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Synthesis of Novel 26-Substituted Milbemycin A_4 Derivatives and Their Acaricidal Activities

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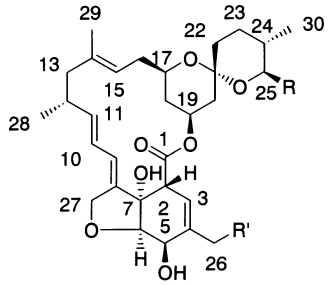
A series of novel 26-substituted milbemycin A_4 derivatives was synthesized from 5-*O*-*t*-butyldimethylsilyl-26-hydroxymilbemycin A_4 prepared by selenium dioxide oxidation of 5-*O*-*t*-butyldimethylsilyl-milbemycin A_4. Their acaricidal activities were assessed against the organophosphorus-sensitive two-spotted spider mite (*Tetranychus urticae*) on the primary leaves of cowpea plants (*Vigna sinesis Savi* species) by spraying.

Milbemycins[1~7] are a family of 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 milbemycin A_3 (**1**) and A_4 (**2**) (Figure 1)] was developed as an agricultural acaricide. Since the discovery of milbemycins, enormous efforts have been made to search for homologues that possess the same sixteen-membered macrolide moieties from nature. These efforts have been fruitful and have led to the isolation and documentation of numerous congeners, including Merck's avermectins[9,10] and Cyanamide's LL-F28249 (nemadectins).[11] Milbemycins α_{11} (**3**) and α_{14} (**4**) (Figure 1), another pair of congeners, have been reported as natural products.[12] The structures of milbemycins α_{11} (**3**) and α_{14} (**4**) are characterized by the presence of 3-methyl-2-butenoyloxy groups at their C-26 positions. Milbemycins α_{11} (**3**) and α_{14} (**4**) also have been found to possess potent acaricidal activities superior to those of milbemycin A_3 (**1**) and A_4 (**2**), and the effects of the substituent at the C-26 position of the milbemycin framework have captured the interest of researchers working with these agents. To clarify these effects, we prepared a series of 26-substituted milbemycin A_4 derivatives from 5-*O*-*t*-butyldimethylsilyl-26-hydroxymilbemycin A_4 (5-OTBDMS-26-OH-milbemycin A_4, **5**, Scheme 1) as a key intermediate,[13] and assessed their acaricidal activities. In this paper we report the results

Fig. 1. Structures and numbering of milbemycins.



Milbemycin A_3 (**1**): R=$C^{(31)}H_3$, R'=H
Milbemycin A_4 (**2**): R=$C^{(31)}H_2C^{(32)}H_3$, R'=H
Milbemycin α_{11} (**3**): R=Me, R'=$OCOCHCMe_2$
Milbemycin α_{14} (**4**): R=Et, R'=$OCOCHCMe_2$

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and preliminary structure-activity relationships of these newly synthesized derivatives.

Results and Discussion

Chemistry

The 26-substituted milbemycin A_4 derivatives were prepared as follows (Scheme 1). First of all, a primary hydroxy group of 5-OTBDMS-26-OH-milbemycin A_4 (**5**) derived from milbemycin A_4 (**2**)[13] was acylated with acetyl chloride (AcCl) and benzoyl chloride (BzCl) in the presence of triethylamine (Et_3N) to afford corresponding 26-*O*-acylated products (**6**, **7**) in good yields.[13] The 5-OTBDMS groups were removed by hydrogen fluoride-pyridine (HF/Py) to give non-natural 26-acyloxymilbemycin A_4 derivatives (**12**, **13**) in good yields.[13]

Etherifications of **5** were carried out with methyl iodide (MeI)[14] and benzyl bromide (BnBr)[15] in the presence of silver(I) oxide (Ag_2O) to afford **8** and **9**, respectively, and subsequent deprotections of **8** and **9** yielded 26-alkoxymilbemycin A_4 derivatives (**14**, **15**).

Diethyl phosphate derivative **10** was prepared from **5** with diethyl chlorophosphate [$ClPO(OEt)_2$] and pyridine (Py),[16] then subsequent deprotection of **10** afforded 26-diethylphospholyloxymilbemycin A_4 (**16**) in good yield.

The fluorine atom was introduced at the C-26 position of milbemycin A_4 (**2**) by the following method. Fluorination of the C-26 primary hydroxy group of **5** by (diethylamino)sulfur trifluoride (DAST)[17] afforded **11**, and subsequent deprotection of **11** gave 26-fluoromilbemycin A_4 (**17**).

The C-26 methyl group of milbemycin A_4 (**2**) was transformed to an oxime moiety by the following method. Oxidation of C-26 allyl alcohol of **5** with manganese dioxide (MnO_2)[18] produced 5-OTBDMS-4-formylmilbemycin A_4 (**18**) in good yield. Oximation[18] of this formyl group with hydroxylamine hydrochloride ($HONH_2 \cdot HCl$) or *O*-methylhydroxylamine hydrochloride ($MeONH_2 \cdot HCl$) and subsequent deprotection provided two corresponding oxime derivatives (**21**, **22**).

In separate experiments, we also examined the introduction of substituents to the C-26 position of the milbemycin framework by nucleophilic substitution. The reactions of various sodium salts of thiocarboxylic acids with 5-OTBDMS-26-methanesulfonyloxymilbemycin A_4 (**23**) derived from **5**[13] afforded corresponding 26-acylthio-5-OTBDMS-milbemycin A_4 derivatives (**24**, **25**, **26**), and subsequent deprotection of these derivatives gave corresponding 26-acylthiomilbemycin A_4 derivatives (**28**, **29**, **30**).

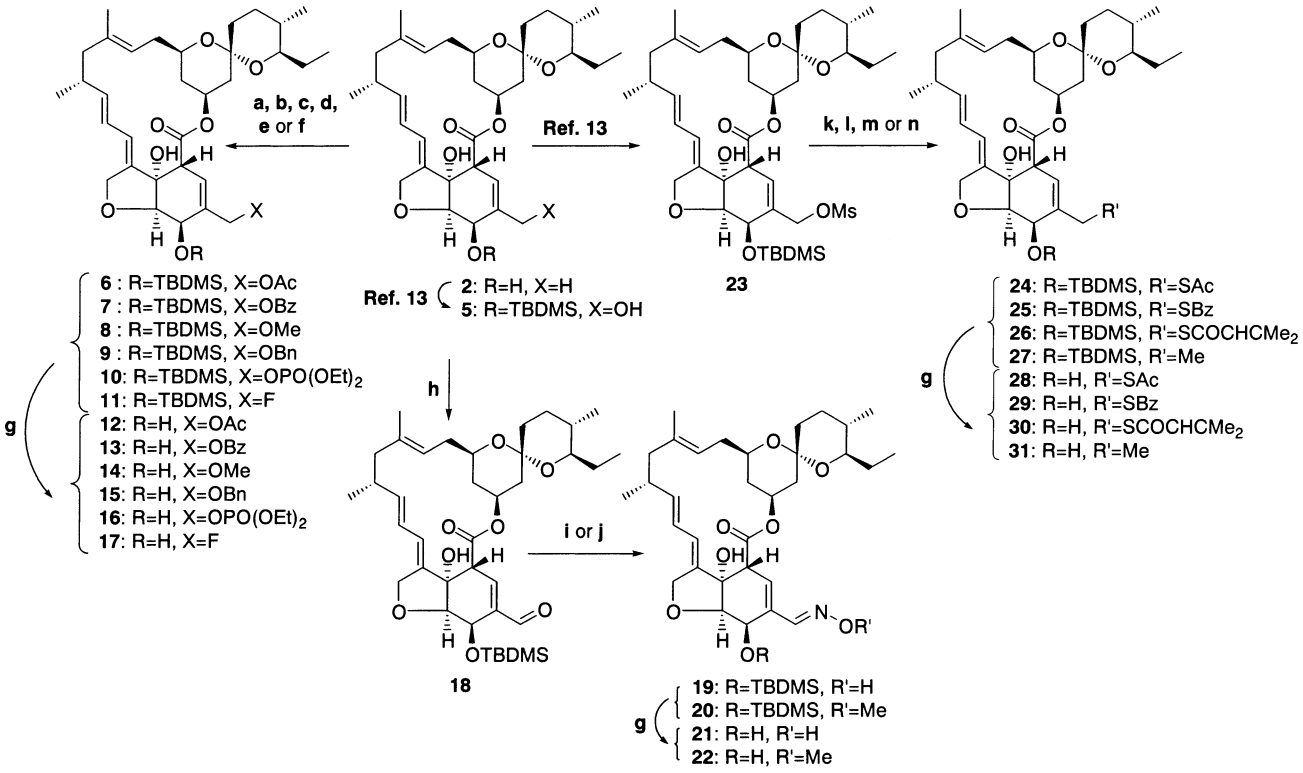
Carbon chain elongation at the C-26 position of milbemycin A_4 (**2**) was achieved by reacting **23** with trimethylaluminum (Me_3Al)[19] to produce 5-OTBDMS-26-methylmilbemycin A_4 (**27**), then deprotecting the C-5 hydroxy group to afford 26-methylmilbemycin A_4 (**31**).

Acaricidal Activities

The acaricidal activities of the prepared milbemycin A_4 C-26 derivatives were assessed against the organophosphorus-sensitive two-spotted spider mite (*Tetranychus urticae*) on the primary leaves of cowpea plants (*Vigna sinesis Savi* species) by spraying. The results are listed in the Table 1. Just as milbemycin α_{14} (**4**) possessed higher acaricidal activity than milbemycin A_4 (**2**), the non-natural type 26-acyloxymilbemycin derivatives (**12**, **13**) individually showed higher acaricidal activities than their parent compound (**2**). Moreover, the activity of **13** was superior to that of **12**, hence the substituent of the acyloxy group at the C-26 position possessing a certain range of steric bulkiness was deemed to be preferable. The increase of the activity of 26-benzyloxymilbemycin A_4 (**15**) and the small decrease of the activity of the 26-methoxymilbemycin A_4 (**14**) also supported this contention. Similarly, the drop of the activity of 26-acetylthiomilbemycin A_4 (**28**) and the enhancement of the activities of 26-benzoylthiomilbemycin A_4 (**29**) and 26-(3-methyl-2-butenoylthio)-milbemycin A_4 (**30**) were also consistent with this trend. At the same time, these results showed that ester moiety was not always essential for high acaricidal activity as a substituent at the C-26 position.

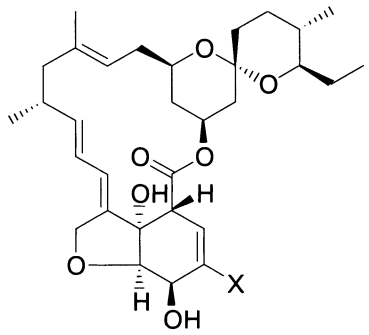
On the other hand, the activity of 26-diethylphosphnyloxymilbemycin A_4 (**16**) was reduced. We speculated that the increased molecular polarity of **16** might inhibit the migration of **16** to the target site. Nevertheless, 26-fluoromilbemycin A_4 (**17**) and 26-methylmilbemycin A_4 (**31**), a pair of derivatives that possessed lower molecular polarities (increased lipophilicities) than milbemycin A_4 (**2**), did not significantly increase the activities. These results might suggest that the steric bulkiness of the fluorine atom and the methyl group was not adequate as a substituent at the C-26 position to increase activities. Introductions of oxime moieties to the C-26 positions did not effectively increase the acaricidal activities of the derivatives (**21**, **22**). We also speculated that the high molecular polarity of the free oxime derivative **21** might have explained the markedly decrease in the activity of **21** compared to that of the *O*-methyloxime derivative **22**.

Scheme 1. Synthesis of milbemycin derivatives at C-26 positions.



Reagents: (a) AcCl, Et_3N; 77 % for **6**; (b) BzCl, Et_3N; 84 % for **7**; (c) MeI, Ag_2O; 74 % for **8**; (d) BnBr, Ag_2O; 27 % for **9**; (e) $ClPO(OEt)_2$, Py; 64 % for **10**; (f) DAST; 46 % for **11**; (g) HF/Py; 62 % for **12**, 46 % for **13**, 87 % for **14**, 59 % for **15**, 53 % for **16**, 38 % for **17**, 43 % for **21**, 39 % for **22**, 50 % for **28**, 65 % for **29**, 65 % for **30**, 54 % for **31**; (h) MnO_2; 75 % for **18**; (i) $HONH_2$·HCl; 61 % for **19**; (j) $MeONH_2$·HCl; 53 % for **20**; (k) AcSH, NaH, NaI; 78 % for **24**; (l) BzSH, NaH, NaI; 75 % for **25**; (m) $Me_2CCHCOSH$, NaH, NaI; 81 % for **26**; (n) Me_3Al; 43 % for **27**.

Table 1. Acaricidal activities of milbemycin derivatives against the two-spotted spider mite.



Compound	X	Mortality(%) 10ppm	1ppm
12	CH_2OAc	100	38
13	CH_2OBz	100	100
14	CH_2OMe	95	5
15	CH_2OBn	100	100
16	$CH_2OPO(OEt)_2$	67	15
17	CH_2F	100	40
21	CHNOH	52	0
22	$CHNOCH_3$	90	42
28	CH_2SAc	87	23
29	CH_2SBz	100	100
30	$CH_2SCOCHCMe_2$	100	100
31	Et	100	53
Milbemycin A_4 (2)	Me	100	32
Milbemycin α_{14} (4)	$CH_2OCOCHCMe_2$	100	100

Conclusion

In conclusion, we established versatile methods to prepare various 26-substituted milbemycin A_4 derivatives using 5-OTBDMS-26-OH-milbemycin A_4 (**5**), a compound derived from milbemycin A_4 (**2**), as a key intermediate. In assessing the acaricidal activities of the synthesized derivatives, we discovered that some of them possessed high acaricidal activity equivalent to that of milbemycin α_{14} (**4**). Evaluation of the structure-activity relationships of the synthesized compounds indicated that an adequate steric bulkiness and suitable lipophilicity are preferable as properties of the substituent at the C-26 position.

We would like to continue comparing the practical performance of milbemycin α_{14} (**4**) and the high active compounds reported in this paper. We also would like to continue researching new derivatives that possess improved activity based on the structure-activity relationship information clarified in this study.

Experimental

NMR spectra were measured on a Varian Gemini-200 FT NMR Spectrometer (200 MHz) or a JEOL JNM-GX-270 FT NMR Spectrometer (270 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 Shimadzu FTIR-8400.

5-OTBDMS-26-acetoxymilbemycin A_4 (**6**). To a stirred solution of 50 mg (0.07 mmol) of 5-OTBDMS-26-hydroxymilbemycin A_4 (**5**) in dichloromethane (CH_2Cl_2, 2 ml) was added 8 μl (0.11 mmol) of AcCl and 15 μl (0.11 mmol) of Et_3N at ambient temperature. After stirring for 15 minutes, the reaction mixture was poured into water and extracted with ethyl acetate (EtOAc). The extract was successively washed with water and brine, dried over magnesium sulfate ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC to give 41 mg (77%) of **6** as a colorless amorphous solid.

6: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2930, 2860, 1740, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.66～5.81 (3H, m, H-3, H-9, H-10), 5.31～5.43 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.54～4.71 (5H, m, H-5, H_2-26, H_2-27), 4.18 (1H, s, 7-OH), 3.84 (1H, d, J=5.4 Hz, H-6), 3.58 (1H, m, H-17), 3.39 (1H, m, H-2), 3.07 (1H, m, H-25), 2.40 (1H, m, H-12), 2.15～2.28 (3H, m, H-13, H_2-16), 2.07 (3H, s, 26-OAc), 2.02 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.00 (3H, d, J=6.2 Hz, H_3-28), 0.98 (3H, t, J=7.4 Hz, H_3-32), 0.91 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.2 Hz, H_3-30), 0.13 (3H, s, CH_3Si), 0.12 (3H, s, CH_3Si), 0.80～1.90 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m*/*z*): 714 (M^+), 696, 654, 639, 597, 579, 564, 195, 167, 151; HREI-MS (*m*/*z*): [M^+]: calcd. for $C_{40}H_{62}O_9Si$, 714.4163; found, 714.4162.

5-OTBDMS-26-benzoyloxymilbemycin A_4 (**7**). To a stirred solution of 50 mg (0.07 mmol) of **5** in CH_2Cl_2 (2 ml) was added 13 μl (0.11 mmol) of BzCl and 15 μl (0.11 mmol) of Et_3N at ambient temperature. After stirring for 40 minutes, the reaction mixture was poured into water and extracted with EtOAc. The extract was successively washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC to give 49 mg (84%) of **7** as a colorless amorphous solid.

7: IR ν_{max} (film) cm^{-1}: 3460, 2955, 2930, 2860, 1720; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.03 (2H, d, J=7.4 Hz, Ar), 7.55 (1H, d, J=7.4 Hz, Ar), 7.44 (2H, t, J=7.4 Hz, Ar), 5.72～5.83 (3H, m, H-3, H-9, H-10), 5.30～5.45 (2H, m, H-11, H-19), 4.80～4.96 (3H, m, H-15, H_2-26), 4.57～4.78 (3H, m, H-5, H_2-27), 4.23 (1H, s, 7-OH), 3.88 (1H, d, J=5.5 Hz, H-6), 3.58 (1H, m, H-17), 3.43 (1H, m, H-2), 3.07 (1H, m, H-25), 2.45 (1H, m, H-12), 2.15～2.30 (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=6.6 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=6.3Hz, H_3-30), 0.12 (3H, s, CH_3Si), 0.11 (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*): 776 (M^+), 719, 701, 654, 597, 414, 245, 195, 167, 151; HREI-MS (*m*/*z*): [M^+]: calcd. for $C_{45}H_{64}O_9Si$, 776.4320; found, 776.4319.

5-OTBDMS-26-methoxymilbemycin A_4 (**8**). To a stirred solution of 100 mg (0.15 mmol) of **5** in 1,2-dichloroethane (CH_2ClCH_2Cl, 2 ml) was added 460 μl (7.45 mmol) of MeI and 345 mg (1.49 mmol) of Ag_2O at ambient temperature. After stirring overnight, the reaction mixture was filtered with Celite®, and the resulting filtrate was evaporated under reduced pressure. The residue was purified by preparative TLC to give 75 mg (74%) of **8** as a colorless amorphous solid.

8: IR ν_{max} (film) cm^{-1}: 3460, 2955, 2930, 2860, 1735, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.67～5.82 (2H, m, H-9, H-10), 5.62 (1H, br, H-3), 5.28～5.42 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.55～4.71 (3H, m, H-26, H_2-27), 4.22 (1H, s, 7-OH), 4.14 (1H, d, J=13.6 Hz, H-26), 3.81 (2H, m, H-5, H-6), 3.58 (1H, m, H-17), 3.39 (1H, m, H-2), 3.30 (3H, s, 26-OMe), 3.05 (1H, dt, J_t=9.2 Hz, J_d=2.7 Hz, H-25), 2.40 (1H, m, H-12), 2.12～2.28 (3H, m,

H-13, H_2-16), 1.98 (1H, m, H-20), 1.54 (3H, br, H_3-29), 0.99 (3H, d, J=6.4 Hz, H_3-28), 0.92 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.4 Hz, H_3-30), 0.13 (6H, s, $(CH_3)_2Si$), 0.70～1.90 (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*): 686 (M^+), 654, 629, 611, 536, 414, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{39}H_{62}O_8Si$, 686.4214; found, 686.4213.

5-OTBDMS-26-benzyloxymilbemycin A_4 (**9**). To a stirred solution of 152 mg (0.23 mmol) of **5** in CH_2ClCH_2Cl (2 ml) was added 270 μl (2.27 mmol) of BnBr and 513 mg (2.21 mmol) of Ag_2O at ambient temperature. After stirring overnight, the reaction mixture was filtered with Celite®, and the resulting filtrate was evaporated under reduced pressure. The residue was purified by preparative TLC to give 46 mg (27%) of **9** as a colorless amorphous solid.

9: IR ν_{max} (film) cm^{-1}: 3460, 2955, 2930, 2855, 1745, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.27～7.40 (5H, m, Ar), 5.72～5.83 (2H, m, H-9, H-10), 5.67 (1H, br, H-3), 5.30～5.45 (2H, m, H-11, H-19), 4.95 (1H, m, H-15), 4.62 (3H, m, H-5, H_2-27), 4.49 (2H, s, 26-OCH_2), 4.20 (1H, d, J=12.8 Hz, H-26), 4.18 (1H, s, 7-OH), 3.94 (1H, d, J=12.8 Hz, H-26), 3.83 (1H, d, J=5.7 Hz, H-6), 3.58 (1H, m, H-17), 3.40 (1H, m, H-2), 3.03 (1H, m, H-25), 2.41 (1H, m, H-12), 2.10～2.28 (3H, m, H-13, H_2-16), 2.02 (1H, m, H-20), 1.54 (3H, br, H_3-29), 0.99 (3H, d, J=6.4 Hz, H_3-28), 0.90 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.4 Hz, H_3-30), 0.11 (3H, s, CH_3Si), 0.10 (3H, s, CH_3Si), 0.70～1.95 (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*): 762 (M^+), 705, 687, 654, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{45}H_{66}O_8Si$, 762.4527; found, 762.4527.

5-OTBDMS-26-diethylphospholyloxymilbemycin A_4 (**10**). To a stirred solution of 100 mg (0.15 mmol) of **5** in CH_2Cl_2 (4 ml) was added 65 μl (0.45 mmol) of $ClPO(OEt)_2$ and 36 μl (0.45 mmol) of Py at ambient temperature. After stirring overnight, the reaction mixture was poured into water and extracted with EtOAc. The extract was successively washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC to give 77 mg (64%) of **10** as a colorless amorphous solid.

10: IR ν_{max} (film) cm^{-1}: 3325, 2955, 2930, 2860, 1740; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.68～5.87 (3H, m, H-3, H-9, H-10), 5.22～5.43 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.50～4.75 (5H, m, H-5, H_2-26, H_2-27), 4.02～4.22 (5H, m, 7-OH, 26-$OPO(OC\underline{H}_2CH_3)_2$), 3.84 (1H, d, J=5.5 Hz, H-6), 3.58 (1H, m, H-17), 3.38 (1H, br, H-2), 3.07 (1H, m, H-25), 2.42 (1H, m, H-12), 2.15～2.30 (3H, m, H-13, H_2-16), 2.08 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.35 (3H, t, J=6.9 Hz, 26-$OPO(OCH_2C\underline{H}_3)$), 1.32 (3H, t, J=6.9 Hz, 26-$OPO(OCH_2C\underline{H}_3)$), 1.00 (3H, d, J=6.6 Hz, H_3-28), 0.96 (3H, t, J=7.1 Hz, H_3-32), 0.92 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.3 Hz, H_3-30), 0.14 (6H, s, $(CH_3)_2Si$), 0.75～1.90 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 808 (M^+), 751, 654, 597, 414, 195, 167; HREI-MS (*m/z*): [M^+]: calcd. for $C_{42}H_{69}O_{11}PSi$, 808.4347; found, 808.4346.

5-OTBDMS-26-fluoromilbemycin A_4 (**11**). To a stirred solution of 150 mg (0.22 mmol) of **5** in CH_2Cl_2 (6 ml) was added 32 μl (0.24 mmol) of DAST under a nitrogen atmosphere while cooling with a dry ice-acetone bath. After stirring for 20 minutes, the reaction mixture was poured into water and extracted with EtOAc. The extract was successively washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC to give 69 mg (46%) of **11** as a colorless amorphous solid.

11: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2930, 2860, 1715, 1180; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.68～5.83 (3H, m, H-3, H-9, H-10), 5.30～5.48 (2H, m, H-11, H-19), 5.15 (1H, m, H-26), 4.85～5.03 (3H, m, H-15, H-26), 4.55～4.75 (3H, m, H-5, H_2-27), 4.18 (1H, s, 7-OH), 3.86 (1H, d, J=5.2 Hz, H-6), 3.58 (1H, m, H-17), 3.38 (1H, br, H-2), 3.07 (1H, dt, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 2.42 (1H, m, H-12), 2.12～2.28 (3H, m, H-13, H_2-16), 2.00 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.00 (3H, d, J=6.7 Hz, H_3-28), 0.92 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.3 Hz, H_3-30), 0.14 (6H, s, $(CH_3)_2Si$), 0.75～1.95 (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*): 674 (M^+), 654, 617, 599, 195, 167; HREI-MS (*m/z*): [M^+]: calcd. for $C_{38}H_{59}FO_7Si$, 674.4014; found, 674.4013.

26-Acetoxymilbemycin A_4 (**12**). To a stirred solution of 40 mg (0.06 mmol) of **6** in acetonitrile (2 ml) was added HF/Py (HF=70%, 500 μl) at ambient temperature. After stirring for 2 hours, the reaction mixture was poured into saturated aqueous sodium bicarbonate solution and extracted with EtOAc. The extract was successively 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 mg (62%) of **12** as a colorless amorphous solid.

12: IR ν_{max} (film) cm^{-1}: 3460, 2955, 2930, 2875, 1735; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.75～5.84 (2H, m, H-9, H-10), 5.73 (1H, br, H-3), 5.35～5.45 (2H, m, H-11, H-19), 4.99 (1H, m, H-15), 4.63～4.80 (3H, m, H-26, H_2-27), 4.50 (1H, m, H-5), 4.12 (1H, s, 7-OH), 4.08～4.16 (1H, m, H-26), 4.00 (1H, d, J=6.2 Hz, H-6), 3.58 (1H, m, H-17), 3.32 (1H, t, J=2.0 Hz, H-2), 3.07 (1H, dt, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 2.62 (1H, d, J=7.7 Hz, 5-OH), 2.42 (1H, m, H-12), 2.15～2.28 (3H, m, H-13, H_2-16), 2.09 (3H, s, 26-OAc),

2.01 (1H, m, H-20), 1.53 (3H, br, H_3-29), 1.00 (3H, d, J=7.4 Hz, H_3-28), 0.99 (3H, t, J=7.7 Hz, H_3-32), 0.83 (3H, d, J=6.7 Hz, H_3-30), 0.80～1.90 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 600 (M^+), 540, 414, 396, 356, 314, 264, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{34}H_{48}O_9$, 600.3298; found, 600.3299.

Using the same procedure described for the preparation of **12**, the other 5-OTBDMS-26-substituted-milbemysins A_4 derivatives (**7**, **8**, **9**, **10**, **11**, **19**, **20**, **24**, **25**, **26** and **27**) were deprotected to give corresponding milbemycins A_4 derivatives (**13**, **14**, **15**, **16**, **17**, **21**, **22**, **28**, **29**, **30** and **31**) as colorless amorphous solids. Yields are described in Scheme 1.

26-Benzoyloxymilbemycin A_4 (**13**): IR ν_{max} (film) cm^{-1}: 3460, 2955, 2925, 2870, 1720; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.06 (2H, d, J=7.4 Hz, Ar), 7.56 (1H, t, J=7.4 Hz, Ar), 7.44 (2H, t, J=7.4 Hz, Ar), 5.71～5.88 (3H, m, H-3, H-9, H-10), 5.32～5.48 (2H, m, H-11, H-19), 4.91～5.05 (3H, m, H-15, H_2-26), 4.71 (2H, br, H_2-27), 4.56 (1H, m, H-5), 4.13 (1H, s, 7-OH), 4.02 (1H, d, J=6.1 Hz, H-6), 3.58 (1H, m, H-17), 3.35 (1H, br, H-2), 3.07 (1H, m, H-25), 2.70 (1H, d, J=7.1 Hz, 5-OH), 2.43 (1H, m, H-12), 2.13～2.28 (3H, m, H-13, H_2-16), 2.00 (1H, m, H-20), 1.53 (3H, br, H_3-29), 1.01 (3H, d, J=7.1 Hz, H_3-28), 0.99 (3H, t, J=8.2 Hz, H_3-32), 0.82 (3H, d, J=6.3 Hz, H_3-30), 0.78～1.90 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 662 (M^+), 540, 414, 264, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{39}H_{50}O_9$, 662.3455; found, 662.3455.

26-Methoxymilbemycin A_4 (**14**): IR ν_{max} (film) cm^{-1}: 3460, 2955, 2925, 2875, 1730, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.70～5.85 (3H, m, H-3, H-9, H-10), 5.31～5.48 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.70 (2H, br, H_2-27), 4.52 (1H, t, J=6.4 Hz, H-5), 4.08～4.20 (2H, m, H_2-26), 4.03 (1H, s, 7-OH), 3.98 (1H, d, J=6.4 Hz, H-6), 3.58 (1H, m, H-17), 3.36 (3H, s, 26-OMe), 3.34 (1H, br, H-2), 3.06 (1H, dt, J_t=9.2 Hz, J_d=2.5 Hz, H-25), 2.73 (1H, d, J=6.4 Hz, 5-OH), 2.42 (1H, m, H-12), 2.12～2.30 (3H, m, H-13, H_2-16), 1.98 (1H, m, H-20), 1.53 (3H, br, H_3-29), 1.00 (3H, d, J=6.9 Hz, H_3-28), 0.98 (3H, t, J=7.4 Hz, H_3-32), 0.82 (3H, d, J=6.2 Hz, H_3-30), 0.75～1.90 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 572 (M^+), 414, 396, 356, 314, 264, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{33}H_{48}O_8$, 572.3349; found, 572.3350.

26-Benzyloxymilbemycin A_4 (**15**): IR ν_{max} (film) cm^{-1}: 3445, 2955, 2925, 2860, 1730, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.27～7.40 (5H, m, Ar), 5.70～5.88 (3H, m, H-3, H-9, H-10), 5.33～5.48 (2H, m, H-11, H-19), 4.97 (1H, m, H-15), 4.70 (2H, br, H_2-27), 4.57 (1H, m, H-5), 4.54 (2H, s, 26-OCH_2), 4.19 (1H, d, J=12.0 Hz, H-26), 4.13 (1H, s, 7-OH), 4.12 (1H, d, J=12.0 Hz, H-26), 3.99 (1H, d, J=6.2 Hz, H-6), 3.58 (1H, m, H-17), 3.35 (1H, m, H-2), 3.07 (1H, dt, J_t=9.2 Hz, J_d=2.6 Hz, H-25), 2.67 (1H, d, J=6.4 Hz, 5-OH), 2.41 (1H, m, H-12), 2.12～2.28 (3H, m, H-13, H_2-16), 2.00 (1H, m, H-20), 1.53 (3H, br, H_3-29), 1.01 (3H, d, J=6.7 Hz, H_3-28), 0.99 (3H, t, J=7.2 Hz, H_3-32), 0.82 (3H, d, J=6.7 Hz, H_3-30), 0.75～1.90 (11H, m, H-13, H_2-18, H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 648 (M^+), 612, 540, 414, 314, 264, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{39}H_{52}O_8$, 648.3662; found, 648.3662.

26-Diethylphospholyloxymilbemycin A_4 (**16**): IR ν_{max} (film) cm^{-1}: 3360, 2960, 2930, 2875, 1735; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.70～5.92 (3H, m, H-3, H-9, H-10), 5.28～5.45 (2H, m, H-11, H-19), 4.97 (1H, m, H-15), 4.69～4.82 (3H, m, H-26, H_2-27), 4.48～4.59 (2H, m, H-5, H-26), 4.06～4.22 (5H, m, 7-OH, 26-OPO($OC\underline{H}_2CH_3$)$_2$), 4.01 (1H, d, J=6.1 Hz, H-6), 3.58 (1H, m, H-17), 3.32 (1H, br, H-2), 3.08 (2H, m, H-25, 5-OH), 2.41 (1H, m, H-12), 2.15～2.30 (3H, m, H-13, H_2-16), 2.05 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.36 (3H, t, J=6.9 Hz, 26-OPO($OCH_2C\underline{H}_3$)), 1.33 (3H, t, J=6.9 Hz, 26-OPO($OCH_2C\underline{H}_3$)), 1.01 (3H, d, J=6.9 Hz, H_3-28), 0.99 (3H, t, J=7.7 Hz, H_3-32), 0.82 (3H, d, J=6.6 Hz, H_3-30), 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*): 694 (M^+), 540, 522, 414, 264, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{36}H_{55}O_{11}P$, 694.3482; found, 694.3482.

26-Fluoromilbemycin A_4 (**17**): IR ν_{max} (film) cm^{-1}: 3455, 2955, 2930, 2875, 1715, 1180; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.70～5.90 (3H, m, H-3, H-9, H-10), 5.32～5.51 (2H, m, H-11, H-19), 4.82～5.18 (3H, m, H-15, H_2-26), 4.70 (2H, br, H_2-27), 4.52 (1H, m, H-5), 4.14 (1H, s, 7-OH), 4.00 (1H, d, J=6.2 Hz, H-6), 3.58 (1H, m, H-17), 3.32 (1H, br, H-2), 3.07 (1H, dt, J_t=9.2 Hz, J_d=2.5 Hz, H-25), 2.35～2.50 (2H, m, H-12, 5-OH), 2.15～2.28 (3H, m, H-13, H_2-16), 1.99 (1H, m, H-20), 1.53 (3H, br, H_3-29), 1.01 (3H, d, J=6.9 Hz, H_3-28), 0.99 (3H, t, J=7.7 Hz, H_3-32), 0.82 (3H, d, J=6.4 Hz, H_3-30), 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*): 560 (M^+), 414, 264, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{32}H_{45}FO_7$, 560.3149; found, 560.3150.

26-Hydroxyiminomilbemycin A_4 (**21**): IR ν_{max} (film) cm^{-1}: 3365, 2955, 2925, 2855, 1755; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.78 (1H, s, H-26), 7.28 (1H, br, NOH), 5.99 (1H, d, J=2.5 Hz, H-3), 5.73～5.89 (2H, m, H-9, H-10), 5.33～5.50 (2H, m, H-11, H-19), 4.92～5.00 (2H, m, H-5, H-15), 4.73 (2H, br, H_2-27), 4.07 (1H, d, J=6.4 Hz, H-6),

4.02 (1H, s, 7-OH), 3.50～3.68 (2H, m, H-2, H-17), 3.07 (1H, m, H-25), 2.42 (1H, m, H-12), 2.15～2.30 (4H, m, H-13, H_2-16, 5-OH), 2.01 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.01 (3H, d, J=7.2 Hz, H_3-28), 0.99 (3H, t, J=7.7 Hz, H_3-32), 0.83 (3H, d, J=6.4 Hz, H_3-30), 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*): 571 (M^+), 553, 535, 414, 195, 167, 151; HREI-MS (*m*/*z*): [M^+]: calcd. for $C_{32}H_{45}NO_8$, 571.3145; found, 571.3145.

26-Methoxyiminomilbemycin A_4 (**22**): IR ν_{max} (film) cm^{-1}: 3450, 2955, 2930, 2875, 1730, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.71 (1H, s, H-26), 5.95 (1H, d, J=2.3 Hz, H-3), 5.72～5.88 (2H, m, H-9, H-10), 5.35～5.48 (2H, m, H-11, H-19), 4.91～5.02 (2H, m, H-5, H-15), 4.73 (2H, br, H_2-27), 4.06 (1H, d, J=5.9 Hz, H-6), 3.99 (1H, s, 7-OH), 3.92 (3H, s, NOMe), 3.70 (1H, d, J=2.3 Hz, H-2), 3.58 (1H, m, H-17), 3.08 (1H, m, H-25), 2.42 (1H, m, H-12), 2.12～2.28 (4H, m, H-13, H_2-16, 5-OH), 2.01 (1H, m, H-20), 1.54 (3H, br, H_3-29), 1.01 (3H, d, J=7.3 Hz, H_3-28), 0.99 (3H, t, J=7.6 Hz, H_3-32), 0.82 (3H, d, J=6.4 Hz, H_3-30), 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*): 585 (M^+), 567, 414, 245, 195, 167, 151; HREI-MS (*m*/*z*): [M^+]: calcd. for $C_{33}H_{47}NO_8$, 585.3302; found, 585.3303.

26-Acetylthiomilbemycin A_4 (**28**): IR ν_{max} (film) cm^{-1}: 3455, 2960, 2925, 2875, 1735, 1695; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.68～5.87 (3H, m, H-3, H-9, H-10), 5.32～5.48 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.68 (2H, br, H_2-27), 4.40 (1H, m, H-5), 4.10 (1H, s, 7-OH), 3.97 (1H, d, J=5.8 Hz, H-6), 3.82 (1H, d, J=14.3 Hz, H-26), 3.62 (1H, d, J=14.3 Hz, H-26), 3.55 (1H, m, H-17), 3.30 (1H, br, H-2), 3.07 (1H, m, H-25), 2.74 (1H, d, J=7.4 Hz, 5-OH), 2.41 (1H, m, H-12), 2.35 (3H, s, 26-SAc), 2.15～2.28 (3H, m, H-13, H_2-16), 2.02 (1H, m, H-20), 1.53 (3H, br, H_3-29), 1.01 (3H, d, J=6.9 Hz, H_3-28), 0.98 (3H, t, J=7.7 Hz, H_3-32), 0.82 (3H, d, J=6.6 Hz, H_3-30), 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*): 616 (M^+), 541, 414, 396, 264, 245, 195, 167, 151; HREI-MS (*m*/*z*): [M^+]: calcd. for $C_{34}H_{48}O_8S$, 616.3070; found, 616.3071.

26-Benzoylthiomilbemycin A_4 (**29**): IR ν_{max} (film) cm^{-1}: 3465, 2955, 2925, 2875, 1710, 1665; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.97 (2H, d, J=7.7 Hz, Ar), 7.58 (1H, m, Ar), 7.45 (2H, t, J=7.7 Hz, Ar), 5.70～5.85 (3H, m, H-3, H-9, H-10), 5.31～5.45 (2H, m, H-11, H-19), 4.95 (1H, m, H-15), 4.69 (2H, br, H_2-27), 4.50 (1H, m, H-5), 3.95～4.20 (3H, m, H-6, H-26, 7-OH), 3.82 (1H, d, J=14.3 Hz, H-26), 3.55 (1H, m, H-17), 3.32 (1H, br, H-2), 3.07 (1H, m, H-25), 2.86 (1H, br, 5-OH), 2.42 (1H, m, H-12), 2.12～2.28 (3H, m, H-13, H_2-16), 2.01 (1H, m, H-20), 1.53 (3H, br, H_3-29), 1.00 (3H, d, J=6.9 Hz, H_3-28), 0.98 (3H, t, J=7.9 Hz, H_3-32), 0.82 (3H, d, J=6.3 Hz, H_3-30), 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*): 678 (M^+), 414, 264, 245, 195, 167, 151; HREI-MS (*m*/*z*): [M^+]: calcd. for $C_{39}H_{50}O_8S$, 678.3226; found, 678.3227.

26-(3-Methyl-2-butenoylthio)-milbemycin A_4 (**30**): IR ν_{max} (film) cm^{-1}: 3470, 2960, 2925, 2875, 1715, 1675, 1630; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 6.00 (1H, br, 26-SCOCH), 5.68～5.90 (3H, m, H-3, H-9, H-10), 5.30～5.48 (2H, m, H-11, H-19), 4.95 (1H, m, H-15), 4.68 (2H, br, H_2-27), 4.46 (1H, m, H-5), 4.07 (1H, s, 7-OH), 3.97 (1H, d, J=6.1 Hz, H-6), 3.88 (1H, d, J=14.3 Hz, H-26), 3.58 (2H, m, H-17, H-26), 3.32 (1H, br, H-2), 3.07 (2H, m, H-25, 5-OH), 2.42 (1H, m, H-12), 2.11～2.29 (3H, m, H-13, H_2-16), 2.16 (3H, s, 26-SCOCHCC$\underline{H}_3$), 2.02 (1H, m, H-20), 1.88 (3H, s, 26-COCHCC$\underline{H}_3$), 1.53 (3H, br, H_3-29), 1.00 (3H, d, J=6.9 Hz, H_3-28), 0.97 (3H, t, J=7.7 Hz, H_3-32), 0.82 (3H, d, J=6.3 Hz, H_3-30), 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*): 656 (M^+), 522, 414, 264, 245, 195, 167, 151; HREI-MS (*m*/*z*): [M^+]: calcd. for $C_{37}H_{52}O_8S$, 656.3383; found, 656.3383.

26-Methylmilbemycin A_4 (**31**): IR ν_{max} (film) cm^{-1}: 3465, 2965, 2925, 2875, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.70～5.88 (2H, m, H-9, H-10), 5.33～5.50 (3H, m, H-3, H-11, H-19), 4.97 (1H, m, H-15), 4.70 (2H, br, H_2-27), 4.36 (1H, m, H-5), 4.09 (1H, s, 7-OH), 3.96 (1H, d, J=6.3 Hz, H-6), 3.58 (1H, m, H-17), 3.32 (1H, br, H-2), 3.08 (1H, m, H-25), 2.46 (1H, d, J=6.9 Hz, 5-OH), 1.53 (3H, br, H_3-29), 1.08 (3H, t, J=7.4 Hz, 26-CH3), 1.00 (3H, d, J=6.6 Hz, H_3-28), 0.98 (3H, t, J=6.9 Hz, H_3-32), 0.82 (3H, d, J=6.6 Hz, H_3-30), 0.75～2.42 (18H, m, H-12, H_2-13, H_2-16, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-26, H_2-31); EI-MS (*m*/*z*): 556 (M^+), 414, 314, 245, 195, 167, 151; HREI-MS (*m*/*z*): [M^+]: calcd. for $C_{33}H_{48}O_7$, 556.3400; found, 556.3400.

5-OTBDMS-4-formylmilbemycin A_4 (**18**). To a stirred solution of 300 mg (0.45 mmol) of **5** in CH_2Cl_2 (6 ml) was added 2.00 g (22.3 mmol) of MnO_2 at ambient temperature. After stirring for 90 minutes, the reaction mixture was filtered with Celite® and the resulting filtrate was evaporated under reduced pressure. The residue was purified by silica gel chromatography [*n*-hexane (Hex)-EtOAc gradient] to give 225 mg (75%) of **18** as a pale yellow amorphous solid.

18: IR ν_{max} (film) cm^{-1}: 3475, 2955, 2855, 1735, 1685; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.54 (1H, s, CHO), 6.90 (1H, d, J=3.3 Hz, H-3), 5.76～5.90 (2H, m, H-9, H-10), 5.38～5.54 (2H, m, H-11, H-19), 5.03 (1H, d, J=5.5 Hz, H-5), 4.97 (1H, m, H-15), 4.67 (2H, m, H_2-27), 3.89 (1H, s, 7-OH), 3.85 (2H, m, H-2, H-6), 3.60 (1H, m, H-17), 3.08 (1H, dt, J_t=9.2 Hz, J_d=2.6 Hz, H-25), 2.45 (1H, m, H-12),

2.18～2.31 (3H, m, H-13, H_2-16), 2.07 (1H, m, H-20), 1.55 (3H, br, H_3-29), 1.01 (3H, d, J=6.6 Hz, H_3-28), 1.00 (3H, t, J=7.3 Hz, H_3-32), 0.86 (9H, s, $(CH_3)_3CSi$), 0.83 (3H, d, J=6.6 Hz, H_3-30), 0.17 (3H, s, CH_3Si), 0.09 (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*): 670 (M^+), 613, 595, 195, 167; HREI-MS (*m/z*): [M^+]: calcd. for $C_{38}H_{58}O_8Si$, 670.3901; found, 670.3900.

5-OTBDMS-26-hydroxyiminomilbemycin A_4 (**19**). To a stirred solution of 60 mg (0.09 mmol) of **18** in 1,4-dioxane (1 ml), methanol (MeOH, 0.6 ml) and water (0.6 ml) was added 19 mg (0.27 mmol) of $HONH_2 \cdot HCl$ at ambient temperature. After stirring for 40 minutes, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified with preparative TLC to give 37 mg (61%) of **19** as a colorless amorphous solid.

19: IR ν_{max} (film) cm^{-1}: 3365, 2955, 2930, 2855, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.72 (1H, s, H-26), 7.58 (1H, s, NOH), 6.15 (1H, d, J=3.2 Hz, H-3), 5.78～5.90 (2H, m, H-9, H-10), 5.33～5.52 (2H, m, H-11, H-19), 5.06 (1H, d, J=5.2 Hz, H-5), 4.95 (1H, m, H-15), 4.70 (1H, d, J=13.1 Hz, H-27), 4.63 (1H, d, J=13.1 Hz, H-27), 4.06 (1H, s, 7-OH), 3.88 (1H, d, J=5.2 Hz, H-6), 3.83 (1H, d, J=3.2 Hz, H-2), 3.60 (1H, m, H-17), 3.08 (1H, m, H-25), 2.42 (1H, m, H-12), 2.13～2.30 (3H, m, H-13, H_2-16), 2.02 (1H, m, H-20), 1.55 (3H, br, H_3-29), 1.02 (3H, d, J=6.2 Hz, H_3-28), 1.00 (3H, t, J=6.9 Hz, H_3-32), 0.87 (9H, s, $(CH_3)_3CSi$), 0.83 (3H, d, J=6.4 Hz, H_3-30), 0.14 (3H, s, CH_3Si), 0.08 (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*): 685 (M^+), 628, 414, 264, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{38}H_{59}NO_8Si$, 685.4010; found, 685.4010.

5-OTBDMS-26-methoxyiminomilbemycin A_4 (**20**). To a stirred solution of 55 mg (0.08 mmol) of **18** in 1,4-dioxane (1 ml), methanol (MeOH, 0.6 ml) and water (0.6 ml) was added 21 mg (0.25 mmol) of $MeONH_2 \cdot HCl$ at ambient temperature. After stirring for 30 minutes, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified with preparative TLC to give 30 mg (53%) of **20** as a colorless amorphous solid.

20: IR ν_{max} (film) cm^{-1}: 3475, 2955, 2930, 2855, 1730, 1710; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.66 (1H, s, H-26), 6.11 (1H, d, J=3.5 Hz, H-3), 5.78～5.86 (2H, m, H-9, H-10), 5.37～5.51 (2H, m, H-11, H-19), 5.09 (1H, d, J=5.7 Hz, H-5), 4.97 (1H, m, H-15), 4.70 (1H, d, J=12.9 Hz, H-27), 4.62 (1H, d, J=12.9 Hz, H-27), 3.94 (1H, s, 7-OH), 3.89 (3H, s, NOMe), 3.86 (1H, d, J=5.7 Hz, H-6), 3.81 (1H, d, J=3.5 Hz, H-2), 3.59 (1H, m, H-17), 3.08 (1H, m, H-25), 2.45 (1H, m, H-12), 2.13～2.30 (3H, m, H-13, H_2-16), 2.01 (1H, m, H-20), 1.55 (3H, br, H_3-29), 1.00 (3H, d, J=6.4 Hz, H_3-28), 0.99 (3H, t, J=6.9 Hz, H_3-32), 0.88 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.4 Hz, H_3-30), 0.17 (3H, s, CH_3Si), 0.11 (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*): 699 (M^+), 642, 549, 414, 264, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{39}H_{61}NO_8Si$, 699.4166; found, 699.4168.

26-Acetylthio5-OTBDMS-milbemycin A_4 (**24**). To a stirred solution of 14 μl (0.20 mmol) of thiolacetic acid (AcSH) in *N*,*N*-dimethylformamide (DMF, 2 ml) was added 6 mg (0.15 mmol) of sodium hydride (NaH, 60%) at ambient temperature. After stirring for 20 minutes, 15 mg (0.10 mmol) of sodium iodide (NaI) and 75 mg (0.10 mmol) of **23** were added to the reaction mixture. After stirring for an additional 30 minutes at ambient temperature, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified with preparative TLC to give 57 mg (78%) of **24** as a pale yellow amorphous solid.

24: IR ν_{max} (film) cm^{-1}: 3475, 2955, 2930, 2850, 1735, 1700; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.65～5.82 (3H, m, H-3, H-9, H-10), 5.30～5.48 (2H, m, H-11, H-19), 4.96 (1H, m, H-15), 4.52～4.72 (3H, m, H-5, H_2-27), 4.22 (1H, s, 7-OH), 3.81 (1H, d, J=5.3 Hz, H-6), 3.51～3.78 (3H, m, H-17, H_2-26), 3.36 (1H, br, H-2), 3.07 (1H, m, H-25), 2.41 (1H, m, H-12), 2.32 (3H, s, 26-SAc), 2.13～2.28 (3H, m, H-13, H_2-16), 2.02 (1H, dd, J=12.1 Hz, 4.4 Hz, H-20), 1.54 (3H, br, H_3-29), 1.00 (3H, d, J=6.9 Hz, H_3-28), 0.93 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.3 Hz, H_3-30), 0.15 (6H, s, $(CH_3)_2Si$), 0.75～1.92 (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*): 730 (M^+), 654, 597, 414, 245, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{40}H_{62}O_8SSi$, 730.3955; found, 730.3934.

26-Benzoylthio-5-OTBDMS-milbemycin A_4 (**25**). To a stirred solution of 16 μl (0.13 mmol) of thiobenzoic acid (BzSH) in DMF (2 ml) was added 4 mg (0.10 mmol) of NaH (60%) at ambient temperature. After stirring for 20 minutes, 10 mg (0.07 mmol) of NaI and 50 mg (0.07 mmol) of **23** were added to the reaction mixture. After stirring for an additional 35 minutes at ambient temperature, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with water and brine, dried over $MgSO_4$, filtered, and evaporated

under reduced pressure. The residue was purified with preparative TLC to give 40 mg (75%) of **25** as a pale yellow amorphous solid.

25: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2930, 2855, 1710, 1670; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.95 (2H, d, J=7.7 Hz, Ar), 7.56 (1H, m, Ar), 7.44 (2H, t, J=7.7 Hz, Ar), 5.68~5.83 (3H, m, H-3, H-9, H-10), 5.29~5.47 (2H, m, H-11, H-19), 4.95 (1H, m, H-15), 4.55~4.73 (3H, m, H-5, H_2-27), 4.23 (1H, s, 7-OH), 3.80~3.97 (3H, m, H-6, H_2-26), 3.57 (1H, m, H-17), 3.40 (1H, br, H-2), 3.07 (1H, m, H-25), 2.41 (1H, m, H-12), 2.12~2.28 (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=7.1 Hz, H_3-28), 0.95 (9H, s, $(CH_3)_3CSi$), 0.81 (3H, d, J=6.3 Hz, H_3-30), 0.18 (3H, s, CH_3Si), 0.17 (3H, s, CH_3Si), 0.75~1.95 (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*): 792 (M^+), 735, 654, 636, 597, 414, 264, 245, 195, 167; HREI-MS (*m/z*): [M^+]: calcd. for $C_{45}H_{64}O_8SSi$, 792.4091; found, 792.4091.

5-OTBDMS-26-(3-Methyl-2-butenoylthio)-milbemycin A_4 (**26**). To a stirred suspension of 4.17 g (52.1 mmol) of sodium hydrosulfide, *n*-hydrate ($NaSH \cdot nH_2O$, 70%) in 25 ml of ethanol (EtOH) was added dropwise 2.0 ml (18.0 mmol) of 3-methylcrotonyl chloride while cooling with an ice bath. After the addition was complete, the reaction mixture was removed from the ice bath, stirred at ambient temperature for 2.5 hours, filtered with Celite®,and evaporated under reduced pressure. The residue was dissolved in 80 ml of 0.5 N aqueous sodium hydroxide solution and washed twice with 30 ml of toluene. The water layer was acidified to pH=1~2 with hydrochloric acid and extracted three times with 80 ml of ether. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give 1.84 g of crude 3-methylcrotonoylthiol. To a stirred solution of 77 mg of this crude 3-methylcrotonoylthiol in 2 ml of DMF was added 16 mg (0.40 mmol) of NaH (60%) at ambient temperature. After stirring for 20 minutes, 20 mg (0.13 mmol) of NaI and 100 mg (0.13 mmol) of **23** were added to the reaction mixture. After stirring for an additional 30 minutes at ambient temperature, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified with preparative TLC to give 83 mg (81%) of **26** as a pale yellow amorphous solid.

26: IR ν_{max} (film) cm^{-1}: 3465, 2955, 2930, 1715, 1680, 1630; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.96 (1H, br, 26-SCOCH), 5.65~5.82 (3H, m, H-3, H-9, H-10), 5.28~5.47 (2H, m, H-11, H-19), 4.97 (1H, m, H-15), 4.52~4.73 (3H, m, H-5, H_2-27), 4.20 (1H, s, 7-OH), 3.81 (1H, d, J=5.5 Hz, H-6), 3.55~3.80 (2H, m, H_2-26), 3.54 (1H, m, H-17), 3.38 (1H, br, H-2), 3.07 (1H, m, H-25), 2.42 (1H, m, H-12), 2.18~2.28 (3H, m, H-13, H_2-16), 2.15 (3H, s, 26-SCOCHCC$\underline{H}_3$), 2.05 (1H, m, H-20), 1.87 (3H, s, 26-COCHCC$\underline{H}_3$), 1.53 (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=6.3 Hz, H_3-30), 0.15 (6H, s, $(CH_3)_2Si$), 0.75~1.95 (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*): 770 (M^+), 713, 695, 655, 637, 414, 264, 167; HREI-MS (*m/z*): [M^+]: calcd. for $C_{43}H_{66}O_8SSi$, 770.4248; found, 770.4249.

5-OTBDMS-26-methylmilbemycin A_4 (**27**). To a stirred solution of 53 mg (0.07 mmol) of **23** in 2 ml of Hex was added dropwise 400 μl (0.68 mmol) of 1.7 N solution of Me_3Al in Hex while cooling with an ice bath under a nitrogen atmosphere. After stirring for 15 minutes, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified with preparative TLC to give 20 mg (43%) of **27** as a pale yellow amorphous solid.

27: IR ν_{max} (film) cm^{-1}: 3465, 2960, 2930, 2860, 1715; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.67~5.82 (2H, m, H-9, H-10), 5.25~5.42 (3H, m, H-3, H-11, H-19), 4.97 (1H, m, H-15), 4.65 (1H, d, J=14.1 Hz, H-27), 4.58 (1H, d, J=14.1 Hz, H-27), 4.48 (1H, m, H-5), 4.14 (1H, s, 7-OH), 3.81 (1H, d, J=5.6 Hz, H-6), 3.58 (1H, m, H-17), 3.39 (1H, br, H-2), 3.08 (1H, dt, J_t=11.3 Hz, J_d=2.4 Hz, H-25), 2.42 (1H, m, H-12), 2.17~2.32 (3H, m, H-13, H_2-16), 1.54 (3H, br, H_3-29), 1.04 (3H, t, J=7.7 Hz, 26-C$\underline{H}_3$), 1.00 (3H, d, J=6.3 Hz, H_3-28), 0.98 (3H, t, J=6.9 Hz, H_3-32), 0.92 (9H, s, $(CH_3)_3CSi$), 0.82 (3H, d, J=6.3 Hz, H_3-30), 0.13 (6H, s, $(CH_3)_2Si$), 0.75~2.15 (14H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-26, H_2-31); EI-MS (*m/z*): 670 (M^+), 613, 595, 414, 396, 264, 195, 167, 151; HREI-MS (*m/z*): [M^+]: calcd. for $C_{39}H_{62}O_7Si$, 670.4265; found, 670.4264.

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 1.

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