Synthesis of Novel 26-Substituted Milbemycin A₄ Derivatives and Their Acaricidal Activities

TAKAHIRO TSUKIYAMA,* HISAKI KAJINO, YOSHIHISA TSUKAMOTO, HARUMI NAKAGAWA, TOSHIKAI YANAI, KAZUO SATO, SHINJI YOKOI, REIJI ICHINOSE and KEIJI TANAKA

Agroscience Research Laboratories, Sankyo Co., Ltd.
894, Yasu, Yasu-cho, Yasu-gun, Shiga 520-2342, Japan
1-12-1, Shinomiya, Hiratsuka-Shi, Kanagawa 254-8560, Japan
Intellectual Property Department, Sankyo Co., Ltd.
2-58, Hiromachi 1-Chome, Shinagawa-ku, Tokyo 140-8710, Japan
Crop Protection Department, Sankyo Agro Co., Ltd.
Kasuga Bldg., 4-23-14, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

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A series of novel 26-substituted milbemycin A₄ derivatives was synthesized from 5-O-t-butyldimethylsilyl-26-hydroxymilbemycin A₄ prepared by selenium dioxide oxidation of 5-O-t-butyldimethylsilyl-milbemycin A₄. Their acaricidal activities were assessed against the organophosphorus-sensitive two-spotted spider mite (Tetranychus urticae) on the primary leaves of cowpea plants (Vigna sinensis Savi species) by spraying.

Milbemycins¹⁻⁷ 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, milbemectin⁸ [a mixture of milbemycin A₃ (1) and A₄ (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 avermectins⁹,¹⁰ and Cyanamide's LL-F28249 (nemadectins).¹¹ Milbemycins α₁₁ (3) and α₁₄ (4) (Figure 1), another pair of congeners, have been reported as natural products.¹² The structures of milbemycins α₁₁ (3) and α₁₄ (4) are characterized by the presence of 3-methyl-2-butenoyloxy groups at their C-26 positions. Milbemycins α₁₁ (3) and α₁₄ (4) also have been found to possess potent acaricidal activities superior to those of milbemycin A₃ (1) and A₄ (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 A₄ derivatives from 5-O-t-butyldimethylsilyl-26-hydroxymilbemycin A₄ (5-OTBDMS-26-OH-milbemycin A₄, 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.

* Corresponding author: tukiya@Sankyo-agro.co.jp
and preliminary structure-activity relationships of these newly synthesized derivatives.

Results and Discussion

Chemistry

The 26-substituted milbemycin A₄ derivatives were prepared as follows (Scheme 1). First of all, a primary hydroxy group of 5-OTBDMS-26-OH-milbemycin A₄ (5) derived from milbemycin A₄ (2)¹³ was acylated with acetyl chloride (AcCl) and benzoyl chloride (BzCl) in the presence of triethylamine (Et₃N) 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 A₄ 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 (Ag₂O) to afford 8 and 9, respectively, and subsequent deprotections of 8 and 9 yielded 26-alkoxymilbemycin A₄ 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-diethylphosphoryloxymilbemycin A₄ (16) in good yield.

The fluorine atom was introduced at the C-26 position of milbemycin A₄ (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 A₄ (17).

The C-26 methyl group of milbemycin A₄ (2) was transformed to an oxime moiety by the following method. Oxidation of C-26 allyl alcohol of 5 with manganese dioxide (MnO₂)¹⁸) produced 5-OTBDMS-4-formylmilbemycin A₄ (18) in good yield. Oximation¹⁸) of this formyl group with hydroxylamine hydrochloride (HONH₂·HCl) or O-methylhydroxylamine hydrochloride (MeONH₂·HCl) and subsequent deprotection provided two corresponding oxime derivatives (21, 22).

Acaricidal Activities

The acaricidal activities of the prepared milbemycin A₄ 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 Table 1. Just as milbemycin A₄ (4) possessed higher acaricidal activity than milbemycin A₄ (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 A₄ (15) and the small decrease of the activity of the 26-methoxymilbemycin A₄ (14) also supported this contention. Similarly, the drop of the activity of 26-acetylthiomilbemycin A₄ (28) and the enhancement of the activities of 26-benzoylthiomilbemycin A₄ (29) and 26-(3-methyl-2-butenoylthio)-milbemycin A₄ (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-diethylphosphonyloxymilbemycin A₄ (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 A₄ (17) and 26-methylmilbemycin A₄ (31), a pair of derivatives that possessed lower molecular polarities (increased lipophilicities) than milbemycin A₄ (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$_3$N; 77% for 6; (b) BzCl, Et$_3$N; 84% for 7; (c) Mel, Ag$_2$O; 74% for 8; (d) BnBr, Ag$_2$O; 27% for 9; (e) ClPO(OEt)$_2$, Py; 64% for 10; (f) DAST; 46% for 11; (g) HF/Py; 66% 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, Nal; 78% for 24; (l) BzSH, NaH, Nal; 75% for 25; (m) Me$_2$CCOCH$_3$, NaH, Nal; 61% for 26; (n) Me$_3$Al; 43% for 27.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10ppm</td>
<td>1ppm</td>
</tr>
<tr>
<td>12</td>
<td>CH$_2$OAc</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>CH$_2$OBz</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>CH$_3$Ome</td>
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<tr>
<td>15</td>
<td>CH$_3$OBn</td>
<td>100</td>
</tr>
<tr>
<td>16</td>
<td>CH$_2$OP(OEt)$_2$</td>
<td>67</td>
</tr>
<tr>
<td>17</td>
<td>CH$_2$F</td>
<td>100</td>
</tr>
<tr>
<td>21</td>
<td>CHNOH</td>
<td>52</td>
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<tr>
<td>22</td>
<td>CHNOCH$_3$</td>
<td>90</td>
</tr>
<tr>
<td>28</td>
<td>CH$_3$SCa</td>
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<td>29</td>
<td>CH$_3$SBz</td>
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<td>30</td>
<td>CH$_3$SCOCH$_2$Me$_2$</td>
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<tr>
<td>31</td>
<td>Et</td>
<td>100</td>
</tr>
<tr>
<td>Milbemycin A$_4$ (2)</td>
<td>Me</td>
<td>100</td>
</tr>
<tr>
<td>Milbemycin A$_{14}$ (4)</td>
<td>CH$_3$CCOCH$_2$Me$_2$</td>
<td>100</td>
</tr>
</tbody>
</table>
Conclusion

In conclusion, we established versatile methods to prepare various 26-substituted milbemycin A₄ derivatives using 5-OTBDMS-26-OH-milbemycin A₄ (5), a compound derived from milbemycin A₄ (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 A₁₄ (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 A₁₄ (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 (200MHz) or a JEOL JNM-GX-270 FT NMR Spectrometer (270MHz). Chemical shifts (δ) were expressed in parts per million relative to internal tetramethylsilane. Mass spectra were measured on a Fisons Instruments VG Autospec. IR spectra were measured on a Shimadzu FTIR-8400.

5-OTBDMS-26-acetoxymilbemycin A₄ (6). To a stirred solution of 50mg (0.07mmol) of 5-OTBDMS-26-hydroxymilbemycin A₄ (5) in dichloromethane (CH₂Cl₂, 2ml) was added 8μl (0.11mmol) of AcCl and 15μl (0.11mmol) of Et₃N 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 (MgSO₄), filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC to give 41mg (77%) of 6 as a colorless amorphous solid.

6: IR νmax (film) cm⁻¹: 3465, 2955, 2930, 2860, 1740, 1715; ¹H-NMR (270MHz, CDCl₃) 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, H₂-26, H₂-27), 4.18 (1H, s, J=5.4Hz, 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-16), 2.07 (3H, s, 26-OAc), 2.02 (1H, m, H-20), 1.54 (3H, br, H-29), 1.00 (3H, d, J=6.2 Hz, H₂-28), 0.98 (3H, t, J=7.4 Hz, H-32), 0.91 (9H, s, (CH₃)₃CSi), 0.82 (3H, d, J=6.2 Hz, H-30), 0.13 (3H, s, CH₃Si), 0.12 (3H, s, CH₃Si), 0.80-1.19 (11H, m, H-13, H₂-18, H-20, H₂-22, H₂-23, H-24, H₂-31); EI-MS (m/z): 714 (M⁺), 696, 654, 639, 597, 579, 564, 195, 167, 151; HREI-MS (m/z): [M⁺]: calcd. for C₄₀H₆₂O₉Si, 714.4163; found, 714.4162.

5-OTBDMS-26-benzoyloxymilbemycin A₄ (7). To a stirred solution of 50mg (0.07mmol) of 5 in CH₂Cl₂ (2ml) was added 13μl (0.11mmol) of BzCl and 15μl (0.11mmol) of Et₃N 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 MgSO₄, filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC to give 49mg (84%) of 7 as a colorless amorphous solid.

7: IR νmax (film) cm⁻¹: 3460, 2955, 2930, 2860, 1720; ¹H-NMR (270 MHz, CDCl₃) 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, H₂-26), 4.57-4.78 (3H, m, H-5, H₂-27), 4.23 (1H, s, 7-OH), 3.88 (1H, d, J=5.5Hz, 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, H₂-16), 1.54 (3H, br, H-29), 1.00 (3H, d, J=6.6 Hz, H₂-28), 0.98 (3H, t, J=7.7 Hz, H-32), 0.91 (9H, s, (CH₃)₃CSi), 0.82 (3H, d, J=6.3Hz, H-30), 0.12 (3H, s, CH₃Si), 0.11 (3H, s, CH₃Si), 0.75~1.95 (11H, m, H-13, H-18, H-20, H₂-22, H₂-23, H-24, H₂-31); EI-MS (m/z): 776 (M⁺), 719, 701, 654, 597, 414, 245, 195, 167, 151; HREI-MS (m/z): [M⁺]: calcd. for C₄₅H₆₄O₉Si, 776.4320; found, 776.4319.

5-OTBDMS-26-methoxymilbemycin A₄ (8). To a stirred solution of 100mg (0.15mmol) of 5 in 1,2-dichloroethane (CH₂ClCH₂Cl, 2ml) was added 460μl (7.45mmol) of MeI and 345mg (1.49mmol) of Ag₂O at ambient temperature. After stirring overnight, 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 (MgSO₄), filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC to give 41mg (77%) of 6 as a colorless amorphous solid.

8: IR νmax (film) cm⁻¹: 3465, 2955, 2930, 2860, 1740, 1715; ¹H-NMR (270 MHz, CDCl₃) 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, H₂-26, H₂-27), 4.18 (1H, s, 7-OH), 3.84 (1H, d, J=5.4Hz, 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-16), 2.07 (3H, s, 26-OAc), 2.02 (1H, m, H-20), 1.54 (3H, br, H-29), 1.00 (3H, d, J=6.2 Hz, H₂-28), 0.98 (3H, t, J=7.4 Hz, H-32), 0.91 (9H, s, (CH₃)₃CSi), 0.82 (3H, d, J=6.3Hz, H-30), 0.12 (3H, s, CH₃Si), 0.11 (3H, s, CH₃Si), 0.75~1.95 (11H, m, H-13, H-18, H-20, H₂-22, H₂-23, H-24, H₂-31); EI-MS (m/z): 776 (M⁺), 719, 701, 654, 597, 414, 245, 195, 167, 151; HREI-MS (m/z): [M⁺]: calcd. for C₄₅H₆₄O₉Si, 776.4320; found, 776.4319.
H-13, H-16), 1.98 (1H, m, H-20), 1.54 (3H, br, H-29), 0.99 (3H, d, J=6.4 Hz, H-28), 0.92 (9H, s, (CH₃)₂CSi), 0.82 (3H, d, J=6.4 Hz, H-30), 0.13 (6H, s, (CH₃)₂Si), 0.70-1.90 (14H, m, H-13, H-18, H-20, H-22, H-23, H-24, H-27, H-31, H-32); EI-MS (m/z): 686 (M⁺). 654, 629, 611, 536, 414, 195, 167, 151; HREI-MS (m/z): [M⁺]: calcd. for C₄₅H₆₆O₈Si, 686.4214; found, 686.4213.

5-OTBDBMS-26-benzoyloxymilbemycin A₄ (9). To a stirred solution of 152 mg (0.23 mmol) of 5 in CH₂Cl₂ (2 ml) was added 270 µl (2.27 mmol) of BrNH₂ and 513 mg (2.21 mmol) of Ag₂O 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 νmax (film) cm⁻¹: 3460, 2955, 2930, 2875, 1745, 1715; ¹H-NMR (270 MHz, CDCl₃) δ: 7.27-7.40 (SH, m, H-3, 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-27), 4.49 (2H, s, 26-OCH₂), 4.20 (1H, d, J=6.4 Hz, H-6), 3.58 (1H, m, H-17), 3.32 (1H, m, H-2), 3.07 (1H, dt, Jt=9.3Hz, Jd=2.4Hz, H-25), 2.42 (1H, m, H-12), 2.15-2.28 (3H, m, H-13, H₂-16), 2.09 (3H, s, 26-OAc), 1.54 (3H, br, H3-29), 1.00 (3H, d, J=6.6 Hz, H-26), 0.96 (3H, t, J=7.1 Hz, H-32), 0.92 (9H, s, (CH₃)₂CSi), 0.82 (3H, d, J=6.3 Hz, H-30), 0.14 (6H, s, (CH₃)₂Si), 0.75-1.90 (11H, m, H-13, H-18, H-20, H-22, H-23, H-24, H-31, H-32); EI-MS (m/z): [M⁺]: calcd. for C₄₅H₆₆O₈Si, 686.4214; found, 686.4213.

To a stirred solution of 150 mg (0.22 mmol) of 5 in CH₂Cl₂ (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 MgSO₄, 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 νmax (film) cm⁻¹: 3465, 2955, 2930, 2860, 1715, 1180; ¹H-NMR (270 MHz, CDCl₃) 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-27), 4.18 (1H, s, H-3), 3.58 (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=9.3 Hz, J₂=2.4 Hz, H-25), 2.42 (1H, m, H-12), 2.12-2.28 (3H, m, H-13, H-16), 2.00 (1H, m, H-20), 1.54 (3H, br, H-29), 1.00 (3H, d, J=6.7 Hz, H-28), 0.92 (9H, s, (CH₃)₂CSi), 0.82 (3H, d, J=6.3 Hz, H-30), 0.14 (6H, s, (CH₃)₂Si), 0.75-1.95 (14H, m, H-13, H-18, H-20, H-22, H-23, H-24, H-31, H-32); EI-MS (m/z): 762 (M⁺). 654, 617, 599, 195, 167; HREI-MS (m/z): [M⁺]: calcd. for C₃₉H₆₂O₈Si, 674.4014; found, 674.4013.

26-Acetoxymilbemycin A₃ (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 MgSO₄, 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 νmax (film) cm⁻¹: 3325, 2955, 2930, 2860, 1740; ¹H-NMR (270 MHz, CDCl₃) δ: 5.68-5.83 (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-26, H-27), 4.02-4.22 (5H, m, 7-OH, 26-OPO(OCH₂CH₃)), 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-16), 2.08 (1H, m, H-20), 1.54 (3H, br, H-29), 1.35 (3H, t, J=6.9 Hz, 26-OPO(OCH₂CH₃)), 1.32 (3H, t, J=6.9 Hz, 26-OPO(OCH₂CH₃)), 1.00 (3H, d, J=6.6 Hz, H-28), 0.96 (3H, t, J=7.1 Hz, H-32), 0.92 (9H, s, (CH₃)₂CSi), 0.82 (3H, d, J=6.3 Hz, H-30), 0.14 (6H, s, (CH₃)₂Si), 0.75-1.90 (11H, m, H-13, H-18, H-20, H-22, H-23, H-24, H-31, H-32); EI-MS (m/z): 808 (M⁺). 751, 654, 597, 414, 195, 167; HREI-MS (m/z): [M⁺]: calcd. for C₄₂H₆₉O₁₁PSi, 808.4347; found, 808.4346.

5-OTBDBMS-26-fluoromilbemycin A₄ (11). To a stirred solution of 150 mg (0.22 mmol) of 5 in CH₂Cl₂ (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 MgSO₄, 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.
2.01 (1H, m, H-20), 1.53 (3H, br, H3-29), 1.00 (3H, d, J=7.4 Hz, H-28), 0.99 (3H, t, J=7.7 Hz, H2-32), 0.83 (3H, d, J=6.7 Hz, H-32), 0.80~1.90 (11H, m, H-13, H-18, H-20, H2-22, H2-23, H-24, H2-31); EI-MS (m/z): 600 (M+), 540, 414, 396, 356, 314, 264, 245, 195, 167, 151; HREI-MS (m/z): [M+]: calcd. for C32H45FO7, 560.3149; found, 560.3150.

Using the same procedure described for the preparation of 12, the other 5-OTBDMS-26-substituted-milbemycins A4 derivatives (7, 8, 9, 10, 11, 19, 20, 24, 25, 26 and 27) were deprotected to give corresponding milbemycins A4 derivatives (13, 14, 15, 16, 17, 21, 22, 28, 29, 30 and 31) as colorless amorphous solids. Yields are described in Scheme 1.

26-Benzoyloxyamilbemycin A4 (13): IR ƒËmax (film) cm⁻¹: 3460, 2955, 2925, 2870, 1720; 1H-NMR (270 MHz, CDCl3) 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, d, J=7.4 Hz, H-26, H-15, H-16), 4.71 (2H, br, H2-27), 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-16), 2.00 (1H, m, H-20), 1.53 (3H, br, H3-29), 1.01 (3H, d, J=7.1 Hz, H-28), 0.99 (3H, d, J=8.2 Hz, H2-32), 0.82 (3H, d, J=6.3 Hz, H3-30), 0.78~1.90 (11H, m, H-13, H-18, H-20, H2-22, H2-23, H-24, H2-31); EI-MS (m/z): 662 (M+), 540, 414, 264, 245, 195, 167, 151; HREI-MS (m/z): [M+]: calcd. for C33H48O8, 648.3662; found, 648.3662.

26-Diethylphosphoryloxyamilbemycin A4 (16): IR ƒËmax (film) cm⁻¹: 3360, 2960, 2930, 2875, 1735; 1H-NMR (270 MHz, CDCl3) 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-27), 4.48~4.59 (2H, m, H-5, H-26), 4.06~4.22 (5H, m, 7-OH, 26-OP(OCH2CH3)), 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-16), 2.05 (1H, m, H-20), 1.54 (3H, br, H3-29), 1.36 (3H, t, J=6.9 Hz, 26-OP(OCH2CH3)), 1.33 (3H, t, J=6.9 Hz, 26-OP(OCH2CH3)), 1.01 (3H, d, J=6.9 Hz, H-28), 0.99 (3H, t, J=7.7 Hz, H3-32), 0.82 (3H, d, J=6.6 Hz, H3-30), 0.75~1.95 (11H, m, H-13, H-18, H-20, H2-22, H2-23, H-24, H2-31); EI-MS (m/z): 694 (M+), 540, 522, 414, 264, 245, 195, 167, 151; HREI-MS (m/z): [M+]: calcd. for C35H52O11P, 694.3482; found, 694.3482.

26-Fluoromilbemycin A4 (17): IR ƒËmax (film) cm⁻¹: 3455, 2955, 2930, 2875, 1715, 1180; 1H-NMR (270 MHz, CDCl3) 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-26), 4.70 (2H, br, H2-27), 4.52 (1H, m, H-5), 4.14 (1H, s, 7-OH), 4.00 (1H, d, J=6.4 Hz, H-6), 3.58 (1H, m, H-17), 3.32 (1H, br, H-2), 3.07 (1H, dt, J=9.2 Hz, J=2.5 Hz, H-25), 2.35~2.50 (2H, m, H-12, 5-OH), 2.15~2.28 (3H, m, H-13, H-16), 1.99 (1H, m, H-20), 1.53 (3H, br, H3-29), 1.01 (3H, d, J=6.9 Hz, H-28), 0.99 (3H, t, J=7.7 Hz, H3-32), 0.82 (3H, d, J=6.6 Hz, H3-30), 0.75~1.95 (11H, m, H-13, H-18, H-20, H2-22, H2-23, H-24, H2-31); EI-MS (m/z): 560 (M+), 414, 264, 245, 195, 167, 151; HREI-MS (m/z): [M+]: calcd. for C33H45FO7, 560.3149; found, 560.3150.

26-Hydroxyaminomilbemycin A4 (21): IR ƒËmax (film) cm⁻¹: 3365, 2955, 2925, 2855, 1755; 1H-NMR (270 MHz, CDCl3) 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, H2-27), 4.07 (1H, d, J=6.4 Hz, H-6),
26-Methoxyiminomilbemycin A4 (22): IR νmax (film) cm⁻¹: 3450, 2955, 2930, 2875, 1730, 1715; ¹H-NMR (270 MHz, CDCl₃) 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, H₂-27), 4.06 (1H, d, J = 5.9 Hz, H-6), 3.99 (1H, s, 7-OH), 3.92 (3H, s, NOE), 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, H₂-16, 5-OH), 2.01 (1H, m, H-20), 1.54 (3H, br, H₃-29), 1.01 (3H, d, J = 6.9 Hz, H₃-28), 0.97 (3H, t, J = 7.6 Hz, H₃-32), 0.82 (3H, d, J = 6.6 Hz, H₂-23, H₂-24, H₃-31); EI-MS (m/z): 585 (M⁺), 567, 414, 245, 195, 167, 151; HREI-MS (m/z): [M⁺]: calcd. for C₃₉H₅₀O₈S, 656.3383; found, 656.3383.

26-Acetylthiomilbemycin A₄ (28): IR νmax (film) cm⁻¹: 3455, 2960, 2925, 2875, 1735, 1695; ¹H-NMR (270 MHz, CDCl₃) 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, H₂-27), 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-SCOCH₃), 2.15~2.28 (3H, m, H-13, H₂-16), 2.02 (1H, m, H-20), 1.53 (3H, br, H₃-29), 1.01 (3H, d, J = 6.9 Hz, H₂-28), 0.98 (3H, t, J = 7.7 Hz, H₃-32), 0.82 (3H, d, J = 6.6 Hz, H₂-30), 0.75~1.95 (11H, m, H-13, H₂-18, H-20, H₂-22, H₂-23, H-24, H₃-31); EI-MS (m/z): 566 (M⁺), 522, 414, 245, 195, 167, 151; HREI-MS (m/z): [M⁺]: calcd. for C₃₉H₅₀O₈S, 656.3383; found, 656.3383.

26-Methylmilbemycin A₄ (31): IR νmax (film) cm⁻¹: 3465, 2965, 2925, 2875, 1715; ¹H-NMR (270 MHz, CDCl₃) 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, H₂-27), 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, H₃-29), 1.08 (3H, t, J = 7.4 Hz, 26-CH₃), 1.00 (3H, d, J = 6.6 Hz, H₂-28), 0.98 (3H, t, J = 6.9 Hz, H₂-32), 0.82 (3H, d, J = 6.6 Hz, H₂-30), 0.75~2.42 (18H, m, H-12, H-13, H-16, H-18, H₂-20, H₂-22, H₂-23, H-24, H₂-23, H-31); EI-MS (m/z): 556 (M⁺), 514, 314, 245, 195, 167, 151; HREI-MS (m/z): [M⁺]: calcd. for C₃₉H₅₀O₈S, 656.3380; found, 556.3400.

5-OTBDMS-4-formylmilbemycin A₄ (18). To a stirred solution of 300 mg (0.45 mmol) of 5 in CH₂Cl₂ (6 ml) was added 2.00 g (22.3 mmol) of MnO₂ 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 νmax (film) cm⁻¹: 3475, 2955, 2855, 1735, 1685; ¹H-NMR (200 MHz, CDCl₃) δ: 9.54 (1H, s, CHO), 6.90 (1H, d, J = 3.3 Hz, H-3), 5.75~5.90 (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, H₂-27), 3.89 (1H, s, 7-OH), 3.85 (2H, m, H-2, H-6), 3.60 (1H, m, H-17), 3.08 (1H, dt, J₁ = 9.2 Hz, J₂ = 2.6 Hz, H-25), 2.45 (1H, m, H-12), 2.10 (1H, m, H-20), 1.53 (3H, br, H₃-29), 1.00 (3H, d, J = 6.9 Hz, H₂-28), 0.98 (3H, t, J = 7.9 Hz, H₂-32), 0.82 (3H, d, J = 6.3 Hz, H₂-30), 0.75~1.95 (11H, m, H-13, H-18, H₂-20, H₂-22, H₂-23, H-24, H₃-31); EI-MS (m/z): 678 (M⁺), 414, 264, 245, 195, 167, 151; HREI-MS (m/z): [M⁺]: calcd. for C₃₉H₅₀O₈S, 678.3226; found, 678.3227.
5-OTBDMS-26-hydroxyiminomilbemycin A₄ (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 21 mg (0.25 mmol) of MeONH₂·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 MgSO₄, 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.

5-OTBDMS-26-hydroxyiminomilbemycin A₄ (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 HONH₂·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 MgSO₄, 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.

5-OTBDMS-26-hydroxyiminomilbemycin A₄ (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 21 mg (0.25 mmol) of MeONH₂·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 MgSO₄, 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.

5-OTBDMS-26-methoxyiminomilbemycin A₄ (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 MeONH₂·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 MgSO₄, 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.

5-OTBDMS-26-methoxyiminomilbemycin A₄ (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 MeONH₂·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 MgSO₄, 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.

5-OTBDMS-26-methoxyiminomilbemycin A₄ (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 MeONH₂·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 MgSO₄, 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.

5-OTBDMS-26-methoxyiminomilbemycin A₄ (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 MeONH₂·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 MgSO₄, 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.
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 $\nu_{\text{max}}$ (film) cm$^{-1}$: 3465, 2955, 2930, 2855, 1710, 1670; $^1$H-NMR (270 MHz, CDCl$_3$) $\delta$: 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$_2$-29), 1.00 (3H, d, $J=7.1$ Hz, H$_2$-28), 0.95 (9H, s, (CH$_3$)$_3$Si), 0.81 (3H, br, H$_3$-30), $J=6.3$ Hz, H$_2$-30), 0.13 (6H, s, (CH$_3$)$_2$Si), 0.75–1.95 (14H, m, H-13, H$_2$-18, H-20, H$_2$-22, H-23, H-24, H$_2$-31, H$_3$-32); EI-MS (m/z): 792 (M$^+$), 735, 654, 636, 597, 414, 245, 195, 167; HREI-MS (m/z): [M$^+$]: calcd. for C$_{39}$H$_{62}$O$_7$Si, 792.4091; found, 792.4091.

5-OTBDS-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$\cdot$H$_2$O, 70%) in 30 ml of DMF was added dropwise 2.0 ml of 1.7 N solution of Me$_3$Al in Hex while cooling with an ice bath. After addition was complete, 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 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 an additional 30 minutes at ambient temperature, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed twice with 30 ml of toluene. The water layer was evaporated under reduced pressure and the residue was purified with preparative TLC to give 8 mg (43%) of 26 as a pale yellow amorphous solid.

26: IR $\nu_{\text{max}}$ (film) cm$^{-1}$: 3465, 2955, 2930, 2855, 1715, 1630; $^1$H-NMR (270 MHz, CDCl$_3$) $\delta$: 5.96 (1H, br, 26-SOCH), 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-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-SOCHCCH$_3$), 2.05 (1H, m, H-20), 1.87 (3H, s, 26-SOCHCCH$_3$), 1.53 (3H, br, H$_2$-29), 1.00 (3H, d, $J=6.6$ Hz, H$_2$-28), 0.93 (9H, s, (CH$_3$)$_3$Si), 0.82 (3H, d, $J=6.3$ Hz, H$_2$-30), 0.15 (6H, s, (CH$_3$)$_2$Si), 0.75–1.95 (14H, m, H-13, H$_2$-18, H-20, H$_2$-22, H-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$_7$Si, 770.4248; found, 770.4249.

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
The results are reported in Table 1.

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References and Notes


