13β-Hydroxylation of Milbemycins by Selenium Dioxide[1)]

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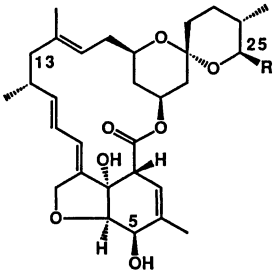
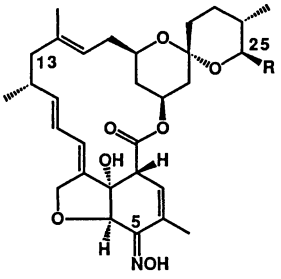
Introduction of a hydroxyl group to milbemycin nucleus was examined. Starting from 5-oxo-5-deoxymilbemycins, 13β-hydroxy-5-oxo-5-deoxymilbemycins were obtained stereo- and regioselectively by selenium dioxide oxidation in formic acid and subsequent acidic hydrolysis. The stereochemistry of the hydroxyl group was elucidated by ^{1}H NMR study.

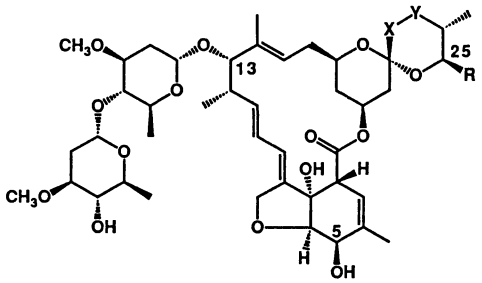
Milbemycins[2,3)] are sixteen-membered ring macrolides isolated from *Streptomyces hygroscopicus*. They demonstrate potent and broad spectrum activity as anthelmintics, acaricides, and insecticides. Among them, a mixture of (6*R*, 25*R*)-5-*O*-demethyl-28-deoxy-6,8-epoxy-25-methylmilbemycin B (milbemycin A_3) (**1a**) and (6*R*, 25*R*)-5-*O*-demethyl-28-deoxy-6,8-epoxy-25-ethylmilbemycin B (milbemycin A_4) (**1b**) was recently developed and commercialized as an agricultural miticide under the name Milbeknock®. On the other hand, (6*R*, 25*R*)-5-*O*-demethyl-28-deoxy-6,8-epoxy-25-(1-methylethyl)milbemycin B (milbemycin D) (**1c**) was put on the market as a parasiticide for dogs. In the wake of it, a mixture of 5-hydroxyimino-5-deoxymilbemycin A_3 (**1d**) and A_4 (**1e**) was just launched to the same market.[4)] Avermectins,[5,6)] which have similar structures to milbemycins, also exhibit high anthelmintic, acaricidal, and insecticidal activities. Out of them, a mixture of avermectin B_{1a} (**2a**) and B_{1b} (**2b**) has been used as insecticide, and a mixture of 22,23-dihydroavermectin B_{1a} (**2c**) and B_{1b} (**2d**) was commercialized as a parasiticide named ivermectin in animal





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

1d 5-Hydroxyimino-5-deoxymilbemycin A_3 (R=Me)
1e 5-Hydroxyimino-5-deoxymilbemycin A_4 (R=Et)



		X–Y	R
2a	Avermectin B_{1a}	CH=CH	*s*-Bu
2b	Avermectin B_{1b}	CH=CH	*i*-Pr
2c		CH_2-CH_2	*s*-Bu
2d		CH_2-CH_2	*i*-Pr

Scheme 1.

health.[7] As shown in Scheme 1, the major structural difference between avermectins and milbemycins is that the former have α-L-oleandrosyl-α-L-oleandrosyl group at the 13-position, while the latter are unsubstituted at that position. The introduction of substituents on the 13-position of milbemycins, therefore, has been paid much attention from the point of view of their biological activities. A hydroxylated milbemycin at the 13-position is thought to be a key intermediate for the synthesis of various 13-substituted milbemycins.[8]

There are some reports concerning hydroxylation of the 13-position of milbemycins. Tombo et al. reported that diastereoselective microbial hydroxylation of milbemycins at the 13β-position was achieved by cultures of *Streptomyces violascens* (ATCC 31560).[9] Nakagawa et al. reported that a culture of *Cunninghamella echinulata* ATCC 9244 also had an efficient 13β-hydroxylating ability on milbemycins.[10] There has been only one report relating to the chemical transformation of milbemycins into 13β-hydroxymilbemycins.[11] The transformation consists of the following four reactions. Epoxidation of a milbemycin gives an epoxide of the C(14)–C(15) double bond. After the protection of the 5-hydroxyl group, the epoxide undergoes acidic ring opening to give an allylic alcohol, which has a 15-hydroxyl group. Subsequent allylic rearrangement of the allylic alcohol produces a 5-O-protected 13β-hydroxymilbemycin.

We describe here alternative chemical 13β-hydroxylation of milbemycins by stereo- and regioselective direct oxidation at the 13-position.

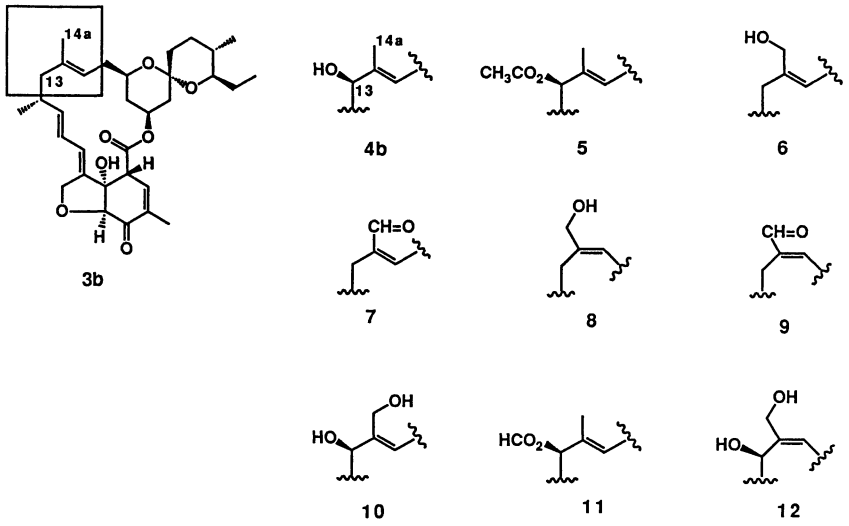
Results and Discussion

The C(13)-methylene of a milbemycin is corresponding to the allylic position. Thus, for gaining 13-hydroxylated milbemycins, initial efforts were made to survey the possibility of carrying out the allylic hydroxylation of milbemycin nucleus in the reaction conditions using selenium dioxide,[12] which is known as a mild allylic oxidizing reagent. The results are summarized in Table 1. Treatment of milbemycin A_4 (**1b**) with selenium dioxide (1.36 molar amount) in acetic acid gave 13β-hydroxy-5-oxo-5-deoxymilbemycin A_4 (**4b**) in 4.8% yield, accompanied with the starting material **1b** (4.8%), 5-oxo-5-deoxymilbemycin A_4 (**3b**) (20.4%), 13β-acetoxy-5-oxo-5-deoxymilbemycin A_4 (**5**) (1.7%), 14a-hydroxy-5-oxo-5-deoxymilbemycin A_4 (**6**) (4.7%),[13] 5,14a-dioxo-5-deoxymilbemycin A_4 (**7**) (0.6%), and (14*E*)-14a-hydroxy-5-oxo-5-deoxymilbemycin A_4 (**8**) (2.5%). In the course of the oxidation, we could not get the desired oxidized products only at the 13-position, but the double oxidized products at the 5- and 13-position were obtained (**4b** and **5**). The simultaneous isolation of 5-oxo-5-deoxymilbemycin A_4 (**3b**), which was oxidized only at the 5-position, indicated that the 5-hydroxyl group was more easily oxidized than the 13-methylene carbon. These observation led us to the idea of using a 5-oxo-5-deoxymilbemycin as a starting material for the selenium dioxide oxidation. Thus, 5-oxo-5-deoxymilbemycin A_4 (**3b**),[2] which was prepared from milbemycin A_4 (**1b**) in 70% yield by manganese dioxide oxidation, was treated with selenium dioxide (1.36 molar amount) in acetic acid at room temperature for 12 h. The desired products **4b** (17.7%) and **5** (9.6%) were obtained along with undesired 14a-oxidized products, **6** (0.7%), **7** (3.2%), **8** (2.4%), (14*E*)-5,14a-dioxo-5-deoxymilbemycin A_4 (**9**) (3.4%), and 13β,14a-dihydroxy-5-oxo-5-deoxymilbemycin A_4 (**10**) (1.5%). When the oxidation reaction was carried out at a higher temperature for a shorter time, the yield of **4b** and **5** was slightly improved to 30.3% and 18.6%, respec-

Table 1. Selenium Dioxide Oxidation of Milbemycins

Entry	Starting[a] material	Conditions	Products[a] (yield/%)
1	**1b**	SeO_2 (1.36 molar amount), AcOH, r.t., 2 h	**4b** (4.8)+**1b** (4.8)[b]+**3b** (20.4)+**5** (1.7)+**6** (4.7)+**7** (0.6)+**8** (2.5)
2	**3b**	SeO_2 (1.36 molar amount), AcOH, r.t., 12 h	**4b** (17.7)+**5** (9.6)+**6** (0.7)+**7** (3.2)+**8** (2.4)+**9** (3.4)+**10** (1.5)
3	**3b**	SeO_2 (1.36 molar amount), AcOH, 40 °C, 3.5 h	**4b** (30.3)+**5** (18.6)+**6** (1.1)+**7** (8.8)+**8** (3.1)+**9** (2.9)+**10** (2.2)+**12** (1.0)
4	**3b**	SeO_2 (1.36 molar amount), HCO_2H, r.t., 1.5 h	**4b** (2.4)+**11** (33.9)
5	**3b**	SeO_2 (1.36 molar amount), HCO_2H, 40 °C, 1 h	**4b** (4.1)+**11** (44.0)
6	**3b**	SeO_2 (1.36 molar amount), HCO_2H, 60 °C, 0.5 h	**11** (19.4)
7	**3b**	SeO_2 (1.36 molar amount), $EtCO_2H$, 40 °C, 1.5 h	**4b** (5.5)+**7** (6.9)+**9** (4.4)+**10** (1.9)+**12** (0.7)
8	**3b**	SeO_2 (1 molar amount), *t*-BuOOH (6 molar amount), CH_2Cl_2, r.t., 23 h	**4b** (6.1)+**6** (13.6)+**8** (3.3)+**12** (3.5)

a) The structures of starting materials and products are shown in Schemes 1 and 2. b) The starting material was recovered.



Scheme 2.

tively, without the significant increase of the side products: **6** (1.1%), **7** (8.8%), **8** (3.1%), **9** (2.9%), **10** (2.2%), and (14Z)-13β,14a-dihydroxy-5-oxo-5-deoxymilbemycin A_4 (**12**) (1.0%). Formic acid was then adopted in place of acetic acid as a solvent. The selenium dioxide (1.36 molar amount) oxidation of **3b** in formic acid at 40 °C for one hour afforded **4b** (4.1%) and 13β-formyloxy-5-ketone **11** (44.0%) without the side products. The same desired products were obtained at a lower and a higher reaction temperature (room temperature and 60 °C), but their yields decreased. Among the other solvents tested, propionic acid could be also used for the oxidation, but the yield of **4b** was only 5.5%. The use of neutral protic and aprotic solvents such as ethanol, 95% ethanol, methanol, acetone, tetrahydrofuran, 1,4-dioxane, and dichloromethane resulted in the recovery of starting material and/or the formation of polar unidentified products. Then, we examined a mixed oxidizing reagent of selenium dioxide and *t*-butyl hydroperoxide, which had been used to convert an avermectin into the hydroxylated compound at the 4a-allylic position.[13,14] Oxidation of **3b** under the similar conditions with selenium dioxide (1 molar amount) and *t*-butyl hydroperoxide (6 molar amount) in dichloromethane at room temperature for 23 h also gave **4b** in 6.1% yield, together with 14a-hydroxylated milbemycins, **6** (13.6%), **8** (3.3%), and **12** (3.5%).

By using selenium dioxide in formic acid, consequently, the 13β-oxidized products of **4b** and **11** could be prepared from 5-oxo-5-deoxymilbemycin A_4 (**3b**) totally in approximate 50% yield.

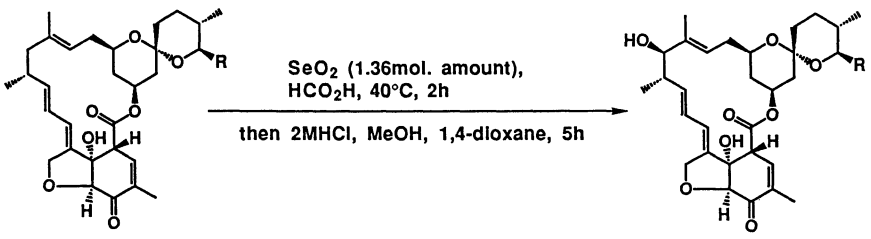
The transformation of the acetate **5** and the formate **11** into the free hydroxy compound **4b** was examined under basic and acidic conditions. These results are summarized in Table 2. The basic hydrolysis of the acetate **5** failed to afford **4b** and many unidentified polar compounds were formed. In the case of the formate **11**, **4b** was obtained under the similar basic conditions,

Table 2. Hydrolysis of **5** and **11**

Entry	Compound	Conditions	Isolated yield of **4b** (%)
1	**5**	K_2CO_3 (0.5 molar amount), MeOH, H_2O, 0 °C, 3 h	0
2	**5**	KOH (1 molar amount), MeOH, H_2O, 0 °C, 1 h	0
3	**11**	$KHCO_3$ (1 molar amount), MeOH, H_2O, r.t., 6 h	4.9
4	**11**	K_2CO_3 (0.5 molar amount), MeOH, H_2O, 0 °C, 70 min	19
5	**11**	KOH (1 molar amount), MeOH, H_2O, 0 °C, 30 min	25
6	**5**	2 M HCl, MeOH, 1,4-Dioxane, r.t., 3 d	54.3
7	**11**	2 M HCl, MeOH, 1,4-Dioxane, r.t., 5 h	80.5

but the yields were low (4.9—25%). On the other hand, the acidic hydrolysis was smoothly performed in good yields. The acetate **5** and the formate **11** were hydrolyzed with 2 M (1 M=1 mol dm^{-3}) hydrochloric acid to afford **4b** in 54.3% and 80.5% yields, respectively. Eventually, the optimum procedure for procuring the 13β-hydroxy compound was the successive treatment of **3b** with selenium dioxide in formic acid followed by acid hydrolysis, and after silica-gel chromatography **4b** was obtained from **3b** in 49.5% yield (Scheme 3). 5-Oxo-5-deoxymilbemycin A_3 (**3a**) and 5-oxo-5-deoxymilbemycin D (**3c**) were similarly subjected to the same two-step transformation to afford the corresponding 13β-hydroxy-5-ketones **4a** (48.6%) and **4c** (45.4%), respectively.

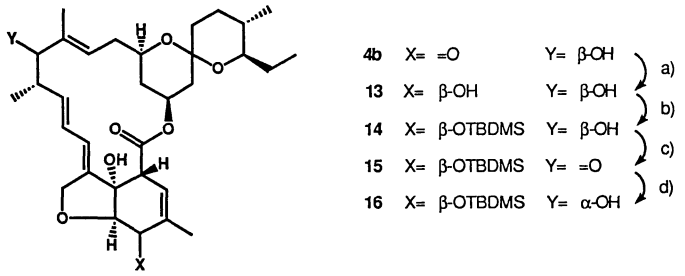
The configurational assignment of the introduced hydroxyl group was based on the comparison of the coupling constants at the 13-position in ^{1}H NMR spectra. The coupling constant of the hydrogen at the 13-position of 22,23-dihydroavermectin B_{1b} aglycon 5-silyl



3a (R=Me)
3b (R=Et)
3c (R=*i*-Pr)

4a (R=Me), 48.6%
4b (R=Et), 49.5%
4c (R=*i*-Pr), 45.4%

Scheme 3.



a) $NaBH_4$, MeOH, 0 °C, 5 min, 86.8% b) TBDMSOTf, 2,6-lutidine, DMF, r.t., 15 min, 31.0% c) Swern Oxidation, 73.8% d) $NaBH_4$, MeOH, −5 °C, 10 min, 92.9%

TBDMS=*t*-butyldimethylsilyl

Scheme 4.

ether is 0 Hz at 4.03 ppm.[8e] On the other hand, the hydrogen at the 13-position of **4b** had a large coupling constant (9.7 Hz at 3.73 ppm), which let us elucidate that **4b** had α-hydrogen (β-hydroxy) at the 13-position. To confirm it, 13α-hydroxy compound **16** was synthesized as follows (Scheme 4); the reduction of **4b** with sodium borohydride gave 13β-hydroxymilbemycin A_4 (**13**), of which 5-hydroxyl group was selectively protected to give 5-silyl ether **14**. The coupling constant of the 13-position of **14** was 9.7 Hz at 3.71 ppm. The subsequent oxidation and reduction of **14** yielded 13α-hydroxy-5-*O*-*t*-butyldimethylsilylmilbemycin A_4 (**16**), in which the coupling constant at the 13-position was 0 Hz at 4.00 ppm. According to these results, it was concluded that **4b** had a 13β-hydroxyl group.

In conclusion, the allylic oxidation of 5-oxo-5-deoxymilbemycins with selenium dioxide in formic acid followed by acidic hydrolysis was ascertained to be a useful method for gaining the 13β-hydroxymilbemycins, which are key intermediates for chemical modification. The transformation of the key intermediates to various 13-substituted milbemycins will be soon issued.

Experimental

IR spectra were recorded on a Perkin-Elmer 1600 Series FT IR spectrometer. ^1H NMR spectra were measured on a JOEL JNM-GX 270 FT NMR spectrometer with tetramethylsilane as an internal standard. MS spectra were measured on a JEOL JMS-D 300 spectrometer. Column chromatography were performed on silica gel (Merck silica gel 60, 230—400 mesh or Wakogel C-100, 40—100 mesh). Preparative TLC were performed on silica gel (Merck Kieselgel $60F_{254}$) of 0.5 or 2 mm thickness.

5-Oxo-5-deoxymilbemycin A_4 (3b).[2] To a solution of milbemycin A_4 (**1b**) (2.00 g) in acetone (50 ml) was added active MnO_2 (15.6 g). The mixture was vigorously stirred for 2 h at room temperature and the reaction mixture was filtered through a Celite®. The filtrate was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography (hexane-ethyl acetate gradient) to afford 1.39 g (69.8%) of 5-ketone **3b**.

5-Oxo-5-deoxymilbemycin A_3 (3a)[2] and 5-Oxo-5-deoxymilbemycin D (3c).[15] By using the same procedure described for the preparation of **3b**, milbemycin A_3 (**1a**) and milbemycin D (**1c**) were oxidized with MnO_2 to give **3a** in 77.6% yield and **3c** in 80.2% yield, respectively.

Oxidation of 3b with Selenium Dioxide in Formic Acid. 13β-Hydroxy-5-oxo-5-deoxymilbemycin A_4 (4b) and 13β-Formyloxy-5-oxo-5-deoxymilbemycin A_4 (11). To a solution of **3b** (2.00 g) in formic acid (25 ml) was added SeO_2 (0.616 g) and the mixture was stirred at 40 °C for 1 h. A Celite® filter aid was added to the reaction mixture and the selenium compound was filtered off. The filtrate was poured onto water and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated under reduced pressure. The

residue was purified by silica-gel column chromatography (hexane–ethyl acetate gradient) to afford 0.082 g (4.1%) of **4b** and 0.951 g (44.0%) of **11**.

Hydrolysis of 11. A solution of **11** (0.200 g) in methanol (25 ml), 1,4-dioxane (15 ml), and 2M HCl (10 ml) was stirred at room temperature for 8 h. The reaction mixture was poured onto water and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (hexane–ethyl acetate gradient) to afford 0.153 g (80.5%) of **4b**.

Typical Procedure for the Synthesis of 13β-Hydroxy-5-oxo-5-deoxymilbemycin A_4 (4b). To a solution of **3b** (2.00 g) in formic acid (25 ml) was added SeO_2 (0.559 g) and the mixture was stirred at 40 °C for 2 h. A Celite® filter aid was added to the reaction mixture and the selenium compound was filtered off. The filtrate was poured onto water and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product containing **11** and a smaller amount of **4b**. To the crude product was added methanol (120 ml), 2 M HCl (20 ml) and 1,4-dioxane (30 ml), and the mixture was stirred at room temperature for 5 h. The reaction mixture was poured onto water and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (hexane–ethyl acetate gradient) to afford 1.02 g (49.5%) of **4b**.

13β-Hydroxy-5-oxo-5-deoxymilbemycin A_4 (4b): IR (KBr) 3480, 2961, 2929, 2873, 1733, 1681, 1640, 1456, 1436, 1376, 1337, 1311, 1273, 1245, 1214, 1185, 1102, 1066, 1045, 1030, 988, 964, 894, 862, 754 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.05 (1H, m, H-18), 0.83 (3H, d, *J*=6.4 Hz, 24-CH_3), 1.00 (3H, t, *J*=7.3 Hz, 25-$CH_2C\underline{H}_3$), 1.13 (3H, d, *J*=6.5 Hz, 12-CH_3), 1.23—1.80 (10H, m, HO-13, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.58 (3H, s, 14-CH_3), 1.89—1.90 (3H, m, 4-CH_3), 2.01—2.07 (1H, m, H-20), 2.26—2.44 (3H, m, H-12, H_2-16), 3.08 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.53—3.63 (2H, m, H-2, H-17), 3.73 (1H, d, *J*=9.7 Hz, H-13), 3.85 (1H, s, H-6), 3.98 (1H, s, HO-7), 4.68—4.82 (2H, m, 8-CH_2-O-), 5.21—5.28 (1H, m, H-15), 5.36—5.48 (2H, m, H-11, H-19), 5.74—5.88 (2H, m, H-9, H-10), 6.54—6.55 (1H, m, H-3); MS *m/z* 556 (M^+, $C_{32}H_{44}O_8$), 538, 520, 279, 259, 241, 195, 167. HR-MS Found: *m/z* 556.3065. Calcd for $C_{32}H_{44}O_8$: M, 556.3036.

13β-Formyloxy-5-oxo-5-deoxymilbemycin A_4 (11): IR (KBr) 3474, 2962, 2929, 2874, 1728, 1682, 1642, 1456, 1436, 1373, 1337, 1310, 1274, 1238, 1177, 1102, 1067, 1045, 1032, 989, 966, 892, 862, 756 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.05 (1H, m, H-18), 0.83 (3H, d, *J*=6.6 Hz, 24-CH_3), 0.97—1.02 (6H, m, 12-CH_3, 25-$CH_2C\underline{H}_3$), 1.24—1.76 (9H, m, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.57 (3H, s, 14-CH_3), 1.89—1.90 (3H, m, 4-CH_3), 2.01—2.07 (1H, m, H-20), 2.20—2.38 (2H, m, H_2-16), 2.53—2.67 (1H, m, H-12), 3.04 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.55—3.63 (2H, m, H-2, H-17), 3.86 (1H, s, H-6), 4.02 (1H, s, HO-7), 4.69—4.81 (2H, m, 8-CH_2-O-), 5.05 (1H, d, *J*=10.5 Hz, H-13), 5.37—5.49 (3H, m, H-11, H-15, H-19), 5.80—5.91 (2H, m, H-9, H-10), 6.54—6.55 (1H, m, H-3), 8.09 (1H, s, HCO_2); MS *m/z* 584 (M^+, $C_{33}H_{44}O_9$), 566, 538, 279, 195, 167, 151. HR-MS Found: *m/z* 584.2985. Calcd for $C_{33}H_{44}O_9$: M, 584.2985.

13β-Hydroxy-5-oxo-5-deoxymilbemycin A_3 (4a) and 13β-Hydroxy-5-oxo-5-deoxymilbemycin D (4c). According to the typical procedure, 5-oxo-5-deoxymilbemycin A_3 (**3a**) and D (**3c**) were converted to **4a** in 48.6% yield and **4c** in 45.4% yield, respectively.

13β-Hydroxy-5-oxo-5-deoxymilbemycin A_3 (4a): IR (KBr) 3482, 2967, 2928, 2870, 1735, 1681, 1642, 1451, 1436, 1378, 1337, 1311, 1272, 1245, 1214, 1183, 1117, 1096, 1085, 1062, 1043, 997, 965, 890, 852, 797 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.84 (3H, d, *J*=6.4 Hz, 24-CH_3), 0.85—1.00 (1H, m, H-18), 1.13—1.17 (6H, m, 12-CH_3, 25-CH_3), 1.37 (1H, t, *J*=11.7 Hz, H-20), 1.51—1.77 (7H, m, HO-13, H-18, H_2-22, H_2-23, H-24), 1.59 (3H, s, 14-CH_3), 1.89—1.90 (3H, m, 4-CH_3), 2.00—2.07 (1H, m, H-20), 2.27—2.45 (3H, m, H-12, H_2-16), 3.22—3.33 (1H, m, H-25), 3.52—3.61 (2H, m, H-2, H-17), 3.73 (1H, d, *J*=9.7 Hz, H-13), 3.86 (1H, s, H-6), 3.98 (1H, br.s, HO-7), 4.69—4.81 (2H, m, 8-CH_2-O-), 5.23—5.30 (1H, m, H-15), 5.34—5.50 (2H, m, H-11, H-19), 5.74—5.87 (2H, m, H-9, H-10), 6.53—6.54 (1H, m, H-3); MS *m/z* 542 (M^+, $C_{31}H_{42}O_8$), 524, 506, 265, 259, 241, 181, 153. HR-MS Found: *m/z* 542.2866. Calcd for $C_{31}H_{42}O_8$: M, 542.2880.

13β-Hydroxy-5-oxo-5-deoxymilbemycin D (4c): IR (KBr) 3481, 2960, 2930, 2871, 1732, 1717, 1682, 1456, 1383, 1367, 1338, 1310, 1274, 1244, 1214, 1186, 1171, 1119, 1069, 1046, 1009, 965, 889, 863, 846 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.05 (1H, m, H-18), 0.81 (3H, d, *J*=5.6 Hz, 24-CH_3), 0.87 (3H, d, *J*=6.9 Hz, H$_3$-25-CH($C\underline{H}_3$)$_2$), 1.05 (3H, d, *J*=6.9 Hz, H$_3$-25-CH($C\underline{H}_3$)$_2$), 1.14 (3H, d, *J*=6.4 Hz, 12-CH_3), 1.32—1.75 (8H, m, HO-13, H-18, H-20, H_2-22, H_2-23, H-24), 1.58 (3H, s, 14-CH_3), 1.80—1.95 (1H, m, 25-$C\underline{H}(CH_3)_2$), 1.89—1.90 (3H, m, 4-CH_3), 2.01—2.07 (1H, m, H-20), 2.26—2.45 (3H, m, H-12, H_2-16), 3.08 (1H, br.d, *J*=9.7 Hz, H-25), 3.53—3.63 (2H, m, H-2, H-17), 3.73 (1H, d, *J*=9.7 Hz, H-13), 3.85 (1H, s, H-6), 3.97 (1H, s, HO-7), 4.68—4.82 (2H, m, 8-CH_2-O-), 5.21—5.27 (1H, m, H-15), 5.33—5.45 (2H, m, H-11, H-19), 5.74—5.88 (2H, m, H-9, H-10), 6.57—6.58 (1H, m, H-3); MS *m/z* 570 (M^+, $C_{33}H_{46}O_8$), 552, 534, 293, 259, 241, 209, 181. HR-MS Found: *m/z* 570.3181. Calcd for $C_{33}H_{46}O_8$: M, 570.3193.

Oxidation of 3b with Selenium Dioxide in Acetic Acid. To a solution of **3b** (200 mg) in acetic acid (3 ml) was added SeO_2 (56 mg) and the mixture was stirred at 40 °C for 3.5 h. The reaction mixture was filtered through Celite® and the filtrate was poured onto water. The resulting mixture was extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica-gel chromatography (hexane–ethyl acetate gradient), preparative TLC (chloroform : acetone=30 : 1—6 : 1), and HPLC (YMC Pack, A-323, S-5, 120A, ODS, 10 mm×250 mm; MeOH : H_2O=5 : 1) to give 62.4 mg (30.3%) of **4b**, 41.2 mg (18.6%) of 13β-acetoxy-5-oxo-5-deoxymilbemycin A_4 (**5**), 2.3 mg (1.1%) of 14a-hydroxy-5-oxo-5-deoxymilbemycin A_4 (**6**), 18.1 mg (8.8%) of 5,14a-dioxo-5-deoxymilbemycin A_4 (**7**), 6.3 mg (3.1%) of (14*E*)-14a-hydroxy-5-oxo-5-deoxymilbemycin A_4 (**8**), 6.0 mg (2.9%) of (14*E*)-5,14a-dioxo-5-deoxymilbemycin A_4 (**9**), 4.7 mg (2.2%) of 13β,14a-dihydroxy-5-oxo-5-deoxymilbemycin A_4 (**10**), and 2.1 mg (1.0%) of (14*Z*)-13β,14a-dihydroxy-5-oxo-5-deoxymilbemycin A_4 (**12**).

13β-Acetoxy-5-oxo-5-deoxymilbemycin A_4 (5): IR (KBr) 3450, 2964, 2930, 2874, 1738, 1682, 1639, 1456, 1436, 1372, 1336, 1310, 1272, 1238, 1190, 1172, 1101, 1067, 1045, 1030, 989, 894, 863, 845, 733 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.05 (1H, m, H-18), 0.83 (3H, d, *J*=6.5 Hz, 24-CH_3), 0.97—1.02 (6H, m, 12-CH_3, 25-$CH_2C\underline{H}_3$), 1.24—1.76 (9H, m, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.55 (3H, s, 14-CH_3), 1.89—1.90 (3H, m, 4-CH_3), 2.01—2.06 (1H, m, H-20), 2.06 (3H, s, CH_3CO_2), 2.19—2.38 (2H, m, H_2-16), 2.48—2.63 (1H, m, H-12), 3.05 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.55—3.63

(2H, m, H-2, H-17), 3.86 (1H, s, H-6), 4.02 (1H, br.s, HO-7), 4.69—4.81 (2H, m, 8-CH_2-O-), 4.95 (1H, d, J=10.5 Hz, H-13), 5.37—5.49 (3H, m, H-11, H-15, H-19), 5.78—5.90 (2H, m, H-9, H-10), 6.54—6.55 (1H, m, H-3); MS m/z 598 (M^+, $C_{34}H_{46}O_9$), 580, 538, 520, 412, 279, 195, 167, 151. HR-MS Found : m/z 598.3138. Calcd for $C_{34}H_{46}O_9$: M, 598.3141.

14a-Hydroxy-5-oxo-5-deoxymilbemycin A_4 (6): IR (KBr) 3469, 2960, 2929, 2873, 1734, 1681, 1457, 1436, 1376, 1338, 1315, 1273, 1244, 1189, 1180, 1102, 1032, 990, 907, 732 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.05 (1H, m, H-18), 0.83 (3H, d, J=6.5 Hz, 24-CH_3), 0.98—1.05 (6H, m, 12-CH_3, 25-$CH_2C\underline{H}_3$), 1.23—1.80 (11H, m, H-13, 14-$CH_2O\underline{H}$, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.88—1.90 (3H, m, 4-CH_3), 2.00—2.05 (1H, m, H-20), 2.29—2.36 (2H, m, H_2-16), 2.51—2.61 (2H, m, H-12, H-13), 3.08 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.55—3.67 (2H, m, H-2, H-17), 3.85 (1H, s, H-6), 3.94 (1H, d, J=12.1 Hz, H-14-$C\underline{H}_2$OH), 4.04 (1H, s, HO-7), 4.27 (1H, d, J=12.1 Hz, H-14-$C\underline{H}_2$OH), 4.66—4.79 (2H, m, 8-CH_2-O-), 5.10—5.15 (1H, m, H-15), 5.41—5.51 (2H, m, H-11, H-19), 5.77 (1H, dd, J=14.5 and 11.3 Hz, H-10), 5.87 (1H, dt, J_d=11.3 Hz, J_t=2.4 Hz, H-9), 6.54—6.55 (1H, m, H-3); MS m/z 556 (M^+, $C_{32}H_{44}O_8$), 538, 520, 412, 279, 195, 167, 151. HR-MS Found: m/z 556.3032. Calcd for $C_{32}H_{44}O_8$: M, 556.3036.

5,14a-Dioxo-5-deoxymilbemycin A_4 (7): IR (KBr) 3464, 2961, 2928, 2871, 1737, 1715, 1682, 1456, 1435, 1374, 1338, 1317, 1276, 1245, 1181,1166, 1102, 1065, 1032, 991, 907, 872, 733 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.08 (1H, m, H-18), 0.84 (3H, d, J=6.5 Hz, 24-CH_3), 0.99—1.05 (6H, m, 12-CH_3, 25-$CH_2C\underline{H}_3$), 1.24—1.82 (10H, m, H-13, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.88—1.90 (3H, m, 4-CH_3), 2.03—2.08 (1H, m, H-20), 2.52—2.62 (2H, m, H-12, H-16), 2.76 (1H, d, J=12.9 Hz, H-13), 2.98—3.12 (2H, m, H-16, H-25), 3.56—3.59 (1H, m, H-2), 3.71—3.80 (1H, m, H-17), 3.83 (1H, s, H-6), 3.99 (1H, br.s, HO-7), 4.65—4.77 (2H, m, 8-CH_2-O-), 5.38 (1H, dd, J=14.5 and 10.1 Hz, H-11), 5.43—5.55 (1H, m, H-19), 5.66 (1H, dd, J=14.5 and 11.3 Hz, H-10), 5.86 (1H, dt, J_d=11.3 Hz, J_t=2.4 Hz, H-9), 6.18 (1H, dd, J=12.9 and 3.6 Hz, H-15), 6.54—6.55 (1H, m, H-3), 10.07 (1H, s, 14-CH=O); MS m/z 554 (M^+, $C_{32}H_{42}O_8$), 536, 518, 496, 410, 371, 277, 259, 241, 195, 167, 151. HR-MS Found : m/z 554.2883. Calcd for $C_{32}H_{42}O_8$: M, 554.2879.

(14*E*)-14a-Hydroxy-5-oxo-5-deoxymilbemycin A_4 (8): IR (KBr) 3466, 2958, 2929, 2873, 1731, 1712, 1638, 1453, 1377, 1337, 1318, 1280, 1245, 1198, 1182, 1103, 1057, 1030, 988, 907, 884, 732 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.83 (3H, d, J=6.4 Hz, 24-CH_3), 0.97 (3H, t, J=7.3 Hz, 25-$CH_2C\underline{H}_3$), 1.03—1.17 (1H, m, H-18), 1.06 (3H, d, J=6.4 Hz, 12-CH_3), 1.23—1.75 (10H, m, 14-$CH_2O\underline{H}$, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.88—1.90 (3H, m, 4-CH_3), 1.88—1.94 (1H, m, H-20), 2.07—2.27 (2H, m, H_2-13), 2.30—2.34 (2H, m, H_2-16), 2.40—2.55 (1H, m, H-12), 3.05 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.53—3.55 (1H, m, H-2), 3.82—3.90 (1H, m, H-17), 3.86 (1H, s, H-6), 4.05 (1H, d, J=12.1 Hz, H-14-$C\underline{H}_2$OH), 4.10 (1H, d, J=12.1 Hz, H-14-$C\underline{H}_2$OH), 4.52 (1H, s, HO-7), 4.71 (1H, dd, J=14.5 and 2.0 Hz, H-8-CH_2-O-), 4.75 (1H, dd, J=14.5 and 2.0 Hz, H-8-CH_2-O-), 5.33 (1H, dd, J=14.1 and 10.1 Hz, H-11), 5.40—5.51 (1H, m, H-19), 5.61—5.65 (1H, m, H-15), 5.69 (1H, dd, J=14.1 and 11.3 Hz, H-10), 5.79 (1H, dt, J_d=11.3 Hz, J_t=2.4 Hz, H-9), 6.48—6.50 (1H, m, H-3); MS m/z 556 (M^+, $C_{32}H_{44}O_8$), 538, 498, 456, 430, 241, 195, 167, 151. HR-MS Found: m/z 556.3031. Calcd for $C_{32}H_{44}O_8$: M, 556.3036.

(14*E*)-5,14a-Dioxo-5-deoxymilbemycin A_4 (9): IR (KBr) 3462, 2960, 2928, 2871, 1736, 1712, 1686, 1641, 1455, 1435, 1375, 1337, 1319, 1281, 1244, 1232, 1200, 1183, 1102, 1069, 1057, 1032, 1001, 988, 970, 882, 731 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.84 (3H, d, J=6.5 Hz, 24-CH_3), 0.92—1.05 (1H, m, H-18), 0.98 (3H, t, J=7.3 Hz, 25-$CH_2C\underline{H}_3$), 1.09 (3H, d, J=6.9 Hz, 12-CH_3), 1.25—1.80 (9H, m, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.88—1.89 (3H, m, 4-CH_3), 1.93—2.08 (2H, m, H-13, H-20), 2.41—2.62 (4H, m, H-12, H-13, H_2-16), 3.06 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.53—3.57 (1H, m, H-2), 3.84 (1H, s, H-6), 3.93—4.01 (1H, m, H-17), 4.41 (1H, s, HO-7), 4.68 (1H, dd, J=14.9 and 2.4 Hz, H-8-CH_2-O-), 4.71 (1H, dd, J=14.9 and 2.4 Hz, H-8-CH_2-O-), 5.29 (1H, dd, J=14.5 and 10.5 Hz, H-11), 5.40—5.54 (1H, m, H-19), 5.62 (1H, dd, J=14.5 and 11.3 Hz, H-10), 5.76 (1H, dt, J_d=11.3 Hz, J_t=2.4 Hz, H-9), 6.48—6.50 (1H, m, H-3), 6.72 (1H, dd, J=8.9 and 5.6 Hz, H-15), 9.41 (1H, d, J=1.2 Hz, 14-CH=O); MS m/z 554 (M^+, $C_{32}H_{42}O_8$), 536, 496, 428, 195, 167, 149. HR-MS Found: m/z 554.2891. Calcd for $C_{32}H_{42}O_8$: M, 554.2880.

13β,14a-Dihydroxy-5-oxo-5-deoxymilbemycin A_4 (10): IR (KBr) 3450, 2960, 2929, 2873, 1735, 1680, 1458, 1438, 1377, 1338, 1312, 1274, 1244, 1214, 1186, 1101, 1065, 1045, 1031, 989, 967, 898, 733 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.79—0.92 (1H, m, H-18), 0.84 (3H, d, J=6.4 Hz, 24-CH_3), 0.99 (3H, t, J=7.3 Hz, 25-$CH_2C\underline{H}_3$), 1.19 (3H, d, J=6.4 Hz, 12-CH_3), 1.23—1.80 (11H, m, HO-13, 14-$CH_2O\underline{H}$, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.89—1.90 (3H, m, 4-CH_3), 2.01—2.07 (1H, m, H-20), 2.23—2.45 (2H, m, H_2-16), 2.51—2.65 (1H, m, H-12), 3.08 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.53—3.68 (2H, m, H-2, H-17), 3.80 (1H, d, J=10.1 Hz, H-13), 3.86 (1H, s, H-6), 3.99 (1H, s, HO-7), 4.13 (1H, d, J=12.1 Hz, H-14-$C\underline{H}_2$OH), 4.13 (1H, d, J=12.1 Hz, H-14-$C\underline{H}_2$OH), 4.74 (1H, d, J=14.5 Hz, H-8-CH_2-O-), 4.75 (1H, d, J=14.5 Hz, H-8-CH_2-O-), 5.33—5.48 (3H, m, H-11, H-15, H-19), 5.75—5.88 (2H, m, H-9, H-10), 6.53—6.55 (1H, m, H-3); MS m/z 572 (M^+, $C_{32}H_{44}O_9$), 554, 536, 295, 277, 259, 242, 195, 167. HR-MS Found: m/z 572.3005. Calcd for $C_{32}H_{44}O_9$: M, 572.2985.

(14*Z*)-13β,14a-Dihydroxy-5-oxo-5-deoxymilbemycin A_4 (12): IR (KBr) 3438, 2960, 2929, 2874, 1731, 1679, 1453, 1435, 1377, 1329, 1311, 1280, 1245, 1195, 1183, 1102, 1060, 1030, 988, 910, 733 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.05 (1H, m, H-18), 0.83 (3H, d, J=6.4 Hz, 24-CH_3), 0.96 (3H, t, J=7.3 Hz, 25-$CH_2C\underline{H}_3$), 1.18 (3H, d, J=6.4 Hz, 12-CH_3), 1.23—1.80 (11H, m, HO-13, 14-$CH_2O\underline{H}$, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.88—1.90 (3H, m, 4-CH_3), 1.88—1.95 (1H, m, H-20), 2.23—2.45 (2H, m, H_2-16), 2.51—2.65 (1H, m, H-12), 3.04 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.51—3.55 (1H, m, H-2), 3.83—3.92 (1H, m, H-17), 3.86 (1H, s, H-6), 4.13 (1H, d, J=12.1 Hz, H-14-$C\underline{H}_2$OH), 4.27 (1H, d, J=10.1 Hz, H-13), 4.45 (1H, d, J=12.1 Hz, H-14-$C\underline{H}_2$OH), 4.53 (1H, s, HO-7), 4.71 (1H, d, J=14.5 Hz, H-8-CH_2-O-), 4.74 (1H, d, J=14.5 Hz, H-8-CH_2-O-), 5.27—5.50 (2H, m, H-11, H-19), 5.70—5.81 (3H, m, H-9, H-10, H-15), 6.48—6.49 (1H, m, H-3); MS m/z 572 (M^+, $C_{32}H_{44}O_9$), 554, 536, 295, 277, 259, 241, 205, 195, 167, 149. HR-MS Found: m/z 572.2960. Calcd for $C_{32}H_{44}O_9$: M, 572.2985.

Oxidation of 3b with Selenium Dioxide and *t*-Butyl Hydroperoxide. To a mixture of *t*-butyl hydroperoxide (3 M solution in toluene, 2 ml) and dichloromethane (8 ml) was added SeO_2 (121 mg). The mixture was stirred at room temperature for 15 min and a solution of **3b** (543 mg) in dichloromethane (12 ml) was added. After stirring at room temperature for 23 h, the reaction mixture was poured onto water and extracted with ethyl acetate. The extract was washed with water and

brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica-gel chromatography (hexane–ethyl acetate gradient) and preparative TLC (chloroform: acetone=7 : 1—5 : 1 and hexane : ethyl acetate=2 : 1—3 : 2) to give 33.8 mg (6.1%) of **4b**, 76.4 mg (13.6%) of **6**, 18.4 mg (3.3%) of **8**, and 20.5 mg (3.5%) of **12**.

13β-Hydroxymilbemycin A_4 (13). To a solution of **4b** (556 mg) in methanol (30 ml) at 0 °C was added $NaBH_4$ (38 mg) and the reaction mixture was stirred at 0 °C for 5 min. The reaction mixture was poured onto ice cold water and extracted with ethyl acetate. The extract was washed with water and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica-gel chromatography (hexane–ehyl acetate gradient) to afford 484 mg (86.8%) of **13**. IR (KBr) 3468, 2961, 2930, 2873, 1715, 1642, 1456, 1376, 1338, 1309, 1273, 1245, 1214, 1182, 1117, 1101, 1066, 1044, 1029, 991, 963, 895, 864 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.05 (1H, m, H-18), 0.83 (3H, d, *J*=6.4 Hz, 24-CH_3), 0.99 (3H, t, *J*=7.3 Hz, 25-$CH_2C\underline{H}_3$), 1.13 (3H, d, *J*=6.4 Hz, 12-CH_3), 1.23—1.75 (11H, m, HO-5, HO-13, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.58 (3H, s, 14-CH_3), 1.87 (3H, s, 4-CH_3), 1.98—2.05 (1H, m, H-20), 2.26—2.44 (3H, m, H-12, H_2-16), 3.07 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.25—3.28 (1H, m, H-2), 3.53—3.63 (1H, m, H-17), 3.72 (1H, d, *J*=9.3 Hz, H-13), 3.96 (1H, d, *J*=6.0 Hz, H-6), 4.03 (1H, s, HO-7), 4.30 (1H, d, *J*=6.0 Hz, H-5), 4.61—4.75 (2H, m, 8-CH_2-O-), 5.21—5.48 (4H, m, H-3, H-11, H-15, H-19), 5.73—5.85 (2H, m, H-9, H-10); MS *m/z* 558 (M^+, $C_{32}H_{46}O_8$), 540, 522, 430, 412, 279, 261, 195, 167. HR-MS Found: *m/z* 558.3198. Calcd for $C_{32}H_{46}O_8$: M, 558.3193.

13β-Hydorxy-5-*O*-*t*-butyldimethylsilylmilbemycin A_4 (14). To a solution of **13** (0.300 g) in dry DMF (5 ml) at 0 °C were added 2,6-lutidine (63.2 mg) and *t*-butyldimethylsilyl trifluoromethanesulfonate (156 mg). After stirring at 0 °C for 15 min, the reaction mixture was poured onto water, and extracted with ethyl acetate. The extract was washed with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (hexane–ehyl acetate gradient) to yield 112 mg (31.0%) of **14**. IR (KBr) 3466, 2958, 2929, 2858, 1713, 1630, 1461, 1371, 1337, 1307, 1289, 1273, 1250, 1214, 1181, 1169, 1125, 1102, 1087, 1045, 1029, 990, 963, 865, 837 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.13 (6H, s, $(CH_3)_2$-Si-O), 0.80—1.05 (1H, m, H-18), 0.82 (3H, d, *J*=6.4 Hz, 24-CH_3), 0.92 (9H, s, *t*-C_4H_9-Si-O), 0.98 (3H, t, *J*=7.3 Hz, 25-$CH_2C\underline{H}_3$), 1.12 (3H, d, *J*=6.5 Hz, 12-CH_3), 1.23—1.75 (10H, m, HO-13, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.59 (3H, s, 14-CH_3), 1.79 (3H, d, *J*=1.2 Hz, 4-CH_3), 2.00—2.06 (1H, m, H-20), 2.25—2.43 (3H, m, H-12, H_2-16), 3.07 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.34—3.37 (1H, m, H-2), 3.51—3.62 (1H, m, H-17), 3.71 (1H, d, *J*=9.7 Hz, H-13), 3.81 (1H, d, *J*=5.6 Hz, H-6), 4.04 (1H, s, HO-7), 4.42—4.44 (1H, m, H-5), 4.59 (1H, dd, *J*=14.5 and 1.6 Hz, H-8-CH_2-O-), 4.76 (1H, dd, *J*=14.5 and 1.6 Hz, H-8-CH_2-O-), 5.20—5.37 (4H, m, H-3, H-11, H-15, H-19), 5.70—5.84 (2H, m, H-9, H-10); MS *m/z* 672 (M^+, $C_{38}H_{60}O_8Si$), 654, 597, 430, 412, 375, 279, 261, 195, 167. HR-MS Found: *m/z* 672.4071. Calcd for $C_{38}H_{60}O_8Si$: M, 672.4058.

13-Oxo-5-*O*-*t*-butyldimethylsilylmilbemycin A_4 (15). To a solution of oxalyl chloride (43.5 μl) in dichloromethane (2.5 ml) at −60 °C was added dimethyl sulfoxide (97.4 μl). The mixture was stirred for 5 min at −60 °C and a solution of **14** (264 mg) in dichloromethane (2 ml) was added. After stirred at −60 °C for 40 min, triethylamine (393 μl) was added to the mixture. The cooling bath was removed and the reaction mixture was stirred at ambient temperature for 30 min. Water was added to the mixture and extracted with ethyl acetate. The extract was washed with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by preparative TLC (hexane : ethyl acetate=4 : 1) to give 194 mg (73.8%) of **15**. IR (KBr) 3467, 2955, 2930, 2857, 1713, 1677, 1643, 1462, 1373, 1335, 1308, 1293, 1277, 1251, 1215, 1168, 1126, 1101, 1087, 1048, 1030, 988, 966, 871, 837 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.14 (6H, s, $(CH_3)_2$-Si-O), 0.84 (3H, d, *J*=6.4 Hz, 24-CH_3), 0.90—1.10 (1H, m, H-18), 0.92—0.97 (12H, m, 25-$CH_2C\underline{H}_3$, *t*-C_4H_9-Si-O), 1.16 (3H, d, *J*=6.8 Hz, 12-CH_3), 1.25—1.75 (9H, m, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.80 (3H, s, 4-CH_3), 1.83 (3H, s, 14-CH_3), 2.00—2.05 (1H, m, H-20), 2.18—2.30 (1H, m, H-16), 2.50—2.58 (1H, m, H-16), 3.04 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.40—3.42 (1H, m, H-2), 3.55—3.67 (2H, m, H-12, H-17), 3.83 (1H, d, *J*=5.6 Hz, H-6), 4.35 (1H, s, HO-7), 4.42—4.45 (1H, m, H-5), 4.62 (1H, dd, *J*=14.5 and 2.4 Hz, H-8-CH_2-O-), 4.73 (1H, dd, *J*=14.5 and 2.4 Hz, H-8-CH_2-O-), 5.31—5.44 (3H , m, H-3, H-11, H-19), 5.81 (1H, d, *J*=11.3 Hz, H-9), 6.03 (1H, dd, *J*=14.9 and 11.3 Hz, H-10), 6.19—6.25 (1H, m, H-15); MS *m/z* 670 (M^+, $C_{38}H_{58}O_8Si$), 613, 595, 453, 428, 375, 295, 277, 225, 195, 167. HR-MS Found: *m/z* 670.3894. Calcd for $C_{38}H_{58}O_8Si$: M, 670.3901.

13α-Hydroxy-5-*O*-*t*-butyldimethylsilylmilbemycin A_4 (16). To a solution of **15** (185.7 mg) in methanol (10 ml) at −5 °C was added $NaBH_4$ (19 mg). The reaction mixture was stirred at −5 °C for 10 min and evaporated under reduced pressure. The residue was purified by preparative TLC (hexane : ethyl acetate=4 : 1) to afford 173.3 mg (92.9%) of **16**. IR (KBr) 3486, 2955, 2930, 2857, 1713, 1633, 1462, 1373, 1337, 1308, 1289, 1272, 1250, 1197, 1181, 1171, 1126, 1101, 1087, 1043, 1030, 992, 966, 869, 837 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.13 (6H, s, $(CH_3)_2$-Si-O-), 0.75—1.00 (1H, m, H-18), 0.82 (3H, d, *J*=6.5 Hz, 24-CH_3), 0.92 (9H, s, *t*-C_4H_9-Si-O), 0.98 (3H, t, *J*=7.3 Hz, 25-$CH_2C\underline{H}_3$), 1.16 (3H, d, *J*=7.3 Hz, 12-CH_3), 1.23—1.75 (10H, m, HO-13, H-18, H-20, H_2-22, H_2-23, H-24, 25-$C\underline{H}_2CH_3$), 1.53 (3H, s, 14-CH_3), 1.76 (3H, s, 4-CH_3), 1.98—2.04 (1H, m, H-20), 2.27—2.33 (2H, m, H_2-16), 2.45—2.57 (1H, m, H-12), 3.08 (1H, td, J_t=9.3 Hz, J_d=2.4 Hz, H-25), 3.34—3.36 (1H, m, H-2), 3.59—3.69 (1H, m, H-17), 3.81 (1H, d, *J*=5.6 Hz, H-6), 4.00 (1H, s, H-13), 4.12 (1H, br.s, HO-7), 4.42—4.45 (1H, m, H-5), 4.58 (1H, d, *J*=14.5 Hz, H-8-CH_2-O-), 4.66 (1H, d, *J*=14.5 Hz, H-8-CH_2-O-), 5.30—5.40 (3H, m, H-3, H-15, H-19), 5.63—5.77 (3H, m, H-9, H-10, H-11); MS *m/z* 672 (M^+, $C_{38}H_{60}O_8Si$), 654, 597, 430, 412, 375, 279, 261, 195, 167. HR-MS Found: *m/z* 672.4047. Calcd for $C_{38}H_{60}O_8Si$: M, 672.4058.

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