Application of Dibutyltin Oxide Method to Regioselective Acylation and Alkylation of Tylosin at C-4″

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4″-*O*-Acyl-, 4″-*O*-alkyl- and 4″-deoxy-tylosin derivatives were synthesized using 2′-*O*-acetyl-3″,4″-*O*-(dibutylstannio)tylosin as a synthetic intermediate. The *in vitro* biological evaluation showed that the new derivatives were active against macrolide-resistant clinical isolates of bacteria and mycoplasmas, and that they were resistant to hepatic esterase.

Keywords tylosin; *O*-dibutylstannio acetal; regioselective modification; acylation; alkylation; deoxygenation; macrolide-resistant pathogen

Tylosin (**1**), a 16-membered basic macrolide, seems to be interesting as a starting material for derivation of so-called second-generation macrolide antibiotics, as 4″-*O*-acyltylosin derivatives possess significantly improved activity against macrolide-resistant clinical isolates of bacteria and mycoplasmas (*e.g.* erythromycin-resistant staphylococci, erythromycin and oleandomycin-resistant staphylococci, and all macrolides-resistant staphylococci). 4″-*O*-(4-Methoxyphenylacetyl)tylosin (**4**), for example, was found to be useful in the treatment of mice experimentally infected with macrolide-resistant staphylococci[1,2] and is now under clinical evaluation.

For 4″-*O*-acylation of tylosin, a microbiological acylation method[3] and several chemical acylation methods[4,5] are known. Initially, 4″-*O*-acyltylosin derivatives were prepared through 2′-*O*-acetyl-4‴-*O*-chloroacetyltylosin (**6**),[4] but in poor reaction yields. Recently an improved method for 4″-*O*-acylation was described *via* 2′-*O*-acetyl-4″,4‴-di-*O*-acyltylosin,[2] and the yields of most 4″-*O*-acyltylosin derivatives were reported to be satisfactory. Contrary to the authors' expectation, however, only **4** was obtained in a poor yield even by this improved chemical method.

A large amount of **4** is required for animal experiments and clinical evaluations, which motivated the authors to develop a more efficient and practical route for preparation of 4″-*O*-acyltylosin derivatives.

In recent years, regioselective acylation and alkylation of polyol compounds have been investigated using *O*-dibutylstannio acetal or tributylstannyl ether intermediate.[6] In this paper, we describe the preparation of 4″-*O*-acyl-, 4″-*O*-alkyl- and 4″-deoxy-tylosin derivatives using 2′-*O*-acetyl-3″,4″-*O*-(dibutylstannio)tylosin as a synthetic intermediate. The *in vitro* biological evaluation showed that, in addition to 4″-*O*-acyltylosin derivatives, 4″-deoxytylosin and 4″-*O*-alkyltylosin compounds were not only antimicrobially active against macrolide-sensitive and -resistant pathogens but also stable to hepatic esterase.

Results and Discussion

Modification of Tylosin at C-4″ *via* 3″,4″-*O*-Dibutylstannio Acetal Figure 1 illustrates a process for modification of tylosin (**1**) at C-4″ by employing dibutyltin oxide. 2′-*O*-Acetyltylosin (**2**)[4] and dibutyltin oxide are refluxed in toluene, yielding stannio acetal (**3**), while the water is removed azeotropically. As reported previously,[6e] the progress of this reaction cannot be monitored by silica gel thin layer chromatography (TLC), because silica gel reverts the product **3** to the parent compound **2**. Accordingly, the yield of this step was estimated from the yield of the product observed in the subsequent acylation or alkylation reaction.

Benzene was found to be an unsuitable solvent for **3**. Even with toluene, more than 3 mol eq of dibutyltin oxide was necessary for complete reaction. The stannio acetal (**3**) can be stored in powder form at room temperature under a dry atmosphere for at least one month without difficulty, and is used *in situ* or in powder form in the subsequent reaction step.

A toluene solution of **3** without any additional base was 4″-acylated with 1.75 mol eq of acid chloride at 0 °C—room temperature. The resulting reaction mixture contained a small amount of 2′-*O*-acetyl-3,4″-di-*O*-acyltylosin as a by-product, but neither 2′-*O*-acetyl-3″-*O*-acyltylosin nor 2′-*O*-acetyl-4‴-*O*-acyltylosin. 4-Methoxyphenylacetyl chloride and **3** give 4″-*O*-(4-methoxyphenylacetyl)tylosin (**4**) in 52% yield after silica gel column chromatography. Proton nuclear magnetic resonance (^{1}H-NMR) showed that the 4″-proton of **4** resonated at 4.58 ppm which was 1.64 ppm lower than that of tylosin (6.22 ppm).[2] The phenylacetyl derivative (**5**) was similarly obtained in 34% yield.

3″,4″-Stannio acetal (**3**) also provides selective alkylation[6] of the 4″-hydroxyl group of tylosin. The acetal (**3**) and excess alkyl halides in the presence of tetrabutylammonium iodide[6f] or sodium iodide as a catalyst were allowed to react in benzene at ambient or reflux temperature, giving the 4″-*O*-alkyl derivatives (**7**—**11**) (Fig. 2). The 4″-proton signal, which is observed at 2.95 ppm as double doublets in tylosin (**1**), was located at around 2.9 ppm as a doublet in the 4″-*O*-alkyl derivatives.

Furthermore, the stannio acetal (**3**) can be employed in a facile synthesis of 4″-deoxytylosin (**14**) (Fig. 3). In particular, the acetal (**3**) was led to the 4″-*O*-triflate (**12**) on treatment with 2.0 mol eq of trifluoromethanesulfonic anhydride in methylene chloride. The labile triflate (**12**) was immediately treated at 60 °C for 60 min with tetrabutylammonium iodide in acetonitrile. The iodination gave two iodine-containing products in a densitometric ratio of 3 : 1 on a silica gel TLC plate. The major product was determined to be 2′-*O*-acetyl-4″-deoxy-4″-iodotylosin (**13**)

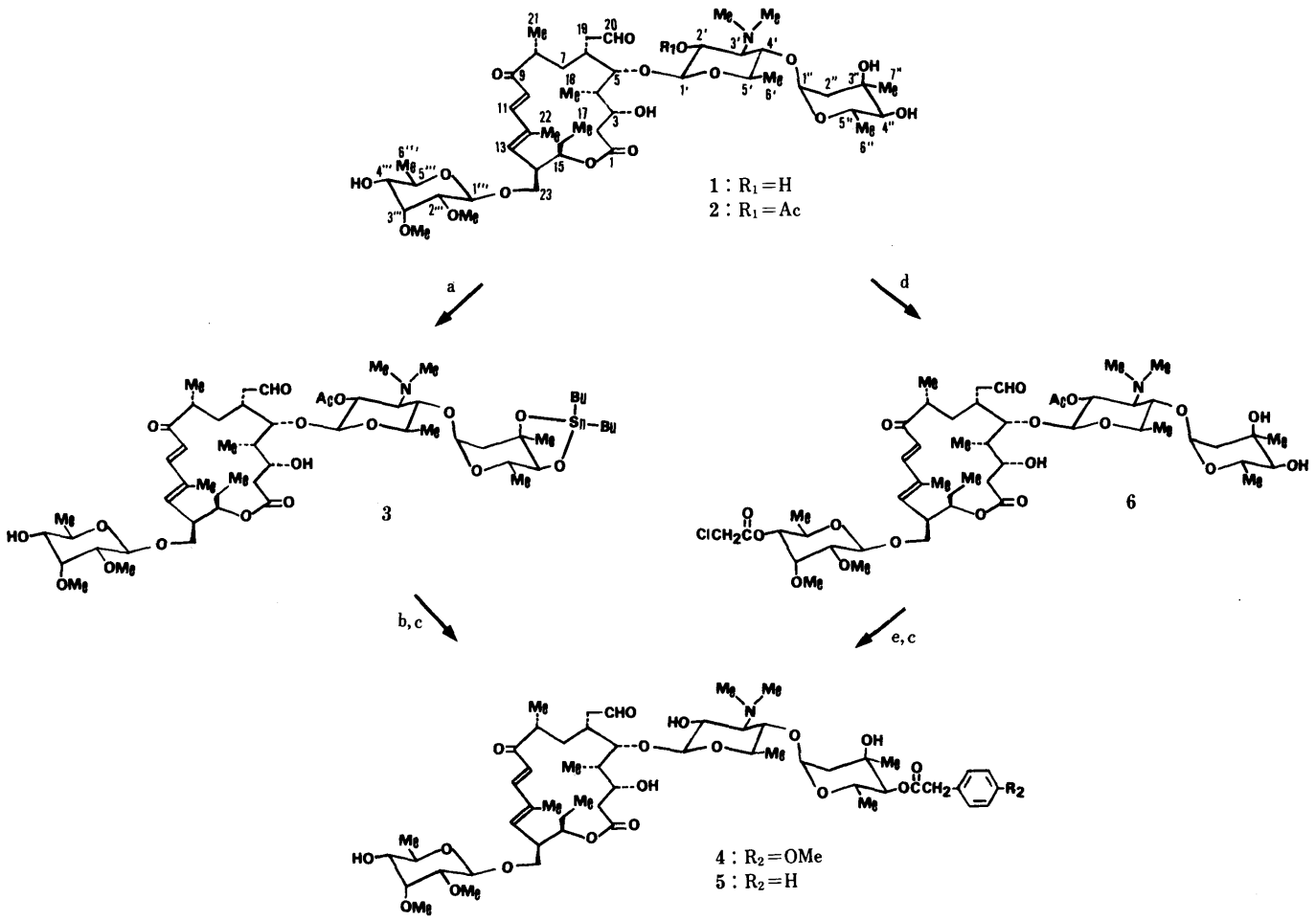

Fig. 1. Synthesis of 4″-*O*-Acyltylosin Derivatives Using 3″,4″-*O*-Dibutylstannio Acetal Intermediate

(a) Bu_2SnO/toluene/reflux; (b) $R_2C_6H_4CH_2COCl$/room temperature; (c) MeOH/reflux; (d) $ClCH_2COCl/NEt_3$/EtOAc/−25 °C; (e) $(R_2C_6H_4CH_2CO)_2O/NEt_3$/DMAP/$CH_2Cl_2$/−25 °C.

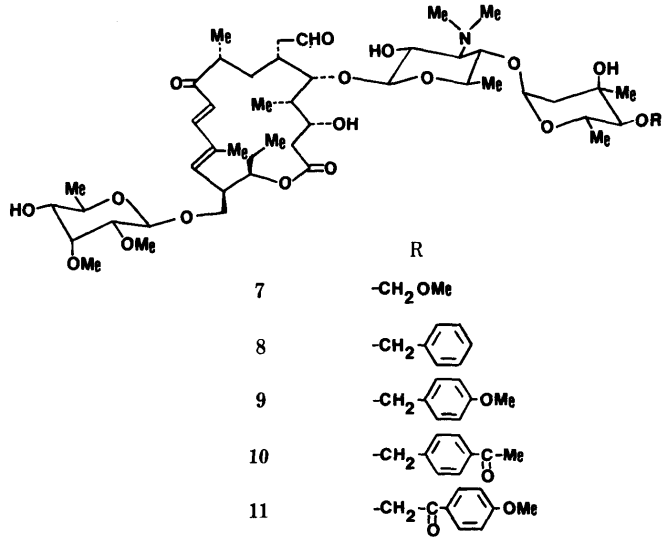

Fig. 2. 4″-*O*-Alkyl Derivatives of Tylosin

on the basis of the NMR spectrum. Since irradiation at δ 1.43 (6″-H) caused the multiplet signal of δ 3.94 (5″-H) to collapse to a doublet ($J_{4'',5''}=10$ Hz), the 4″-iodine atom appeared to have an equatorial configuration. The minor product could not be successfully purified for structure elucidation, and was supposed to be the 4″-epimer of **13**. The yield of iodination was as low as 10%, presumably because the 4″-*O*-triflate **12** was unstable and the C-3″-substituents caused steric hindrance to 4″-iodination. Compound **13** was radically reduced at 75 °C for 2.5 h with 3 mol eq of tributyltin hydride[7] in benzene under a nitrogen atmosphere, and subsequent 2′-*O*-deacetylation in refluxing methanol yielded 4″-deoxytylosin (**14**).

Antimicrobial Activity and Esterase Stability of 4″-*O*-Alkyltylosin Derivatives (7—11) and 4″-Deoxytylosin (14)

As reported in previous papers,[1,2,8,9] most of the 4″-*O*-acyltylosin derivatives hitherto synthesized were found to be antimicrobially active *in vitro* against macrolide-resistant clinical isolates of bacteria and mycoplasmas, but few remained active *in vivo* for a sufficient period of time to express their antimicrobial activities, because hepatic esterase reverted them to tylosin by 4″-deacylation. Among these 4″-*O*-acyltylosin derivatives, 4″-*O*-(4-methoxyphenylacetyl)tylosin (**4**) is the most promising from the viewpoints of antimicrobial activity and stability to esterase. Accordingly, it is interesting to determine the comparative antimicrobial activities and esterase stabilities of these new derivatives **7**—**11** and **14**, 4″-*O*-(4-methoxyphenylacetyl)tylosin (**4**) and 4″-*O*-phenylacetyltylosin (**5**).

Tables I, II and III summarize the antibacterial and antimycoplasmal activities of the new and reference tylosin derivatives, tylosin and josamycin. Generally speaking, the new tylosin derivatives possess good antibacterial activity

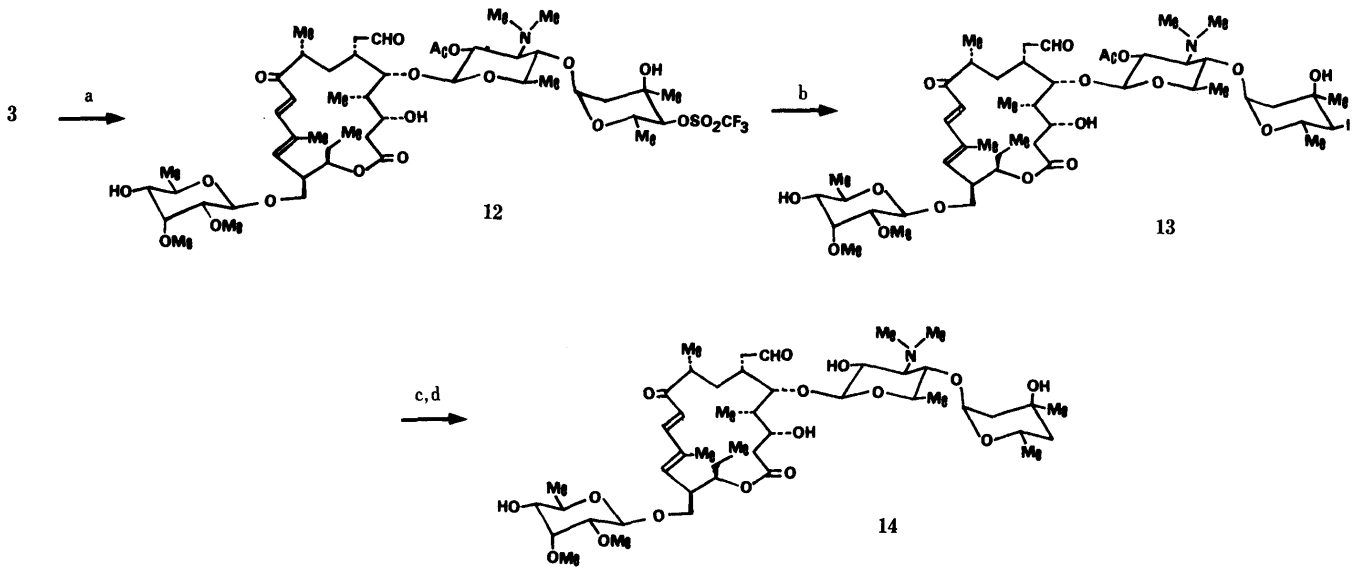

Fig. 3. Synthesis of 4''-Deoxytylosin

(a) $(CF_3SO_2)_2O/CH_2Cl_2/0\,°C$; (b) $Bu_4N^+I^-/CH_3CN/55\,°C$; (c) $Bu_3SnH/AIBN/benzene/N_2$ atmosphere/75 °C; (d) MeOH/reflux.

TABLE I. Antibacterial Activity of 4''-*O*-Alkyltylosin Derivatives and 4''-Deoxytylosin against Gram-Positive Bacteria (MIC in μg/ml)

Bacterium	Reference compounds		4''-*O*-Alkyltylosin derivatives					4''-Deoxytylosin	Controls	
	4	**5**	**7**	**8**	**9**	**10**	**11**	**14**	Tylosin	Josamycin
Aerococcus catalasicus NCIB 9642	0.10	<0.05	0.10	0.10	0.10	0.20	0.20	3.13	<0.05	0.10
Arthrobacter viscosus ATCC 15294	0.20	0.20	0.78	0.39	0.39	0.78	0.39	0.20	0.39	0.39
Bacillus circulans ATCC 9966	<0.05	<0.05	0.20	0.10	0.10	0.10	0.10	0.10	0.10	<0.05
Bacillus licheniformis ATCC 25972	0.20	0.20	0.20	0.20	0.39	0.39	0.10	0.20	0.20	0.39
Bacillus subtilis ATCC 6633	0.20	0.20	0.20	0.20	0.39	0.20	0.20	0.10	0.10	0.20
Corynebacterium equi IAM 1038	0.20	0.20	0.78	0.39	0.78	0.78	0.39	25	0.78	0.39
Micrococcus luteus ATCC 9341	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Staphylococcus aureus FDA209P	0.10	0.39	0.20	0.20	0.39	0.39	0.39	0.39	0.20	0.10
Staphylococcus aureus SMITH	0.10	0.39	0.39	0.39	0.39	0.39	0.39	0.78	0.39	0.20
Staphylococcus aureus RUSSELL	0.39	0.39	0.39	0.39	0.39	0.78	0.39	0.39	0.39	0.20
Staphylococcus epidermidis ATCC 12228	0.39	0.78	0.39	0.39	0.39	0.78	0.39	0.78	0.20	0.20

TABLE II. Antimycoplasmal Activity of 4''-*O*-Alkyltylosin Derivatives and 4''-Deoxytylosin (MIC in μg/ml)

Mycoplasma	Reference compounds		4''-*O*-Alkyltylosin derivatives					4''-Deoxytylosin	Controls	
	4	**5**	**7**	**8**	**9**	**10**	**11**	**14**	Tylosin	Josamycin
Macrolide-sensitive strains										
Mycoplasma gallisepticum S-6	0.0004	0.012	0.012	0.003	0.006	0.006	0.012	0.003	0.024	0.024
Mycoplasma gallisepticum KP-13	0.0008	<0.0002	0.0016	<0.0002	<0.0002	0.0016	0.0008	0.003	0.006	0.006
Mycoplasma gallisepticum TS-18	0.0004	0.0016	0.0004	0.0004	0.0008	0.0004	0.0016	0.003	0.012	0.012
Mycoplasma pneumoniae MAC	0.012	0.003	0.006	0.006	0.006	0.006	0.003	0.012	0.006	0.012
Macrolide-resistant strains										
Mycoplasma gallisepticum S4A	0.0004	0.012	0.20	0.024	0.006	0.006	0.39	3.13	0.39	6.25
Mycoplasma gallisepticum E7	0.012	0.0008	0.39	0.012	0.003	0.024	0.39	6.25	0.39	6.25
Mycoplasma gallisepticum E11	0.006	0.0016	0.39	0.05	0.012	0.006	0.39	6.25	0.20	3.13
Mycoplasma gallisepticum E40	0.024	0.012	0.39	0.0008	0.012	0.05	0.39	6.25	0.39	6.25
Mycoplasma gallisepticum E57	<0.0002	0.0008	0.012	0.0016	0.0008	0.0008	0.05	0.024	0.024	0.024
Mycoplasma gallisepticum E103	0.012	0.0008	0.39	0.024	0.003	0.024	0.39	6.25	0.39	6.25
Mycoplasma gallisepticum E112	0.006	0.003	0.39	0.05	0.012	0.006	0.39	6.25	0.39	3.13
Mycoplasma gallisepticum A69	0.024	0.012	0.39	0.0008	0.012	0.05	0.39	6.25	0.39	6.25
Mycoplasma gallisepticum A72	0.012	0.0008	0.39	0.024	0.006	0.024	0.39	6.25	0.39	6.25
Mycoplasma pulmonis PG-22	0.05	0.05	0.20	0.20	0.05	0.05	0.78	6.25	0.78	12.5

comparable to those of the reference compounds **4** and **5**. In particular, it is noteworthy that the 4''-*O*-alkyltylosin derivatives (**8**—**10**) exhibit antimycoplasmal activity as high as those of the reference compounds **4** and **5** against macrolide-sensitive and -resistant strains, whereas the 4''-*O*-alkyltylosin derivatives (**7** and **11**) show the same level of antimycoplasmal activity as tylosin against macrolide-resistant strains, indicating that no improvement is attainable by 4''-*O*-methoxymethylation or 4''-*O*-phenacylation (Table II). Unexpectedly, 4''-deoxytylosin (**14**), although

TABLE III. Cumulative MIC's (μg/ml) of 4''-*O*-Alkyltylosin Derivatives and 4''-Deoxytylosin against Macrolide-Resistant Staphylococci (15 Clinical Isolates)

Macrolide	Cumulative percentage (%) of strains susceptible at a concentration of								
	0.78	1.56	3.13	6.25	12.5	25	50	100	(μg/ml)
Reference compounds **4**	7	47	73	100	100	100	100	100	
5	0	0	20	67	67	100	100	100	
Derivatives **7**	0	0	0	13	13	13	13	20	
8	0	0	7	7	7	7	20	47	
9	0	0	0	0	7	7	33	60	
10	0	0	0	7	7	20	67	73	
11	0	7	13	20	20	20	80	100	
14	0	0	0	13	13	13	27	47	
Tylosin	0	0	0	13	13	13	13	13	
Josamycin	0	0	0	0	0	7	7	7	

TABLE IV. *In Vitro* Stability of 4''-*O*-Alkyltylosin Derivatives and 4''-Deoxytylosin to Mouse Liver Homogenate

Macrolide	Percent (%) unchanged macrolide remaining after an incubation period of				
	0	30	60	120	(min)
Reference compounds **4**	99	97	95	91	
5	100	92	85	68	
Derivatives **7**	100	100	100	98	
8	100	100	100	99	
9	100	99	99	99	
10	99	99	98	97	
11	100	97	96	96	
14	100	100	100	100	

active against macrolide-sensitive strains (Tables I and II), is inferior to tylosin in antimycoplasmal activity against macrolide-resistant strains (Table II). Table III lists the cumulative minimum inhibitory concentrations (MIC's) of the reference and new tylosin derivatives, tylosin and josamycin against recent macrolide-resistant clinical isolates of staphylococci. It is apparent that reference compound **4** is the best, followed by the 4''-*O*-alkyltylosin derivative (**11**). Derivative **11** seems able to differentiate the microbial and mycoplasmal resistance mechanisms to macrolide antibiotics.

The *in vitro* esterase stability of the new tylosin derivatives is summarized in Table IV, indicating that 4''-*O*-alkylation (derivatives **7**—**11**) and 4''-deoxygenation (derivative **14**) are more effective than 4''-*O*-acylation (reference compounds **4** and **5**) in conferring esterase resistance.

In conclusion, 4''-*O*-alkyltylosin derivatives (**7**—**11**) and 4''-deoxytylosin (**14**), although more stable to hepatic esterase, are slightly inferior in antimicrobial activity to 4''-*O*-(4-methoxyphenylacetyl)tylosin (reference compound **4**) which is now considered to be the most promising tylosin derivative for potential clinical application.

Experimental

General Methods Ultraviolet (UV) absorption spectra, optical rotations and mass spectra (MS) were measured with a Hitachi 200-20 UV/visible spectrophotometer, a JASCO DIP-181 digital polarimeter and a Hitachi RMU-7M MS spectrometer, respectively. ^{1}H-NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer using tetramethylsilane as an internal standard. Wakogel C-200 (Wako Pure Chemical Industries, Ltd.) was used for silica gel column chromatography. Silica gel TLC was run on pre-coated Silica gel 60 F plates (E. Merck, Darmstadt) and the amounts of macrolide compounds were read by UV densitometry with a Shimadzu CS-920 chromato-scanner.

2'-*O*-Acetyl-3'',4''-*O*-(dibutylstannio)tylosin (3) A suspension of 1 g of **2** and 1.3 g of dibutyltin oxide in 50 ml of toluene was heated under reflux for 3 h with azeotropic removal of water. The resulting clear solution was evaporated to dryness *in vacuo*, yielding 2.3 g of the stannylated mixture. This preparation was used in subsequent reactions without further purification. For preparation of 4''-*O*-acyltylosin derivatives, the stannylated mixture was dissolved in toluene.

4''-*O*-(4-Methoxyphenylacetyl)tylosin (4) 4-Methoxyphenylacetyl chloride (0.355 g) in 20 ml of toluene was added dropwise to 2.3 g of the stannylated mixture in 10 ml of toluene and the mixture was stirred for 1 h at room temperature. The solution was poured into 80 g of ice-water and then the toluene layer was recovered and washed with a saturated sodium bicarbonate solution and brine. The toluene layer was dried over anhydrous sodium sulfate, and evaporated. The residue was subjected to silica gel column chromatography (silica gel 50 g; toluene–acetone = 4 : 1) to give 0.91 g of 2'-*O*-acetyl-4''-*O*-(4-methoxyphenylacetyl)tylosin. The 2'-*O*-acetyl derivative was dissolved in 30 ml of methanol and heated under reflux for 16 h to remove the 2'-*O*-acetyl group. Evaporation of the methanol left a residue, which was purified by silica gel column chromatography (silica gel 35 g; toluene–acetone = 3 : 1) to provide 0.60 g of **4**. *Rf* 0.25 (silica gel TLC; benzene : acetone = 2 : 1). $[\alpha]_D^{24}$ −43.6° (c = 1.0, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (24200), 226 (11300). ^{1}H-NMR ($CDCl_3$) δ: 1.80 (3H, s, 22-H), 2.49 (6H, s, NMe_2), 3.49 (3H, s, 2'''-OMe), 3.62 (3H, s, 3'''-OMe), 3.65 (2H, s, 4''-$OCOCH_2$–), 3.79 (3H, s, phenyl-O-Me), 4.58 (1H, d, $J_{4'',5''}$ = 10 Hz, H-4''), 6.27 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 6.85 (2H, d, J = 9 Hz, α-phenyl), 7.24 (2H, d, J = 9 Hz, β-phenyl), 7.32 (1H, d, J = 15.5 Hz, H-11), 9.68 (1H, s, H-20). SI-MS m/z: 1064 $(M+H)^+$.

4''-*O*-Phenylacetyltylosin (5) Phenylacetyl chloride (0.69 g) was added dropwise to an ice-cold solution of 5.7 g of the stannylated mixture in 60 ml of toluene, and the mixture was stirred at 0 °C for 1 h. The resulting solution was treated in a manner similar to that used for the preparation of **4**. Silica gel column chromatography (silica gel 150 g; toluene–acetone = 3 : 1) gave 0.9 g of **5**. *Rf* 0.27 (silica gel TLC; benzene : acetone = 2 : 1). $[\alpha]_D^{24}$ −43.8° (c = 1.76, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (21300). ^{1}H-NMR ($CDCl_3$) δ: 1.79 (3H, s, H-22), 2.49 (6H, s, NMe_2), 3.50 (3H, s, 2'''-OMe), 3.62 (3H, s, 3'''-OMe), 3.70 (2H, s, 4''-$OCOCH_2$–), 4.21 (1H, d, $J_{1',2'}$ = 7.5 Hz, H-1'), 4.51 (1H, d, $J_{4''',5'''}$ = 10 Hz, H-4'''), 4.53 (1H, d, $J_{1''',2'''}$ = 8 Hz, H-1'''), 5.00 (1H, d, $J_{1''',2'''}$ = 3.5 Hz, H-1'''), 5.87 (1H, d, $J_{13,14}$ = 10.5 Hz, H-13), 6.22 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 7.32 (1H, d, J = 15.5 Hz, H-11), 7.33 (5H, s, phenyl), 9.63 (1H, s, H-20). SI-MS m/z: 1034 $(M+H)^+$.

4''-*O*-Methoxymethyltylosin (7) A mixture of 1.5 g of the stannylated mixture, 0.25 g of tetrabutylammonium iodide and 0.17 g of chloromethyl methyl ether in 20 ml of dry benzene was stirred at room temperature for 15 h. The reaction mixture was diluted with 20 ml of toluene and washed successively with water, a saturated sodium bicarbonate solution and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was dissolved in a small volume of chloroform and dropped into *n*-hexane to yield 0.31 g of a crude precipitate of 2'-*O*-acetyl-4''-*O*-methoxymethyltylosin. The crude preparation

(0.30 g) was dissolved in 30 ml of methanol and subjected to removal of the 2′-*O*-acetyl group under reflux for 11 h. The methanol was removed by evaporation and the solid residue was purified by silica gel column chromatography (silica gel 25 g; toluene–acetone = 5 : 2) to give 0.18 g of **7**. *Rf* 0.45 (silica gel TLC; $CHCl_3$: MeOH : 25% ammonia = 15 : 1 : 0.1). $[\alpha]_D^{24}$ −51.1° (*c* = 0.93, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (22100). ^{1}H-NMR ($CDCl_3$) δ: 2.57 (6H, s, NMe_2), 3.41 (3H, s, 4″-OCH_2OCH_3), 3.53 (3H, s, 2‴-OMe), 3.66 (3H, s, 3‴-OMe), 4.63 and 4.93 (2H, *J* = 7 Hz, 4″-O–CH_2–), 5.94 (1H, d, $J_{13,14}$ = 10.5 Hz, H-13), 6.30 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 7.37 (1H, d, *J* = 15.5 Hz, H-11), 9.76 (1H, s, H-20). SI-MS *m/z*: 960 $(M+H)^+$.

4″-*O*-Benzyltylosin (8) A mixture of 1.5 g of the stannylated mixture, 0.25 g of tetrabutylammonium iodide and 0.35 g of benzyl bromide in 20 ml of dry benzene was heated under reflux for 15 h and then treated as described above for **7**. Silica gel column chromatography (silica gel 50 g; toluene–acetone = 7 : 2) afforded 0.2 g of **8**. *Rf* 0.16 (benzene : acetone = 2 : 1). $[\alpha]_D^{24}$ −41.3° (*c* = 1.25, MeOH). UV λ_{max}^{MeOH} nm (ε): 285 (20800). ^{1}H-NMR ($CDCl_3$) δ: 1.80 (3H, s, H-22), 2.53 (6H, s, NMe_2), 2.94 (1H, d, $J_{4'',5''}$ = 10 Hz, H-4″), 3.51 (3H, s, 2‴-OMe), 3.63 (3H, s, 3‴-OMe), 4.23 (1H, d, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.57 (1H, d, $J_{1''',2'''}$ = 7.5 Hz, H-1‴), 4.69 (2H, s, 4″-O–CH_2), 5.01 (1H, d, $J_{1'',2''}$ = 3.5 Hz, H-1″), 5.92 (1H, d, $J_{13,14}$ = 10.5 Hz, H-13), 6.28 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 7.25—7.41 (6H, m, H-11 and phenyl), 9.72 (1H, s, H-20). SI-MS *m/z*: 1006 $(M+H)^+$.

4″-*O*-(4-Methoxybenzyl)tylosin (9) The stannylated mixture (1.5 g), tetrabutylammonium iodide (0.25 g) and 4-methoxybenzyl chloride (0.53 g) were suspended in 20 ml of dry benzene, and treated as described for **7** (reflux for 23 h). Silica gel column chromatography (silica gel 50 g; toluene–acetone = 2 : 1) of the product afforded 0.35 g of **9**. *Rf* 0.22 (silica gel TLC; benzene : acetone = 2 : 1). $[\alpha]_D^{24}$ −32.7° (*c* = 1.25, MeOH). UV λ_{max}^{MeOH} nm (ε): 282 (22800), 227 (14300). ^{1}H-NMR ($CDCl_3$) δ: 2.45 and 2.52 (3H × 2, s × 2, NMe_2), 2.91 (1H, d, $J_{4'',5''}$ = 10 Hz, H-4″), 3.51 (3H, s, 2‴-OMe), 3.63 (3H, s, 3‴-OMe), 3.82 (3H, s, phenyl-OMe), 6.86 (2H, d, *J* = 8.5 Hz, phenyl), 7.28 (2H, d, *J* = 8.5 Hz, phenyl), 9.71 (1H, s, H-20). SI-MS *m/z*: 1036 $(M+H)^+$.

4″-*O*-(4-Acetylbenzyl)tylosin (10) A mixture of 1.5 g of the stannylated mixture, 0.25 g of tetrabutylammonium iodide and 3 g of 4-acetylbenzyl bromide in 20 ml of dry benzene was heated under reflux for 16 h and then diluted with 20 ml of toluene. After being washed successively with water, a saturated sodium bicarbonate solution and brine, followed by drying over anhydrous sodium sulfate, the organic layer was concentrated to dryness. The residue was chromatographed on silica gel (40 g) with a 4 : 1 mixture of toluene and acetone to yield 0.12 g of 2′-*O*-acetyl-4″-*O*-(4-acetylbenzyl)tylosin. The product was dissolved in 15 ml of methanol and 2′-deprotected by heating under reflux for 16 h. After removal of the methanol, the residue was subjected to silica gel column chromatography (silica gel 15 g; toluene : acetone = 3 : 1) to afford 20 mg of **10**. *Rf* 0.16 (silica gel TLC; benzene–acetone = 2 : 1). $[\alpha]_D^{24}$ −41.5° (*c* = 0.53, MeOH). UV λ_{max}^{MeOH} nm (ε): 282 (19900), 255 (17000). ^{1}H-NMR ($CDCl_3$) δ: 1.81 (3H, s, H-22), 2.54 (6H, s, NMe_2), 2.61 (3H, s, COMe), 2.95 (1H, d, $J_{4'',5''}$ = 10 Hz, H-4″), 3.51 (3H, s, 2‴-OMe), 3.64 (3H, s, 3‴-OMe), 5.92 (1H, d, $J_{13,14}$ = 10.5 Hz, H-13), 6.28 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 7.49 (2H, d, *J* = 8.5 Hz, phenyl), 7.96 (2H, d, *J* = 8.5 Hz, phenyl), 9.74 (1H, s, H-20). SI-MS *m/z*: 1048 $(M+H)^+$.

4″-*O*-(4-Methoxyphenacyl)tylosin (11) A mixture of 1.5 g of the stannylated mixture, 1.5 g of sodium iodide and 0.78 g of (4-methoxy)phenacyl bromide was heated in 20 ml of dry benzene under reflux for 17 h, and worked up as described for **10** to yield 2′-*O*-acetyl-4″-*O*-(4-methoxyphenacyl)tylosin, and the derivative **11**. They were purified by silica gel column chromatography eluting with 4 : 1 and 7 : 2 mixtures of toluene–acetone, respectively. The yield of **11** was 37 mg. *Rf* 0.14 (silica gel TLC; benzene : acetone = 2 : 1). $[\alpha]_D^{24}$ −37.5° (*c* = 0.92, MeOH). UV λ_{max}^{MeOH} nm (ε): 284 (34000), 221 (12300). ^{1}H-NMR ($CDCl_3$) δ: 2.57 (6H, s, NMe_2), 3.51 (3H, s, 2‴-OMe), 3.63 (3H, s, 3‴-OMe), 3.90 (3H, s, phenyl-OMe), 5.91 (1H, d, $J_{13,14}$ = 10.5 Hz, H-13), 6.28 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 6.94 (2H, d, *J* = 8.5 Hz, phenyl), 7.35 (1H, d, *J* = 15.5 Hz, H-11), 8.07 (2H, d, *J* = 8.5 Hz, phenyl), 9.72 (1H, s, H-20). SI-MS *m/z*: 1064 $(M+H)^+$.

2′-*O*-Acetyl-4″-*O*-trifluoromethanesulfonyltylosin (12) Trifluoromethanesulfonic anhydride (0.5 g) in 5 ml of dry methylene chloride was added dropwise into an ice-cold solution of 1.5 g of the stannylated mixture in 7 ml of dry methylene chloride, and the mixture was stirred for 1 h at 0 °C. After the excess of trifluoromethanesulfonic anhydride was decomposed with ice-water, the solution was extracted with 50 ml of toluene. The organic extract was washed with a saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and then filtered. Evaporation of the toluene yielded 0.85 g of a crude preparation of **12**. As **12** was unstable, the crude preparation was immediately used for subsequent reactions without further purification. *Rf* 0.40 (silica gel TLC; benzene : acetone = 2 : 1). ^{1}H-NMR ($CDCl_3$) δ: 1.81 (3H, s, H-22), 2.07 (3H, s, 2′-OCOMe), 2.42 (6H, s, NMe_2). 3.52 (3H, s, 2‴-OMe), 3.65 (3H, s, 3‴-OMe), 4.42 (1H, d, $J_{4'',5''}$ = 10.5 Hz, H-4″), 4.57 (1H, d, $J_{1''',2'''}$ = 7.5 Hz, H-1‴), 5.92 (1H, d, $J_{13,14}$ = 10.5 Hz, H-13), 6.29 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 7.34 (1H, d, *J* = 15.5 Hz, H-11), 9.71 (1H, s, H-20).

2′-*O*-Acetyl-4″-deoxy-4″-iodotylosin (13) Tetrabutylammonium iodide (0.27 g) and 0.4 g of **12** in 10 ml of acetonitrile were heated at 55 °C for 1 h, then diluted with 30 ml of toluene. The solution was washed with a saturated sodium bicarbonate solution and brine, and dried over anhydrous sodium sulfate. The organic layer was concentrated to dryness and the residue was purified by silica gel column chromatography (silica gel 40 g; toluene : acetone = 5 : 1) to yield 40 mg of **13**. *Rf* 0.43 (silica gel TLC; benzene–acetone = 2 : 1). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, d, $J_{5'',6''}$ = 5 Hz, H-6″), 1.80 (3H, s, H-22), 2.06 (3H, s, 2′-OCOMe), 2.43 (6H, s, NMe_2), 3.52 (3H, s, 2‴-OMe), 3.65 (3H, s, 3‴-OMe), 3.87 (1H, d, $J_{4'',5''}$ = 10 Hz, H-4″), 3.94 (1H, m, H-5″), 5.88 (1H, d, $J_{13,14}$ = 10.5 Hz, H-13), 6.27 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 7.32 (1H, d, *J* = 15.5 Hz, H-11), 9.71 (1H, s, H-20).

4″-Deoxytylosin (14) A mixture of 0.12 g of **13**, 0.11 g of tributyltin hydride and a catalytic amount of α,α′-azobisisobutyronitrile (AIBN) in 7 ml of dry benzene was heated at 75 °C for 2.5 h under a nitrogen atmosphere, and then diluted with 30 ml of toluene. After being washed with water and brine, the organic layer was dried over anhydrous sodium sulfate and filtered. Removal of the solvent provided a residue, which was subjected to Sephadex LH-20 gel (150 ml) filtration using methanol as the eluent to give 0.1 g of 2′-*O*-acetyl-4″-*O*-deoxytylosin. This was dissolved in 20 ml of methanol and heated under reflux for 11 h. Silica gel column chromatography (silica gel 15 g; chloroform–methanol = 30 : 1) of the product yielded 45 mg of **14**. *Rf* 0.28 (chloroform–methanol–25% ammonia = 15 : 1 : 0.1). $[\alpha]_D^{24}$ +0.1° (*c* = 1.18, MeOH). UV λ_{max}^{MeOH} nm (ε): 283 (20200). ^{1}H-NMR ($CDCl_3$) δ: 1.79 (3H, s, H-22), 2.49 (6H, s, NMe_2), 3.47 (3H, s, 2‴-OMe), 3.60 (3H, s, 3‴-OMe), 4.22 (1H, d, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.55 (1H, d, $J_{1''',2'''}$ = 7.5 Hz, H-1‴), 4.84 (1H, dd, $J_{1'',2''a}$ = 9 Hz, $J_{1'',2''b}$ = 2.5 Hz, H-1″), 5.89 (1H, d, $J_{13,14}$ = 10.5 Hz, H-13), 6.25 (1H, d, $J_{10,11}$ = 15.5 Hz, H-10), 7.33 (1H, d, *J* = 15.5 Hz, H-11), 9.74 (1H, s, H-20). SI-MS *m/z*: 900 $(M+H)^+$.

Antimicrobial Activity Antibacterial and antimycoplasmal activities of macrolide derivatives were evaluated as previously described.[8]

Esterase Stability Freshly prepared mouse liver homogenate was employed as hepatic esterase. The amounts of unchanged and 4″-*O*-deacylated tylosin derivatives were measured by UV-chromato-scanning.[2,9]

References

1) T. Takeuchi, T. Sawa, H. Naganawa, M. Hamada, H. Umezawa, T. Yoshioka, K. Kiyoshima, H. Iguchi, M. Sakamoto, H. Tone, Y. Fukagawa and T. Ishikura, *J. Antibiot.*, **40**, 1358 (1987).
2) T. Yoshioka, K. Kiyoshima, M. Maeda, M. Sakamoto, T. Ishikura, Y. Fukagawa, T. Sawa, M. Hamada, H. Naganawa and T. Takeuchi, *J. Antibiot.*, **41**, 1617 (1988).
3) R. Okamoto, H. Nomura, M. Tsuchiya, H. Tsunekawa, T. Fukumoto, T. Inui, T. Sawa, T. Takeuchi and H. Umezawa, *J. Antibiot.*, **32**, 542 (1979).
4) M. Tsuchiya, M. Hamada, T. Takeuchi, H. Umezawa, K. Yamamoto, H. Tanaka, K. Kiyoshima, S. Mori and R. Okamoto, *J. Antibiot.*, **35**, 661 (1982).
5) H. A. Kirst, M. Debono, K. E. Willard, B. A. Truedell, J. E. Toth, J. R. Turner, D. R. Berry, B. B. Briggs, D. S. Fukuda, V. M. Daupert, A. M. Felty-Duckworth, J. L. Ott and F. T. Counter, *J. Antibiot.*, **39**, 1724 (1986).
6) *a*) S. David and S. Hanessian, *Tetrahedron*, **41**, 643 (1985); *b*) Y. Tsuda, M. E. Haque and K. Yoshimoto, *Chem. Pharm. Bull.*, **31**, 1612 (1983); *c*) M. E. Haque, T. Kikuchi, K. Yoshimoto and Y. Tsuda, *ibid.*, **33**, 2243 (1985); *d*) M. E. Haque, T. Kikuchi, K. Kanemitsu and Y. Tsuda, *ibid.*, **35**, 1016 (1987); *e*) M. A. Nashed and L. Anderson, *Tetrahedron Lett.*, **1976**, 3503; *f*) S. David, A. Thieffry and A. Veyrieres, *J. Chem. Soc., Perkin Trans. 1*, **1981**, 1796.
7) W. Hartwig, *Tetrahedron*, **39**, 2609 (1983).
8) R. Okamoto, M. Tsuchiya, H. Nomura, H. Iguchi, K. Kiyoshima, S. Hori, T. Inui, T. Sawa, T. Takeuchi and H. Umezawa, *J. Antibiot.*, **33**, 1309 (1980).
9) M. Tsuchiya, Studies on the mechanism of macrolide resistance and tylosin derivatives active against macrolide-resistant pathogens (in Japanese), Ph. D. Thesis, Univ. Osaka Prefecture, 1982.