MILBEMYCIN DERIVATIVES: EPOXIDATION OF MILBEMYCINS

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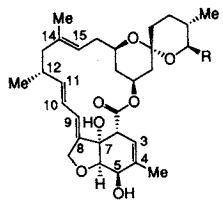
Epoxidation reactions (MCPBA epoxidation and Sharpless epoxidation) were examined as a means of chemically modifying milbemycins as part of our program for discovering anthelmintics. 8,9-Epoxy-, 14,15-epoxy-, 8,9-14,15-diepoxy-, and 3,4-8,9-14,15-triepoxymilbemycin A_4 were selectively obtained from milbemycin A_4 and its derivatives, in which either the C-5 and C-7 hydroxyl groups or C-5 alone were protected as appropriate by a silyl ether (in the former case) or a carbonyl group. Further silylation or epoxidation on these epoxidized compounds indicated that the configuration of each epoxide moiety of the mono- and diepoxides is in accord with that of the corresponding epoxide moiety of the triepoxide. Furthermore, in order to confirm the absolute configurations of these epoxide functionalities, an X-ray analysis of a carbamate derivative from the triepoxymilbemycin was conducted.

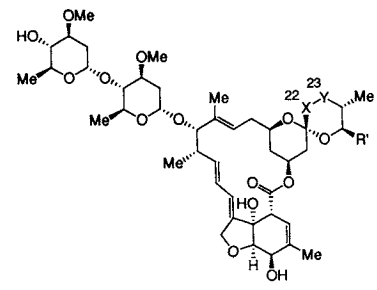
Milbemycins are a family of naturally-occurring 16-membered ring macrolides which were first isolated from *Streptomyces hydroscopicus* subsp. *aureolacrimosus* by Sankyo chemists.[1] Thereafter, the structurally-related avermectins were isolated by a group from Merck[2] (Fig. 1). These families of compounds have attracted considerable interest from several laboratories due to their potent anthelmintic, acaricidal and insecticidal activities, as well as their structural uniqueness.[3~6] In our laboratories, efforts to develop milbemycins as anthelmintics have been made. However, since milbemycins were found to be inferior in anthelmintic activity, though less toxic, than avermectins, this prompted us to carry out chemical modifications on these compounds.

Epoxidation is an important method for modifying the chemical and/or physical characteristics of

Fig. 1. Structures of milbemycins and avermectins.



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



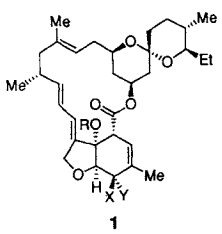
Avermectin	R′	X–Y
B_{1a}	*s*-Bu	CH=CH
B_{1b}	*i*-Pr	CH=CH
B_{2a}	*s*-Bu	CH_2–CHOH
B_{2b}	*i*-Pr	CH_2–CHOH

compounds bearing double bonds. This conversion preserves the overall molecular shape, but changes the electronic characteristics of a molecule. Moreover, improvements in the photostability of milbemycins by epoxidation of its 8,9-double bond can be expected on the basis of similar observations being reported elsewhere.[7~9] In this paper, the effect of epoxidation reactions on milbemycin substrates[7,8,10~17] and the stereochemistry of the products are described.

Results and Discussion

Firstly, epoxidation of naturally-occurring milbemycin A_4 (**1a**) was examined.[18] When **1a** was treated with 1.5 equiv of *m*-chloroperbenzoic acid (MCPBA), and then purification by chromatography on silica gel, 14,15-epoxide **2a** was obtained as the major product (59% yield). In the remaining impure fraction, at least 3 other unidentifiable components could be detected by TLC analysis. Of these unknown compounds, 8,9-14,15-diepoxide **3a** and 3,4-8,9-14,15-triepoxide **4a** were identified to be present by simply comparing the ^{1}H NMR data and TLC Rf values of the compounds **3a** and **4a** which could be obtained

Table 1. Epoxidation of Milbemycins.



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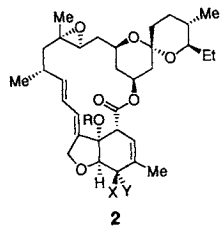

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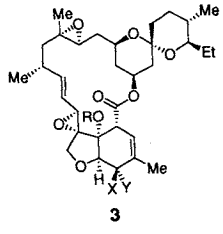

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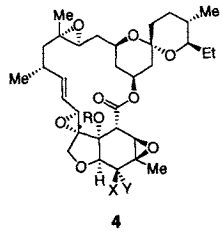

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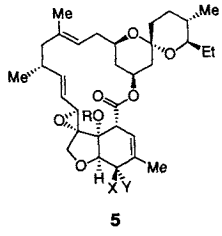

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Substrate	R	X	Y	Conditions	Products (yield)
1a	H	OH	H	1.5 equiv MCPBA, 0°C, 1 hour	**2a** (59%)
1a	H	OH	H	3.0 equiv MCPBA, rt, 1 hour	**4a** (76%)
1d	H	–O–		1.5 equiv MCPBA, 0°C, 1 hour[a]	**2a** (56%)+**3a** (36%)
1d	H	–O–		3.0 equiv MCPBA, rt, 1 hour[a]	**3a** (82%)
1d	H	–O–		1.5 equiv TBHP/cat. VO $(acac)_2$, $-20\sim0$°C, 1 hour[a]	**5a** (65%)
1d	H	–O–		30% H_2O_2-1 N NaOH	[b]
1e	H	OTBS[d]	H	1.5 equiv MCPBA, 0°C, 1 hour	**2e** (59%)+**3e** (35%)
1e	H	OTBS[d]	H	2.0 equiv MCPBA, 0°C, 1 hour	**3e** (73%)
1e	H	OTBS[d]	H	1.3 equiv TBHP/cat. VO $(acac)_2$, $-20\sim0$°C, 3 hours	**5e** (78%)
1f	TMS[c]	OTBS[d]	H	1.5 equiv MCPBA, 0°C, 1 hour	**2f** (90%)+**3f** (7%)

[a] Reduction with $NaBH_4$ was subsequently carried out.
[b] Decomposition of the starting material.
[c] TMS: trimethylsilyl.
[d] TBS: *tert*-butyldimethylsilyl.

in pure form from the reactions described below. On the other hand, the reaction with 3 equiv of the oxidant at room temperature (rt) preferentially afforded the triepoxide **4a** in 76% yield as a single diastereomer. Other possible diastereomers which the triepoxide conversion could generate were not identified in this reaction.

Next, 5-oxomilbemycin A_4 (**1d**)[19,20] was chosen as the substrate for epoxidation reactions. The 3,4-double bond was expected to be unreactive to electrophilic reagents because of its electron-deficient nature. As 5-oxomilbemycins are unstable in silica gel chromatography, epoxidized 5-oxomilbemycins were subsequently treated with $NaBH_4$ to reduce the carbonyl group at C-5 into a hydroxyl group.[20] Reaction of **1d** with 1.5 equiv of MCPBA at 0°C for 1 hour, and the following reduction with $NaBH_4$ provided the 14,15-epoxide **2a** and the 8,9-14,15-diepoxide **3a** in 56% and 36% yield, respectively. In the case of 3 equiv of MCPBA being employed, the diepoxide **3a** was produced predominantly (82% yield). In these reactions, epoxidation did not take place at the 3,4-double bond as expected. Epoxidation of 5-oxomilbemycin A_4 (**1d**) under basic conditions (NaOH, H_2O_2)[21] was attempted in expectation of obtaining a 3,4-epoxy derivative. The reaction, however, resulted in the decomposition of the starting material due to its instability in basic conditions. Regioselective epoxidation of the 8,9-double bond was achieved by Sharpless epoxidation. Treatment of **1d** with 1.5 equiv of *tert*-butyl hydroperoxide in the presence of 10 mol% of $VO(acac)_2$ at $-20 \sim 0$°C for 1 hour, and subsequent reduction of the carbonyl group with $NaBH_4$, afforded the 8,9-epoxide **5a** in 65% yield.

The participation of a hydroxyl group during a MCPBA-promoted epoxidation of allyl alcohols is exemplified in the literature.[22~25] Therefore, epoxidation of *O*-silyl-protected milbemycins was carried out with the expectation of the reaction products being different to those produced when the same epoxidation conditions were applied to the non-silylated milbemycin substrate **1a**. Indeed, when 5-*O*-(*tert*-butyldimethylsilyl)milbemycin A_4 (**1e**) was treated with 1.5 equiv of MCPBA at 0°C for 1 hour, 14,15-epoxide **2e**[18] and 8,9-14,15-diepoxide **3e** were isolated as the sole products in 59% and 35% yield, respectively. The formation of **2e** and **3e** is significant in that it is in accordance with the premise asserted above concerning epoxidation of allyl alcohols. It was interesting to note also that the proportion of the products was similar to that in the reaction of 5-oxomibemycin **1d** with 1.5 equiv of MCPBA. Moreover, reaction of **1e** with 2 equiv of MCPBA at 0°C afforded **3e** in 73% yield. In these reactions, protection of the C-5 hydroxyl group with the *tert*-butyldimethylsilyl group, or the C-5 hydroxyl group being in the form of a "latent" carbonyl group, retarded epoxidation of the 3,4-double bond. (Reaction of diepoxide **3e** with excess MCPBA at room temperature gave a mixture of compounds which was assumed to be a pair of diastereomers of the corresponding 3,4-8,9-14,15-triepoxide with respect to the 3,4-epoxide moiety.) Sharpless epoxidation was also performed on the 5-*O*-(*tert*-butyldimethylsilyl)milbemycin A_4 (**1e**).[14~16] This reaction afforded the expected 8,9-epoxide **5e** in good yield (78%).

In the epoxidation reactions using MCPBA described above, the 14,15-double bond had greater facility over other olefinic moieties in being converted to an epoxide moiety. In these reactions, however, considerable amounts of 8,9-epoxidation products were isolated. It is reported that the protection of the C-7 hydroxyl group in an avermectin derivative retards epoxidation of the 8,9-double bond.[17] Then, 5-*O*-(*tert*-butyldimethylsilyl)-7-*O*-(trimethylsilyl)milbemycin A_4 (**1f**) was selected as the substrate for examining the selectivity of 14,15-epoxidation. Indeed, the reaction of **1f** with 1.5 equiv of MCPBA at 0°C proceeded selectively to give the corresponding 14,15-epoxide **2f** in 90% yield, and only 7% of the 8,9-14,15-diepoxide **3f** was isolated.

Structure Determination of the Epoxide Groups

The transformations shown in Scheme 1 revealed that the configurations of the 8,9- and 14,15-epoxide groups in all derivatives were identical to those in the triepoxide **4a**. The stereochemistry of the 8,9- and 14,15-epoxide moieties was assumed to be the α-configuration from precedent literature.[7,8,15,16,18] The 3,4-epoxide group[17] was the remaining moiety with undetermined stereochemistry, To resolve this, crystalline derivatives from the triepoxide **4a** were sought for X-ray analysis. After experimentation with several carbamate derivatives of **4a**, *p*-bromophenylcarbamate **4g** was found to afford a sufficient crop of crystals with a morphology suitable for X-ray analysis (for crystal data, see experimental section). As shown in Fig. 2, the X-ray data both confirms that the 8,9- and 14,15-epoxide moieties have the α-configuration and also reveals that the 3,4-epoxide group is positioned on the β-face of the cyclohexane ring.

Scheme 1. Transformations of epoxymilbemycins.

a: MCPBA, b: TBSCl/imidazole, c: TMSCl/DMAP.

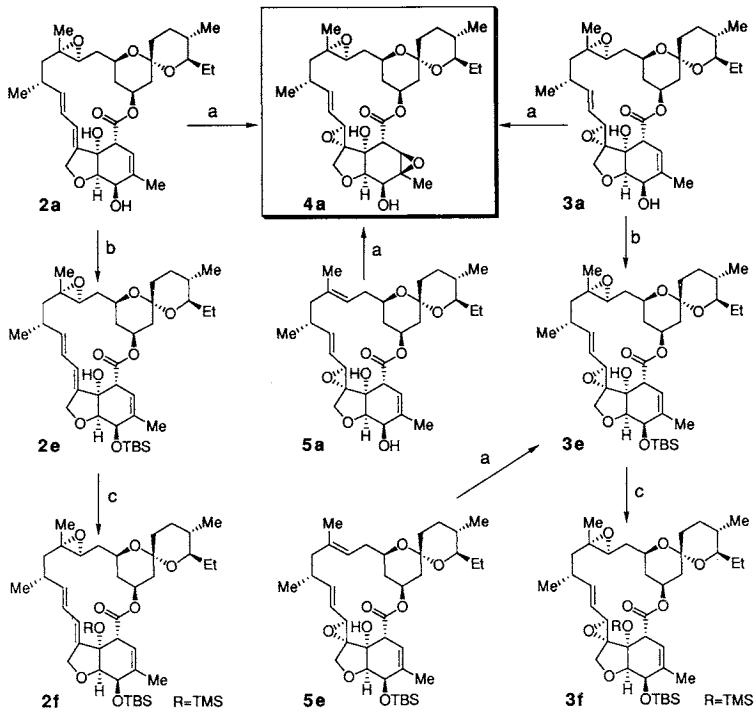
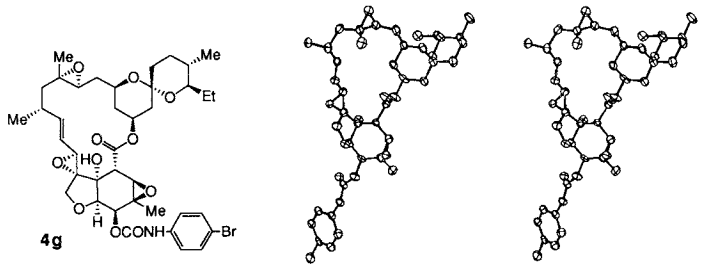


Fig. 2. Molecular structure and stereoscopic drawing of *p*-bromophenylcarbamate **4g** (hydrogen atoms are omitted).



This β-face orientation may be a result of the participation of the C-5 hydroxyl group during epoxidation using MCPBA, despite the reaction taking place from a concave face.

In summary, from these experiments the 14,15-double bond can be singled out as a facile one toward MCPBA epoxidation on the milbemycin skeleton. Protection of the C-5 and C-7 hydroxyl group retards epoxidation at the 3,4- and 8,9-double bonds, respectively. However, it is further established that the 10,11-double bond is insufficiently reactive toward MCPBA. It can be argued that the equatorial C-12 methyl group seems to impede the 10,11-double bond from undergoing epoxidation on the basis of steric crowding. Sharpless epoxidation selectively converts 5-*O*-protected milbemycins to the corresponding 8,9-epoxides. Structural evidence for the stereochemistry of the 8,9- and 14,15-epoxide moieties to be α and that of the 3,4-epoxide position to be β was provided by X-ray crystallographic data.

Experimental

General Methods

The natural milbemycin A_4 isolated from *Streptomyces hygroscopicus* subsp. *aureolacrimosus* was used as the starting material, which was purified by flash chromatography prior to experimentation and showed >96% purity by HPLC analysis. All compounds were characterized by NMR spectra on a JEOL GSX 400 or a JEOL GX 270 spectrometer in $CDCl_3$ solution with tetramethylsilane as internal reference, by mass spectra on a JEOL JMS-AX505H model and by IR spectra on a JASCO FT/IR-830 and were in full agreement with the assigned structures. Melting points were obtained on a YANACO MP-S3 micro-melting point apparatus and are uncorrected. Unless otherwise indicated all common reagents and solvents were used as obtained from commercial suppliers without further purification.

5-Oxomilbemycin A_4 (**1d**)

1d was prepared according to the literature procedure[19] from milbemycin A_4 (**1a**) by oxidation with active MnO_2.

5-*O*-(*tert*-Butyldimethylsilyl)milbemycin A_4 (**1e**)

To a solution of milbemycin A_4 (2.97 g, 5.47 mmol) and imidazole (0.82 g, 12.0 mmol) in DMF (14 ml) was added *tert*-butyldimethylsilyl chloride (TBSCl; 1.81 g, 12.0 mmol) in one portion at room temperature under a nitorgen atmosphere. The reaction mixture was stirred for 3 hours, poured into water, extracted with EtOAc, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from hexane to give 2.23 g of **1e** (62%) as colorless needles: mp 208~211°C; IR (KBr) cm^{-1} 3423, 1699, 1079, 997; MS *m/z* 656 (M^+), 195, 167; partial ^1H NMR (270 MHz, $CDCl_3$) δ 5.68~5.80 (2H, m, 9-H and 10-H), 5.28~5.40 (3H, m, 3-H, 11-H and 19-H), 4.93 (1H, m, 15-H), 4.67 (1H, br d, J=14.4 Hz, 8a-H), 4.57 (1H, br d, J=14.4 Hz, 8a-H), 4.42~4.44 (1H, m, 5-H), 4.13 (1H, s, 7-OH), 3.81 (1H, d, J=5.4 Hz, 6-H), 3.52~3.61 (1H, m, 17-H), 3.36 (1H, m, 2-H), 3.07 (1H, dt, J=2.5, 9.3 Hz, 25-H), 1.79 (3H, br s, 4a-H), 1.54 (3H, s, 14a-H), 0.93 (9H, s, *t*-BuSi), 0.13 (6H, s, Me_2Si).

5-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-(trimethylsilyl)milbemycin A_4 (**1f**)

To a solution of 5-*O*-(*tert*-butyldimethylsilyl)milbemycin A_4 (1.31 g, 2.0 mmol) and imidazole (0.41 g, 6.0 mmol) in DMF (6 ml), chlorotrimethylsilane (TMSCl; 0.38 ml, 3.0 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 40 hours, poured into water, extracted with EtOAc and washed with water. The extract was dried over Na_2SO_4, evaporated under reduced pressure and purified by silica gel chromatography (hexane-EtOAc, 15:1) to give 1.40 g of **1f** (96%): IR (KBr) cm^{-1} 1743, 1176, 1169, 1088, 990, 839; MS *m/z* 728 (M^+), 223, 167, 150; partial ^1H NMR (270 MHz, $CDCl_3$) δ 5.79 (1H, dd, J=11.2, 14.7 Hz, 10-H), 5.66 (1H, br d, J=11.2 Hz, 9-H), 5.45~5.47 (1H, m, 3-H), 5.36 (1H, dd, J=9.7, 14.7 Hz, 11-H), 5.02~5.08 (1H, m, 15-H), 4.77~4.90 (1H, m, 19-H), 4.66 (1H, dd, J=2.4, 14.2 Hz, 8a-H), 4.55 (1H, dd, J=2.0, 14.4 Hz, 8a-H), 4.38~4.40 (1H, m, 5-H), 3.79 (1H, d, J=4.9 Hz, 6-H), 3.57~3.61 (1H, m, 17-H), 3.23 (1H, m, 2-H), 3.05 (1H, dt, J=2.5, 9.3 Hz, 25-H), 1.79 (3H, br s,

4a-H), 1.55 (3H, s, 14a-H), 0.93 (9H, s, *t*-BuSi), 0.14 (3H, s, MeSi), 0.13 (3H, s, MeSi), 0.12 (9H, s, Me_3Si).

Reaction of Milbemycin A_4 (**1a**) with MCPBA (1.5 equiv)

To a solution of **1a** (108 mg, 0.2 mmol) in CH_2Cl_2 (1.5 ml), MCPBA (80% purity, 65 mg, 0.3 mmol) was added in one portion at 0°C and stirred for 1 hour. The reaction mixture was poured into 10% of $Na_2S_2O_3$ solution, extracted with EtOAc and washed with satd $NaHCO_3$ solution. The extract was dried over Na_2SO_4, concentrated *in vacuo* and chromatographed (hexane-EtOAc, 1:1~1:2) to give 66 mg of 14,15-epoxide **2a** (59%) and 32 mg of inseparable mixture of compounds. 14,15-Epoxymilbemycin A_4 (**2a**): IR (KBr) cm^{-1} 3481, 1737, 1179, 990; MS *m/z* 558 (M^+), 430, 195; partial ^1H NMR (400 MHz, $CDCl_3$) δ 5.91 (1H, dd, J=11.5, 14.5 Hz, 10-H), 5.82 (1H, dt, J=2.2, 11.5 Hz, 9-H), 5.48 (1H, dd, J=9.8, 14.5 Hz, 11-H), 5.46 (1H, br s, 3-H), 5.32~5.40 (1H, m, 19-H), 4.74 (1H, dd, J=2.2, 14.3 Hz, 8a-H), 4.70 (1H, dd, J=2.2, 14.3 Hz, 8a-H), 4.30 (1H, m, 5-H), 3.99 (1H, d, J=6.2 Hz, 6-H), 3.71~3.78 (1H, m, 17-H), 3.55 (1H, s, 7-OH), 3.31~3.29 (1H, m, 2-H), 3.06 (1H, dt, J=2.4, 9.2 Hz, 25-H), 2.59 (1H, d, J=9.2 Hz, 15-H), 1.89 (3H, s, 4a-H), 1.24 (3H, s, 14a-H).

Reaction of Milbemycin A_4 (**1a**) with MCPBA (3.0 equiv)

1a (108 mg, 0.2 mmol) was reacted with MCPBA (80% purity, 130 mg, 0.6 mmol) in CH_2Cl_2 (1.5 ml) at room temperature for 1 hour. Purification of the crude mixture gave 89 mg of 3,4-8,9-14,15-triepoxide **4a** (76%) as colorless crystals: mp 201~205°C; IR (KBr) cm^{-1} 3474, 1744, 1170, 991; MS *m/z* 590 (M^+), 195, 167; partial ^1H NMR (400 MHz, $CDCl_3$) δ 5.98 (1H, dd, J=8.8, 15.4 Hz, 11-H), 5.18~5.39 (1H, m, 19-H), 5.13 (1H, dd, J=9.5, 15.4 Hz, 10-H), 4.41 (1H, d, J=11.5 Hz, 8a-H), 4.14~4.19 (1H, m, 5-H), 3.96 (1H, d, J=6.1 Hz, 6-H), 3.90 (1H, d, J=11.5 Hz, 8a-H), 3.50 (1H, d, J=9.5 Hz, 9-H), 3.44 (1H, s, 3-H), 3.01 (1H, s, 2-H), 3.04~3.10 (1H, m, 25-H), 2.84 (1H, s, 7-OH), 2.65 (1H, d, J=9.6 Hz, 15-H), 1.05~2.63 (1H, m, 17-H), 1.52 (3H, s, 4a-H), 1.26 (3H, s, 14a-H).

Reaction of 4-Oxomilbemycin A_4 (**1d**) with MCPBA (1.5 equiv)

To a solution of **1d** (108 mg, 0.2 mmol) in CH_2Cl_2 (1.5 ml) MCPBA (80% purity, 65 mg, 0.3 mmol) was added in one portion at 0°C. The reaction mixture was stirred at 0°C for 1 hour, and then poured into 10% of $Na_2S_2O_3$ solution. The organic layer was extracted with EtOAc, washed with satd $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated *in vacuo*. The crude mixture was dissolved in MeOH (1.5 ml) and reacted with $NaBH_4$ (11 mg, 0.3 mmol) at 0°C for 20 minutes. Then, the reaction mixture was poured into satd NaCl solution, extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated *in vacuo*. Purification of the mixture on silica gel column chromatography (hexane-EtOAc, 2:3) gave 62 mg of 14,15-epoxide **2a** (56%) and 41 mg of 8,9-14,15-diepoxide **3a** (36%). 8,9-14,15-Diepoxymilbemycin A_4 (**3a**): mp 227~229°C; IR (KBr) cm^{-1} 3440, 1746, 1167, 989; MS *m/z* 574 (M^+), 195, 167, 95; partial ^1H NMR (400 MHz, $CDCl_3$) δ 5.97 (1H, dd, J=8.7, 15.3 Hz, 11-H), 5.39 (1H, m, 3-H), 5.19~5.27 (1H, m, 19-H), 5.15 (1H, dd, J=9.5, 15.3 Hz, 10-H), 4.49 (1H, d, J=1H, d, J=11.1 Hz, 8a-H), 4.30~4.33 (1H, m, 5-H), 4.10 (1H, d, J=5.7 Hz, 6-H), 3.98 (1H, d, J=11.1 Hz, 8a-H), 3.77~3.88 (1H, m, 17-H), 3.52 (1H, d, J=9.5 Hz, 9-H), 3.37~3.39 (1H, m, 2-H), 3.12 (1H, s, 7-OH), 3.04~3.09 (1H, m, 25-H), 2.66 (1H, br d, J=9.2 Hz, 15-H), 1.87 (3H, s, 4a-H), 1.26 (3H, s, 14a-H).

Reaction of 5-Oxomilbemycin A_4 (**1d**) with MCPBA (3.0 equiv)

1d (54 mg, 0.1 mmol) and MCPBA (80% purity, 65 mg, 0.3 mmol) were reacted at room temperature for 1 hour and subsequent reduction with $NaBH_4$ (11 mg, 0.3 mmol) gave 47 mg of 8,9-14,15-diepoxide **3a** (82%).

Reaction of 5-Oxomilbemycin A_4 (**1d**) with *tert*-Butyl Hydroperoxide (TBHP)

To a solution of **1d** (137 mg, 0.25 mmol) in toluene (1.5 ml) containing $VO(acac)_2$ (13 mg, 0.05 mmol), 5 M toluene solution of TBHP (0.13 ml, 0.38 mmol) was added dropwise at −20°C. The reaction temperature was gradually raised to 0°C for 1 hour, and the reaction mixture was poured into 10% Na_2SO_3 solution, stirred for 20 minutes and extracted with EtOAc. The extract was washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was dissolved with MeOH (1.5 ml), and reacted with $NaBH_4$ (excess amount) at 0°C for 20 minutes. The reaction mixture was diluted with satd NaCl solution and

extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, evaporated *in vacuo* and chromatographed (hexane - EtOAc, 3 : 2) to give 92 mg of 8,9-epoxide **5a** (65%): IR (KBr) cm^{-1} 3486, 1744, 1713, 1165, 989; MS *m/z* 558 (M^+), 195, 167; partial ^1H NMR (400 MHz, $CDCl_3$) δ 5.88 (1H, dd, J=8.9, 15.3 Hz, 11-H), 5.28~5.36 (1H, m, 19-H), 5.33 (1H, m, 3-H), 5.06~5.10 (1H, m, 15-H), 4.99 (1H, dd, J=9.5, 15.3 Hz, 10-H), 4.39 (1H, d, J=11.3 Hz, 8a-H), 4.28~4.32 (1H, m, 5-H), 4.16 (1H, d, J=5.6 Hz, 6-H), 4.02 (1H, s, 7-OH), 3.94 (1H, d, J=11.3 Hz, 8a-H), 3.60~3.67 (1H, m, 17-H), 3.57 (1H, d, J=9.5 Hz, 9-H), 3.29~3.31 (1H, m, 2-H), 3.09 (1H, dt, J=2.5, 9.4 Hz, 25-H), 1.86 (3H, s, 4a-H), 1.55 (3H, s, 14a-H).

Reaction of 5-*O*-(*tert*-Butyldimethylsilyl)milbemycin A_4 (**1e**) with MCPBA (1.5 equiv)

Reaction of **1e** (131 mg, 0.2 mmol) with MCPBA (80% purity, 65 mg, 0.3 mmol) in CH_2Cl_2 (1.5 ml) at 0°C for 1 hour gave 79 mg, of 14,15-epoxide **2e** (59%) and 48 mg of 8,9-14,15-diepoxide **3e** (35%). 14,15-Epoxy-5-*O*-(*tert*-butyldimethylsilyl)milbemycin A_4 (**2e**): mp 220~225°C; IR (KBr) cm^{-1} 3425, 1699, 1082, 995; MS *m/z* 672 (M^+), 597, 430, 195, 167; partial ^1H NMR (400 MHz, $CDCl_3$) δ 5.91 (1H, dd, J=11.5, 14.7 Hz, 10-H), 5.77 (1H, br d, J=11.5 Hz, 9-H), 5.45 (1H, dd, J=9.7, 14.7 Hz, 11-H), 5.36 (1H, m, 3-H), 5.26~5.34 (1H, m, 19-H), 4.71 (1H, dd, J=2.3, 14.7 Hz, 8a-H), 4.62 (1H, dd, J=2.2, 14.5 Hz, 8a-H), 4.42~4.44 (1H, m, 5-H), 3.83 (1H, d, J=5.4 Hz, 6-H), 3.71~3.78 (1H, m, 17-H), 3.54 (1H, br s, 7-OH), 3.38~3.40 (1H, m, 2-H), 3.06 (1H, dt, J=2.4, 9.2 Hz, 25-H), 2.60 (1H, d, J=9.2 Hz, 15-H), 1.81 (3H, s, 4a-H), 1.25 (3H, s, 14a-H), 0.93 (9H, s, *t*-BuSi), 0.14 (6H, s, Me_2Si). 8,9-14,15-Diepoxy-5-*O*-(*tert*-butyldimethylsilyl)milbemycin A_4 (**3e**): IR (KBr) cm^{-1} 3488, 1748, 1715, 1169, 1110, 990; MS *m/z* 688 (M^+), 631, 195, 167; partial ^1H NMR (400 MHz, $CDCl_3$) δ 5.97 (1H, dd, J=8.6, 15.4 Hz, 11-H), 5.33~5.34 (1H, m, 3-H), 5.17~5.25 (1H, m, 19-H), 5.15 (1H, dd, J=9.5, 15.4 Hz, 10-H), 4.48 (1H, d, J=11.6 Hz, 8a-H), 4.47 (1H, m, 5-H), 3.95 (1H, d, J=5.4 Hz, 6-H), 3.92 (1H, d, J=11.6 Hz, 8a-H), 3.77~3.84 (1H, m, 17-H), 3.52 (1H, d, J=9.5 Hz, 9-H), 3.44~3.45 (1H, m, 2-H), 3.18 (1H, s, 7-OH), 3.06 (1H, dt, J=2.5, 9.2 Hz, 25-H), 2.66 (1H, br d, J=9.3 Hz, 15-H), 1.81 (3H, s, 4a-H), 1.28 (3H, s, 14a-H), 0.94 (9H, s, *t*-BuSi), 0.15 (6H, s, Me_2Si).

Reaction of 5-*O*-(*tert*-Butyldimethylsilyl)milbemycin A_4 (**1e**) with *tert*-Butyl Hydroperoxide (TBHP)

To a solution of **1e** (131 mg, 0.2 mmol) and VO $(acac)_2$ (11 mg, 0.04 mmol) in toluene (1.5 ml), 3 M toluene solution of TBHP (0.09 ml, 0.26 mmol) was added dropwise at −20°C. The reaction mixture was stirred at −20~0°C for 3 hours and poured into 10% $Na_2S_2O_3$, and then extracted with EtOAc. The extract was dried over Na_2SO_4, evaporated *in vacuo* and purified by column chromatography to give 104 mg of 8,9-epoxide **5e** (78%): IR (KBr) cm^{-1} 3491, 1746, 1714, 1166, 1118, 990; MS *m/z* 672 (M^+), 195, 167; partial ^1H NMR (400 MHz, $CDCl_3$) δ 5.87 (1H, dd, J=8.8, 15.3 Hz, 11-H), 5.30 (1H, m, 3-H), 5.27~5.33 (1H, m, 19-H), 5.05 (1H, m, 15-H), 4.99 (1H, dd, J=9.5, 15.3 Hz, 10-H), 4.46 (1H, m, 5-H), 4.38 (1H, d, J=11.3 Hz, 8a-H), 4.10 (1H, s, 7-OH), 3.99 (1H, d, J=5.0 Hz, 6-H), 3.89 (1H, d, J=11.3 Hz, 8a-H), 3.59~3.67 (1H, m, 17-H), 3.56 (1H, d, J=9.5 Hz, 9-H), 3.35 (1H, m, 2-H), 3.08 (1H, dt, J=2.4, 11.3 Hz, 25-H), 1.79 (3H, br s, 4a-H), 1.57 (3H, s, 14a-H), 0.93 (9H, s, *t*-BuSi), 0.14 (6H, s, Me_2Si).

Reaction of 5-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-(trimethylsilyl)milbemycin A_4 (**1f**) with MCPBA (1.5 equiv)

To a stirred solution of **1f** (146 mg, 0.2 mmol) in CH_2Cl_2 (1.5 ml), MCPBA (80% purity, 65 mg, 0.3 mmol) was added in one portion at 0°C, and the mixture was stirred for 1 hour, poured into 10% Na_2SO_3 solution, extracted with EtOAc and washed with satd $NaHCO_3$ solution. The extract was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by chromtography (hexane - EtOAc, 10 : 1) to give 135 mg, of 14,15-epoxide **2f** (90%) and 10 mg of 8,9-14,15-diepoxide **3f** (7%). 14,15-Epoxy-5-*O*-(*tert*-butyldimethylsilyl)-7-*O*-(trimethylsilyl)milbemycin A_4 (**2f**): IR (KBr) cm^{-1} 1744, 1251, 1169, 1088, 989, 840; MS *m/z* 744 (M^+), 222, 167, 73; partial ^1H NMR (270 MHz, $CDCl_3$) δ 5.93 (1H, dd, J=11.7, 14.6 Hz, 10-H), 5.69 (1H, br d, J=11.7 Hz, 9-H), 5.49 (1H, m, 3-H), 5.45 (1H, dd, J=9.3, 14.6 Hz, 11-H), 4.83~4.95 (1H, m, 19-H), 4.70 (1H, br d, J=14.6 Hz, 8a-H), 4.58 (1H, br d, J=14.6 Hz, 8a-H), 4.39~4.41 (1H, m, 5-H), 3.82 (1H, d, J=5.4 Hz, 6-H), 3.70~3.84 (1H, m, 17-H), 3.23~3.26 (1H, m, 2-H), 3.04 (1H, dt, J=2.4, 9.3 Hz, 25-H), 2.65 (1H, br d, J=9.3 Hz, 15-H), 1.80 (3H, s, 4a-H), 1.26 (3H, s, 14a-H), 0.94 (9H, s, *t*-BuSi), 0.14 (6H, s, Me_2Si), 0.12 (9H, s, Me_3Si). 8,9-14,15-Diepoxy-5-*O*-(*tert*-butyldimethylsilyl)-7-*O*-(trimethylsilyl)milbemycin A_4 (**3f**): IR (KBr) cm^{-1} 1750, 1250, 1167, 1102, 989, 840; MS *m/z* 760

(M^+), 195, 167, 73; partial ^1H NMR (270 MHz, $CDCl_3$) δ 5.95 (1H, dd, J=8.8, 15.6 Hz, 11-H), 5.36~5.38 (1H, m, 3-H), 5.15 (1H, dd, J=9.3, 15.6 Hz, 10-H), 4.85~4.93 (1H, m, 19-H), 4.46 (1H, d, J=10.7 Hz, 8a-H), 4.36~4.38 (1H, m, 5-H), 4.07 (1H, d, J=4.9 Hz, 6-H), 3.87 (1H, d, J=10.7 Hz, 8a-H), 3.77~3.90 (1H, m, 17-H), 3.39 (1H, m, 2-H), 3.36 (1H, d, J=9.3 Hz, 9-H), 3.01~3.09 (1H, m, 25-H), 2.69 (1H, br d, J=9.3 Hz, 15-H), 1.79 (3H, br s, 4a-H), 1.28 (3H, s, 14a-H), 0.94 (9H, s, *t*-BuSi), 0.16 (3H, s, MeSi), 0.15 (3H, s, MeSi), 0.13 (9H, s, Me_3Si).

Transformations of Epoxymilbemycins

Transformations of epoxy derivatives were conducted according to the following representative procedures.

Epoxidation with MCPBA: MCPBA (80% purity, 58 mg, 0.27 mmol) was added to a solution of 8,9-epoxide **5a** (50 mg, 0.09 mmol) in CH_2Cl_2 (1.5 ml) at 0°C, and then the reaction mixture was stirred at room temperature for 2 hours. Following work-up and crystallization from cyclohexane-$CHCl_3$ gave 49 mg of 3,4-8,9-14,15-triepoxide **4a** (93%).

Protection of the C-5 hydroxyl group with a *tert*-butyldimethylsilyl group: 14,15-Epoxide **2a** (22 mg, 0.04 mmol), *tert*-butyldimethylsilyl chloride (12 mg, 0.08 mmol) and imidazole (11 mg, 0.16 mmol) were reacted in DMF (0.5 ml) at room temperature for 6 hours. The reaction mixture was poured into EtOAc, washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. Purification by preparative TLC gave 24 mg of 14,15-epoxide **2e** (90%).

Protection of the C-7 hydroxyl group with a trimethylsilyl group: 14,15-Epoxide **2e** (50 mg, 0.09 mmol), chlorotrimethylsilane (0.06 ml, 0.50 mmol) and dimethylaminopyridine (DMAP) (61 mg, 0.50 mmol) were reacted in DMF (0.5 ml) at room temperature for 1 hour. Following work-up and purification by preparative TLC gave 33 mg of 14,15-epoxide **2f** (94%).

5-*O*-(*p*-Bromophenylcarbamoyl)-3,4-8,9-14,15-triepoxymilbemycin A_4 (**4g**)

3,4-8,9-14,15-Triepoxide **4a** (24 mg, 0.04 mmol) and *p*-bromophenyl isocyanate (12 mg, 0.06 mmol) were reacted in the presence of a catalytic amount of pyridine in CH_2Cl_2 (0.5 ml) at room temperature for 2 hours. The reaction mixture was poured into 1 N HCl solution, extracted with $CHCl_3$, dried over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by preparative TLC (hexane-EtOAc, 3:2) to afford 24 mg of **4g** (75%): mp 255~258°C (dec.); IR (KBr) cm^{-1} 3438, 3301, 1733, 1713, 1226, 1062; MS *m/z* 558 (M^+), 195, 167; partial ^1H NMR (400 MHz, $CDCl_3$) δ 7.44 (2H, d, J=8.8 Hz, H-aromatic), 7.30 (2H, d, J=8.8 Hz, H-aromatic), 6.96 (1H, br, $-NHCO_2$), 5.98 (1H, dd, J=8.8, 15.3 Hz, 11-H), 5.47 (1H, d, J=5.8 Hz, 5-H), 5.16~5.24 (1H, m, 19-H), 5.12 (1H, dd, J=9.5, 15.3 Hz, 10-H), 4.44 (1H, d, J=11.3 Hz, 8a-H), 4.11 (1H, d, J=5.8 Hz, 6-H), 3.89 (1H, d, J=11.3 Hz, 8a-H), 3.78~3.86 (1H, m, 17-H), 3.52 (1H, d, J=9.5 Hz, 9-H), 3.46 (1H, s, 3-H), 3.12 (1H, s, 2-H), 3.07 (1H, dt, J=2.4, 9.5 Hz, 25-H), 2.78 (1H, s, 7-OH), 2.66 (1H, br d, J=10.3 Hz, 15-H), 1.49 (3H, s, 4a-H), 1.27 (3H, s, 14a-H).

X-ray Structure Determination for **4g**

A colorless 0.4×0.4×0.3 mm crystal obtained by recrystallization from methanol was used for data collection on a Rigaku AFC-5 diffractometer with graphite monochromated Cu-$K\alpha$ radiation (λ 1.5418 Å). Crystal data: $C_{39}H_{50}NO_{11}Br$, MW=788.7, orthorhombic, space group $P2_12_12_1$, a=43.365(9) Å, b=11.581(3) Å, c=8.005(2) Å, V=4020.1(14) $Å^3$, Z=4, Dc=1.30/cm^3. The structure elucidation was performed using the heavy atom method and anisotropically refined by the block-diagonal least-squares method to R=0.055 for 2367 observed reflections larger than 3σ (F_0).

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