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Synthesis of Milbemycins α_9, α_{10}, α_{11}, α_{12}, α_{14}, α_{15}, α_{20}, α_{21}, α_{22}, α_{23}, α_{26}, α_{27}, $\Delta^{2,3}$,$\Delta^{4,26}$-Milbemycins A_3, A_4 from Milbemycins A_3, A_4, and Their Acaricidal Activities

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Chemical derivation methods to prepare 26-acyloxy and 26-hydroxymilbemycins, which had been reported as natural products, milbemycins α_9, α_{10}, α_{11}, α_{12}, α_{14}, α_{15}, α_{20}, α_{21}, α_{22}, α_{23}, α_{26}, α_{27} from milbemycins A_3, A_4 were reported. $\Delta^{2,3}$,$\Delta^{4,26}$-Milbemycins A_3, A_4, which had also been reported as natural products, were further prepared from milbemycins A_3, A_4. Their acaricidal activities were also assessed against the organophosphorus-sensitive two-spotted spider mite (*Tetranychus urticae*) on primary leaves of cowpea plants (*Vigna sinesis Savi* species) by spraying.

Milbemycins[1~7] are sixteen membered ring macrolides that have been isolated from *Streptomyces hygroscopicus*. They exhibit notable activities as acaricides, insecticides and anthelmintics. Among them, milbemectin[8] [a mixture of milbemycins A_3 (**1a**) and A_4 (**1b**) (Figure 1)] was developed as an agricultural acaricide. Abamectin[9] [a mixture of avermectins[10] B_{1a} (**8a**) and B_{1b} (**8b**) (Figure 1)] has a similar structural and biological features, and that was developed as an agricultural acaricide and an insecticide. And ivermectin[11] [a mixture of 22,23-dihydroavermectins B_{1a} (**9a**) and B_{1b} (**9b**) (Figure 1)], that was derived from abamectin, was developed as an anthelmintic for livestock. The principal difference between milbemycins and avermectins is the absence or presence of the side chain moiety (α-L-oleandosyl-α-L-oleandosyl group) at the C-13 position.

On the other hand, a series of milbemycins,[3,12] which had acyloxy moieties at their C-26 position, namely α_9 (**2a**), α_{10} (**2b**), α_{11} (**3a**), α_{12} (**4a**), α_{14} (**3b**) and α_{15} (**4b**) (Figure 1) were reported as natural products, and some of them possessed potent acaricidal activities superior to those of milbemycins A_3 (**1a**) and A_4 (**1b**). Among them, milbemycins α_{11} (**3a**) and α_{14} (**3b**) received remarks for the highest activities in the series. That promoted enormous efforts focused on improvement of their bio productivity,[13] microbiological introduction of 3-methyl-2-butenoyloxy moieties to the C-26 positions of the related compounds[14] and clarification of their biosynthetic pathways.[15] In the course of these investigations, new congeners of 26-acyloxymilbemycins [milbemycins α_{20} (**5a**), α_{21} (**5b**), α_{22} (**6a**) and α_{23} (**6b**) (Figure 1)] and their biosynthetic precursors [milbemycins α_{26} (**7a**) and α_{27} (**7b**) (Figure 1)] were isolated.[16] The low bio productivities of most of the 26-acyloxy and hydroxymilbemycins, the necessity to supply them as reference materials for further investigations and interest in their biological activities required the development of versatile derivation methods to access the C-26 positions of milbemycins A_3 (**1a**) and A_4 (**1b**). In this paper, we would like to report the preparation of these milbemycin derivatives at the C-26 position.

On the other hand, $\Delta^{2,3}$,$\Delta^{4,26}$-milbemycins A_3 (**10a**) and A_4 (**10b**) (Figure 2) were also reported as natural products.[17] The low bio productivity and requirements as reference materials of them also stimulated the development of derivation methods from milbemycins A_3 (**1a**) and A_4 (**1b**). We also disclose herein an efficient

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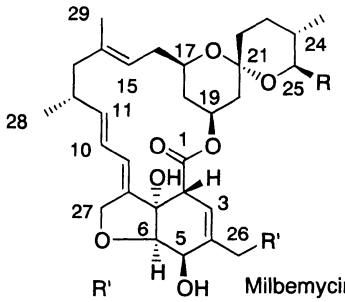
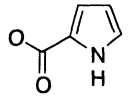
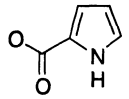
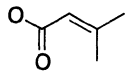
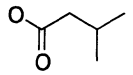
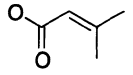
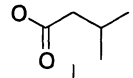
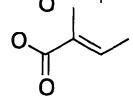
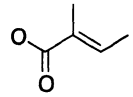
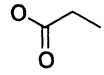
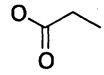
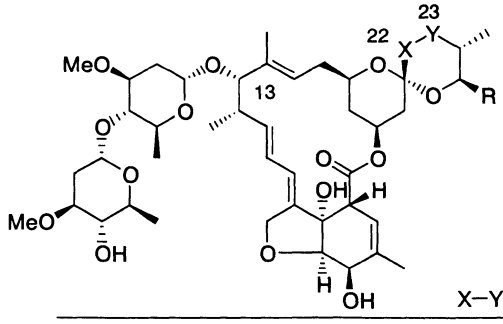
Fig. 1. Structures of milbemycins and avermectins.



Milbemycin	R	R'
A_3 (**1a**)	$C^{(31)}H_3$	H
A_4 (**1b**)	$C^{(31)}H_2C^{(32)}H_3$	H
α_9 (C_1) (**2a**)	Me	
α_{10} (C_2) (**2b**)	Et	
α_{11} (**3a**)	Me	
α_{12} (**4a**)	Me	
α_{14} (**3b**)	Et	
α_{15} (**4b**)	Et	
α_{20} (**5a**)	Me	
α_{21} (**5b**)	Et	
α_{22} (**6a**)	Me	
α_{23} (**6b**)	Et	
α_{26} (**7a**)	Me	OH
α_{27} (**7b**)	Et	OH



	X–Y	R
Avermectin B_{1a} (**8a**)	HC=CH	*s*-Bu
Avermectin B_{1b} (**8b**)	HC=CH	*i*-Pr
22,23-Dihydroavermectin B_{1a} (**9a**)	H_2C-CH_2	*s*-Bu
22,23-Dihydroavermectin B_{1b} (**9b**)	H_2C-CH_2	*i*-Pr

method for **10a** and **10b** from the same intermediates as those prepared for **2a**~**7b**.

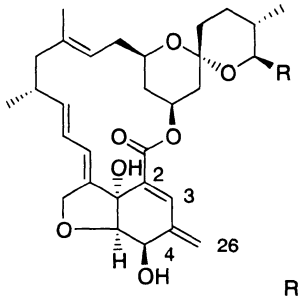
Results and Discussion

Chemistry

The method to introduce a hydroxy moiety to the C-26 position of avermectins was reported by Merck

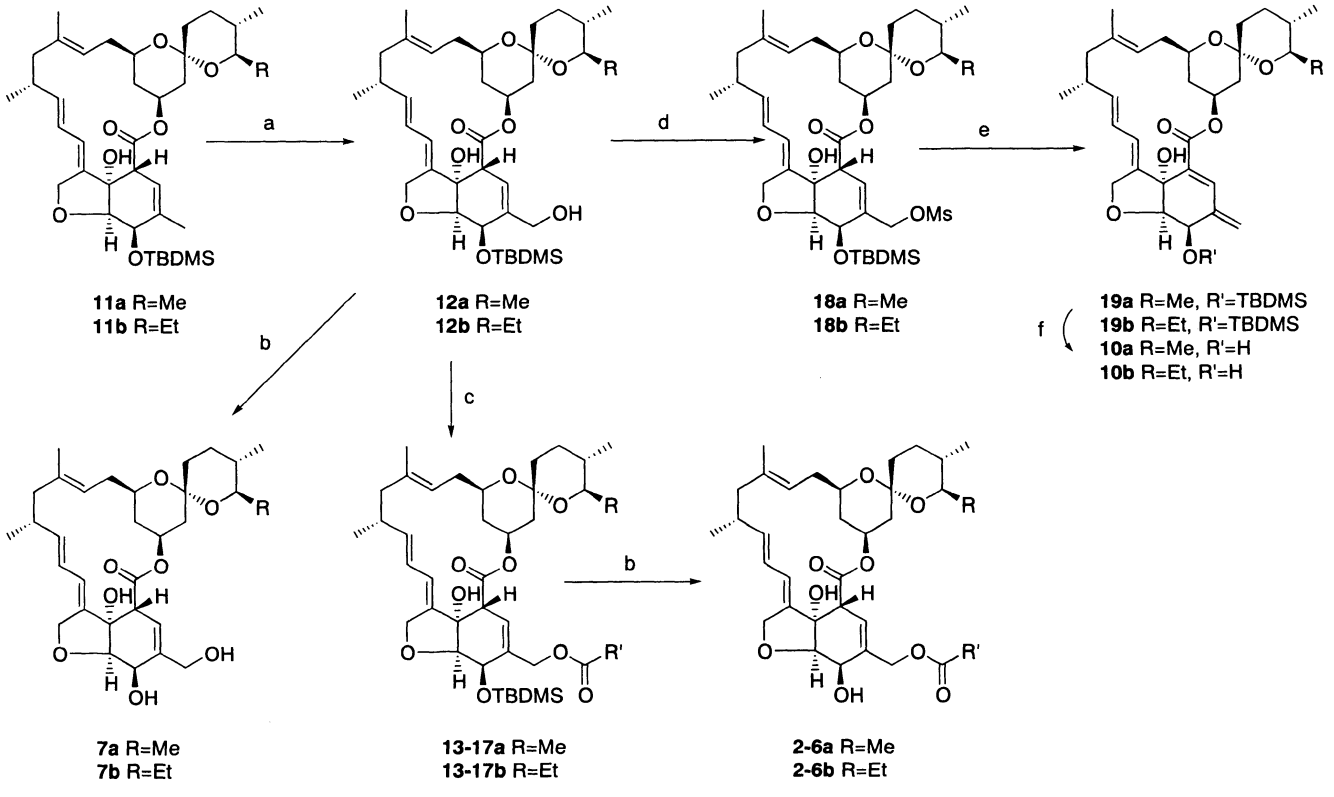
chemists[18,19] [catalytic amount of selenium dioxide (SeO_2)/ *t*-butylhydroperoxide (t-BuOOH)]. Later, the subsequent conversion of the hydroxy moiety to the acyloxy moiety under Mitsunobu conditions was also reported.[18,19] To prepare a variety of 26-acyloxy and 26-hydroxymilbemycins and $\Delta^{2,3}$,$\Delta^{4,26}$-milbemycins from milbemycins A_3 (**1a**) and A_4 (**1b**), we postulated that 26-hydroxy-5-*O*-silylprotected milbemycins A_3 (**12a**) and A_4 (**12b**) could be used as common key intermediates (Scheme 1). Because the products obtained by Merck's methods necessitated tedious exchanges of protecting groups[20,21] to accomplish our aim. To resolve this problem, we attempted to employ 5-*O*-*t*-butyldimethylsilyl (TBDMS)-milbemycins[22] A_3 (**11a**), A_4 (**11b**) as substrates for the oxidation at the C-26 position. The TBDMS group is inert to acylation conditions and is removable to react under the conditions that do not affect the acyloxy moieties at the C-26 positions. At first, we attempted 5-*O*-TBDMS-milbemycins A_3 (**11a**), A_4 (**11b**) under Merck's conditions, but messy complex mixtures were obtained as products[19] due to the elimination of the TBDMS group under the acidic reaction conditions, the subsequent oxidation of the C-5 hydroxy group into a ketone and so on. Fortunately, we succeeded in suppressing the elimination of the TBDMS group by adding potassium hydrogen carbonate ($KHCO_3$) to the reaction media to quench the acidity (Scheme 1). Thus, upon treatment of 5-

Fig. 2. Structures of $\Delta^{2,3}$,$\Delta^{4,26}$-milbemycins.



	R
$\Delta^{2,3}$,$\Delta^{4,26}$-milbemycin A_3 (**10a**)	Me
$\Delta^{2,3}$,$\Delta^{4,26}$-milbemycin A_4 (**10b**)	Et

Scheme 1. Synthesis of milbemycin derivatives.



Reagents: (a) SeO_2, $KHCO_3$, t-BuOOH; 45.9% for **12a**, 34.5% for **12b**; (b) HF/Pyridine; see Table 1.; (c) R'COCl, base; see Table 1.; (d) MsCl, Pyridine; 95.2% for **18a**, 85.7% for **18b**; (e) Li_2CO_3; 42.0% for **19a**, 49.4% for **19b**; (f) p-TsOH·H_2O; 91.1% for **10a**, 98.1% for **10b**.

O-TBDMS-milbemycins A_3 (**11a**) and A_4 (**11b**) with SeO_2 and t-BuOOH in the presence of $KHCO_3$ produced 5-*O*-TBDMS-26-hydroxymilbemycins A_3 (**12a**) and A_4 (**12b**) in moderate yields. The obtained key intermediates **12a** and **12b** were deprotected to afford milbemycins α_{26} (**7a**) and α_{27} (**7b**) in good yields, respectively. To prepare a variety of 26-acyloxymilbemycins, the corresponding acylation[23] of the C-26 hydroxy groups of **12a** and **12b**, and the subsequent deprotection were achieved in good to moderate yields, which are summarized in Table 1.

Next, we examined a method to derive $\Delta^{2,3},\Delta^{4,26}$-milbemycins A_3 (**10a**) and A_4 (**10b**) from **12a** and **12b** (Scheme 1). The C-26 hydroxy groups of **12a** and **12b** were converted to good leaving groups by methanesulufonylation to afford **18a** and **18b**. Treatment of **18a** and **18b** with lithium carbonate (Li_2CO_3) successfully induced δ-conjugate elimination, thereby affording 5-*O*-TBDMS-$\Delta^{2,3},\Delta^{4,26}$-milbemycins A_3 (**19a**) and A_4 (**19b**) in moderate yields, respectively. The TBDMS groups were deprotected with acidic media to afford $\Delta^{2,3},\Delta^{4,26}$-milbemycins A_3 (**10a**) and A_4 (**10b**) in good yields.

All spectral data of synthesized milbemycins α_9 (**2a**), α_{10} (**2b**), α_{11} (**3a**), α_{14} (**3b**), α_{12} (**4a**), α_{15} (**4b**), α_{20} (**5a**), α_{21} (**5b**), α_{22} (**6a**), α_{23} (**6b**), α_{26} (**7a**), α_{27} (**7b**), $\Delta^{2,3},\Delta^{4,26}$-milbemycins A_3 (**10a**), A_4 (**10b**) were in accord with those of corresponding natural products.

Acaricidal Activities

The acaricidal activities[12] of milbemycins α_9 (**2a**),

Table 1. Yields of C-26 acylation and C-5 deprotection.

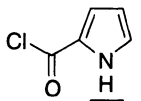
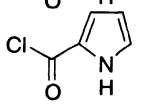
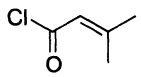
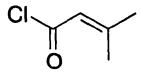
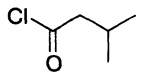
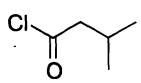
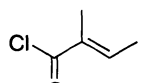
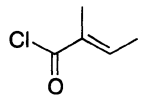
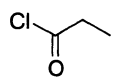
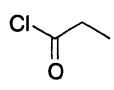
Substrate	R'COCl	Product	Yield	Product (HF / Pyridine)	Yield
12a		**13a**	79.8%	**2a**	83.3%
12b		**13b**	75.2%	**2b**	79.8%
12a		**14a**	89.6%	**3a**	90.9%
12b		**14b**	78.3%	**3b**	85.0%
12a		**15a**	95.2%	**4a**	71.2%
12b		**15b**	79.0%	**4b**	72.0%
12a		**16a**	44.1%	**5a**	66.0%
12b		**16b**	47.7%	**5b**	63.0%
12a		**17a**	95.8%	**6a**	70.5%
12b		**17b**	80.4%	**6b**	72.1%
		12a	—	**7a**	73.0%
		12b	—	**7b**	77.3%

Table 2. Acaricidal activities of milbemycins against the two-spotted spider mites.

Milbemycin	Mortality (%) 10ppm	1ppm
α_9 (C_1) (2a)	100	100
α_{10} (C_2) (2b)	100	100
α_{11} (3a)	100	100
α_{14} (3b)	100	100
α_{12} (4a)	100	97
α_{15} (4b)	100	100
α_{20} (5a)	100	100
α_{21} (5b)	100	100
α_{22} (6a)	59	21
α_{23} (6b)	100	22
α_{26} (7a)	15	10
α_{27} (7b)	33	15
$\Delta^{2,3}$,$\Delta^{4,26}$-milbemycin A_3 (10a)	0	4
$\Delta^{2,3}$,$\Delta^{4,26}$-milbemycin A_4 (10b)	7	4
A_3 (1a)	69	3
A_4 (1b)	100	32

α_{10} (**2b**), α_{11} (**3a**), α_{14} (**3b**), α_{12} (**4a**), α_{15} (**4b**), α_{20} (**5a**), α_{21} (**5b**), α_{22} (**6a**), α_{23} (**6b**), α_{26} (**7a**), α_{27} (**7b**), $\Delta^{2,3}$,$\Delta^{4,26}$-milbemycins A_3 (**10a**), A_4 (**10b**) were assessed against the organophosphorus-sensitive two-spotted spider mite (*Tetranychus urticae*) on primary leaves of cowpea plants (*Vigna sinesis Savi* species) by spraying. Results are listed in Table 2. All 26-acyloxy derivatives exhibited good to excellent activities, and most of them were superior to those of their parent compounds, milbemycins A_3 (**1a**) and A_4 (**1b**). On the other hand, 26-hydroxy derivatives **7a** and **7b** had decreased the activities. The deactivation of **7a** and **7b** was speculated to be due to an inhibition of the migration of the molecules to target sites because of the increased polarities. And $\Delta^{2,3}$,$\Delta^{4,26}$-derivatives **10a** and **10b** also showed poor activities. These results suggested that the conformations of the hexahydrobenzofuran moieties are important for the activities.

Conclusion

In conclusion, we established a versatile method to prepare a series of 26-acyloxy and 26-hydroxymilbemycins A_3, A_4 (milbemycin α-series) from milbemycins A_3 (**1a**), A_4 (**1b**). The derivation method to prepare $\Delta^{2,3}$,$\Delta^{4,26}$-milbemycins A_3 (**10a**), A_4 (**10b**) was devised from the same key intermediates, **12a**, **12b**. Most of the 26-acyloxy derivatives exhibited more excellent acaricidal activities than their parent compounds, milbemycins A_3 (**1a**), and A_4 (**1b**). On the other hand, milbemycins α_{26} (**7a**), α_{27} (**7b**) and $\Delta^{2,3}$,$\Delta^{4,26}$-milbemycins A_3 (**10a**), A_4 (**10b**) showed only poor acaricidal activities.

Experimental

NMR spectra were measured on a Varian Gemini-200 FT NMR Spectrometer (200 MHz). Chemical shifts (δ) were expressed in parts per million relative to internal tetramethylsilane. Mass spectra were measured on a Fisions Instruments VG Autospec. IR spectra were measured on a Perkin Elmer 1600 series FT IR.

5-*O*-TBDMS-26-hydroxymilbemycin A_3 (**12a**)

To a stirred suspension of SeO_2 (6.01 g, 54.13 mmol) in dichloromethane (CH_2Cl_2) (200 ml) was added $KHCO_3$ (4.88 g, 48.71 mmol) and t-BuOOH (5.0 M solution in decane, 10.83 ml, 54.13 mmol). The reaction was then stirred at ambient temperature for 20 minutes under a nitrogen atmosphere. To the reaction mixture was added 5-*O*-TBDMS-milbemycin A_3 (**11a**) (3.48 g, 5.41 mmol) and the solution was stirred at ambient temperature for 4.5 hours. The reaction mixture was poured into a saturated aqueous solution of sodium thiosulfate ($Na_2S_2O_3$), stirred for several minutes and filtered through Celite®. After separating the aqueous layer, the organic layer was washed with a saturated aqueous solution of sodium hydrogen carbonate ($NaHCO_3$ aq) and brine, dried over magnesium sulfate ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography [*n*-hexane (Hex) - ethyl acetate (EtOAc) gradient] to give 1.64 g (45.9%) of **12a** and 0.89 g (25.6%) of recovered **11a** as colorless amorphous solids, respectively.

12a: IR ν_{max} (film) cm^{-1}: 3460, 2925, 2915, 2880, 2860, 1730, 1715; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.73~5.82 (2H, m, H-9, H-10), 5.64 (1H, br, H-3), 5.25~5.44 (2H, m, H-11, H-19), 4.99 (1H, m, H-15), 4.52~4.74 (3H, m, H_2-27, H-26), 4.10~4.30 (2H, m, H-5, H-26), 4.19 (1H, s, 7-OH), 3.82 (1H, d, J=5.6 Hz, H-6), 3.55 (1H, m, H-17), 3.38 (1H, m, H-2), 3.26 (1H, dd, J=9.5, 5.9 Hz, H-25), 2.42 (1H, m, H-12), 2.13~2.31 (3H, m, H-13, H_2-16), 2.03 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.14 (3H, d, J=6.2 Hz, H_3-28), 1.00 (3H, d, J=6.6 Hz, H_3-31), 0.93 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.2 Hz, H_3-30), 0.15 (6H, s, $(CH_3)_2Si$), 0.78~1.95 (10H, m, H-13, H_2-18, H-20, H_2-22,

H_2-23, H-24, 26-OH); EI-MS (*m/z*): 658 (M^+), 640, 601, 583, 565; HREI-MS (*m/z*): [M^+]: calcd. for $C_{37}H_{58}O_8Si$, 658.3901; found, 658.3902.

5-*O*-TBDMS-26-hydroxymilbemycin A_4 (**12b**)

Using the same procedure described for the preparation of **12a**, **12b** was prepared from **11b** in 34.5% yield as a colorless amorphous solid and 56.9% of **11b** was recovered.

12b: IR ν_{max} (film) cm^{-1}: 3455, 2935, 2925, 2850, 1730, 1710; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.70~5.85 (2H, m, H-9, H-10), 5.67 (1H, m, H-3), 5.25~5.44 (2H, m, H-11, H-19), 4.97 (1H, m, H-15), 4.55~4.75 (3H, m, H_2-27, H-26), 4.08~4.29 (3H, m, H-5, H-26, 7-OH), 3.82 (1H, d, J=5.5 Hz, H-6), 3.57 (1H, m, H-17), 3.39 (1H, m, H-2), 3.07 (1H, m, H-25), 2.42 (1H, m, H-12), 2.13~2.30 (3H, m, H-13, H_2-16), 2.01 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.00 (3H, d, J=6.6 Hz, H_3-28), 0.93 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.2 Hz, H-30), 0.62 (6H, s, $(CH_3)_2Si$), 0.75~1.95 (15H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31, H_3-32, 26-OH); EI-MS (*m/z*): 672 (M^+), 654, 636, 615, 597; HREI-MS (*m/z*): [M^+]: calcd. for $C_{38}H_{60}O_8Si$, 672.4057; found, 672.4057.

Milbemycin α_{26} (**7a**)

To a stirred solution of 5-*O*-TBDMS-26-hydroxymilbemycin A_3 (**12a**) (100 mg, 0.15 mmol) in acetonitrile (1 ml) was added hydrogen fluoride-pyridine (HF=70%, 0.2 ml) at ambient temperature. After stirring for 2 hours, the reaction mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with water, $NaHCO_3$ aq, water, brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC to give 60.4 mg (73.0%) of **7a** as a colorless amorphous solid.

Using the same procedure described for the preparation of **7a**, the other milbemycin α-series (**2a**, **2b**, **3a**, **3b**, **4a**, **4b**, **5a**, **5b**, **6a**, **6b** and **7b**) were prepared from corresponding precursors as colorless amorphous solids, respectively. Yields are summarized in Table 1.

5-*O*-TBDMS-milbemycin α_9 (**13a**)

To a stirred solution of pyrrole-2-carboxylic acid (674.4 mg, 6.07 mmol) in 1,2-dimethoxyethane (DME) (16 ml) was added triethylamine (Et_3N) (1.4 ml, 10.04 mmol) at ambient temperature. After stirring for 15 minutes, the reaction mixture was evaporated under reduced pressure. The residue was dissolved in DME (14 ml) and to the solution was added a solution of thionyl chloride ($SOCl_2$) (10 ml, 137.09 mmol) in DME (10 ml) was added dropwise cooling with an ice bath under a nitrogen atmosphere. After stirring for 15 minutes cooling with an ice bath, then for 30 minutes at ambient temperature, the reaction mixture was evaporated under reduced pressure. To the solution of **12a** (2.00 g, 3.04 mmol) in CH_2Cl_2 (16 ml) was added Et_3N (1.4 ml, 10.04 mmol) and a solution of the residue prepared by the above method in CH_2Cl_2 (5 ml) dropwise while cooling with an ice bath under a nitrogen atmosphere. After the addition was complete, the ice bath was removed and the reaction mixture was stirred at ambient temperature for 30 minutes. The reaction mixture was poured into water, extracted with EtOAc, and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography (Hex-EtOAc gradient) to give 1.82 g (79.8%) of **13a** as a colorless amorphous solid.

13a: IR ν_{max} (film) cm^{-1}: 3445, 3320, 2955, 2925, 2880, 2860, 1705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.28 (1H, br, NH), 6.94 (2H, m, H_2-Ar), 6.26 (1H, m, H-Ar), 5.66~5.84 (3H, m, H-3, H-9, H-10), 5.25~5.48 (2H, m, H-11, H-19), 4.99 (1H, m, H-15), 4.52~4.92 (5H, m, H-5, H_2-26, H_2-27), 4.25 (1H, br, 7-OH), 3.86 (1H, d, J=5.5 Hz, H-6), 3.55 (1H, m, H-17), 3.41 (1H, br, H-2), 3.26 (1H, dd, J=9.5, 6.6 Hz, H-25), 2.42 (1H, m, H-12), 2.13~2.30 (3H, m, H-13, H_2-16), 2.05 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.14 (3H, d, J=6.2 Hz, H_3-28), 1.00 (3H, d, J=6.6 Hz, H_3-31), 0.91 (9H, s, $(CH_3)_3CSi$), 0.83 (3H, d, J=6.6 Hz, H_3-30), 0.13 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.78~1.95 (9H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 751 (M^+), 733, 694, 640, 622, 583, 565, 508; HREI-MS (*m/z*): [M^+]: calcd. for $C_{42}H_{61}NO_9Si$, 751.4116; found, 751.4114.

5-*O*-TBDMS-milbemycin α_{10} (**13b**)

Using the same procedure described for the preparation of **13a**, **13b** was prepared from **12b** in 75.2% yield as a colorless amorphous solid.

13b: IR ν_{max} (film) cm^{-1}: 3455, 3320, 2955, 2925, 2850, 1705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.27 (1H, br, NH), 6.96 (2H, m, H_2-Ar), 6.26 (1H, m, H-Ar), 5.65~5.87 (3H, m, H-3, H-9, H-10), 5.23~5.50 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.83 (2H, m, H_2-26), 4.55~4.78 (3H, m, H-5, H_2-27), 4.25 (1H, br, 7-OH), 3.86 (1H, d, J=5.5 Hz, H-6), 3.58 (1H, m, H-17), 3.41 (1H, m, H-2), 3.06 (1H, m, H-25), 2.40 (1H, m, H-12), 2.11~2.30 (3H, m, H-13, H_2-16), 2.02 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.00 (3H, d, J=7.0 Hz, H_3-28), 0.98 (3H, t, J=7.7 Hz, H_3-32), 0.91 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.2 Hz, H_3-30), 0.14 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.70~1.95 (11H, m, H-13,

H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 765 (M^+), 747, 708, 654, 636, 597, 579; HREI-MS (*m/z*): [M^+]: calcd. for $C_{43}H_{63}NO_9Si$, 765.4272; found, 765.4271.

5-*O*-TBDMS-milbemycin α_{11} (**14a**)

To a stirred solution of **12a** (100 mg, 0.15 mmol) in CH_2Cl_2 (2 ml) was added pyridine (49 μl, 0.61 mmol) and 3,3-dimethylacryloyl chloride (68 μl, 0.61 mmol) while cooling with an ice bath under a nitrogen atmosphere. After stirring for 30 minutes, the reaction mixture was poured into water and extracted with EtOAc, and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC to give 100.8 mg (89.6%) of **14a** as a colorless amorphous solid.

14a: IR ν_{max} (film) cm^{-1}: 3450, 2955, 2915, 2880, 2860, 1715, 1645; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.62~5.85 (4H, m, H-3, H-9, H-10, C(26)OCOC*H*), 5.28~5.47 (2H, m, H-11, H-19), 4.98 (1H, m, H-15), 4.51~4.79 (5H, m, H-5, H_2-26, H_2-27), 4.15 (1H, br, 7-OH), 3.85 (1H, d, *J*=5.5 Hz, H-6), 3.55 (1H, m, H-17), 3.40 (1H, m, H-2), 3.25 (1H, dd, *J*=9.5, 6.6 Hz, H-25), 2.42 (1H, m, H-12), 2.16 (3H, d, *J*=1.5 Hz, C(26)OCOCHC(CH_3)$_2$), 2.10~2.32 (3H, m, H-13, H_2-16), 2.02 (1H, m, H-20), 1.90 (3H, d, *J*=1.5 Hz, C(26)OCOCHC(CH_3)$_2$), 1.54 (3H, br, H_3-29), 1.14 (3H, d, *J*=6.6 Hz, H_3-31), 1.00 (3H, d, *J*=6.6 Hz, H_3-28), 0.91 (9H, s, $(CH_3)_3CSi$), 0.83 (3H, d, *J*=6.6 Hz, H_3-30), 0.13 (3H, s, CH_3Si), 0.11 (3H, s, CH_3Si), 0.75~1.94 (9H, m, H-13, H_2-18. H-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 740 (M^+), 722, 683, 665, 640, 622, 583, 565; HREI-MS (*m/z*): [M^+]: calcd. for $C_{42}H_{64}O_9Si$, 740.4320; found, 740.4321.

Using the same procedure described for the preparation of **14a**, the other 5-*O*-*t*-butyldimethylsilyl-milbemycin α-series (**14b**, **15a**, **15b**, **17a** and **17b**) was prepared from **12a** and **12b** as colorless amorphous solids, respectively. Yields are summarized in Table 1.

14b: IR ν_{max} (film) cm^{-1}: 3485, 2955, 2920, 2855, 1715, 1635; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.60~5.85 (4H, m, H-3, H-9, H-10, C(26)OCOC*H*), 5.25~5.47 (2H, m, H-11, H-19), 4.97 (1H, m, H-15), 4.52~4.78 (5H, m, H-5, H_2-26, H_2-27), 4.21 (1H, br, 7-OH), 3.84 (1H, d, *J*=5.5 Hz, H-6), 3.58 (1H, m, H-17), 3.40 (1H, br, H-2), 3.07 (1H, m, H-25), 2.17 (3H, br, C(26)OCOCHC(CH_3)$_2$), 1.90 (3H, br, C(26)OCOCHC(CH_3)$_2$), 1.54 (3H, br, H_3-29), 1.00 (3H, d, *J*=6.6 Hz, H_3-28), 0.98 (3H, t, *J*=6.6 Hz, H_3-32), 0.91 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, *J*=6.6 Hz, H_3-30), 0.13 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.78~2.55 (16H, m, H-12, H_2-13, H_2-16, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 754 (M^+), 697, 679, 654, 597, 579; HREI-MS (*m/z*): [M^+]: calcd. for $C_{43}H_{66}O_9Si$, 754.4476; found, 754.4478.

15a: IR ν_{max} (film) cm^{-1}: 3450, 2960, 2925, 2880, 2860, 1735, 1715; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.70~5.87 (2H, m, H-9, H-10), 5.66 (1H, br, H-3), 5.25~5.48 (2H, m, H-11, H-19), 4.98 (1H, m, H-15), 4.52~4.75 (5H, m, H-5, H_2-26, H_2-27), 4.12 (1H, br, 7-OH), 3.85 (1H, d, *J*=5.9 Hz, H-6), 3.55 (1H, m, H-17), 3.39 (1H, br, H-2), 3.26 (1H, dd, *J*=9.5, 6.2 Hz, H-25), 2.47 (1H, m, H-12), 1.98~2.34 (7H, m, H-13, H_2-16, H-20, C(26)OCOCH_2C*H*), 1.54 (3H, br, H_3-29), 1.13 (3H, d, *J*=6.2 Hz, H_3-31), 1.00 (3H, d, *J*=6.6 Hz, H_3-28), 0.95 (6H, d, *J*=5.9 Hz, C(26)-OCOCH$_2$CH(CH_3)$_2$), 0.91 (9H, s, $(CH_3)_3CSi$), 0.83 (3H, d, *J*=6.6 Hz, H_3-30), 0.14 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.81~1.95 (9H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 742 (M^+), 724, 685, 667, 640, 583, 565; HREI-MS (*m/z*): [M^+]: calcd. for $C_{42}H_{66}O_9Si$, 742.4476; found, 742.4476.

15b: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2925, 2880, 2860, 1735, 1710; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.62~5.88 (3H, m, H-3, H-9, H-10), 5.25~5.47 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.53~4.75 (5H, m, H-5, H_2-26, H_2-27), 4.15 (1H, br, 7-OH), 3.84 (1H, d, *J*=5.9 Hz, H-6), 3.57 (1H, m, H-17), 3.39 (1H, m, H-2), 3.07 (1H, m, H-25), 1.97~2.55 (8H, m, H-12, H-13, H_2-16, H-20, C(26)-OCOCH_2C*H*), 1.54 (3H, br, H_3-29), 1.00 (3H, d, *J*=6.2 Hz, H_3-28), 0.99 (3H, t, *J*=6.6 Hz, H_3-32), 0.95 (6H, d, *J*=6.6 Hz, C(26)OCOCH$_2$CH(CH_3)$_2$), 0.92 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, *J*=6.2 Hz, H_3-30), 0.14 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.75~1.95 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 756 (M^+), 738, 699, 681, 654, 636, 597, 579; HREI-MS (*m/z*): [M^+]: calcd. for $C_{43}H_{68}O_9Si$, 756.4633; found, 756.4632.

17a: IR ν_{max} (film) cm^{-1}: 3460, 2955, 2925, 2885, 2855, 1735, 1715; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.70~5.87 (2H, m, H-9, H-10), 5.65 (1H, br, H-3), 5.25~5.47 (2H, m, H-11, H-19), 4.98 (1H, m, H-15), 4.55~4.73 (5H, m, H-5, H_2-26, H_2-27), 4.12 (1H, br, 7-OH), 3.85 (1H, d, *J*=5.5 Hz, H-6), 3.55 (1H, m, H-17), 3.39 (1H, m, H-2), 3.26 (1H, dd, *J*=9.5, 6.6 Hz, H-25), 2.36 (2H, q, *J*=7.7 Hz, C(26)-OCOCH$_2$), 2.12~2.52 (4H, m, H-12, H-13, H_2-16), 2.05 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.16 (3H, d, *J*=6.2 Hz, H_3-28), 1.15 (3H, t, *J*=7.7 Hz, C(26)OCOCH$_2$CH_3), 1.00 (3H, d, *J*=6.6 Hz, H_3-31), 0.91 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, *J*=6.6 Hz, H_3-30), 0.13 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.80~1.95 (9H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 714 (M^+), 657, 640, 562; HREI-MS (*m/z*): [M^+]: calcd. for $C_{40}H_{62}O_9Si$, 714.4163; found, 714.4163.

17b: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2925, 2880, 2850, 1735, 1710; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.61~5.85 (3H, m, H-3, H-9, H-10), 5.25~5.50 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.52~4.73 (5H, m, H-5, H_2-26, H_2-27), 4.19 (1H, s, 7-OH), 3.84 (1H, d, J=5.5 Hz, H-6), 3.58 (1H, m, H-17), 3.39 (1H, br, H-2), 3.07 (1H, m, H-25), 2.12~2.55 (4H, m, H-12, H-13, H_2-16), 2.34 (2H, q, J=7.7Hz, C(26)OCOCH_2), 2.05 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.15 (3H, t, J=7.7 Hz, C(26)OCOCH$_2$CH_3), 1.00 (3H, d, J=6.6 Hz, H_3-28), 0.98 (3H, t, J=7.0 Hz, H_3-32), 0.91 (9H, s, $(CH_3)_3$CSi), 0.82 (3H, d, J=6.2 Hz, H_3-30), 0.13 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.81~1.95 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 728 (M^+), 710, 671, 654, 636, 597, 579; HREI-MS (*m/z*): [M^+]: calcd. for $C_{41}H_{64}O_9Si$, 728.4320; found, 728.4319.

5-*O*-TBDMS-milbemycin α_{20} (**16a**)

To a stirred solution of tiglic acid (30.4 mg, 0.30 mmol) in CH_2Cl_2 (1 ml) was added oxalyl chloride (33 μl, 0.38 mmol) and *N*,*N*-dimethylformamide (DMF) (one drop) at ambient temperature under a nitrogen atmosphere. After stirring for 1 hour, the reaction mixture was evaporated under reduced pressure to give a crude residue of tiglyl chloride. To a solution of **12a** (50 mg, 0.08 mmol), pyridine (25 μl, 0.30 mmol) and 4-(dimethylamino)pyridine (9.8 mg, 0.08 mmol) in CH_2Cl_2 (1 ml) was added a solution of a crude residue of tiglyl chloride prepared by the above method in CH_2Cl_2 (1 ml) dropwise at ambient temperature under a nitrogen atmosphere. After stirring for 2 hours, the reaction mixture was poured into water, extracted with EtOAc, and the organic layer was washed with saturated $NaHCO_3$ aqueous solution, water and brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC to give 24.8 mg (44.1%) of **16a** as a colorless amorphous solid.

16a: IR ν_{max} (film) cm^{-1}: 3455, 2955, 2880, 2860, 1710, 1650; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 6.83~6.92 (1H, m, C(26)OCOC(CH$_3$)CH), 5.73~5.83 (2H, m, H-9, H-10), 5.66 (1H, m, H-3), 5.27~5.42 (2H, m, H-11, H-19), 4.99 (1H, m, H-15), 4.55~4.79 (5H, m, H-5, H_2-26, H_2-27), 4.21 (1H, br, 7-OH), 3.85 (1H, d, J=5.5 Hz, H-6), 3.55 (1H, m, H-17), 3.41 (1H, m, H-2), 3.26 (1H, dd, J=9.5, 6.2 Hz, H-25), 2.43 (1H, m, H-12), 2.15~2.30 (3H, m, H-13, H_2-16), 2.02 (1H, dd, J=11.7, 3.3 Hz, H-20), 1.84 (3H, d, J=1.5 Hz, C(26)OCOCCH$_3$), 1.80 (3H, d, J=7.0 Hz, C(26)OCOC(CH$_3$)CHCH_3), 1.54 (3H, br, H_3-29), 1.14 (3H, d, J=6.2 Hz, H_3-31), 1.00 (3H, d, J=6.6 Hz, H_3-28), 0.91 (9H, s, $(CH_3)_3$CSi), 0.83 (3H, d, J=6.6 Hz, H_3-30), 0.13 (3H, s, CH_3Si), 0.11 (3H, s, CH_3Si), 0.89~1.95 (9H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 740 (M^+), 722, 683, 665, 640, 622, 583, 565; HREI-MS (*m/z*): [M^+]: calcd. for $C_{42}H_{64}O_9Si$, 740.4320; found, 740.4321.

5-*O*-TBDMS-milbemycin α_{21} (**16b**)

Using the same procedure described for the preparation of **16a**, **16b** was prepared from **12b** in 47.7% yield as a colorless amorphous solid.

16b: IR ν_{max} (film) cm^{-1}: 3465, 2960, 2925, 2880, 2860, 1710, 1645; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 6.83~6.92 (1H, m, C(26)OCOC(CH$_3$)CH), 5.71~5.83 (2H, m, H-9, H-10), 5.66 (1H, br, H-3), 5.25~5.48 (2H, m, H-11, H-19), 4.99 (1H, m, H-15), 4.55~4.79 (5H, m, H-5, H_2-26, H_2-27), 4.21 (1H, br, 7-OH), 3.84 (1H, d, J=5.5 Hz, H-6), 3.56 (1H, m, H-17), 3.41 (1H, m, H-2), 3.08 (1H, dt, J_t=9.2 Hz, J_d=2.2 Hz, H-25), 2.42 (1H, m, H-12), 2.15~2.30 (3H, m, H-13, H_2-16), 2.02 (1H, m, H-20), 1.84 (3H, d, J=1.5 Hz, C(26)OCOCCH$_3$), 1.80 (3H, d, J=7.3 Hz, C(26)OCOC(CH$_3$)CHCH_3), 1.55 (3H, br, H_3-29), 1.01 (3H, d, J=6.6 Hz, H_3-28), 0.99 (3H, t, J=7.0 Hz, H_3-32), 0.91 (9H, s, $(CH_3)_3$CSi), 0.83 (3H, d, J=6.6 Hz, H_3-30), 0.13 (3H, s, CH_3Si), 0.11 (3H, s, CH_3Si), 0.89~1.95 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 754 (M^+), 736, 697, 679, 654, 636, 597, 579; HREI-MS (*m/z*): [M^+]: calcd. for $C_{43}H_{66}O_9Si$, 754.4476; found, 754.4477.

5-*O*-TBDMS-26-methansulfonyloxymilbemycin A_3 (**18a**)

To a stirred solution of **12a** (100 mg, 0.15 mmol) in CH_2Cl_2 (2 ml) was added methanesulfonyl chloride (MsCl) (29 μl, 0.38 mmol) and Et_3N (68 μl, 0.61 mmol) while cooling with an ice bath under a nitrogen atmosphere. After the addition was complete, the ice bath was removed and the reaction mixture was stirred at ambient temperature for 30 minutes. The reaction mixture was poured into water and extracted with EtOAc, and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC to give 106.5 mg (95.2%) of **18a** as a colorless amorphous solid.

18a: IR ν_{max} (film) cm^{-1}: 3480, 2955, 2925, 2880, 2855, 1730, 1355, 1335, 1170; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.67~5.85 (3H, m, H-3, H-9, H-10), 5.25~5.44 (2H, m, H-11, H-19), 5.00 (1H, m, H-15), 4.89 (1H, d, J=11.7 Hz, H-26), 4.73 (1H, d, J=11.7 Hz, H-26), 4.70 (1H, d, J=14.9 Hz, H-27), 4.59 (1H, d, J=14.9 Hz, H-27), 4.15 (1H, m, H-5), 3.85 (1H, d, J=5.5 Hz, H-6), 3.69 (1H, s, 7-OH), 3.55 (1H,,m, H-17), 3.48 (1H, br, H-2), 3.25 (1H, dd, J=9.5, 6.2 Hz, H-25), 3.02 (3H, s, OSO_2CH_3), 2.45 (1H, m, H-12), 2.13~2.31 (3H, m, H-13, H_2-16), 2.05 (1H, m, H-

20), 1.54 (3H, br, H_3-29), 1.14 (3H, d, J=6.2 Hz, H_3-28), 1.00 (3H, d, J=6.6 Hz, H_3-31), 0.93 (9H, s, $(CH_3)_3CSi$), 0.83 (3H, d, J=6.2 Hz, H_3-30), 0.16 (3H, s, CH_3Si), 0.15 (3H, s, CH_3Si), 0.77~1.95 (9H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 736 (M^+), 718, 679, 661, 640, 622, 583; HREI-MS (*m/z*): [M^+−57]: calcd. for $C_{34}H_{51}O_{10}SSi$, 679.2972; found, 679.2962.

5-*O*-TBDMS-26-methansulfonyloxymilbemycin A_4 (**18b**)

Using the same procedure described for the preparation of **18a**, **18b** was prepared from **12b** in 85.7% yield as a colorless amorphous solid.

18b: IR ν_{max} (film) cm^{-1}: 3470, 2960, 2920, 2855, 1730, 1710, 1360, 1335, 1170; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.65~5.87 (3H, m, H-3, H-9, H-10), 5.25~5.52 (2H, m, H-11, H-19), 4.55~5.02 (6H, m, H-5, H-15, H_2-26, H_2-27), 4.18 (1H, s, 7-OH), 3.86 (1H, d, J=5.5 Hz, H-6), 3.57 (1H, m, H-17), 3.39 (1H, br, H-2), 3.08 (1H, m, H-25), 3.02 (3H, s, OSO_2CH_3), 2.44 (1H, m, H-12), 2.15~2.32 (3H, m, H-13, H_2-16), 2.05 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.00 (3H, d, J=6.6 Hz, H_3-28), 0.93 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.2 Hz, H_3-30), 0.16 (3H, s, CH_3Si), 0.15 (3H, s, CH_3Si), 0.78~1.95 (14H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31, H_3-32); EI-MS (*m/z*): 750 (M^+), 732, 693, 654, 636, 597; HREI-MS (*m/z*): [M^+]: calcd. for $C_{39}H_{62}O_9SSi$, 750.3833; found, 750.3835.

5-*O*-TBDMS-$\Delta^{2,3}$,$\Delta^{4,26}$-milbemycin A_3 (**19a**)

To a stirred solution of **18a** (60 mg, 0.08 mmol) in DMF (1 ml) was added Li_2CO_3 (18.1 mg, 0.24 mmol) at ambient temperature under a nitrogen atmosphere. After the addition was complete, the reaction temperature was increased to 120°C, and the reaction mixture was stirred for 20 minutes. The reaction mixture was poured into water, extracted with EtOAc, and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC to give 21.9 mg (42.0%) of **19a** as a colorless amorphous solid.

19a: IR ν_{max} (film) cm^{-1}: 3500, 2955, 2925, 2880, 2845, 1690; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 6.65 (1H, s, H-3), 6.11 (1H, dt, J_d=11.0 Hz, J_t=2.5 Hz, H-9), 5.61~5.79 (2H, m, H-10, H-27), 5.31~5.49 (3H, m, H-11, H-19, H-27), 4.95 (1H, t, J=6.7 Hz, H-15), 4.73 (1H, s, 7-OH), 4.61 (1H, d, J=2.4 Hz, H-5), 4.53 (1H, dd, J=14.3, 2.4 Hz, H-26), 4.43 (1H, dd, J=14.3, 2.4 Hz, H-26), 4.03 (1H, d, J=2.4 Hz, H-6), 3.62 (1H, m, H-17), 3.28 (1H, dd, J=9.5, 6.5 Hz, H-25), 2.40 (1H, m, H-12), 2.11~2.29 (3H, m, H-13, H_2-16), 1.49 (3H, br, H_3-29), 1.14 (3H, d, J=6.2 Hz, H_3-28), 0.99 (3H, d, J=7.0 Hz, H_3-31), 0.96 (9H, s, $(CH_3)_3CSi$), 0.84 (3H, d, J=6.6 Hz, H_3-30), 0.16 (3H, s, CH_3Si), 0.15 (3H, s, CH_3Si), 0.68~2.02 (10H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 640 (M^+), 622, 583, 565, 547; HREI-MS (*m/z*): [M^+]: calcd. for $C_{37}H_{56}O_7Si$, 640.3795; found, 640.3795.

5-*O*-TBDMS-$\Delta^{2,3}$,$\Delta^{4,26}$-milbemycin A_4 (**19b**)

Using the same procedure described for the preparation of **19a**, **19b** was prepared from **18b** in 49.4% yield as a colorless amorphous solid.

19b: IR ν_{max} (film) cm^{-1}: 3500, 2955, 2935, 2885, 2845, 1690; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 6.66 (1H, s, H-3), 6.13 (1H, dt, J_d=11.0 Hz, J_t=2.4 Hz, H-9), 5.63~5.76 (2H, m, H-10, H-27), 5.31~5.48 (3H, m, H-11, H-19, H-27), 4.93 (1H, m, H-15), 4.74 (1H, s, 7-OH), 4.62 (1H, m, H-5), 4.58 (1H, dd, J=13.9, 2.2 Hz, H-26), 4.43 (1H, dd, J=13.9, 2.2 Hz, H-26), 4.02 (1H, d, J=2.6 Hz, H-6), 3.63 (1H, m, H-17), 3.09 (1H, dt, J_t=9.2 Hz, J_d=2.4 Hz, H-25), 2.38 (1H, m, H-12), 2.10~2.30 (3H, m, H-13, H_2-16), 1.49 (3H, br, H_3-29), 1.01 (3H, d, J=6.6 Hz, H_3-28), 0.99 (3H, t, J=7.3 Hz, H_3-32), 0.96 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.2 Hz, H_3-30), 0.16 (3H, s, CH_3Si), 0.15 (3H, s, CH_3Si), 0.65~2.01 (12H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 654 (M^+), 636, 597, 579, 561; HREI-MS (*m/z*): [M^+]: calcd. for $C_{38}H_{58}O_7Si$, 654.3952; found, 654.3950.

$\Delta^{2,3}$,$\Delta^{4,26}$-Milbemycin A_3 (**10a**)

To a stirred solution of **19a** (11 mg, 0.02 mmol) in methanol (MeOH) (1 ml) was added *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) (4.9 mg, 0.03 mmol) at ambient temperature. After stirring for 2 hours, the reaction mixture was poured into water, extracted with EtOAc, and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC to give 8.2 mg (91.1%) of **10a** as a colorless amorphous solid.

10a: IR ν_{max} (film) cm^{-1}: 3490, 2955, 2925, 2890, 1690; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 6.66 (1H, s, H-3), 6.13 (1H, m, H-9), 5.62~5.80 (2H, m, H-10, H-27), 5.30~5.49 (3H, m, H-11, H-19, H-27), 4.98 (1H, m, H-15), 4.75 (1H, s, 7-OH), 4.59 (1H, dd, J=14.3, 2.5 Hz, H-26), 4.50 (1H, m, H-5), 4.46 (1H, dd, J=14.3, 2.2 Hz, H-26), 4.14 (1H, d, J=1.5 Hz, H-6), 3.62 (1H, m, H-17), 3.28 (1H, dd, J=9.5, 6.2 Hz, H-25), 2.40 (1H, m, H-12), 2.05~2.30 (4H, m, H-13, H_2-16, 5-OH), 1.48 (3H, br, H_3-29), 1.14 (3H, d, J=6.2 Hz, H_3-28), 0.99 (3H, d, J=6.6 Hz, H_3-31), 0.83 (3H, d, J=6.6 Hz, H_3-30), 0.65~2.05 (10H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 526 (M^+), 508, 490; HREI-MS (*m/z*): [M^+]: calcd. for $C_{31}H_{42}O_7$, 526.2931;

found, 526.2932.

$\Delta^{2,3}$,$\Delta^{4,26}$-Milbemycin A_4 (**10b**)

Using the same procedure described for the preparation of **10a**, **10b** was prepared from **19b** in 98.1% yield as a colorless amorphous solid.

10b: IR ν_{max} (film) cm^{-1}: 3490, 2955, 2925, 2875, 1690; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 6.66 (1H, s, H-3), 6.16 (1H, dt, J_d=11.0 Hz, J_t=2.4 Hz, H-9), 5.70 (2H, m, H-10, H-27), 5.30~5.53 (3H, m, H-11, H-19, H-27), 4.95 (1H, m, H-15), 4.76 (1H, s, 7-OH), 4.61 (1H, dd, J=14.1, 2.4 Hz, H-26), 4.60 (1H, m, H-5), 4.46 (1H, dd, J=14.1, 2.4 Hz, H-26), 4.14 (1H, d, J=2.2 Hz, H-6), 3.61 (1H, m, H-17), 3.09 (1H, dt, J_t=9.0 Hz, J_d=2.4 Hz, H-25), 2.10~2.51 (5H, m, H-12, H-13, H_2-16, 5-OH), 1.48 (3H, br, H_3-29), 1.03 (3H, d, J=6.2 Hz, H_3-28), 1.01 (3H, t, J=7.1 Hz, H_3-32), 0.83 (3H, d, J=6.2 Hz, H_3-30), 0.67~2.05 (12H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 540 (M^+), 522, 504; HREI-MS (*m/z*): [M^+]: calcd. for $C_{32}H_{44}O_7$, 540.3087; found, 540.3086.

Acaricidal Activity against *Tetranychus urticae*

The primary leaves of cowpea plants (*Vigna sinensis Savi* species) were infected with the organic phosphate-sensitive two-spotted spider mites (*Tetranychus urticae*). One day after infection, the infested plants were sprayed (Mizuho rotary sprayer) with 7 ml of a solution containing the test compound at concentrations ranging from 1 to 10 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 numbers of living and dead individuals. Two plants were used for each concentration and each test compound. The plants were kept during the test in green-house compartments at 25°C. The results are reported in Table 2.

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