

Practical Synthesis of 13-Substituted Milbemycin

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A novel and practical method to synthesize 13-alkoxy derivatives from milbemycin A_4 was studied and an efficient approach via the 15-hydroxymilbemycin derivative was established. During the study, a novel conversion of the 15-alkoxy derivative into the 13-alkoxy derivative via allyl cation and a new method for the conversion of allyl iodide into allyl ether using copper(I) trifluoromethanesulfonate as a catalyst were also found.

Milbemycin derivatives are known to be potent anthelmintic products,[1] and have attracted a great deal of interest recently. Milbemycins isolated from *Streptomyces hygroscopicus*[2] are natural products possessing a 16-membered ring in their structure. Avermectin isolated from *Streptomyces avermitilis*[3] is known to possess similar activity to milbemycins against endoparasites. Its structure closely resembles milbemycin, having a 16-membered ring. A significant structural difference is that avermectin has an 4-*O*-α-L-oleandrosyl-α-L-oleandrosyl group at the 13-position, while milbemycin has no functional group in this position (Fig. 1).

Ivermectin, which is a mixture of 22,23-dihydroavermectin B_{1a} and 22,23-dihydroavermectin B_{1b}, is widely used as a potent parasiticide on livestock.[4] It is known that the substituent at the 13-position greatly contributes to the anthelmintic activity of ivermectin.[5] 13-Substituted milbemycin derivatives also have a strong effect on parasites. This activity greatly depends on the substituent at the 13-position. That is why a great number of 13-alkoxy or 13-acyloxy milbemycin derivatives have been synthesized and their activities evaluated.

In a previous study,[6] we synthesized numerous 13-phenethyloxy milbemycin derivatives, which were shown to possess biological activity almost equal to that of ivermectin. However, the synthetic procedure was not really practical, since toxic Hg salt was used in the etherification step[6] (Scheme 1). Thus, it was necessary to develop a new, more efficient and more practical method for synthesizing beneficial 13-alkoxy milbemycin derivatives from milbemycin A_4 (**1**) so that the derivatives could be synthesized much more easily and effectively.

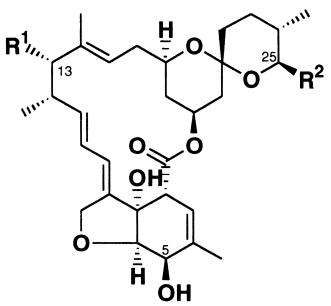
Results and Discussion

An Approach via Urethane Derivative Using $Cu(OTf)_2$ or $Zn(OTf)_2$. As mentioned in a previous report,[6] when the 13-urethane derivative **2a** was treated with ZnI_2, it was converted into 13-iodomilbemycin (**3**). This suggests that the urethane moiety is easily removed in the presence of a metal cation (Scheme 1). Thus, it seemed possible that the urethane derivative **2a** could be converted into the 13-alkoxy derivative **4a** directly by a treatment with an alcohol in the presence of a catalyst having a counter anion with lower nucleophilicity in place of the iodide anion.

When the 13-urethane derivatives, **2a** and **2c**, were individually mixed with an alcohol in the presence of metal trifluoromethanesulfonate, the desired corresponding 13-alkoxy derivatives **4a**, and **4c** were given, as expected. But they were accompanied by undesired corresponding 15-alkoxy derivatives, **5a** and **5c**, which was unavoidable (Fig. 2, Table 1).

Given the result that both the 13- and 15-alkoxy derivatives were obtained from 13-urethane derivative, it was very likely that the reaction could proceed via the allyl cation **6** (Fig. 2). Details of the allyl cation are discussed later in this report.

If the reaction proceeds via the allyl cation, the 15-urethane derivative **7** was also likely to react in the same way. Thus, the 15-urethane derivative was examined as an alternate of the 13-urethane derivative, since 15-hydroxymilbemycin is easier to synthesize than 13-hydroxymilbemycin.[7] The catalysts listed



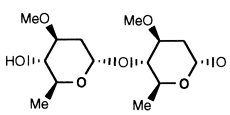
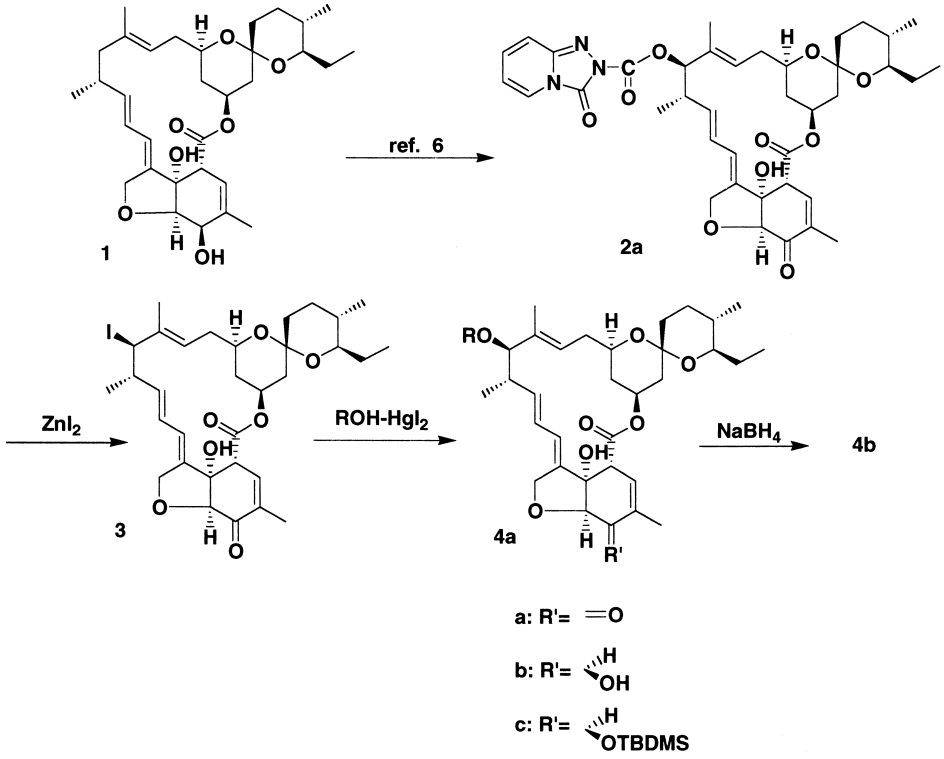
	R^1	R^2
Milbemycin A3	**H**	**Me**
Milbemycin A4	**H**	**Et**
Ivermectin		**mixture of s-Bu and i-Pr**

Fig. 1. The structures of milbemycins and ivermectin.



Scheme 1.

Table 1. Etherification of 13-Urethanemilbemycin

Run	R	Cat.	Solvent	Temp	Time	Yield/%	Ratio
				˚C	min	(4 + 5)	(4:5)
1	$-OSi(Me)_2tBu$	$Zn(OTf)_2$	DCE	r.t.	20	65.0	2:1
2	=O	$Cu(OTf)_2$	DCE	r.t.	20	75.4	3.6:1
3	=O	$Cu(OTf)_2$	THF	r.t.	20	77.1	3.2:1
4	=O	$Cu(OTf)_2$	Toluene	40	20	78.8	3.2:1
5	=O	$Cu(OTf)_2$ + ZnI	DCE	r.t.	20	65.2	3.8:1
6	=O	$Cu(OTf)_2 + CF_3SO_3H$	DCE	r.t.	20	73.4	2.8:1

in Table 2 were tested, and copper(II) trifluoromethanesulfonate turned out to give a good result, although it was still unavoidable to obtain the undesired 15-alkoxy derivative.

A Novel Conversion of 13-Iodomilbemycin (3) Using Copper(I) Trifluoromethanesulfonate. Since we could not obtain a satisfactory result in reactions using the urethane derivatives, we gave up trying to obtain the 13-alkoxy derivative directly and decided instead to use 13-iodomilbemycin (**3**) as a substrate (Fig. 3). According to the HSAB principle, it seemed possible to use a soft acid, Cu(I), in place of Hg(II), so we chose copper(I) trifluoromethanesulfonate as a catalyst. In fact, the reaction of the 13-iodomilbemycin derivative and the alcohol proceeded smoothly in the presence of Cu(I) (Table 3). As this type of reaction has not been reported yet, we thus found a new method for the conversion of allyl iodide into allyl ether using copper(I) trifluoromethanesulfonate as a catalyst.

We obtained some interesting results here. If the reaction was carried out at room temperature, the 13-alkoxy derivative was the only product. On the other hand, if the reaction was carried out at 4 ˚C, the 13- and the 15-alkoxy derivatives were both produced simultaneously, and when the temperature of the mixture was raised to room temperature, the ratio of the 13-alkoxy derivatives increased significantly. The ratio of the yield of the 13- and 15-alkoxy derivatives, therefore, was dependent on the reaction temperature.

These results suggest that 15-alkoxymilbemycin (**5a**) was converted into the thermodynamically more stable 13-alkoxymilbemycin (**4a**) via the allyl cation **6** during the reaction. To confirm this, we isolated the 15-alkoxy derivative **5a** and treated it with copper(I) iodide and trifluoromethanesulfonic acid, which were produced during the reaction of **3**, described in the above paragraph (Fig. 3). At last, as we had expected, the 13-alkoxymilbemycin **4a** was obtained from the 15-alkoxy derivative. Thus, the conversion of the 15-alkoxy derivative into the 13-alkoxy derivative took place without doubt. The possibility for the existence of the allyl cation was also enhanced.

Application to 15-Hydroxymilbemycin. These findings have great significance for our study on synthesizing milbemycin derivatives because these facts suggest that a 15-hydroxy derivative **8** could also be converted into a 13-alkoxy derivative **4a** directly. The 15-hydroxy derivative **8** seemed to produce

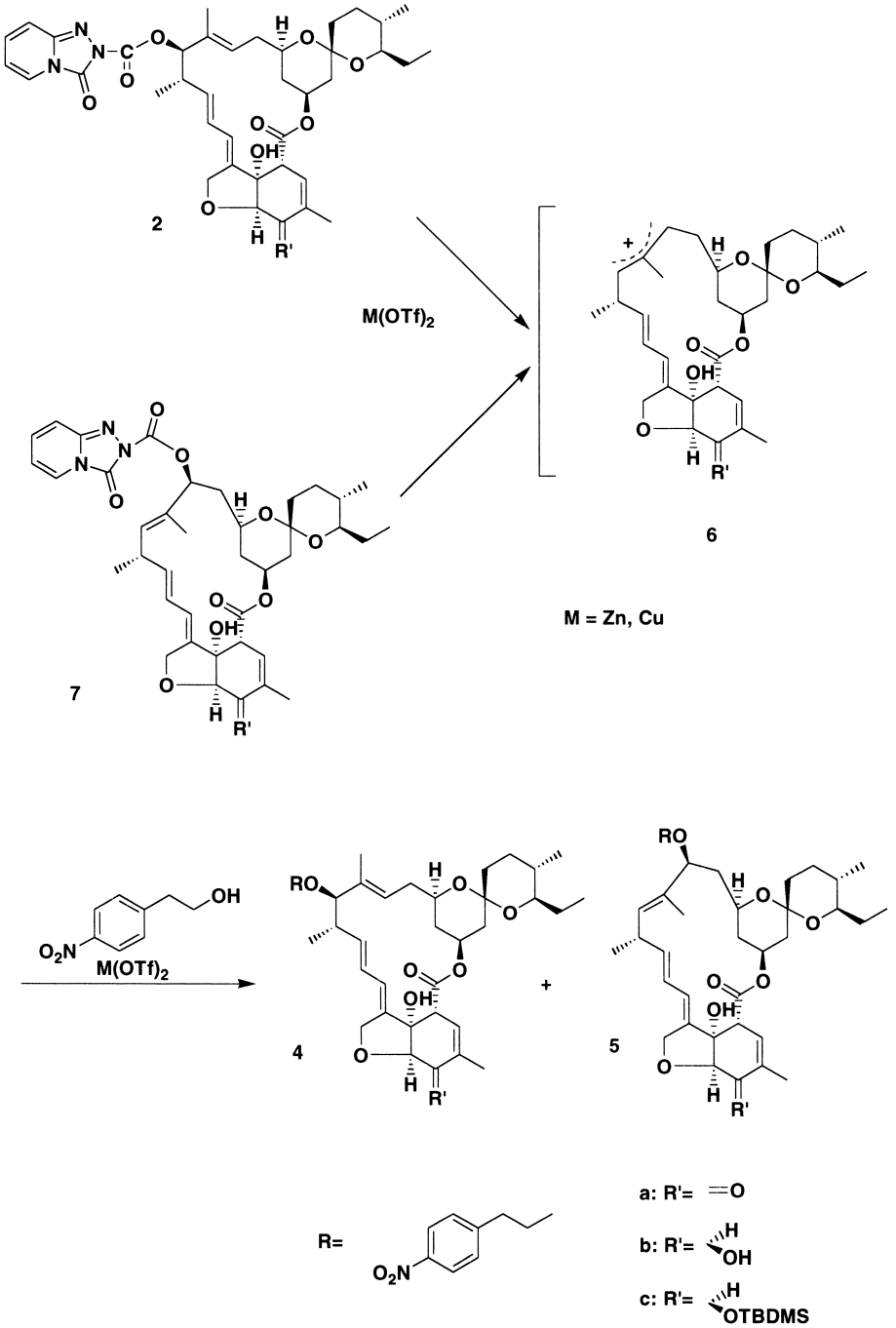


Fig. 2. Conversion of urethane derivatives into the alkoxymilbemycins.

Table 2. Etherification of 15-Urethanemilbemycin

Run	SM	Product	Cat.	Solvent	Temp.	Time	Yield/%	Ratio
	R	R				min	(4 + 5)	(4:5)
1	–OSi(Me)$_2$*t*Bu	–OSi(Me)$_2$*t*Bu	Zn(OTf)$_2$	DCE	r.t.	20	65.2	1.8:1
2	–OSi(Me)$_2$*t*Bu	OH[a)]	Cu(OTf)$_2$	DCE	r.t.	30	55.5	6.6:1
3	OH	OH	Cu(OTf)$_2$	DCE	r.t.	20	86.5	2.5:1

a) As the reaction condition was acidic, the silyl group was removed.

the allyl cation **6** more easily under the reaction conditions with the acidic catalyst (Fig. 4). Since the 15-hydroxy derivative **8** can be easily synthesized from milbemycin A$_4$, using this as the substrate would be advantageous.

To establish this, we then tried the same reaction using 15-hydroxymilbemycin as a substrate to produce the13-alkoxy de-

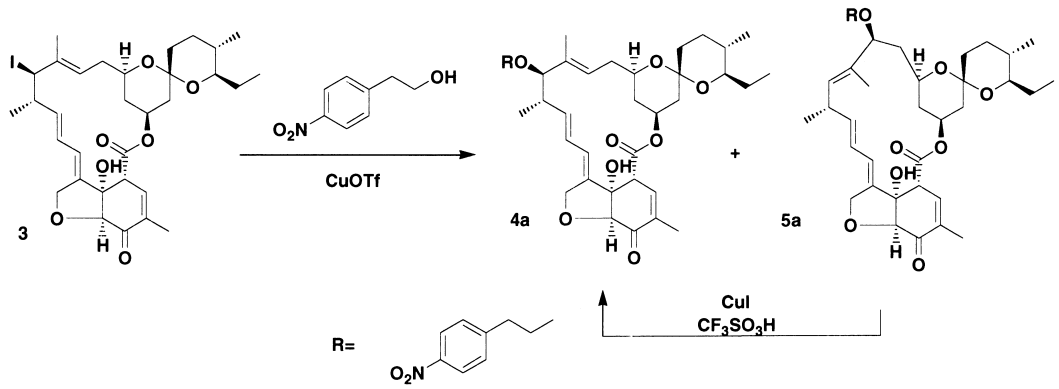


Fig. 3. Conversion of the 13-iodomibemycin into the alkoxymilbemycins.

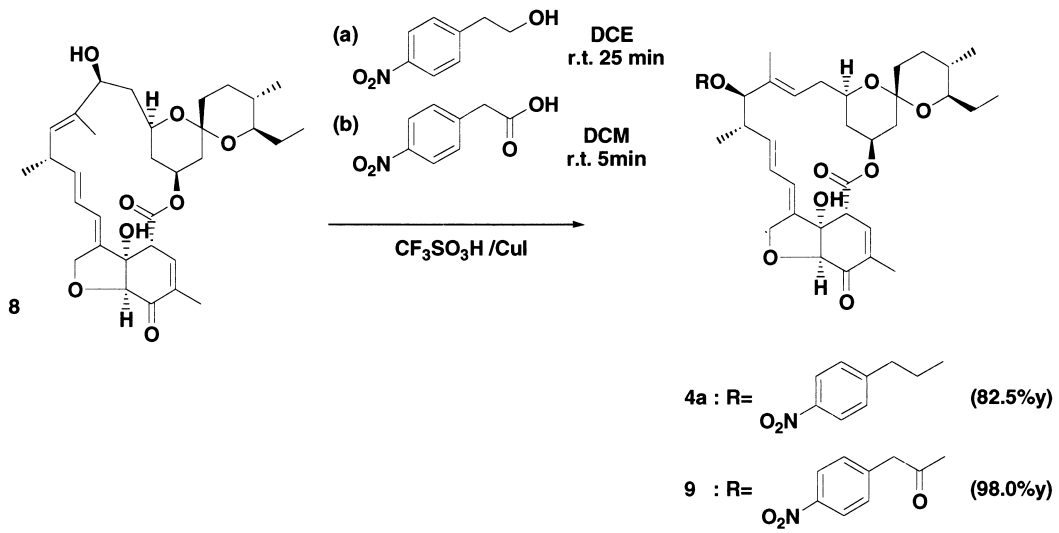


Fig. 4. Direct conversion of 15-hydroxymibemycin into 13-α-alkoxymilbemycin.

Table 3. Etherification of 13-Iodomilbemycin

Run	Reaction Condition	Yield/% (4 + 5)	Ratio (4:5)
1	r.t. 20 min	91.3	20:1
2	4 °C 20 min	90.2	1.5:1
3	4 °C 20 min then r.t. 20 min	92.1	17:1

rivative **4a** directly under the conditions shown below.

To our entire satisfaction, when 15-hydroxymilbemycin **8** was treated with an alcohol in the presence of copper(I) iodide and trifluoromethanesulfonic acid, it gave the desired 13-alkoxy derivative in extremely high yield.

We also tried to obtain the 13-acyloxy derivative **9** from 15-hydroxymilbemycin directly with the same method. As we predicted, a 13-acyloxyderivative was readily obtained in extremely high yield.

Discussion on the Allyl Cation. Since milbemycin has a very rigid 16-membered ring in its structure, the configuration of the reaction site is fixed, as shown in Fig. 5.[8] The configuration was verified by an X-ray crystallographic analysis, and it was very clear that one side of the double bond is much more hindered than the other side to attack by reagents.[7] In fact, the 14,15-double bond is epoxidized only from the less-hindered side to produce exclusively its 14,15-epoxide,[7] which can be cleaved to produce only 15-β-hydroxymilbemycin. The con-

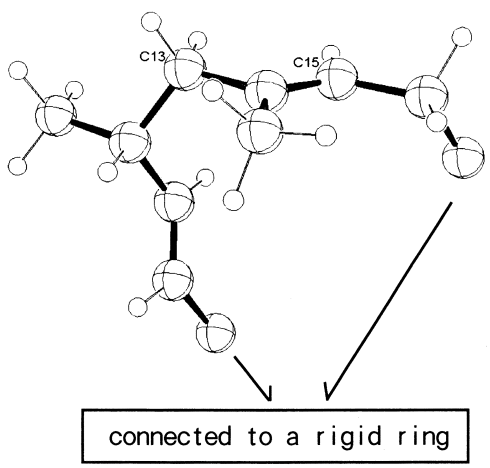


Fig. 5. Partial configuration of milbemycin A_4.
The hydroxyl group at the 13-β position is in the same direction as the π orbital of the adjacent double bond. Thus, it is much easier to eliminate this hydroxy group than the one in the opposite side.

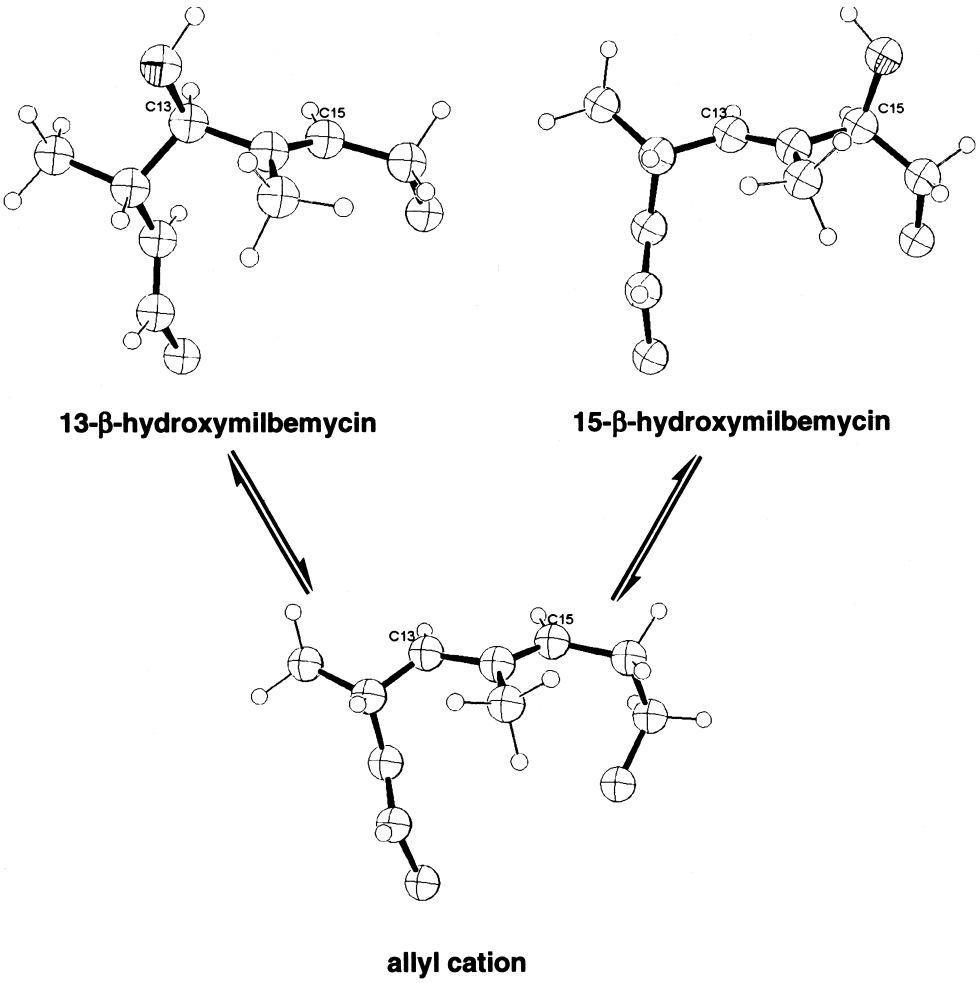


Fig. 6. Conversion of 13- or 15-hydroxymilbemycin into allyl cation.

figuration of the 14,15-epoxymilbemycin was also verified by an X-ray crystallographic analysis.[9]

As for the configuration of the allyl cation, it is supposed to be almost the same as milbemycin. As shown in Fig. 6, the hydroxy groups of 13-β-hydroxymilbemycin and 15-β-hydroxymilbemycin are in the same direction as the π orbital of the adjacent double bond.[10] Thus, those hydroxy groups are likely to be eliminated easily to produce the same allyl cation without changing its configuration. Once the allyl cation is produced, it gives the β-substituted derivative exclusively because of a steric hindrance. In fact, the configuration of the products of the reaction proceeding via allyl cation was always β, which was verified by ^{1}H NMR. The details of the configuration of the 13- and 15-substituted derivatives and analysis of their ^{1}H NMR spectra are discussed by O'Sullivan et al.[7]

On the other hand, the hydroxy groups of 13- or 15-α-hydroxymilbemycin are almost vertical to the π orbital of the adjacent double bond. Thus, it is difficult to produce the allyl cation. In fact, those reactions which used 13- or 15-α-hydroxymilbemycin gave a different result from those given by 13- or 15-β-hydroxymilbemycin. The details of the reactions of the 13-hydroxy derivatives are discussed in another paper, which is under preparation.

Summary. We have established a new, efficient, and practical method to synthesize 13-substituted milbemycin derivatives under the control of regio- and stereoselectivity. We thus no longer need to use toxic HgI_2. We also no longer obtain undesired by-products. In addition, the new method decreases the number of reaction steps. This has great significance for research on milbemycin derivatives by making it easier to produce a greater number of 13-β-substituted derivatives.

Experimental

Synthesis of 4a from 2a. 4-Nitrophenethyl alcohol (418 mg, 2.5 mmol) and copper(II) trifluoromethanesulfonate (217 mg, 0.6 mmol) were dissolved in 1,2-dichloroethane (2.0 mL) and the mixture was stirred for 10 minutes at room temperature. A solution of **2** (360 mg, 0.5 mmol) in 1,2-dichloroethane (1 mL) was added and the mixture was stirred at room temperature for 20 minutes. The reaction mixture was diluted with ethyl acetate and washed with water, a 4% aqueous solution of $NaHCO_3$, and with water again, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel, with the eluent (ethyl acetate:cyclohexane = 1:3) to obtain a mixture (340 mg, 75.4% yield) of the desired **4a** and undesired **5a**. Other derivatives, such as **4c** and **5c**, were synthesized in a similar manner from **2c**. The ratio of **4a** and **5a** was estimated at **4a**:**5a** = 3.6:1.0 by analyzing the area ratio of the NMR spectrum. **2a**: ^{1}H NMR δ 0.84 (3H, d, *J*

= 6.5 Hz, C-24 CH_3), 1.00 (3H, t, J = 7.4 Hz, C-25 CH_2CH_3), 1.15 (3H, d, J = 7.4 Hz, C-12 CH_3), 1.90 (3H, s, C-4 CH_3), 3.07 (1H, m, C-25 H), 3.57 (1H, m, C-2 H), 3.62 (1H, m, C-17 H), 4.02 (1H, broad s, C-7 OH), 4.80 and 4.75 (2H, ABq, J = 14.5 Hz, C-27 H), 5.20 (1H, d, J = 7.6 Hz, C-13 H), 5.54 (1H, m, C-15 H), 6.48 (1H, t, J = 6.7 Hz, pyridine H), 6.56 (1H, s, C-3 H), 7.15 (2H, m, pyridine H), 7.72 (1H, d, J = 7.2 Hz, pyridine H). **2c**: ^{1}H NMR δ 0.83 (3H, d, J = 6.5 Hz, C-24 CH_3), 0.93 (9H, s, *t*Bu), 0.99 (3H, t, J = 7.3 Hz, C-25 CH_2CH_3), 1.14 (3H, d, J = 6.5 Hz, C-12 CH_3), 1.80 (3H, s, C-4 CH_3), 2.85 (1H, m, C-12 H), 3.07 (1H, m, C-25 H), 3.37 (1H, m, C-2 H), 3.60 (1H, m, C-17 H), 3.82 (1H, d, J = 5.4 Hz, C-6 H), 4.06 (1H, broad s, C-7 OH), 4.44 (1H, m, C-5 H), 4.61 and 4.70 (2H, ABq, J = 14.5 Hz, C-27 H), 5.18 (1H, d, J = 10.6 Hz, C-13 H), 5.32 (1H, s, C-3 H), 5.38 (1H, dd, J = 10.2 and 14.6 Hz, C-11 H), 5.53 (1H, m, C-19 H), 5.78 (1H, d, J = 11.4 Hz, C-9 H), 5.89 (1H, dd, J = 11.4 and 14.6 Hz, C-10 H), 6.48 (1H, m, py H), 7.10 (1H, d, J = 9.6 Hz, py H), 7.17 (1H, dd, J = 6.2 and 9.6 Hz, py H), 7.72 (1H, dd, J = 1.1 and 7.1 Hz, py H).

4a: ^{1}H NMR δ 0.83 (3H, d, J = 6.8 Hz, C-24 CH_3), 0.99 (3H, t, J = 7.3 Hz, C-25 CH_2CH_3), 1.01 (3H, d, J = 6.8 Hz, C-12 CH_3), 1.43 (3H, s, C-14 CH_3), 1.89 (3H, s, C-4 CH_3), 2.94 (2H, t, J = 6.4 Hz, $PhCH_2$), 3.06 (1H, m, C-25 H), 3.20 (1H, d, J = 9.8 Hz, C-13 H), 3.39 (1H, m, C-13 OCH), 3.50–3.64 (2H, m, C-13 OCH and C-17 H), 3.55 (1H, m, C-2 H), 3.84 (1H, s, C-6 H), 3.93 (1H, s, C-7 OH), 4.72 and 4.75 (2H, ABq, J = 15.7 Hz, C-27 H), 5.18 (1H, m, C-15 H), 5.35 (1H, dd, J = 10.2 and 13.7 Hz, C-11 H), 5.40 (1H, m, C-19 H), 5.74 (1H, dd, J = 11.2 and 13.7 Hz, C-10 H), 5.84 (1H, td, J = 1.9 and 11.2 Hz, C-9 H), 6.54 (1H, m, C-3 H), 7.37 (2H, d, J = 8.8 Hz, Ph H), 8.14 (2H, d, J = 8.8 Hz, Ph H), **4c**: ^{1}H NMR δ 0.82 (3H, d, J = 6.4 Hz, C-24 CH_3), 0.93 (9H, s, *t*-Bu), 0.99 (3H, d, J = 6.4 Hz, C-12 CH_3), 1.40 (3H, s, C-14 CH_3), 1.79 (3H, s, C-4 CH_3), 2.94 (2H, t, J = 6.3Hz, $PhCH_2$), 3.05 (1H, m, C-25 H), 3.19 (1H, d, J = 9.8 Hz, C-13 H), 3.40 (1H, m, C-13 OCH), 3.50–3.71 (2H, m, C-17 H and C-13 OCH), 3.87 (1H, d, J = 5.9Hz, C-6 H), 3.98 (1H, broad s, C-7 OH), 4.42 (1H, m, C-5 H), 4.66 and 4.58 (2H, ABq, J = 14.6 Hz, C-27 H), 5.31 (1H, m, C-3 H), 7.33(2H, d, J = 8.8 Hz, Ph H), 8.14 (2H, d, J = 8.8 Hz, Ph H).

5a: ^{1}H NMR δ 0.84 (3H, d, J = 6.3 Hz, C-24 CH_3), 0.98 (3H, t, J = 7.3 Hz, C-25 CH_2CH_3), 1.08 (3H, d, J = 6.3 Hz, C-12 CH_3), 1.36 (3H, s, C-14 CH_3), 1.89 (3H, s, C-4 CH_3), 2.94 (2H, t, J = 8.8 Hz, $PhCH_2$), 2.97 (1H, m, C-25 H), 3.09 (1H, m, C-12 H), 3.31 (1H, m, C-25 H), 3.40–3.60 (4H, m, C-2 H, C-15 H and C-13 OCH_2), 3.52 (1H, m, C-2 H), 3.80 (1H, s, C-6 H), 3.92 (1H, s, C-7 OH), 4.72 and 4.76 (2H, ABq, J = 14.6 Hz, C-27 H), 4.91 (1H, m, C-19 H), 5.10 (1H, d, J = 9.3 Hz, C-13 H), 5.26 (1H, m, C-11 H), 6.54 (1H, m, C-3 H), 7.38 (2H, d, J = 8.8 Hz, Ph H), 8.14 (2H, d, J = 8.8 Hz, Ph H), **5c**: ^{1}H NMR δ 0.82 (3H, d, J = 6.6 Hz, C-24 CH_3), 0.92 (9H, s, *t*Bu), 1.07 (3H, d, J = 6.5 Hz, C-12 CH_3), 1.37 (3H, s, C-14 CH_3), 1.79 (3H, s, C-4 CH_3), 2.93 (2H, t, J = 6.6 Hz, $PhCH_2$), 3.05 (1H, m, C-25 H), 3.28 (1H, m, C-17 H), 3.58 (1H, dd, J = 4.4 and 11.0 Hz, C-15 H), 3.86 (1H, d, J = 5.8 Hz, C-6 H), 4.00 (1H, broad s, C-7 OH), 4.42 (1H, d, J = 5.1 Hz, C-5 H), 4.58 and 4.66 (2H, ABq, J = 14.6 Hz, C-27 H), 4.82 (1H, m, C-19 H), 5.08 (1H, d, J = 8.7 Hz, C-14 H), 5.32 (1H, s, C-3 H), 7.37 (2H, d, J = 8.8 Hz, Ph H), 8.14 (2H, d, J = 8.8 Hz, Ph H); MS *m*/*z* 822 (M+1, $C_{46}H_{67}NO_{10}Si$).

Synthesis of 4c from 7c. 15-Urethane derivative **7c** (417 mg, 0.5 mmol) was dissolved in 1,2-dichloroethane (3.0 mL), then 4-nitrophenethyl alcohol (251 mg, 1.5 mmol) and zinc triflate (218 mg, 0.6 mmol) were added. The mixture was stirred at room temperature for 20 minutes. Then the reaction mixture was diluted with 1,2-dichloroethane and filtered. The filtrate was washed with water, a 4% aqueous solution of $NaHCO_3$, and with water again, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel, with the eluent (ethyl acetate:cyclohexane = 1:9) to obtain a mixture (268 mg, 65.2% yield) of the desired **4c** and undesired **5c**. The ratio of **4c** to **5c** was estimated at **4c:5c** = 1.8:1.0 by analyzing the area ratio of the NMR spectrum. Other derivatives, such as **4b** and **5b**, were synthesized in a similar manner from **7b** and also **7c** (Table 2). **4b**: ^{1}H NMR δ 0.82 (3H, d, J = 6.4 Hz, C-24 CH_3), 0.98 (3H, t, J = 7.3 Hz, C-25 CH_2CH_3), 1.00 (3H, d, J = 6.4 Hz, C-12 CH_3), 1.38 (3H, s, C-14 CH_3), 1.83 (3H, s, C-4 CH_3), 2.94 (2H, t, J = 6.3 Hz, $PhCH_2$), 3.05 (1H, m, C-25 H), 3.19 (1H, d, J = 9.8 Hz, C-13 H), 3.26 (1H, m, C-2 H), 3.40 (1H, m, C-13 OCH), 3.50–3.71 (2H, m, C-17 H and C-13 OCH), 3.95 (1H, d, J = 6.4 Hz, C-6 H), 4.29 (1H, m, C-5 H), 4.70 and 4.66 (2H, ABq, J = 15.7 Hz, C-27 H), 5.39 (1H, m, C-3 H), 7.37 (2H, d, J = 8.8 Hz, Ph H), 8.14 (2H, d, J = 8.8 Hz, Ph H), **5b**: ^{1}H NMR δ 0.82 (3H, d, J = 6.4 Hz, C-24 CH_3), 0.98 (3H, t, J = 7.3 Hz, C-25 CH_2CH_3), 1.07 (3H, d, J = 6.3 Hz, C-12 CH_3), 1.36 (3H, s, C-14 CH_3), 1.83 (3H, s, C-4 CH_3), 2.94 (2H, t, J = 6.3 Hz, $PhCH_2$), 3.05 (1H, m, C-25 H), 3.26 (1H, m, C-2 H), 3.58 (1H, dd, J = 4.4 and 11.0 Hz, C-15 H), 4.02 (1H, d, J = 6.3 Hz, C-6 H), 4.29 (1H, m, C-5 H), 4.66 and 4.70 (2H, ABq, J = 15.7 Hz, C-27 H), 4.87 (1H, m, C-19 H), 5.10 (1H, d, J = 8.3 Hz, C-13 H), 5.45 (1H, m, C-3 H), 7.37 (2H, d, J = 8.8 Hz, Ph H), 8.14 (2H, d, J = 8.8 Hz, Ph H); MS *m*/*z* 718 (M+1, $C_{40}H_{53}NO_{10}$). **7b**: ^{1}H NMR δ 0.84 (3H, d, J = 6.4 Hz, C-24 CH_3), 1.03 (3H, t, J = 7.3 Hz, C-25 CH_2CH_3), 1.11 (3H, d, J = 6.5 Hz, C-12 CH_3), 1.72 (3H, s, C-14 CH_3), 1.84 (3H, s, C-4 CH_3), 3.04 (1H, m, C-25 H), 3.12 (1H, m, C-12 H), 3.28 (1H, m, C-2 H), 3.45 (1H, m, C-17 H), 3.79 (1H, broad s, C-7 OH), 4.03 (1H, d, J = 6.4 Hz, C-6 H), 4.31 (1H, d, J = 6.1 Hz, C-5 H), 4.75 and 4.67 (2H, ABq, J = 14.5 Hz, C-27 H), 4.93 (1H, m, C-19 H), 5.28 (1H, dd, J = 10.3 and 14.4 Hz, C-11 H), 5.40–5.44 (2H, m, C-13 H and C-15 H), 5.45 (1H, m, C-2 H), 5.74 (1H, dt, J = 11.3 and 2.3 Hz, C-9 H), 5.84 (1H, dd, J = 11.3 and 14.4 Hz, C-10 H), 6.50 (1H, m, py H), 7.09 (1H, d, J = 9.6 Hz, py H), 7.18 (1H, dd, J = 7.2 and 9.6 Hz, py H), 7.72 (1H, d, J = 7.2 Hz, py H). **7c**: ^{1}H NMR δ 0.82 (3H, d, J = 6.5 Hz, C-24 CH_3), 0.93 (9H, s, *t*Bu), 1.00 (3H, t, J = 6.3Hz, C-25 CH_2CH_3), 1.11 (3H, d, J = 6.4 Hz, C-12 CH_3), 1.62 (3H, s, C-14 CH_3),1.74 (3H, s, C-4 CH_3), 3.03 (1H, m, C-25 H), 3.11 (1H, m, C-12 H), 3.29 (1H, m, C-2 H), 3.51 (1H, m, C-17 H), 3.86 (1H, d, J = 5.7 Hz, C-6 H), 4.37 (1H, d, J = 5.7 Hz, C-5 H), 4.54 and 4.67 (2H, ABq, J = 14.3 Hz, C-27 H), 4.71 (1H, m, C-19 H), 5.26 (1H, dd, J = 10.3 and 14.5 Hz, C-11 H), 5.42 (1H, d, J = 9.8Hz, C-13 H), 5.43 (1H, m, C-15 H), 5.49 (1H, s, C-3 H), 5.66 (1H, dt, J = 11.3 and 2.3 Hz, C-9 H), 5.83 (1H, dd, J = 11.3 and 14.5 Hz, C-10 H), 6.48 (1H, m, py H), 7.10 (1H, d, J = 9.6 Hz, py H), 7.17 (1H, dd, J = 7.1 and 9.6Hz, py H), 7.72 (1H, d, J = 7.1Hz, py H).

Synthesis of 4a from 3. 13-Iodomilbemycin (**3**) (220 mg, 0.33 mmol) was dissolved in 1,2-dichloroethane (3.0 mL). Then, 4-nitrophenethyl alcohol (251 mg, 1.5 mmol) and copper(I) trifluoromethanesulfonate (120 mg, 0.41 mmol) were added under nitrogen atmosphere. The mixture was stirred at room temperature for 20 minutes. Then the reaction mixture was diluted with ethyl acetate and filtered. The filtrate was washed with 1 mol dm^{-3} HCl and twice with water, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel with the eluent (ethyl acetate:cyclohexane = 1:4) to obtain only the desired **4a** (215 mg, 91.3% yield).

Synthesis of 4a from 5a. 15-Alkoxymilbemycin (**5a**) (353 mg, 0.5 mmol) was added into a solution of trifluoromethanesulfonic acid (0.5 mmol), CuI (0.5 mmol), and 4-nitrophenethyl alcohol (2.0 mmol) in 1,2-dichloroethane. The mixture was stirred at room temperature for 20 minutes. Ethyl acetate was added to the reaction mixture and filtered. The filtrate was washed with a 4% aqueous solution of $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated in vacuo. It was confirmed by the NMR spectrum of residue that the desired **4a** was the only product.

Synthesis of 4a from 8. To a solution of 4-nitrophenethyl alcohol (4.35g, 26.0 mmol) in 1,2-dichloroethane (25 mL) were added CuI (1.05g, 5.51 mmol) and trifluoromethanesulfonic acid (0.77 ml). Then, a solution of **8** (3.00g, 5.38 mmol) in 1,2-dichloroethane (5 mL) was added and the mixture was stirred at room temperature for 25 minutes. The reaction mixture was diluted with ethyl acetate and filtered. The filtrate was washed with water, a 4% aqueous solution of $NaHCO_3$, and with water again, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel, with the eluent (ethyl acetate:cyclohexane = 1:3) to obtain the desired **4a** (3.12g, 82.5% yield). **8**: ^{1}H NMR δ 0.83 (3H, d, J = 6.4 Hz, C-24 CH_3), 1.00 (3H, t, J = 7.3 Hz, C-25 CH_2CH_3), 1.09 (3H, d, J = 6.4 Hz, C-12 CH_3), 1.89 (3H, dd, J = 1.5 and 1.8 Hz, C-4 CH_3), 3.00 (1H, m, C-25 H), 3.10 (1H, m, C-12 H), 3.36 (1H, m, C-17 H), 3.53 (1H, m, C-2 H), 3.81 (1H, s, C-6 H), 3.92 (1H, s, C-7 OH), 4.08 (1H, dd, J = 4.4 and 10.8 Hz, C-15 H), 4.78 and 4.70 (2H, ABq, J = 14.5 Hz, C-27 H), 4.95 (1H, m, C-19 H), 5.16 (1H, dd, J = 1.0 and 9.3 Hz, C-13 H), 5.77 (1H, td, J = 2.4 and 11.2 Hz, C-9 H), 5.82 (1H, dd, J = 11.2 and 11.5 Hz, C-10 H), 6.54 (1H, m, C-3 H); MS m/z 556 (M, $C_{32}H_{44}O_8$).

Synthesis of 9 from 8. To a solution of 4-nitrophenylacetic acid (452 mg, 2.5 mmol) in dichloromethane (5 mL) were added **8** (279 mg, 0.5 mmol) and trifluoromethanesulfonic acid (1 drop). Then the mixture was stirred at room temperature for 15 minutes. The reaction mixture was diluted with ethyl acetate, washed with water, a 4% aqueous solution of $NaHCO_3$, and with water again, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel, with the eluent (ethyl acetate:cyclohexane = 1:3) to obtain the desired **9** (353 mg, 98.0% yield). **9**: ^{1}H NMR δ 0.83 (3H, d, J = 6.4 Hz, C-24 CH_3), 0.93 (3H, d, J = 6.4 Hz, C-12 CH_3), 0.98 (3H, t, J = 7.3 Hz, C-25 CH_2CH_3), 1.47 (3H, s, C-14 CH_3), 1.89 (3H, dd, J = 1.5 and 2.4 Hz, C-4 CH_3), 2.54 (1H, m, C-12 H), 3.03 (1H, m, C-25 H), 3.55 (1H, m, C-2 H), 3.58 (1H, m, C-17 H), 3.85 (1H, s, C-6 H), 4.00 (1H, broad s, C-7 OH), 4.71 and 4.76 (2H, ABq, J = 12.5 Hz, C-27 H), 4.97 (1H, d, J = 10.7 Hz, C-13 H), 6.53 (1H, m, C-3 H), 7.44 (2H, d, J = 8.8 Hz, Ph H), 8.19 (2H, d, J = 8.8 Hz, Ph H); MS m/z 719 (M, $C_{40}H_{49}NO_{10}$).

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References

1 Y. Takiguchi, H. Mishima, M.Okuda, M. Terao, A. Aoki, and R. Fukuda, *J. Antibiot.*, **33**, 1120 (1980).

2 H. Mishima, J. Ide, S. Muramatsu, and M. Ono, *J. Antibiot.*, **36**, 980 (1983).

3 M. H. Fisher, and H. Mrozik, "Macrolide Antibiot.," ed by S. Omura, Academic Press (1984), p. 553.

4 J. C. Chabala, H. Mrozik, R. L. Tolman, P. Eskola, A. Lusi, L. H. Peterson, M. F. Woods, and M. H. Fisher, *J. Med. Chem.*, **23**, 1134 (1980).

5 H. Mrozik, B. O. Linn, P. Escola, A. Lusi, A. Matzuk, F. A. Preiser, D. A. Ostlind, J. M. Schaeffer, and M. H. Fisher, *J. Med. Chem.*, **32**, 375 (1989).

6 A. Saito, S. Naito, M. Kobayashi, M. Tsuchiya, T. Toyama, S. Kaneko, T. Nanba, and Y. Morisawa, *J. Antibiot.*, **46**, 1252 (1993).

7 B. Frei, P. Huxley, P. Maienfisch, H. B. Mereyala, G. Rist, and A. C. O'Sullivan, *Helv. Chim. Acta*, **73**, 1905 (1990).

8 J. P. Springer, B. H. Arison, J. M. Hirshfield, and K. Hoogsteen, *J. Am. Chem. Soc.*, **103**, 4221 (1981).

9 S. Naito, A. Saito, Y. Furukawa, T. Hata, Y. Nakada, S. Muramatsu, and J. Ide, *J. Antibiot.*, **47**, 812 (1994).

10 These conformations are the most stable according to the results of MM2 and MOPAC calculations.