

PREPARATION OF 15-HYDROXY- $\Delta^{13,14}$ -MILBEMYCIN A₄ AND ITS TRANSFORMATION TO 13-ALKYLMILBEMYCINS

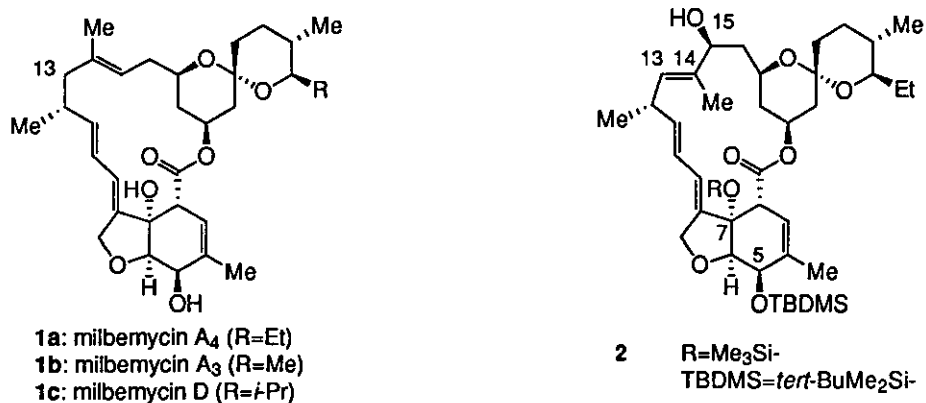
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Abstract—13-Alkylmilbemycins were synthesized regioselectively by Claisen rearrangement of a 15-hydroxy- $\Delta^{13,14}$ -milbemycin derivative, which was prepared selectively from a 14,15-epoxymilbemycin derivative by isomerization of the 14,15-epoxide moiety induced by ethylaluminum bis(2,6-diphenylphenoxide).

Milbemycins (**1**), isolated from *Streptomyces hygroscopicus*, are a family of 16-membered ring macrolides and exhibit acaricidal, insecticidal and anthelmintic activities.¹ During the course of our investigations on chemical modification of milbemycins with the aim of improving their original anthelmintic activities, introduction of substituents at C₁₃ of milbemycins intrigued us because such modification would change their pharmacokinetics. In our preceding paper,² we disclosed the synthesis of 13-alkyloxymilbemycins and reported that such modification was found to be successful in improving the anthelmintic activities of the parent milbemycins. These findings prompted us to synthesize 13-alkylmilbemycins for further investigation. In this report, an efficient procedure for preparing a 15-hydroxymilbemycin derivative and transformation of the 15-hydroxymilbemycin to 13-alkylmilbemycins are described.

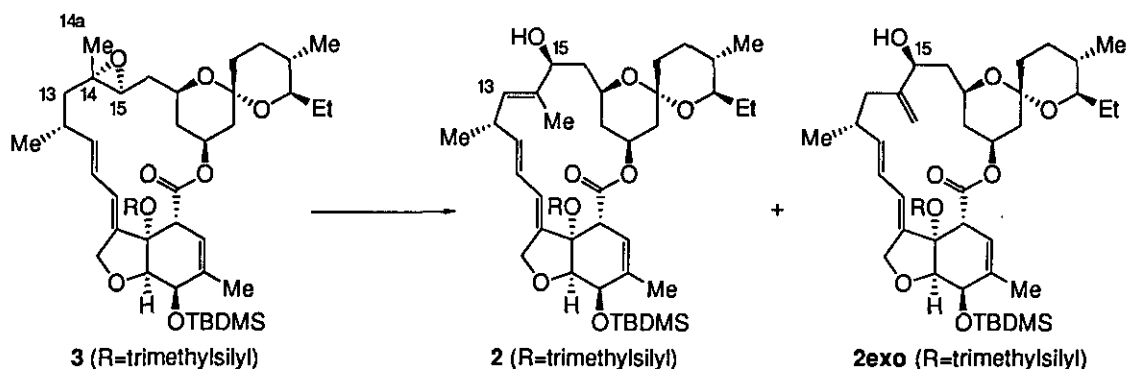
Scheme 1.



Several methods³ are known to introduce substituents at C₁₃ of milbemycins by forming carbon-carbon bonds; albeit, these methods suffer from production of regio- and stereoisomers. For the selective introduction of alkyl substituents at C₁₃ of milbemycin A₄ (**1a**), 15-hydroxy- $\Delta^{13,14}$ -milbemycin A₄ (**2**) has emerged as the key intermediate. It was assumed that Claisen rearrangement at the C₁₃-C₁₅ allylic alcohol moiety of the compound (**2**) would give 13 β -alkylmilbemycins selectively.

Firstly, preparation of **2**⁴ was investigated. Isomerization⁵ of the 14,15-epoxide moiety of **3**⁶ by reactions with 9-borabicyclo[3.3.1]nonyl triflate⁷ (9-BBNOTf) and trimethylsilyl triflate⁸ (TMSOTf) gave **2** in good yields; however, in both reactions *exo*-methylene derivative (**2_{exo}**) was also isolated as a by-product (Table 1, entries 1, 2). Then, organoaluminum reagents⁹ bearing hindered phenoxy substituents on aluminum were examined for the isomerization reaction with the hope that the amphoteric property of aluminum would work well for activation of the carbon-oxygen bond of the epoxide moiety and removal of the hydrogen at C₁₃ or C_{14a}. After surveying a variety of phenoxy groups on aluminum and the reaction conditions, ethylaluminum bis(2,6-diphenylphenoxide),¹⁰ EtAl(DPP)₂, was found to be the reagent of choice for converting the epoxide (**3**) to the allylic alcohol (**2**). In the reaction of **3** with EtAl(DPP)₂, the isomer (**2_{exo}**) was not detected by hplc analysis (Table 1, entry 4).

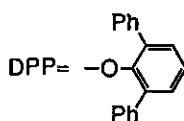
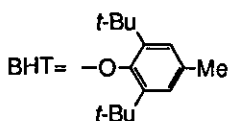
Table 1. Isomerization of epoxide (**3**) to allylic alcohol (**2**).



entry	reagents	solvent	temperature ^b	time (h)	yield (%)	
					2	2_{exo}
1	9-BBNOTf/2,6-lutidine	hexane	0°C~rt	2	84	10
2	TMSOTf/2,6-lutidine ^a	hexane	0°C	19	88	6
3	EtAl(BHT) ₂ (3.0 equiv)	toluene	-78°C~rt	1	72	9
4	EtAl(DPP) ₂ (3.0 equiv)	toluene	0°C~rt	5	91	-

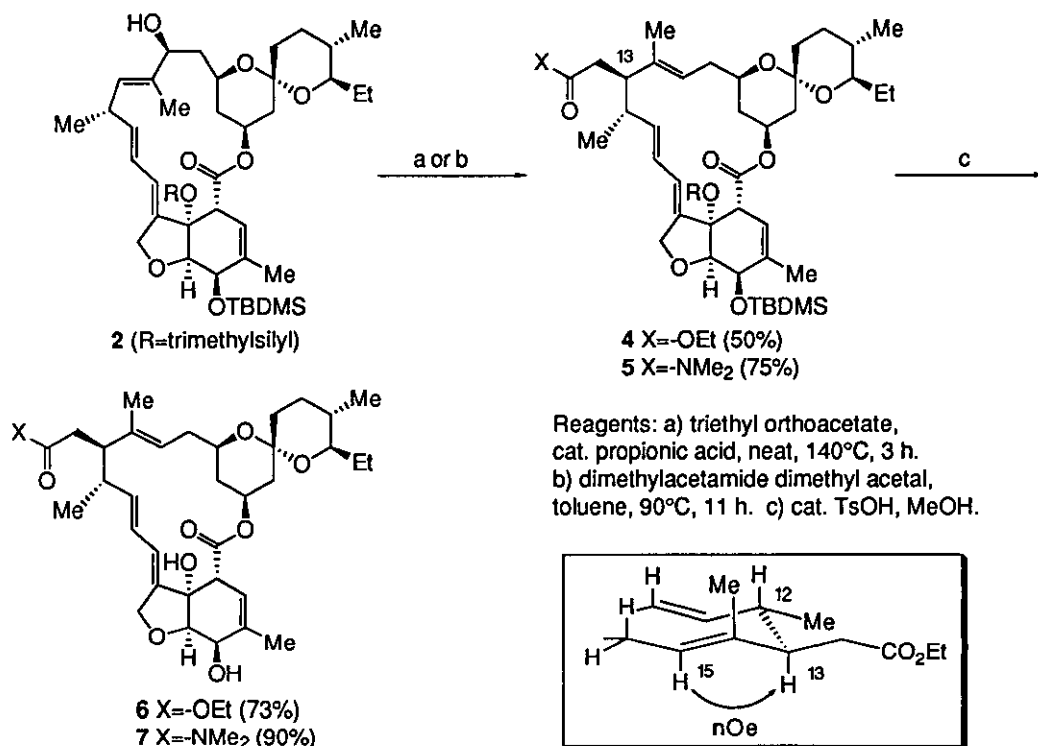
^a Deprotection of the trimethylsilyl group at C₁₅ was carried out after the isomerization reaction.

^b rt: room temperature.



Having secured an efficient procedure to obtain the 15-hydroxide (2), Claisen rearrangement of (2) was next carried out. Meerwein-Eschenmoser-Claisen¹¹ and Johnson-Claisen¹² rearrangements were applied to the compound (2), and the corresponding rearranged products (4) and (5) were isolated in 50 and 75 % yields, respectively. Subsequent deprotection of the silyl groups of the compounds (4) and (5) in methanol containing a catalytic amount of *p*-toluenesulfonic acid afforded 13-alkylmilbemycins (6) and (7) (Scheme 2.).

Scheme 2.



The stereochemistry at C₁₃ of the synthesized 13-alkylmilbemycins was confirmed to be the β -configuration on the basis of nOe difference experiments, taking account of the local structure of the milbemycin skeleton.^{1a,b,e} In the case of the compound (6), for example, irradiation of the C₁₅ hydrogen at δ 5.11 gave an nOe enhancement of the C₁₃ hydrogen at δ 2.36.

In summary, a 14,15-epoxymilbemycin derivative was selectively converted to a 15-hydroxymilbemycin derivative in excellent yield by employing ethylaluminum bis(2,6-diphenylphenoxide) for isomerizing the 14,15-epoxide moiety, and 13 β -alkylmilbemycins were regiospecifically synthesized from the 15-hydroxymilbemycin derivative by Claisen rearrangement.

EXPERIMENTAL

Milbemycin A₄ isolated from *Streptomyces hygroscopicus* was used as the starting material, which was

purified by column chromatography prior to experimentation and showed >96% purity by hplc analysis. All compounds were characterized by nmr spectrometry on a JEOL GSX 400 or a JEOL GX 270 spectrometer in CDCl₃ solution with tetramethylsilane as internal reference; by mass spectrometry on a JEOL JMS-AX 505H model; and by ir spectrometry on a JASCO FT/IR-830, and were in full agreement with the assigned structures. Unless otherwise indicated all common reagents and solvents were used as obtained from commercial suppliers without further purification.

14,15-Epoxy-5-*O*-*tert*-butyldimethylsilyl-7-*O*-trimethylsilylmilbemycin A₄ (3).

3 was prepared according to the literature procedure.⁶

13-Dehydro-15-hydroxy-5-*O*-*tert*-butyldimethylsilyl-7-*O*-trimethylsilylmilbemycin A₄ (2).

To a solution of 3 (75 mg, 0.1 mmol) in toluene (0.3 ml) at 0 °C was added 1M-toluene solution of ethylaluminum bis(2,6-diphenylphenoxide), which was prepared by reacting Et₃Al (1M in toluene) and 2 equiv of 2,6-diphenylphenol.¹⁰ The reaction mixture was stirred at room temperature for an additional 5 h, and poured into 0.1 N-HCl solution, extracted with CH₂Cl₂, and the extract was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by silica gel chromatography to give 70 mg of 2 (91% yield).

2: Ir(KBr)cm⁻¹ 1745, 1250, 1167, 989, 840; ms m/z 744(M⁺), 484, 167, 73; ¹H-nmr(400 MHz, CDCl₃) δ 5.80(1H, dd, *J*=11.4 and 14.7 Hz, H-10), 5.65(1H, br d, *J*=11.4 Hz, H-9), 5.49(1H, br s, H-3), 5.23(1H, dd, *J*=10.2 and 14.7 Hz, H-11), 5.16(1H, d, *J*=8.9 Hz, H-13), 4.64-5.19(2H, m, H-19 and H-8a), 4.54(1H, dd, *J*=2.2 and 14.2 Hz, H-8a'), 4.37(1H, m, H-5), 4.08(1H, m, H-15), 3.86(1H, d, *J*=5.6 Hz, H-6), 3.42(1H, m, H-17), 3.30(1H, m, H-2), 3.06-3.15(1H, m, H-12), 3.00(1H, dt, *J*=2.4 and 9.5 Hz, H-25), 1.78(3H, br s, H-4a), 1.62(3H, s, H-14a), 1.12(3H, d, *J*=6.5 Hz, H-12a), 0.98(3H, t, *J*=7.3 Hz, H-25b), 0.94(9H, s, *t*-butyl-Si), 0.82(3H, d, *J*=6.5 Hz, H-24a), 0.15(3H, s, MeSi), 0.14(3H, s, MeSi), 0.12(9H, s, Me₃Si); *Anal.* Calcd for C₄₁H₆₈O₈Si₂: C,66.09; H,9.20. Found: C,65.64; H,9.21.

13-Ethoxycarbonylmethyl-5-*O*-*tert*-butyldimethylsilyl-7-*O*-trimethylsilylmilbemycin A₄ (4).

A mixture of 2 (75 mg, 0.1 mmol), triethyl orthoacetate (0.2 ml, 1.1 mmol) and propionic acid (2 mg, 0.03 mmol) was heated at 130 °C for 2 h. The resulting mixture was concentrated *in vacuo* and the residue was purified by preparative thin layer chromatography to give 41 mg of 4 (50% yield).

4: Ir(KBr)cm⁻¹ 1741, 1251, 1169, 989, 840; ms m/z 814(M⁺), 572, 223, 167, 73; ¹H-nmr(400 MHz, CDCl₃) δ 5.77(1H, dd, *J*=11.5 and 14.7 Hz, H-10), 5.65(1H, br d, *J*=11.5 Hz, H-9), 5.46(1H, m, H-3), 5.42(1H, dd, *J*=9.9 and 14.7 Hz, H-11), 5.18(1H, m, H-15), 4.78-4.86(1H, m, H-19), 4.65(1H, dd, *J*=2.1 and 14.2 Hz, H-8a), 4.56(1H, dd, *J*=2.1 and 14.2 Hz, H-8a'), 4.39(1H, m, H-5), 3.99-4.16(2H, m, -CO₂CH₂-), 3.79(1H, d, *J*=5.2 Hz, H-6), 3.54-3.60(1H, m, H-17), 3.22(1H, m, H-2), 3.02(1H, dt, *J*=2.3 and 9.5 Hz, H-25), 1.79(3H, s, H-4a), 1.50(3H, s, H-14a), 1.20(3H, t, *J*=7.2 Hz, -CO₂CH₂CH₃), 1.05(3H, d, *J*=6.6 Hz, H-12a), 0.95(3H, t, *J*=7.1 Hz, H-25b), 0.93(9H, s, *t*-butyl-Si),

0.81(3H, d, $J=6.5$ Hz, H-24a), 0.14(3H, s, MeSi), 0.13(3H, s, MeSi), 0.12(9H, s, Me₃Si); *Anal.* Calcd for C₄₅H₇₄O₉Si₂: C,66.30; H,9.15. Found: C,65.50; H,8.90.

13-Dimethylaminocarbonylmethyl-5-*O*-*tert*-butyldimethylsilyl-7-*O*-trimethylsilyl-milbemycin A₄ (5).

A solution of **2** (75 mg, 0.1 mmol) and *N,N*-dimethylacetamide dimethyl acetal (133 mg, 1.0 mmol) in toluene (1 ml) was heated (80~90 °C) for 11 h, and poured into water, extracted with ethyl acetate, and the extract was dried over Na₂SO₄ and evaporated under reduced pressure. Purification by silica gel chromatography gave 61 mg of **5** (75% yield).

5: Ir(KBr)cm⁻¹ 1741, 1653, 1251, 1168, 1129, 1094, 989, 840; ms m/z 813(M⁺), 571, 262, 223, 195, 167, 73; ¹H-nmr(270 MHz, CDCl₃) δ 5.77(1H, dd, $J=11.2$ and 14.2 Hz, H-10), 5.66(1H, br d, $J=11.2$ Hz, H-9), 5.40-5.49(2H, m, H-3 and H-11), 5.22(1H, m, H-15), 4.78-4.86(1H, m, H-19), 4.66(1H, dd, $J=2.0$ and 14.2 Hz, H-8a), 4.55(1H, dd, $J=2.0$ and 14.2 Hz, H-8a'), 4.38-4.40(1H, m, H-5), 3.79(1H, d, $J=5.4$ Hz, H-6), 3.50-3.63(1H, m, H-17), 3.21-3.24(1H, m, H-2), 2.80-3.08(7H, m, H-25 and Me₂N-), 1.79(3H, s, H-4a), 1.51(3H, s, H-14a), 1.06(3H, d, $J=6.4$ Hz, H-12a), 0.95(3H, t, $J=7.3$ Hz, H-25b), 0.93(9H, s, *t*-butyl-Si), 0.80(3H, d, $J=6.3$ Hz, H-24a), 0.13(6H, s, Me₂Si), 0.11(9H, s, Me₃Si).

13-Ethoxycarbonylmethylmilbemycin A₄ (6).

4 (41 mg, 0.05 mmol) was treated with *p*-toluenesulfonic acid (3 mg, 0.015 mmol) in methanol (1 ml) for 2 h. The reaction mixture was poured into saturated NaHCO₃ solution, extracted with CH₂Cl₂, and the extract was dried over Na₂SO₄. The residue obtained on concentration was chromatographed on silica gel to give 23 mg of **6** (73% yield).

6: Ir(KBr)cm⁻¹ 3460, 1736, 1717, 1181, 1169, 991; ms m/z 628(M⁺), 500, 195, 167, 151, 95, 55; HRms(EI) calcd for C₃₆H₅₂O₉(M⁺) 628.3613, found 628.3635; ¹H-nmr(400 MHz, CDCl₃) δ 5.80(1H, br d, $J=11.3$ Hz, H-9), 5.73(1H, dd, $J=11.3$ and 14.0 Hz, H-10), 5.45(1H, dd, $J=9.9$ and 14.0 Hz, H-11), 5.41(1H, br s, H-3), 5.32-5.38(1H, m, H-19), 5.09-5.13(1H, m, H-15), 4.64-4.73(2H, m, H-8a and H-8a'), 4.28-4.30(1H, m, H-5), 3.99-4.15(3H, m, 7-OH and -CH₂CO₂-), 3.96(1H, d, $J=6.0$ Hz, H-6), 3.51-3.58(1H, m, H-17), 3.26-3.28(1H, m, H-2), 3.05(1H, dt, $J=2.4$ and 9.3 Hz, H-25), 2.36(1H, m, H-13), 1.88(3H, br s, H-4a), 1.47(3H, s, H-14a), 1.20(3H, t, $J=7.0$ Hz, -CO₂CH₂CH₃), 1.02(3H, d, $J=6.6$ Hz, H-12a), 0.99(3H, t, $J=7.4$ Hz, H-25b), 0.82(3H, d, $J=6.4$ Hz, H-24a).

13-Dimethylaminocarbonylmethylmilbemycin A₄ (7).

5 (42 mg, 0.05 mmol) was treated as described for **6** to provide **7** (29 mg, 90% yield).

7: Ir(KBr) cm⁻¹ 3450, 1736, 1711, 1645, 1180, 1168, 990; ms m/z 627(M⁺) 195, 167, 87, 72, 55; HRms(EI) calcd for C₃₆H₅₃NO₈(M⁺) 627.3773, found 627.3768; ¹H-nmr(270 MHz, CDCl₃) δ 5.68-5.82(2H, m, H-9 and H-10), 5.33-5.51(3H, m, H-3, H-11 and H-19), 5.16(1H, m, H-15), 4.69(2H, m, H-8a and H-8a'), 4.30(1H, m, H-5), 3.96(1H, d, $J=5.9$ Hz, H-6), 3.49-3.58(1H, m, H-17), 3.27(1H, m, H-2), 2.85-3.09(7H, m, H-25 and -CONMe₂), 2.49(1H, m, H-13), 1.88(3H, br s, H-4a), 1.49(3H, s, H-14a), 1.03(3H, d, $J=6.4$ Hz, H-12a), 0.98(3H, t, $J=7.3$ Hz, H-25b), 0.82(3H, d, $J=6.3$ Hz, H-24a).

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