (2H, m, CH_2-C (8)), 4.96 (1H, t, J = 7.7 Hz, H-C (15)), 5.30–5.43 (3H, m, H-C (3), H-C (11), H-C (19)), 5.69–5.84 (2H, m, H-C (9), H-C (10)). MS *m/z*: 630 (M^+, $C_{35}H_{50}O_{10}$), 612, 594, 502, 484, 352, 333, 283, 255, 193, 165, 151, 149. HR-MS *m/z* (M^+): calcd. for $C_{35}H_{50}O_{10}$, 630.3404; found, 630.3396.

24a-(p-Toluenesulfonyloxy)milbemycin A_4 (**17**). IR ν_{max} (KBr) cm^{-1}: 3480 (OH), 1713 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.79–0.95 (1H, m, H-C (18)), 0.94 (3H, t, J = 7.3 Hz, CH_3-CH_2-C (25)), 1.00 (3H, d, J = 6.4 Hz, CH_3-C (12)), 1.25–1.91 (9H, m, H-C (13), H-C (18), H_2-C (22), H_2-C (23), H-C (24), CH_3-CH_2-C (25)), 1.34 (1H, t, J = 11.7 Hz, H-C (20)), 1.52 (3H, s, CH_3-C (14)), 1.87 (3H, s, CH_3-C (4)), 1.95–2.02 (1H, m, H-C (20)), 2.17–2.28 (3H, m, H-C (13), H_2-C (16)), 2.35–2.51 (2H, m, HO-C (5), H-C (12)), 2.45 (3H, s, CH_3-C_6H_4-SO_3-CH_2-C (24)), 3.24–3.32 (2H, m, H-C (2), H-C (25)), 3.45–3.54 (1H, m, H-C (17)), 3.87 (1H, dd, J = 10.1, 5.2 Hz, CH_3-C_6H_4-SO_3-CH-C (24)), 3.92–4.00 (1H, m, CH_3-C_6H_4-SO_3-CH-C(24)), 3.95 (1H, d, J = 6.1 Hz, H-C (6)), 4.00 (1H, s, HO-C (7)), 4.26–4.31 (1H, m, H-C (5)), 4.62–4.73 (2H, m, CH_2-C (8)), 4.94 (1H, t, J = 7.7 Hz, H-C (15)), 5.26–5.42 (3H, m, H-C (3), H-C (11), H-C (19)), 5.68–5.82 (2H, m, H-C (9), H-C (10)), 7.35 (2H, d, J = 8.1 Hz, 2H of CH_3-C_6H_4-SO_3-CH_2-C (24)), 7.78 (2H, d, J = 8.1 Hz, 2H of CH_3-C_6H_4-SO_3-CH_2-C (24)). MS *m/z*: 712 (M^+, $C_{39}H_{52}O_{10}S$), 694, 676, 584, 540, 512, 504, 434, 412, 365, 337, 261, 243, 225, 193, 165, 151. HR-MS *m/z* (M^+): calcd. for $C_{39}H_{52}O_{10}S$, 712.3281; found, 712.3284.

24a-Methanesulfonyloxymilbemycin A_4 (**18**). IR ν_{max} (KBr) cm^{-1}: 3484 (OH), 1715 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (1H, q, J = 11.7 Hz, H-C (18)), 0.99–1.05 (6H, m, CH_3-C (12), CH_3-CH_2-C (25)), 1.23–1.95 (9H, m, H-C (13), H-C (18), H_2-C (22), H_2-C (23), H-C (24), CH_3-CH_2-C (25)), 1.37 (1H, t, J = 11.7 Hz, H-C (20)), 1.53 (3H, s, CH_3-C (14)), 1.87 (3H, s, CH_3-C (4)), 1.99–2.06 (1H, m, H-C (20)), 2.17–2.30 (3H, m, H-C (13), H_2-C (16)), 2.35–2.51 (2H, m, HO-C (5), H-C (12)), 3.01 (3H, s, CH_3-SO_3-CH_2-C (24)), 3.25–3.29 (1H, m, H-C (2)), 3.31–3.38 (1H, m, H-C (25)), 3.50–3.61 (1H, m, H-C (17)), 3.96 (1H, d, J = 6.4 Hz, H-C (6)), 4.00 (1H, s, HO-C (7)), 4.06 (1H, dd, J = 10.1, 5.2 Hz, CH_3-SO_3-CH-C (24)), 4.16 (1H, dd, J = 10.1, 3.6 Hz, CH_3-SO_3-CH-C (24)), 4.29 (1H, br. s, H-C (5)), 4.62–4.74 (2H, m, CH_2-C (8)), 4.93–4.98 (1H, m, H-C (15)), 5.30–5.43 (3H, m, H-C (3), H-C (11), H-C (19)), 5.69–5.84 (2H, m, H-C (9), H-C (10)). MS *m/z*: 636 (M^+, $C_{33}H_{48}O_{10}S$), 618, 600, 540, 508, 490, 450, 412, 358, 339, 314, 289, 261, 248, 193, 165, 151. HR-MS *m/z* (M^+): calcd. for $C_{33}H_{48}O_{10}S$, 636.2968; found, 636.2977.

Synthesis of 5-deoxy-24a-fluoro-5-oxomilbemycin A_4 (**19**). To a stirred solution of **6** (51 mg) in dichloromethane (5 ml) was added diethylaminosulfur trifluoride (DAST, 19 μl) in an ice bath, and the mixture was stirred for 1 h. The reaction mixture was poured into ice-cold water and then extracted with ethyl acetate. The extract was washed with brine and dried over anhydrous magnesium sulfate. Removal of the solvent under vacuum left a residue which was purified by preparative thin-layer chromatography (hexane–ethyl acetate = 2 : 1) to give 14 mg (27% yield) of **19**.

5-Deoxy-24a-fluoro-5-oxomilbemycin A_4 (**19**). IR ν_{max} (KBr) cm^{-1}: 3472 (OH), 1715 (C=O), 1683 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (1H, q, J = 12.1 Hz, H-C (18)), 1.00–1.06 (6H, m, CH_3-C (12), CH_3-CH_2-C (25)), 1.35–1.92 (9H, m, H-C (13), H-C (18), H_2-C (22), H_2-C (23), H-C (24), CH_3-CH_2-C (25)), 1.40 (1H, t, J = 11.7 Hz, H-C (20)), 1.53 (3H, s, CH_3-C(14)), 1.89–1.90 (3H, m, CH_3-C (4)), 2.01–2.08 (1H, m, H-C (20)), 2.14–2.32 (3H, m, H-C (13), H_2-C (16)), 2.35–2.51 (1H, m, H-C (12)), 3.39 (1H, td, J_t = 9.3 Hz, J_d = 2.4 Hz, H-C (25)), 3.53–3.65 (2H, m, H-C (2), H-C (17)), 3.86 (1H, s, H-C (6)), 3.97 (1H, s, HO-C (7)), 4.33 (2H, dd, J = 47.1, 4.4 Hz, F-CH_2-C (24)), 4.68–4.81 (2H, m, CH_2-C (8)), 4.94–4.99 (1H, m, H-C (15)), 5.38–5.50 (2H, m, H-C (11), H-C (19)), 5.74 (1H, dd, J = 14.5, 11.3 Hz, H-C (10)), 5.87 (1H, dt, J_d = 11.3 Hz, J_t = 2.4 Hz, H-C (9)), 6.55–6.56 (1H, m, H-C (3)). MS *m/z*: 558 (M^+, $C_{32}H_{43}O_7F$), 540, 448, 432, 229, 213, 185, 151. HR-MS *m/z* (M^+): calcd. for $C_{32}H_{43}O_7F$, 558.2993; found, 558.2993.

Synthesis of 24a-fluoromilbemycin A_4 (**20**). To a solution of **19** (14 mg) in methanol (3 ml) was added sodium borohydride (4.0 mg) at room temperature, and the mixture was stirred for 5 min. Brine was added, and the resulting mixture was extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by preparative thin-layer chromatography (hexane–ethyl acetate = 3 : 2) to afford 7.3 mg (52% yield) of **20**.

24a-Fluoromilbemycin A_4 (**20**). IR ν_{max} (KBr) cm^{-1}: 3471 (OH), 1714 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (1H, q, J = 12.1 Hz, H-C (18)), 0.99–1.05 (6H, m, CH_3-C (12), CH_3-CH_2-C (25)), 1.35–1.91 (9H, m, H-C (13), H-C (18), H_2-C (22), H_2-C (23), H-C (24), CH_3-CH_2-C (25)), 1.37 (1H, t, J = 11.7 Hz, H-C (20)), 1.53 (3H, s, CH_3-C (14)), 1.87–1.88 (3H, m, CH_3-C (4)), 1.99–2.05 (1H, m, H-C (20)), 2.15–2.27 (3H, m, H-C (13), H_2-C (16)), 2.35–2.51 (2H, m, HO-C (5), H-C (12)), 3.25–3.29 (1H, m, H-C (2)), 3.38 (1H, td, J_t = 9.3 Hz, J_d = 2.4 Hz, H-C (25)), 3.51–3.62 (1H, m, H-C (17)), 3.96 (1H, d, J = 6.0 Hz, H-C (6)), 4.04 (1H, s, HO-C (7)), 4.29 (1H, t, J = 6.0 Hz, H-C (5)), 4.32 (2H, dd, J = 47.5, 4.4 Hz,

F-CH_2-C (24)), 4.62–4.74 (2H, m, CH_2-C (8)), 4.96 (1H, t, J=7.7 Hz, H-C (15)), 5.30–5.43 (3H, m, H-C (3), H-C (11), H-C (19)), 5.69–5.84 (2H, m, H-C (9), H-C (10)). MS *m/z*: 560 (M^+, $C_{32}H_{45}O_7F$), 542, 448, 432, 414, 341, 282, 263, 248, 229, 213, 185, 151. HR-MS *m/z* (M^+): calcd. for $C_{32}H_{45}O_7F$, 560.3149; found, 560.3141.

Synthesis of 5-O-t-butyldimethylsilyl-24a-methylmilbemycin A_4 (**21**). To a suspension of copper(I) iodide (116 mg) in ether (3 ml) at 0°C was added methyllithium (1.30 ml of a 0.99 M solution in ether), and the mixture was stirred for 30 min at 0°C. A solution of **12** (99.0 mg) in ether (2 ml) was added, and the resulting mixture was stirred for 2 h at 0°C. The reaction mixture was poured into aqueous ammonium chloride, and the resulting mixture was extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate and evaporated under reduced pressure to leave a residue, which was purified by silica-gel chromatography (hexane–ethyl acetate gradient) to give 70.0 mg (87% yield) of **21**.

5-O-t-Butyldimethylsilyl-24a-methylmilbemycin A_4 (**21**). IR ν_{max} (KBr) cm^{-1}: 3423 (OH), 1701 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, $(CH_3)_2$-Si-O-C (5)), 0.81–1.00 (1H, m, H-C (18)), 0.86 (3H, t, J=7.3 Hz, CH_3-CH_2-C (24)), 0.93 (9H, s, $(CH_3)_3$C-Si-O-C (5)), 0.96–1.01 (6H, m, CH_3-C (12), CH_3-CH_2-C (25)), 1.03–1.90 (11H, m, H-C (13), H-C (18), H_2-C (22), H_2-C (23), H-C (24), CH_3-CH_2-C (24), CH_3-CH_2-C (25)), 1.35 (1H, t, J=11.7 Hz, H-C (20)), 1.54 (3H, s, CH_3-C (14)), 1.79 (3H, s, CH_3-C (4)), 1.98–2.05 (1H, m, H-C (20)), 2.16–2.27 (3H, m, H-C (13), H_2-C (16)), 2.34–2.50 (1H, m, H-C (12)), 3.17 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-C (25)), 3.34–3.38 (1H, m, H-C (2)), 3.50–3.63 (1H, m, H-C (17)), 3.81 (1H, d, J=5.2 Hz, H-C (6)), 4.14 (1H, s, HO-C (7)), 4.41–4.46 (1H, m, H-C (5)), 4.58 (1H, d, J=14.5 Hz, CH-C (8)), 4.67 (1H, d, J=14.5 Hz, CH-C (8)), 4.92–4.99 (1H, m, H-C (15)), 5.28–5.43 (3H, m, H-C (3), H-C (11), H-C (19)), 5.68–5.80 (2H, m, H-C (9), H-C (10)). MS *m/z*: 670 (M^+, $C_{39}H_{62}O_7Si$), 652, 613, 595, 520, 439, 428, 278, 259, 243, 225, 209, 181, 167, 151. HR-MS *m/z* (M^+): calcd. for $C_{39}H_{62}O_7Si$, 670.4265; found, 670.4259.

In accordance with the procedure for the synthesis of **14**, compound **21** was converted to **22** (97% yield).

24a-Methylmilbemycin A_4 (**22**). IR ν_{max} (KBr) cm^{-1}: 3475 (OH), 1713 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.80–1.00 (1H, m, H-C (18)), 0.86 (3H, t, J=6.9 Hz, CH_3-CH_2-C (24)), 0.96–1.01 (6H, m, CH_3-C (12), CH_3-CH_2-C (25)), 1.03–1.90 (11H, m, H-C (13), H-C (18), H_2-C (22), H_2-C (23), H-C (24), CH_3-CH_2-C (24), CH_3-CH_2-C (25)), 1.36 (1H, t, J=11.7 Hz, H-C (20)), 1.53 (3H, s, CH_3-C (14)), 1.87 (3H, s, CH_3-C (4)), 1.96–2.03 (1H, m, H-C (20)), 2.16–2.27 (3H, m, H-C (13), H_2-C (16)), 2.32–2.52 (2H, m, HO-C (5), H-C (12)), 3.17 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-C (25)), 3.25–3.28 (1H, m, H-C (2)), 3.50–3.63 (1H, m, H-C (17)), 3.96 (1H, d, J=6.1 Hz, H-C (6)), 4.11 (1H, s, HO-C (7)), 4.26–4.32 (1H, m, H-C (5)), 4.62–4.74 (2H, m, CH_2-C (8)), 4.92–4.99 (1H, m, H-C (15)), 5.33–5.45 (3H, m, H-C (3), H-C (11), H-C (19)), 5.69–5.84 (2H, m, H-C (9), H-C (10)). MS *m/z*: 556 (M^+, $C_{33}H_{48}O_7$), 538, 520, 428, 410, 279, 259, 248, 209, 181, 167, 151, 149. HR-MS *m/z* (M^+): calcd. for $C_{33}H_{48}O_7$, 556.3400; found, 556.3401.

Acaricidal activity against Tetranychus urticae. The primary leaves of plants of the *Vigna sinensis Savi* species were infected with organophosphate-sensitive mites (*Tetranychus urticae*). One day after infection, the infested plants were sprayed, using a Mizuho rotary sprayer, with 7 ml of a test solution containing the compound under test at a concentration ranging from 0.1 to 3 ppm at a rate of 3.5 mg of the test solution per 1 cm^2 of leaf. The plants were assessed after 3 days by examining the adult mites under a binocular microscope to determine the living and dead individuals. Two plants were used for each concentration and each test compound. The plants were kept during the test in individual greenhouse compartments at 25°C. The results are reported in the Table.

Acknowledgments. We are grateful to Dr. Teruomi Jojima, the director of our laboratories, and to Dr. Soji Sugai for their support in this research.

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