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Synthesis of 2-Methyl 16-Membered Macrolide Derived from Tylosin

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Abstract To improve the metabolic stability of a 16-membered macrolide, 2-methylated derivatives of desmycosin were synthesized. Among these derivatives, 2β-methyldesmycosin retained antibacterial activity and showed improved stability in rat serum compared to desmycosin.

Keywords 16-membered macrolide, tylosin, desmycosin, metabolic stability

Introduction

Macrolide antibiotics have played important roles as antibacterial agents for half a century, and years of assiduous efforts have been devoted to developing their derivatives more effectively. This research and development has led to the success of second-generation macrolides, such as clarithromycin and azithromycin [1]. While erythromycin and its derivatives have been extensively prescribed, pathogens resistant to these compounds have become increasingly prevalent. To address the problem of resistant bacteria, third-generation macrolides known as "ketolides" were developed, and the first ketolide antibiotic, telithromycin, was recently approved for use [1].

Sixteen-membered macrolide antibiotics have also been used for the treatment of respiratory tract infections. While not as widely prescribed as erythromycin and its derivatives, these antibiotics are known to have a number of advantages, including better gastrointestinal tolerance, absence of QT increase, and lack of drug-drug interactions. Nevertheless, one of the contributing factors in the low rates of prescription of 16-membered macrolides is their low metabolic stability. In the case of spiramycin, the ring-opening derivative was identified as its metabolite by esterase in rat serum [2]. This result was not reported with erythromycin and its derivatives. Our approach to improving the metabolic stability of 16-membered macrolides is to introduce the methyl group at position 2, as with 14-membered macrolides. 2-Methyl 16-membered macrolides were expected to acquire resistance to esterase caused by steric hindrance around the ester bond. Kageyama *et al.* also reported the synthesis of (2*R* and 2*S*)-3-deoxy-5-*O*-(4-deoxymycaminosyl)-2-*C*-methyltylonolide using the same strategy, but no data on the stability of these compounds are available [3]. Here we describe the synthesis of 2-methyldesmycosin and its stability against rat esterase.

Results

Desmycosin (**2**), prepared from tylosin (**1**), was converted to **3** using a previously reported method [4]. The methylation of **3** yielded mono- and di-methylated derivatives (**4**, **5**) in the ratio 2 : 1. Contrary to expectations,

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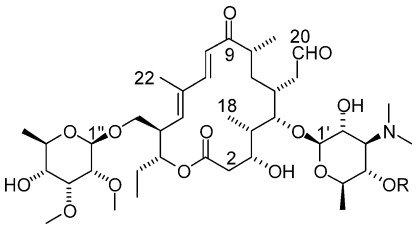


1 : R=β-mycinosyl
2 : R=H

Fig. 1 Structures of tylosin (**1**) and desmycosin (**2**).

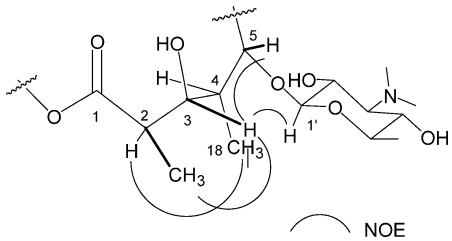


Fig. 2 Stereo-drawing of **6** at positions 2 and 3.

4 was derived as a single isomer and no enol form was detected in ^{1}H and ^{13}C NMR. Reduction of **4** yielded **6**, subsequently only the 9-hydroxyl group was oxidized by Dess-Martin oxidation to produce **7**. The stereochemistry of **6** at positions 2 and 3 was determined by analyzing the coupling constants and NOESY spectrum. The coupling constant between 2-H and 3-H was 2.4 Hz, indicating gauche orientation. And that between 3-H and 4-H was 10.7 Hz, indicating anti orientation. The multiplicity of 3-H could be measured by adding D_2O. Together with the following NOEs, these findings revealed the stereochemistry of **6** at positions **2** and **3** as shown in Fig. 2: 2-H/18-H, 2-Me/3-H, 3-H/5-H and 3-H/1′-H. 3-*epi* configuration was also supported by Furuuchi's data [5]. Recently they reported on the synthesis of 3-*epi*-leucomycin A_7 (3-*epi*-LM-A_7) *via* reduction of 3-keto-LM-A_7.

Initially we planned to produce several stereoisomers of 2-methyldesmycosin by the above route, unfortunately, however, methylation and the reduction process progressed stereoselectively. Furuuchi [5] also reported that the stability of 3-*epi*-LM-A_7 is significantly reduced compared to LM-A_7. Accordingly, we planned to synthesize other isomers of 2-methyldesmycosin.

2β-Methyldesmycosin (**14**) and 2α-methyldesmycosin (**15**) were synthesized as shown in Scheme 1. **1** was converted to protected compound **8** using a previously reported method [4]. Reduction of the 9-keto group of **8** yielded **9** as a single isomer. After deprotection of the formyl group of **9**, the 3-hydroxyl and 20-formyl groups were protected as 3,20-(*O*-silyl)acetal together with a 9-hydroxyl group as silyl ether to produce **10**. Three acetyl groups at sugar moieties were converted to triethylsilyl groups, following methylation in the presence of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) yielded **12** and **13** in the ratio 10 : 1. **12** and **13**, separated by silica gel pTLC, were both deprotected to yield **14** and **15**. The stereochemistry of **14** and **15** were determined through NOESY spectrum analysis. Correlation between 2-H and 22-H of **14** revealed a β configuration of the 2-methyl group. In contrast, a correlation was seen between 2-Me and 22-H of **15**.

The *in vitro* antibacterial activities of **7**, **14**, and **15** are summarized in Table 1. **7** and **15** displayed poor activity compared to the parent macrolide desmycosin (**2**). 2-Methyl-3-keto-desmycosin, the deprotected compound of **4**, and 2,2-dimethyl-3-keto-desmycosin, the deprotected compound of **5**, also displayed poor activity (data not shown). Only **14** retained antibacterial activity approximately equivalent to that of desmycosin (**2**).

Discussion

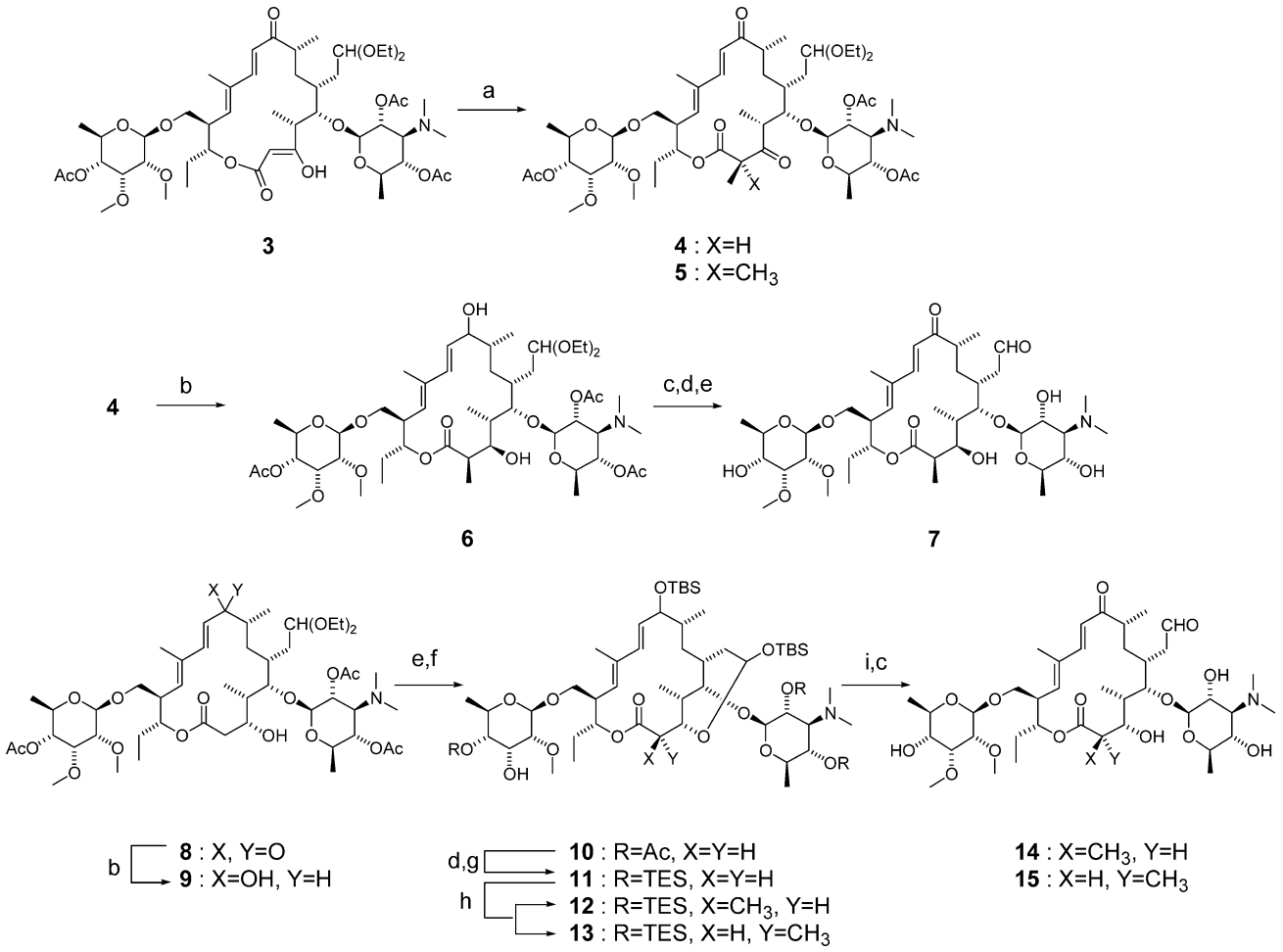
In the antibacterial test of desmycosin derivatives, only **14** showed activity approximately equivalent to that of the parent compound desmycosin (**2**). **14** features the same stereochemistry as erythromycin at positions 2 and 3, indicating the importance of this stereochemistry.

The stability of **2** and **14** in rat serum was estimated by LC/MS. In the case of desmycosin (**2**), the peak corresponding to the ring-opening metabolite (MW 790) was observed after 1-hour incubation in rat serum (Fig. 3b). No ring-opening metabolites were detected after incubation of desmycosin (**2**) in a phosphate buffer (Fig. 3d) and **14** in rat serum (Fig. 3f). These results suggested that **14** acquired resistance to esterase in rat serum due to the steric hindrance of the 2-methyl group. To verify this data, we plan to confirm the structure of the desmycosin metabolite (Fig. 3b) and to prepare **14** using the combinatorial biosynthesis method for the *in vivo* antibacterial test. These results will be reported elsewhere.

Experimental

General Procedure

^{1}H and ^{13}C NMR spectra were measured on a JEOL Alpha



Reagents and conditions: (a) CH_3I, *t*BuOK, THF, 0°C, 2 hours; (b) $CeCl_3 \cdot 7H_2O$, $NaBH_4$, MeOH, 0°C, 30 minutes; (c) Dess-Matin periodinane, CH_2Cl_2, 0°C, 1.5 hours; (d) NH_3 aq., MeOH, r.t., 72 hours; (e) 1 N HCl, CH_3CN, r.t., 1 hour; (f) TBSCl, imidazole, DMF, 45°C, 18 hours; (g) TESCl, imidazole, DMF, r.t., 18 hours; (h) LDA, CH_3I, THF - DMPU 2 : 1, −78°C, 1 hour; (i) $F_2CHCOOH$, $CH_3CN - H_2O$ 1 : 1, 45°C, 72 hours.

Scheme 1

Table 1 *In vitro* antibacterial activities of desmycosin derivatives

Strain	Characteristics	MIC (μg/ml)					
		2	**7**	**14**	**15**	CAM	RKM
Streptococcus pneumoniae IID553		0.5	2	0.5	2	0.03	0.12
S. pneumoniae 205	*ermB*$^+$	>128	>128	>128	32	>128	32
S. pneumoniae 210	*mefA*$^+$	2	4	2	4	4	0.25
Haemophilus influenzae ATCC33533		4	16	8	32	16	8

CAM : clarithromycin, RKM : rokitamycin

500 or JEOL Lambda 500 spectrometer. Chemical shifts are reported in parts per million (ppm) with TMS as an internal standard. Coupling constants (*J*) are given in hertz (Hz). Optical rotations were measured on a JASCO DIP-360 digital polarimeter. Mass spectra were recorded on a Micromass Platform LC or a Micromass Q-Tof 2. LC/MS

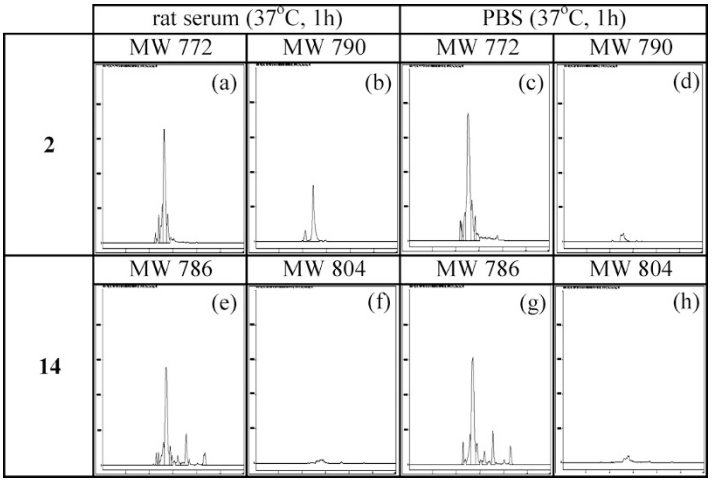


Fig. 3 LC/MS analysis of **2** and **14** after incubation in rat serum or PBS for 1 hour at 37°C[a].

[a] Each compound was detected by expected molecular weight and all data are written in same scale.

spectra were obtained on an Agilent 1100 LC/MSD SL system.

Antibacterial Activity Test

Minimum inhibitory concentration (MIC) was determined by the agar dilution method as recommended by NCCLS [6].

Stability Test in Rat Serum

Test compound was dissolved in DMSO (100 mg/ml). The DMSO solution (10 μl) was added to EDTA-treated rat serum (0.5 ml, prepared from Wister Rat, 5 weeks of age) and incubated for 1 hour at 37°C. CH_3CN (0.5 ml) was added to the reaction mixture and centrifuged for 5 minutes at 10000 rpm. The supernatant was collected and evaporated under reduced pressure. The resulting residue was dissolved to 50% EtOH and analyzed by LC/MS. LC/MS conditions are as follows; ionization method: electron spray ionization (ESI); column: YMC-Pack ODS-AM 100×4.6 mm I.D.; solvent system: 10 mM HCOOH, $HCOONH_4/CH_3CN$ gradient; flow rate: 1.0 ml/minute; temperature: 40°C.

2β-Methyl-3-dehydro-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylacetal (4), 2,2-Dimethyl-3-dehydro-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylacetal (5)

2,3-Dehydro-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylacetal (**3**, 1.54 g, 1.59 mmol), prepared by literature-based procedures [4] from tylosin tartrate (Wako Pure Chemical Industries, Ltd.), was dissolved in THF (75 ml). To this solution, potassium *tert*-butoxide (210.9 mg, 1.88 mmol) and methyl iodide (0.2 ml, 3.21 mmol) were added at 0°C. After stirring for 2 hours, the reaction solution was added to water and extracted with EtOAc. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The resulting residue was chromatographed on silica gel eluting with 50% EtOAc in hexane to yield **4** (595.3 mg, 38.1% yield) and **5** (319.9 mg, 20.2% yield). **4**: ESI-MS *m/z* 984 $(M+H)^+$; $[\alpha]_D^{25}=+4.6°$ (*c* 1.05, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3464, 2978, 2939, 1750, 1666, 1456, 1375, 1232, 1171, 1089, 1056; ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (3H, t, *J*=7.3 Hz; 17-H), 1.11 (3H, d, *J*=6.7 Hz; 21-H), 1.31 (3H, d, *J*=7.0 Hz; 2-Me), 1.80 (3H, s; 22-H), 1.94 (3H, s; OAc), 2.03 (3H, s; OAc), 2.08 (3H, s; OAc), 2.30 (6H, s; 3′-NMe_2), 3.40 (3H, s; 2″-OMe), 3.49 (3H, s; 3″-OMe), 3.55 (1H, q, *J*=7.3 Hz; 2-H), 5.79 (1H, br d; 13-H), 6.08 (1H, br d; 10-H), 7.06 (1H, d, *J*=16.5 Hz; 11-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6 (C-1), 49.5 (C-2), 13.8 (2-Me), 209.0 (C-3). **5**: ESI-MS *m/z* 998 $(M+H)^+$; $[\alpha]_D=-31.1°$ (*c* 1.03, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3444, 2979, 2939, 1752, 1723, 1667, 1456, 1374, 1232, 1169, 1152, 1090, 1056; ^1H NMR (500 MHz, $CDCl_3$) δ 0.92 (3H, t, *J*=7.3 Hz; 17-H), 0.97 (3H, d, *J*=7.0 Hz; 18-H), 1.13 (3H, d, *J*=6.7 Hz; 21-H), 1.33 (6H, s; 2-Me_2), 1.81 (3H, s; 22-H), 1.95 (3H, s; OAc), 2.05 (3H, s; OAc), 2.11 (3H, s; OAc), 2.34 (6H, s; 3′-NMe_2), 3.42 (3H, s; 2″-OMe), 3.51 (3H, s; 3″-OMe), 5.94 (1H, d, *J*=11.0 Hz; 13-H), 6.08 (1H, d, *J*=16.5 Hz; H-10), 7.18 (1H, d, *J*=16.5 Hz; 11-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4 (C-1), 55.8 (C-2), 21.6 (2-Me), 22.6 (2-Me), 213.6 (C-3).

2β-Methyl-3-*epi*-9-hydro-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylacetal (6)

To a solution of **4** (261.4 mg, 0.266 mmol) in methanol

(6.0 ml), cerium chloride heptahydrate (148.4 mg, 0.398 mmol) was added. After stirring for 30 minutes at room temperature, sodium borohydride (400.0 mg, 10.6 mmol) was added slowly at 0°C. After stirring for 30 minutes, 1 M Na_2HPO_4 (50 ml) was added and the mixture was extracted with EtOAc. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The resulting residue was chromatographed on silica gel eluting with 50% EtOAc in hexane to yield **6** (98.7 mg, 37.6% yield). **6**: ESI-MS *m/z* 988 $(M+H)^+$; $[\alpha]_D^{25}=+7.0°$ (*c* 1.14, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3492, 2977, 2938, 1751, 1708, 1457, 1375, 1232, 1173, 1090, 1054; ^1H NMR (500 MHz, $CDCl_3$) δ 0.72 (3H, d, *J*=6.7 Hz; 18-H), 0.88 (3H, t, *J*=7.3 Hz; 17-H), 0.97 (3H, d, *J*=7.3 Hz; 21-H), 1.36 (3H, d, *J*=7.3 Hz; 2-Me), 1.70 (3H, s; 22-H), 1.96 (3H, s; OAc), 2.01 (3H, s; OAc), 2.08 (3H, s; OAc), 2.30 (6H, s; 3′-NMe_2), 2.65 (1H, dq, *J*=1.8, 7.3 Hz; 2-H), 3.08 (1H, br t, *J*=11.0 Hz; 3-H), 3.44 (3H, s; 2″-OMe), 3.49 (3H, s; 3″-OMe), 4.11 (1H, br; 9-H), 5.36 (1H, d, *J*=10.4 Hz; 13-H), 5.67 (1H, dd, *J*=3.7, 15.9 Hz; 10-H), 6.32 (1H, dd, *J*=1.2, 15.9 Hz; 11-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.2 (C-1), 39.8 (C-2), 17.2 (2-Me), 76.7 (C-3), 76.8 (C-9).

2β-Methyl-3-*epi*-desmycosin (7)

To a solution of **6** (158.8 mg, 0.161 mmol) in CH_2Cl_2 (10 ml), Dess-Martin periodinane (81.8 mg, 0.193 mmol) was added at 0°C. After stirring for 1.5 hours, 0.2 N NaOH aq. was added to the reaction mixture and it was extracted with EtOAc. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The resulting residue was dissolved in methanol (10 ml). After 28% NH_3 aq. (0.06 ml) was added to this solution, the reaction mixture was stirred for 3 days at room temperature. The solution was evaporated and the resulting residue was dissolved in CH_3CN (2.0 ml). To this solution, 1 N HCl aq. (0.5 ml) was added and the reaction mixture was stirred for 1 hour at room temperature. The solution was evaporated and the resulting residue was purified by silica gel pTLC ($CHCl_3$-MeOH 4 : 1) to yield **7** (101.0 mg, 80.1% yield over three steps). **7**: HRTOF-MS *m/z* 784.4495 $(M-H)^-$ (calcd for $C_{40}H_{66}NO_{14}$: 784.4483); $[\alpha]_D^{25}=-9.2°$ (*c* 0.95, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3447, 2975, 2935, 1721, 1660, 1632, 1598, 1458, 1380, 1271, 1169, 1084, 1064; ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (3H, d, *J*=6.7 Hz; 18-H), 0.87 (3H, t, *J*=7.3 Hz; 17-H), 1.13 (3H, d, *J*=6.7 Hz; 21-H), 1.19 (3H, d, *J*=6.4 Hz; 6″-H), 1.20 (3H, d, *J*=6.0 Hz; 6′-H), 1.31 (3H, d, *J*=7.3 Hz; 2-Me), 1.73 (3H, s; 22-H), 2.44 (6H, s; 3′-NMe_2), 2.56 (1H, qd, *J*=7.3, 2.1 Hz; 2-H), 3.15 (1H, br; 3-H), 3.41 (3H, s; 2″-OMe), 3.54 (3H, s; 3″-OMe), 4.13 (1H, d, *J*=7.3 Hz; 1′-H), 4.33 (1H, br s; 5-H), 4.46 (1H, d, *J*=7.6 Hz; 1″-H), 4.99 (1H, td, *J*=9.8, 2.7 Hz; 15-H), 5.85 (1H, d, *J*=10.4 Hz; 13-H), 6.16 (1H, d, *J*=15.9 Hz; 10-H), 7.02 (1H, d, *J*=15.9 Hz; 11-H), 9.67 (1H, s; 20-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.1 (C-1), 40.3 (C-2), 17.7 (2-Me), 77.3 (C-3), 43.9 (C-4), 205.0 (C-9), 202.4 (C-20).

9-Hydro-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylacetal (9)

2′,4′,4″-Tri-*O*-acetyldesmycosin-20-diethylacetal (**8**, 50.1 g), prepared by literature-based procedures [4] from tylosin tartrate, was dissolved in methanol (1 liter). To this solution, cerium chloride heptahydrate (28.8 g, 0.077 mol) was added. After stirring at room temperature for 30 minutes, sodium borohydride (9.7 g, 0.26 mol) was added slowly at 0°C. After stirring for 30 minutes, 1 M Na_2HPO_4 (500 ml) was added and the mixture was extracted with EtOAc. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The resulting residue was chromatographed on silica gel eluting with 60% EtOAc in hexane to yield **9** (22.4 g, 49.0% yield over four steps from tylosin tartrate). **9**: ESI-MS *m/z* 974 $(M+H)^+$; $[\alpha]_D^{25}=+6.2°$ (*c* 1.06, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3510, 2978, 2938, 1750, 1714, 1456, 1374, 1232, 1173, 1090, 1051; ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (3H, t, *J*=7.3 Hz; 17-H), 1.69 (3H, s; 22-H), 2.00 (3H, s; OAc), 2.02 (3H, s; OAc), 2.08 (3H, s; OAc), 2.30 (6H, s; 3′-NMe_2) 3.45 (3H, s; 2″-OMe), 3.49 (3H, s; 3″-OMe), 4.91 (1H, dd, *J*=10.1, 2.4 Hz; 9-H), 5.29 (1H, br d, *J*=9.8 Hz; 13-H), 5.77 (1H, br; 10-H), 6.41 (1H, dd, *J*=15.8, 1.5 Hz; 11-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 76.0 (C-9).

9-Hydro-9,20-di-*O*-*tert*-butyldimethylsilyl-2′,4′,4″-tri-*O*-acetyldesmycosin-3,20-acetal (10)

The formyl group of **9** (22.2 g, 22.8 mmol) was deprotected as mentioned above and the resulting residue was dissolved in DMF (250 ml). *tert*-Butyldimethylsilylchloride (10.9 g, 72.3 mmol) and imidazole (9.8 g, 0.15 mol) were added to this solution and the mixture was stirred for 18 hours at 45°C. Water was added and the mixture was extracted with toluene. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The resulting residue was chromatographed on silica gel eluting with 40% EtOAc in hexane to yield **10** (3.98 g, 15.6% yield over two steps). **10**: ESI-MS *m/z* 1128 $(M+H)^+$; $[\alpha]_D^{25}=-8.4°$ (*c* 1.04, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3476, 2936, 2859, 1751, 1464, 1229, 1166, 1091, 1055; ^1H NMR (500 MHz, $CDCl_3$) δ 0.98 (3H, d, *J*=6.7 Hz; 21-H), 1.73 (3H, s; 22-H), 1.97 (3H, s; OAc), 2.04 (3H, s; OAc), 2.09 (3H, s; OAc), 2.33 (6H, s; 3′-NMe_2), 3.46 (3H, s; 2″-OMe), 3.50 (3H, s; 3″-OMe), 3.81 (1H, dd, *J*=7.6, 5.5 Hz; 9-H), 5.36 (1H, d, *J*=9.8 Hz; 13-H), 5.90 (1H, dd, *J*=15.8, 5.5 Hz; 10-H), 6.18 (1H, d,

J=15.8 Hz; 11-H).

9-Hydro-9,20-di-*O*-*tert*-butyldimethylsilyl-2′,4′,4″-tri-*O*-triethylsilyldesmycosin-3,20-acetal (11)

Three hydroxyl groups at sugar moieties of **10** (3.88 g, 3.44 mmol) were deprotected as mentioned above and the resulting residue was dissolved in DMF (60 ml). Triethylsilylchrolide (3.5 ml, 20.6 mmol) and imidazole (2.8 g, 41.3 mmol) were added to this solution and the reaction mixture was stirred for 18 hours at room temperature. Water was added and the mixture was extracted with EtOAc. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The resulting residue was chromatographed on silica gel eluting with 20% EtOAc in hexane to yield **11** (3.37 g, 75.2% yield over two steps). **11**: ESI-MS m/z 1346 $(M+H)^+$; $[\alpha]_D^{25}=-17.5°$ (c 1.00, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3436, 2958, 2879, 1743, 1463, 1382, 1251, 1106, 1082; ^1H NMR (500 MHz, $CDCl_3$) δ 1.18 (3H, d, J=6.1 Hz; 21-H), 1.75 (3H, s; 22-H), 2.42 (6H, s; 3′-NMe_2), 3.47 (3H, s; 2″-OMe), 3.58 (3H, s; 3″-OMe), 5.44 (1H, d, J=10.4 Hz; 13-H), 5.84 (1H, dd, J=15.9, 6.1 Hz; 10-H), 6.14 (1H, d, J=15.9 Hz; 11-H).

2β-Methyl-9-hydro-9,20-di-*O*-*tert*-butyldimethylsilyl-2′,4′,4″-tri-*O*-triethylsilyldesmycosin-3,20-acetal (12), 2α-Methyl-9,20-di-*O*-*tert*-butyldimethylsilyl-2′,4′,4″-tri-*O*-triethylsilyldesmycosin-3,20-acetal (13)

n-BuLi (15% solution in hexane, 1.1 ml, 2.51 mmol) was added to the mixture of $^iPr_2NEt$ (0.35 ml, 2.51 mmol) and THF - DMPU 2 : 1 (3.0 ml) at −78°C. After stirring for 20 minutes, **11** (675.1 mg, 0.502 mmol) in THF - DMPU 2 : 1 (3.0 ml) was added to this solution. Methyl iodide (0.16 ml, 2.51 mmol) was added and the reaction mixture was stirred for 30 minutes. The reaction mixture was added to saturated NH_4Cl aq. and extracted with EtOAc. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated. The resulting residue was separated by silica gel pTLC (hexane - EtOAc 5 : 1) to yield **12** (340.1 mg, 49.8% yield) and **13** (39.2 mg, 5.7% yield). **12**: ESI-MS m/z 1360 $(M+H)^+$; $[\alpha]_D^{25}=-2.1°$ (c 0.84, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3436, 2957, 2879, 1741, 1463, 1381, 1252, 1106; ^1H NMR (500 MHz, $CDCl_3$) δ 1.26 (3H, d, J=6.7 Hz; 2-Me), 1.69 (3H, s; 22-H), 2.41 (6H, s; 3′-NMe_2), 2.52 (1H, dq, J=9.2, 6.7 Hz; 2-H), 3.47 (3H, s; 2″-OMe), 3.58 (3H, s; 3″-OMe), 5.42 (1H, d, J=9.8 Hz; 13-H), 5.71 (1H, dd, J=15.3, 6.7 Hz; 10-H), 5.94 (1H, d, J=15.3 Hz; 11-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3 (C-1), 43.8 (C-2), 75.9 (C-3), 15.8 (2-Me), 80.0 (C-9). **13**: ESI-MS m/z 1360 $(M+H)^+$; $[\alpha]_D^{25}=-63.6°$ (c 0.68, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3436, 2958, 2879, 1743, 1463, 1382, 1251, 1106, 1082; ^1H NMR (500 MHz, $CDCl_3$) δ 1.81 (3H, s; 22-H), 2.42 (6H, s; 3′-NMe_2), 2.46 (1H, m; 2-H), 3.46 (3H, s; 2″-OMe), 3.58 (3H, s; 3″-OMe), 3.82 (1H, br d, J=10.4 Hz; 3-H), 5.46 (1H, d, J=9.8 Hz; 13-H), 5.98 (1H, dd, J=15.9, 5.5 Hz; 10-H), 6.29 (1H, d, J=15.8 Hz; 11-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2 (C-1), 45.3 (C-2), 74.1 (C-3), 13.8 (2-Me), 76.5 (C-9).

2β-Methyldesmycosin (14)

To a solution of **12** (107.3 mg, 0.0789 mmol) in CH_3CN - H_2O 1 : 1 (10 ml), difluoroacetic acid (0.05 ml) was then added and stirred for 72 hours at 45°C. Saturated $NaHCO_3$ aq. was added to the reaction mixture and extracted with EtOAc. The organic solution was dried over anhydrous $MgSO_4$, filtered and evaporated to yield a crude deprotected compound. 9-Hydroxyl group of this compound was oxidized as mentioned above. The resulting crude residue was purified by silica gel pTLC ($CHCl_3$ - MeOH 4 : 1) to yield **14** (25.9 mg, 78.4% yield over two steps). **14**: HRTOF-MS m/z 784.4477 $(M-H)^-$ (calcd for $C_{40}H_{66}NO_{14}$: 784.4483); $[\alpha]_D^{25}=-21.3°$ (c 1.15, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3450, 2975, 2936, 1721, 1595, 1458, 1380, 1317, 1268, 1168, 1083, 1063; ^1H NMR (500 MHz, $CDCl_3$) δ 0.86 (3H, t, J=7.3 Hz; 17-H), 0.95 (3H, d, J=7.0 Hz; 18-H), 1.13 (3H, d, J=6.7 Hz; 21-H), 1.19 (3H, d, J=6.4 Hz; 6″-H), 1.21 (3H, d, J=7.0 Hz; 2-Me), 1.22 (3H, d, J=6.1 Hz; 6′-H), 1.72 (3H, s; 22-H), 2.28 (1H, m; 2-H), 2.51 (6H, s; 3′-NMe_2), 3.41 (3H, s; 2″-OMe), 3.54 (3H, s; 3″-OMe), 3.84 (1H, m; 3-H), 4.22 (1H, d, J=7.6 Hz; 1′-H), 4.48 (1H, d, J=7.6 Hz; 1″-H), 4.88 (1H, td, J=9.8, 2.7 Hz; 15-H), 5.82 (1H, d, J=10.4 Hz; 13-H), 6.15 (1H, d, J=15.8 Hz; 10-H), 7.11 (1H, d, J=15.8 Hz; 11-H), 9.63 (1H, s; 20-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7 (C-1), 43.8 (C-2), 13.7 (2-Me), 73.3 (C-3), 203.9 (C-9), 202.6 (C-20).

2α-Methyldesmycosin (15)

Compound **13** (53.5 mg, 0.0394 mmol) was deprotected in the same method applied to **12**, followed by oxidation and purification by silica gel pTLC ($CHCl_3$ - MeOH 4 : 1), yielding **15** (18.9 mg, 61.0% yield over two steps). **15**: HRTOF-MS m/z 786.4659 $(M+H)^+$ (calcd for $C_{40}H_{68}NO_{14}$: 786.4640); $[\alpha]_D^{25}=+3.8°$ (c 0.43, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3436, 2975, 2932, 1722, 1594, 1457, 1380, 1267, 1168, 1084; ^1H NMR (500 MHz, acetone-d_6) δ 0.94 (3H, t, J=7.3 Hz; 17-H), 0.99 (3H, d, J=7.3 Hz; 2-Me), 1.01 (3H, d, J=6.7 Hz; 18-H), 1.16 (3H, d, J=6.1 Hz; 6″-H), 1.88 (3H, s; 22-H), 2.48 (1H, m; 2-H), 2.48 (6H, s; 3′-NMe_2), 3.45 (3H, s; 2″-OMe), 3.52 (3H, s; 3″-OMe), 5.88 (1H, d, J=10.4 Hz; 13-H), 6.40 (1H, br; 10-H), 7.23 (1H, d, J=15.3 Hz; 11-H), 9.69 (1H, s; 20-H); ^{13}C NMR (125 MHz, acetone-d_6) δ 177.1 (C-1), 43.5 (C-2), 11.6 (2-Me),

203.8 (C-9), 203.5 (C-20).

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