STUDIES OF TYLOSIN DERIVATIVES EFFECTIVE AGAINST MACROLIDE-RESISTANT STRAINS: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS

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The 4″-*O*-substituted tylosin derivatives were prepared by selective esterification of the 4″-OH, and relationships between the substituent groups and antimicrobial activity against macrolide-resistant strains were examined. Introduction of branched-chain aliphatic acyl groups such as 2-methoxyisovaleryl or 4-methylvaleryl group afforded derivatives with good antibacterial activity; MIC values were 12.5 μg/ml against *Staphylococcus aureus* MS-8710. MIC values of tylosin, erythromycin and josamycin against this strain were 800 μg/ml or more. Further improved activity was obtained by introduction of aromatic groups such as phenylthioacetyl, phenylsulfonylacetyl, 4-nitrophenylacetyl, 4-nitrophenylsulfonyl and phenylethanesulfonyl groups; MIC values were 6.25 μg/ml. These derivatives had also an improved antimycoplasmal activity; MIC values were 0.08 μg/ml against macrolide-resistant strains of *Mycoplasma gallisepticum*. MIC values of tylosin against these strains were from 2.5 to 10 μg/ml. Introduction of the groups described above into the 4″-OH was confirmed to increase the uptake by a resistant strain.

In our previous paper[1], the relative significance of the 3-, 2′-, 4″- and 4‴-*O*-acyl groups of tylosin on antibacterial activity against *Staphylococcus aureus* was reported. Acylation of the 4″-hydroxyl group of tylosin resulted in an improved antibacterial activity against macrolide-resistant strains, while acylation of the 2′- and/or 4‴-hydroxyl group(s) of tylosin produced a negative effect. The antibacterial activity of tylosin was not or little affected by the C-3 acylation. Moreover, acylation of the 4″-hydroxyl group also provided derivatives which produced a higher blood level in mice and rabbits than tylosin and a good therapeutic effect against infection after oral administration[1]. From the study of the action of 3-*O*-acetyl-4″-*O*-isovaleryltylosin against a macrolide-resistant strain of *S. aureus*, it became clear that the introduction of an isovaleryl group onto the 4″-hydroxyl group enhances both penetration into the bacterial cells and the binding ability to ribosomes[2,3].

The mycinose moiety attached to the 23-OH of aglycone was also shown to have an important role in the antibacterial activity against macrolide-resistant strains[3]. Therefore, we synthesized a large number of tylosin derivatives by introducing a variety of groups into the C-4″ hydroxyl group, with the aim to obtain derivatives having improved therapeutic activity against infection of macrolide-resistant isolates.

In this paper, we describe the preparation of new tylosin derivatives, and relationships between the structures of the 4″-*O*-substituent groups and their antibacterial effects.

Chemical Synthesis of New Tylosin Derivatives (Table 1)

Table 1. Tylosin derivatives synthesized, methods for synthesis, physicochemical properties and their antibacterial activity against a tylosin-resistant strain MS-8710.

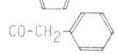
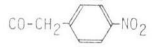
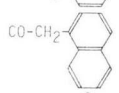
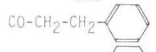
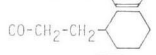
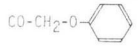
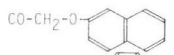
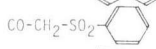
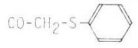
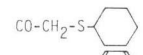
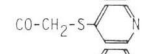
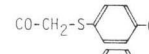
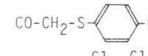
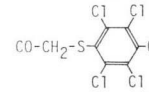
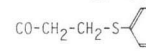
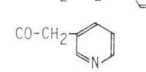
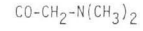
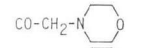
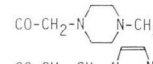
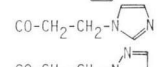
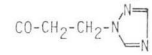
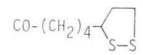
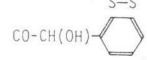
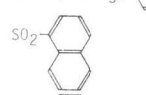
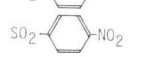
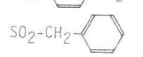
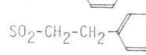
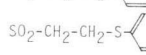
Compound		Structure of substituent group at 4″-*O*-	Method for synthesis*1	Melting point (°C)	$[\alpha]_D^{22}$*2	MIC (μg/ml) MS-8710
No.	Name					
1	Tylosin (parent compound)	H				800
2	2′-*O*-Acetyltylosin	H	b	123～127	−74.8	>200
3	2′-*O*-Acetyl-4‴-*O*-monochloroacetyltylosin	H	b	127～132	−25.6	>200
4	2′-*O*-Acetyl-4‴-*O*-trichloroacetyltylosin	H	b	129～132	−46.4	>200
5	3,2′-Di-*O*-acetyltylosin	H	a	117～119	−41.4	>200
6	3,2′-Di-*O*-acetyl-4‴-*O*-monochloroacetyltylosin	H	b	111～113	−35.8	>200
7	3,2′-Di-*O*-acetyl-4‴-*O*-trichloroacetyltylosin	H	b	116～120	−20.0	>200
8	4″-*O*-Acetyltylosin	$CO-CH_3$	b	124～127	−54.2	100
9	4″-*O*-Butyryltylosin	$CO-CH_2-CH_2-CH_3$	m	147～151	−53.4	25
10	4″-*O*-Isovaleryltylosin	$CO-CH_2-CH(CH_3)_2$	m	154～156	−50.8	25
11	4″-*O*-(2-Methoxy)isovaleryltylosin	$CO-CH(OCH_3)-CH(CH_3)_2$	b	130～135	−45.7	12.5
12	4″-*O*-(4-Methyl)valeryltylosin	$CO-CH_2-CH_2-CH(CH_3)_2$	a	209～214	−50.7	12.5
13	4″-*O*-(2-Methyl)valeryltylosin	$CO-CH(CH_3)-CH_2-CH_2-CH_3$	b	100～104	−31.2	25
14	4″-*O*-Hexanoyltylosin	$CO-(CH_2)_4-CH_3$	a	105～109	−59.0	25
15	4″-*O*-Octanoyltylosin	$CO-(CH_2)_6-CH_3$	a	90～94	−48.8	25
16	4″-*O*-Decanoyltylosin	$CO-(CH_2)_8-CH_3$	a	85～90	−68.6	>200
17	4″-*O*-Benzoyltylosin		b	129～134	−32.6	100
18	4″-*O*-(2-Furoyl)tylosin		b	129～133	−47.8	100
19	4″-*O*-Phenylacetyltylosin		b	104～109	−56.1	50
20	4″-*O*-(4-Nitrophenylacetyl)tylosin		b	115～126	−46.1	6.25
21	4″-*O*-(α-Naphthyl)acetyltylosin		b	118～121	−49.6	12.5
22	4″-*O*-(2-Thienyl)acetyltylosin		b	115～118	−32.4	12.5
23	4″-*O*-(3-Phenylpropionyl)tylosin		b	110～112	−58.6	12.5
24	4″-*O*-(3-Cyclohexylpropionyl)-tylosin		a	102～106.5	−46.8	25
25	4″-*O*-(Phenoxy)acetyltylosin		b	115～117	−50.6	25
26	4″-*O*-(β-Naphthoxy)acetyltylosin		b	122～127	−43.0	25
27	4″-*O*-(Phenylsulfonyl)acetyltylosin		b	110～116	−41.0	6.25
28	4″-*O*-(Phenylthio)acetyltylosin		b	105～107	−49.8	6.25

Table 1. (continued)

Compound No.	Name	Structure of substituent group at 4''-*O*-	Method for synthesis*1	Melting point (°C)	$[\alpha]_D^{22}$*2	MIC (μg/ml) MS-8710
29	4''-*O*-(Cyclohexylthio)acetyltylosin		c	112~116	−37.2	12.5
30	4''-*O*-(4-Pyridylthio)acetyltylosin		c	116~118	−54.2	12.5
31	4''-*O*-(4-Methylphenylthio)-acetyltylosin		b	112~114	−48.9	25
32	4''-*O*-(4-Chlorophenylthio)-acetyltylosin		c	109~113	−43.8	12.5
33	4''-*O*-(Pentachlorophenylthio)-acetyltylosin		b	106~110	−38.6	>200
34	4''-*O*-(3-Phenylthio)-propionyltylosin		b	103~107.5	−40.8	12.5
35	3-*O*-Acetyl-4''-*O*-(3-pyridyl)-acetyltylosin		c	109~112	−28.4	12.5
36	3-*O*-Acetyl-4''-*O*-(dimethylamino)-acetyltylosin		b	96~103	−32.0	>200
37	3-*O*-Acetyl-4''-*O*-(morpholino)-acetyltylosin		c	114~117	−26.8	50
38	3-*O*-Acetyl-4''-*O*-(4-methyl-piperazinoacetyl)tylosin		c	110~114	−29.6	100
39	3-*O*-Acetyl-4''-*O*-(1-imidazolyl-propionyl)tylosin		c	110~115	−29.6	25
40	3-*O*-Acetyl-4''-*O*-(1-triazolyl-propionyl)tylosin		c	108~112	−31.4	>200
41	3-*O*-Acetyl-4''-*O*-(1,2-dithiolane-3-valeryl)tylosin		b	77~ 82	−25.4	25
42	3-*O*-Acetyl-4''-*O*-D-(2-hydroxyphenylacetyl)tylosin		b	115~118	−23.0	12.5
43	3-*O*-Acetyl-4''-*O*-D-(2-acetoxyphenylacetyl)tylosin		b	111~114	−53.8	100
44	4''-*O*-(α-Naphthalenesulfonyl)-tylosin		b	127~139	−43.4	>200
45	4''-*O*-Phenylsulfonyltylosin		b	121~128	−42.4	12.5
46	4''-*O*-(4-Nitrophenylsulfonyl)-tylosin		b	124~129	−44.8	6.25
47	4''-*O*-Phenylmethanesulfonyltylosin		b	117~121	−24.4	12.5
48	4''-*O*-Phenylethanesulfonyltylosin		b	115~121	−35.6	6.25
49	4''-*O*-Phenylthioethanesulfonyl-tylosin		b	94~ 98	−37.4	12.5

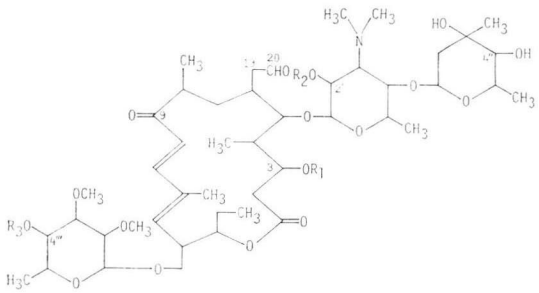
*1 Methods for esterification at the 4''-OH, described in the experimental part.
a; General Method a, b; General Method b, c; General Method c, m; microbial transformation.

*2 *c* 0.5, MeOH

As shown in Fig. 1, tylosin (**1**), a 16-membered macrolide antibiotic, has four secondary hydroxyl groups (C-3, C-2', C-4'' and C-4''') and a tertiary hydroxyl group (C-3''). The latter is far less reactive than the former groups. In order to modify selectively the 4''-OH, the residual secondary hydroxyl groups were successfully protected with suitable blocking agents.

Selective acetylation of the 2'-OH was successful by treatment of **1** in acetone with acetyl chloride in the presence of potassium hydrogen carbonate, giving 2'-*O*-acetyltylosin (**2**) as reported previously[6]. The location of this acetyl group was confirmed by ^{1}H NMR and mass spectrum.

Fig. 1. Intermediates for synthesis of tylosin derivatives and protective groups.



Compound	R_1	R_2	R_3
1	H	H	H
2	H	$COCH_3$	H
3	H	$COCH_3$	$COCH_2Cl$
4	H	$COCH_3$	$COCCl_3$
5	$COCH_3$	$COCH_3$	H
6	$COCH_3$	$COCH_3$	$COCH_2Cl$
7	$COCH_3$	$COCH_3$	$COCCl_3$

The reactivities of other secondary hydroxyl groups were examined and the 4‴-OH was found to be the most reactive. Reaction of **2** in methylene chloride (CH_2Cl_2) with chloroacetyl chloride ($ClCH_2COCl$) or trichloroacetyl chloride (CCl_3COCl) in the presence of pyridine (−15°C, 5 minutes) gave 2′-*O*-acetyl-4‴-*O*-monochloroacetyltylosin (**3**) or 2′-*O*-acetyl-4‴-*O*-trichloroacetyltylosin (**4**).

Modification of the 4″-OH of **3** or **4** performed according to the method reported for synthesis of derivatives of deltamycin[4] *etc.* gave mainly 4″-*O*-substituted-2′,4‴-*O*-protected derivatives. In the reactions, 3,4″-di-*O*-substituted-2′,4‴-*O*-protected derivatives were obtained as a minor product: the yield was dependent on reaction conditions and reagents used for esterification. Removal of the 2′- and 4‴-*O*-protective groups selectively was successful by refluxing in 95% methanol, yielding 4″-*O*-substituted tylosin derivatives. 3-*O*-Acetyl-4″-*O*-substituted derivatives were also chemically synthesized by the same method starting from 3-*O*-acetyltylosin[5] instead of **1**. Synthesis of intermediate compounds (Fig. 1) and new tylosin derivatives is described in detail in experimental section. Physicochemical properties of new tylosin derivatives reported are shown in Table 1 together with their antibacterial activity against a macrolide-resistant strain MS-8710 of *S. aureus*.

Structure-Activity Relationships

1. Antibacterial Activity against Macrolide-resistant Strains of *Staphylococcus aureus*

To examine the effect of aliphatic acyl groups introduced onto the 4″-hydroxyl group on the antibacterial activity, we prepared tylosin derivatives having straight and branched-chain aliphatic acyl groups on the 4″-OH from two to ten carbon atoms. Their MIC values against *Staphylococcus aureus* MS-8710 are shown in Table 1 (compounds **8**～**16** in Table). The derivatives having from four to eight carbon atoms in the straight-chain acyl groups showed the same degree of antibacterial activity. 4″-*O*-Decanoyltylosin had the decreased antibacterial activity. The stronger activity against resistant strains was shown by 4″-*O*-(2-methoxy)isovaleryltylosin and 4″-*O*-(4-methyl)valeryltylosin: MIC values of both compounds were 12.5 μg/ml. For comparison, the strain MS-8710 was resistant to 800 μg/ml of erythromycin, leukomycin, josamycin, spiramycin, tylosin and angolamycin.

We prepared tylosin 4″-*O*-derivatives containing aromatic, alicyclic and heterocyclic groups and tested their antibacterial activity against resistant strains. Structures of substituent groups and MIC values of the derivatives are shown in Table 1 (compounds **17**～**43** in Table). The activity was elevated in the increasing order of 4″-*O*-benzoyl (**17**), phenylacetyl (**19**) and (3-phenylpropionyl) (**23**) tylosins. 4″-*O*-(Phenylthio)acetyl (**28**) and (phenylsulfonyl)acetyl (**27**) tylosins showed a further stronger activity; MIC values of both compounds were 6.25 μg/ml. The substitution of a hydrogen atom with methyl group (**31**) or chlorine group (**32,33**) in the phenyl moiety of these derivatives caused a decrease of the

Table 2. The activity in inhibiting mycoplasma resistant to macrolides and the 4''-*O*-substituents.

Compound	Substituent group at the 4''-*O*-	*Mycoplasma gallisepticum*: MIC (μg/ml)				
		E-5	E-11	A-68	A-72	KP-13
Erythromycin		100	100	100	100	0.1
Spiramycin		100	100	100	100	0.1
Tylosin	H	10	5	2.5	2.5	0.02
Mycaminosyl tylonolide*		>10	>10	>10	>10	0.16
3-*O*-Acetyl-4''-*O*-isovaleryl demycinosyl tylosin*	$CO\text{-}CH_2\text{-}CH(CH_3)_2$	10	2.5	2.5	1.25	0.02
3-*O*-Acetyl-4''-*O*-isovaleryltylosin	$CO\text{-}CH_2\text{-}CH(CH_3)_2$	0.62	0.31	0.31	0.31	0.02
4''-*O*-(Phenylthio)acetyltylosin	$CO\text{-}CH_2\text{-}S$–C$_6$H$_5$	0.08	0.08	0.08	0.08	0.04
4''-*O*-(4-Nitrophenylacetyl)tylosin	$CO\text{-}CH_2$–C$_6$H$_4$–NO_2	0.08	0.08	0.08	0.08	0.04
4''-*O*-(Phenylsulfonyl)acetyltylosin	$CO\text{-}CH_2\text{-}SO_2$–C$_6H_5$	0.08	0.08	0.08	0.08	0.04
Chloramphenicol		10	3	30	10	30
Tetracycline		1	0.3	1	1	1

* Chemical structures were reported in previous papers.[3]

antibacterial activity. This was clearly shown in 4''-*O*-(phenylthio)acetyltylosin derivatives. The replacement of the benzene group of the derivatives with cyclohexane (**29**) and pyridine (**30**) did not increase the activity. The introduction of the groups having the other heterocyclic structures and the different length of methylene-chain (compounds **35**~**43** in Table) did not afford a further increased activity. However, 4''-*O*-(4-nitrophenylacetyl)tylosin (**20**) showed the same degree of antibacterial activity as 4''-*O*-(phenylthio)acetyltylosin. We prepared also 4''-*O*-sulfonyl derivatives as shown in Table 1 (compounds **44**~**49** in Table). The length of the carbon chain in this case did not show any effect on the activity. Among 4''-*O*-sulfonyl derivatives, 4''-*O*-phenylethanesulfonyltylosin (**48**) and 4''-*O*-(4-nitrophenylsulfonyl)tylosin (**46**) showed a strong antibacterial activity (MIC, 6.25 μg/ml).

2. Antimycoplasmal Activity, Especially Activity against Macrolide-resistant Strains of *Mycoplasma gallisepticum*

Tylosin is currently used for the treatment of mycoplasma infections in animals. As shown in Table 2, 4''-*O*-(phenylthio)acetyltylosin, 4''-*O*-(4-nitrophenylacetyl)tylosin and 4''-*O*-phenylsulfonylacetyltylosin showed an improved antimycoplasmal activity against macrolide-resistant strains. MIC values were 0.08 μg/ml. It is interesting that the degree of the activity of derivatives in inhibiting mycoplasma resistant to tylosin is parallel to the antibacterial activity against resistant *Staphylococcus* strains. 3-*O*-Acetyl-4''-*O*-isovaleryltylosin showed a strong inhibition against mycoplasma resistant to tylosin, but 3-*O*-acetyl-4''-*O*-isovaleryl demycinosyl tylosin had no activity. Mycaminosyl tylonolide[7] which lacks both mycarose and mycinose moieties inhibited tylosin-sensitive mycoplasma, but had no activity against tylosin-resistant strains. But, this derivative had a stronger antibacterial activity against Gram-negative bacteria than tylosin: MIC against *E. coli* NIHJ was 50 μg/ml, while that of tylosin was 200 μg/ml.

3. Uptake of Tylosin Derivatives by *Staphylococcus* strain (MS-8710) Resistant to Tylosin and its Correlation to Antibacterial Activity

Introduction of an isovaleryl group onto the 4''-OH of tylosin enhanced the uptake of drug by the strain MS-8710. This alteration correlated well with the antibacterial activity of tylosin derivatives[2]. As reported previously, the ribosomes of *S. aureus* MS-9610 were resistant[3]. In contrast, the ribosome

Table 3. MIC, retention time in reverse phase column chromatography, Rf values in TLC and inhibition of uptake of 3-*O*-[1-^{14}C]acetyl-4″-*O*-isovaleryltylosin and of [^{14}C]leucine incorporation by intact cells of *Staphylococcus aureus* MS-8710.

No.	Derivative Structure at the 4″-*O*-	MIC (μg/ml) *S. aureus* MS-8710	Retention time*1 (minutes)	Rf*2 (1)	Rf*2 (2)	% Inhibition by a compound of Uptake of ^{14}C-labeled tylosin derivative*3	% Inhibition by a compound of [^{14}C]Leucine incorporation*4
1	H (parent compound)	800	3.22	1	1	15	30
8	CO-CH_3	100	3.66	3.5	4.7	34	33
9	CO-CH_2-CH_2-CH_3	25	4.90	5.6	6.3	54	76
10	CO-CH_2-$CH(CH_3)_2$	25	5.47	5.7	6.4	57	79
12	CO-CH_2-CH_2-$CH(CH_3)_2$	12.5	7.15	6.2	6.8	59	85
14	CO-$(CH_2)_4$-CH_3	25	7.45	6.2	6.8	58	81
15	CO-$(CH_2)_6$-CH_3	25	13.35	6.3	7.1	66	62
16	CO-$(CH_2)_8$-CH_3	>200*5	27.91	6.8	7.4	50	11
20	CO-CH_2-  -NO_2	6.25	4.65	—	—	54	88
28	CO-CH_2-S- 	6.25	5.88	—	—	55	82
27	CO-CH_2-SO_2- 	6.25	3.85	—	—	51	87
46	SO_2-  -NO_2	6.25	4.52	—	—	51	80
48	SO_2-CH_2-CH_2- 	6.25	5.20	—	—	53	82

*1 Retention time: determined by HPLC with C_{18} reversed phase column, acetonitrile - 2% monoethanolamine in H_2O=60: 40 (v/v).

*2 Rf: derivative/tylosin; (1) *n*-Hexane - acetone - MeOH - benzene - EtOAc, 30: 10: 8: 25: 20 (v/v)
(2) $CHCl_3$ - MeOH, 10: 1 (v/v)

*3 % Inhibition on uptake: Cells of *S. aureus* MS-8710, cultured in BHI medium for 6 hours with shaking, collected and suspended in 0.05 M phosphate buffer, pH 7.0, to give a cell density of 3.3 OD_{660}. Each 0.4 ml of cell suspension mixed with 0.1 ml of unlabeled tylosin derivatives solution dissolved in 10% MeOH (500 μg/ml). After preincubation at 37°C for 10 minutes, 10 μl of 3-*O*-[1-^{14}C]acetyl-4″-*O*-isovaleryltylosin (1.84 μg, 10.24 mCi/mmole) added and incubated for 20 minutes. Cells filtered through Millipore filter disc (HA, 0.45 μm) and washed three times with each 3 ml of cold buffer saline containing 50 μg/ml of unlabeled 3-*O*-acetyl-4″-*O*-isovaleryltylosin. Radioactivity remaining on the filter disc was determined by the liquid-scintillation method.

*4 % Inhibition of [^{14}C]leucine incorporation into cellular macromolecules: Cells of *S. aureus* MS-8710, cultured in BHI medium for 7 hours (OD_{660}=0.62) with shaking, collected and suspended to one forth of the culture with 0.05 M phosphate buffer, pH 7.0, containing 1.0 M sucrose and 0.15 M NaCl. Each 90 μl of cell suspension mixed with 10 μl of unlabeled tylosin derivative aqueous solution to give a final concentration of 6.25 μg/ml. After preincubation at 37°C for 10 minutes, 10 μl of L-[U-^{14}C]leucine (5 μCi/ml, 351 mCi/mmole) added and incubated at 37°C for 30 minutes. Radioactivity in cold TCA insoluble materials was determined by a toluene scintillation method as reported in a previous paper[2].

*5 Compound No. 16
The data obtained in 3 experiments for MIC and % inhibition of the uptake of ^{14}C-labeled tylosin derivative are as follows: >200, >200, 200 μg/ml for MIC; 55, 49, 46% inhibition.

system of *S. aureus* MS-8710 is sensitive to tylosin and other macrolides and its resistance has been confirmed to be due to decreased permeability (the mechanism of macrolide-resistance of this strain will be reported elsewhere). As a parameter for the uptake of the compounds into the strain MS-8710, the

Table 4. Effect of 4''-*O*-(phenylthio)acetyltylosin and 3-*O*-acetyl-4''-*O*-isovaleryltylosin on *in vitro* protein synthesis with ribosomes of MS-9610 and the binding of 3-*O*-[1-^{14}C]acetyl-4''-*O*-isovaleryltylosin to its ribosomes.

Compound	MIC (μg/ml)	% Inhibition	
		In vitro protein synthesis*1	Binding of ^{14}C-*2
Tylosin	1600	41	20.5
3-*O*-Acetyl-4''-*O*-isovaleryltylosin	100	63	57
4''-*O*-(Phenylthio)acetyltylosin	25	78.5	77

*1 Inhibition of the protein synthesis *in vitro*[2] with 70S-ribosomes at a concentration of 2 μg/ml.
*2 Inhibitory activities of unlabeled drugs on the binding of 3-*O*-[1-^{14}C]acetyl-4''-*O*-isovaleryltylosin (0.92 μg/200 μl) to 70S-ribosomes[3] at a concentration of 2 μg/200 μl.

inhibitory activities of unlabeled tylosin derivatives on uptake of 3-*O*-[1-^{14}C]acetyl-4''-*O*-isovaleryltylosin by cells and on [^{14}C]leucine incorporation into cellular macromolecules were measured. Hydrophobicities of tylosin derivatives were measured by thin-layer chromatography (TLC) using silica gel plates and by high performance liquid chromatography (HPLC) using C_{18} reversed phase column. In the derivatives having the straight and branched-chain aliphatic acyl groups, 4''-*O*-decanoyltylosin (compound **16**) had the decreased antibacterial activity and its retention time in HPLC was much longer than the other derivatives, as shown in Table 3. The activities of the derivatives having the side chain of from four to eight carbon atoms in inhibiting with the uptake of 3-*O*-[1-^{14}C]acetyl-4''-*O*-isovaleryltylosin by cells and [^{14}C]leucine incorporation into cellular macromolecules were more than 50% and these derivatives showed stronger antibacterial activities than tylosin or 4''-*O*-acetyltylosin. 4''-*O*-Octanoyltylosin (compound **15**) showed the highest inhibitory activity on uptake of ^{14}C-labeled tylosin derivative but inhibition of [^{14}C]leucine incorporation into cellular macromolecules was lower than the other derivatives. 4''-*O*-Decanoyltylosin inhibited uptake to the extent of 50% but has an MIC $>$200 μg/ml. It seems that the ribosomes are resistant to this derivative, because the inhibitory activity by this compound on [^{14}C]leucine incorporation was extremely low.

In spite of having more improved antibacterial activities, the inhibitory activities on uptake of ^{14}C-labeled tylosin derivative by the derivatives having the substituent groups such as 4''-*O*-(4-nitrophenylacetyl)tylosin (**20**), 4''-*O*-(phenylsulfonyl)acetyltylosin (**27**), 4''-*O*-(phenylthio)acetyltylosin (**28**), 4''-*O*-(4-nitrophenylsulfonyl)tylosin (**46**) and 4''-*O*-phenylethanesulfonyltylosin (**48**) did not increase as compared with the aliphatic acyl derivatives, but similarly showed the high inhibitory activities on [^{14}C]-leucine incorporation into cellular macromolecules. In the case of derivatives having the substituent groups containing cyclic structures, we could not find the correlation between the hydrophobicity and antibacterial activity of the derivatives. These derivatives having the aromatic groups also showed an improved antibacterial activity against *S. aureus* MS-9610 whose ribosomes were resistant. As shown in Table 4, 4''-*O*-(phenylthio)acetyltylosin strongly inhibited both the protein synthesis *in vivo* and the binding of 3-*O*-[1-^{14}C]acetyl-4''-*O*-isovaleryltylosin to ribosomes of this strain. It indicates that the improved antibacterial activity of this derivative might be due to its strong binding to ribosomes.

Experimental

General Methods

Melting point, optical rotation and ^{1}H NMR ($CDCl_3$) spectra were measured by the same methods

as described in a previous paper[5]. Column chromatography was performed on Wakogel C-200. For analytical TLC plates Merck silica gel 60 F_{254} was used and macrolide spots were detected by spraying with 10% sulfuric acid. High performance liquid chromatography (HPLC) was performed on a reverse phase Radial-pak A column (Waters Associates, Inc.,) at room temperature using a Waters Liquid Chromatograph model ALC/GPC 244. The solvent used was a mixture of acetonitrile - 2% monoethanolamine in H_2O (60: 40, v/v, 1 ml/minute) and effluents were monitered by UV_{280}. Biological tests, such as, the measurement of the minimum inhibitory concentration (MIC, μg/ml), inhibition of the protein synthesis *in vitro* and *in vivo* and the effect on uptake of ^{14}C-labeled tylosin derivative by intact cells of *S. aureus* MS-8710 were performed according to the methods described in previous papers[1,2].

2′-*O*-Acetyltylosin (**2**)

To a solution of tylosin (**1**) (1.0 g) in acetone (7 ml) was added potassium hydrogen carbonate (1.0 g) and under stirring at room temperature, acetyl chloride (0.4 g) in acetone (0.3 ml) was added dropwise. The mixture was kept at room temperature for 5 hours. The mixture was poured into ice-water (30 ml) and then extracted twice each with 10 ml of benzene. The benzene extract was washed twice with a saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution (50 ml), twice with a saturated aqueous sodium chloride (NaCl) solution (50 ml), dried over anhydrous sodium sulfate (Na_2SO_4), and evaporated to dryness to give 2′-*O*-acetyltylosin (**2**) (1.0 g) which was crystallized from toluene. ^{1}H NMR, δ 1.78 (3H, s, 12-CH_3), 2.04, (3H, s, 2′-$OCOCH_3$), 2.37 (6H, s, $N(CH_3)_2$), 3.46 (3H, s, OCH_3), 3.58 (3H, s, OCH_3), 4.24 (1H, d, $J_{1',2'}$=7.5 Hz, H-1′), 4.54 (1H, d, $J_{1''',2'''}$=8 Hz, H-1‴), 5.88 (1H, d, $J_{13,14}$= 10.5 Hz, H-13), 6.24 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.27 (1H, d, $J_{10,11}$=16 Hz, H-11), 9.65 (1H, s, CHO). λ_{max}^{MeOH} 284 nm ($E_{1cm}^{1\%}$ 213).

Anal. Calcd. for $C_{48}H_{79}NO_{13}$ (MW 957): C 60.17, H 8.31, N 1.46.
Found: C 60.50, H 8.72, N 1.43.

2′-*O*-Acetyl-4‴-*O*-monochloroacetyltylosin (**3**)

To a solution of **2** (1.0 g) in a mixture of methylene chloride (20 ml) and pyridine (0.16 g) at $-15\sim-10$°C was added chloroacetyl chloride (0.28 g) under stirring kept for 5 minutes, and then poured into a saturated aqueous toluene solution (140 ml). The toluene layer was washed twice with 50 ml of a saturated aqueous $NaHCO_3$ solution and of a saturated aqueous NaCl solution, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was dissolved in a small amount of benzene, placed on a column of silica gel (1.8×25 cm), and eluted with a mixture of benzene - acetone (10: 1). Effluents were collected in 5-g fractions. Concentration of fractions 45 ~ 55 gave 2′-*O*-acetyl-4‴-*O*-monochloroacetyltylosin (**3**) (0.62 g), as a white powder. ^{1}H NMR, δ 1.80 (3H, s, 12-CH_3), 2.06 (3H, s, 2′-$OCOCH_3$), 2.39 (6H, s, $N(CH_3)_2$), 3.48 (3H, s, OCH_3), 3.52 (3H, s, OCH_3), 4.09 (2H, s, 4‴-$OCOCH_2Cl$), 4.64 (1H, d, $J_{1''',2'''}$=7.5 Hz, H-1‴), 5.91 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.29 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.34 (1H, d, $J_{10,11}$=16 Hz, H-11), 9.70 (1H, s, CHO). λ_{max}^{MeOH} 284 nm ($E_{1cm}^{1\%}$ 165).

Anal. Calcd. for $C_{50}H_{80}NO_{19}Cl$ (MW 1,034): C 58.04, H 7.79, N 1.35.
Found: C 58.24, H 7.57, N 1.39.

2′-*O*-Acetyl-4‴-*O*-trichloroacetyltylosin (**4**)

To a solution of **2** (1.0 g) in a mixture of methylene chloride (20 ml) and pyridine (0.22 g) at $-15\sim-10$°C was added trichloroacetyl chloride (0.70 g), 2′-*O*-acetyl-4‴-*O*-trichloroacetyltylosin (0.75 g) was obtained by the same procedures as described in the preparation of **3**. ^{1}H NMR, δ 1.80 (3H, s, 12-CH_3), 2.06 (3H, s, 2′-$OCOCH_3$), 2.40 (6H, s, $N(CH_3)_2$), 3.52 (3H, s, OCH_3), 3.56 (3H, s, OCH_3), 4.69 (1H, d, $J_{1''',2'''}$=8 Hz, H-1‴), 5.95 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.32 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.37 (1H, d, $J_{10,11}$=16 Hz, H-11), 9.73 (1H, s, CHO). λ_{max}^{MeOH} 283 nm ($E_{1cm}^{1\%}$ 196).

Anal. Calcd. for $C_{50}H_{78}NO_{19}Cl_3$ (MW 1,103): C 54.42, H 7.12, N 1.27.
Found: C 54.21, H 7.43, N 1.31.

3,2′-Di-*O*-acetyltylosin (**5**)

3-*O*-Acetyltylosin (1.0 g) was mixed with acetic anhydride (5 ml), the resulting mixture was stirred to dissolve completely for about 30 seconds at room temperature and thereafter poured into ice-water (45 ml) and extracted twice with 100 ml of benzene, after the pH was adjusted to 8 with $NaHCO_3$. After washing with a saturated aqueous $NaHCO_3$ solution and a saturated NaCl solution, drying over anhydr-

ous Na_2SO_4 and evaporation of the solvent, 3,2′-di-*O*-acetyltylosin (**5**) (1.0 g), obtained and crystallized from carbon tetrachloride. ^1H NMR, δ 1.79 (3H, s, 12-CH_3), 2.04 (3H, s, 2′-$OCOCH_3$), 2.09 (3H, s, 3-$OCOCH_3$), 2.37 (6H, s, $N(CH_3)_2$), 3.43 (3H, s, OCH_3), 3.58 (3H, s, OCH_3), 4.18 (1H, d, $J_{1',2'}$=7.5 Hz, H-1′), 4.53 (1H, d, $J_{1''',2'''}$=7.5 Hz, H-1‴), 5.90 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.25 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.38 (1H, d, $J_{10,11}$=16 Hz, H-11), 9.62 (1H, s, CHO). λ_{max}^{MeOH} 282 nm ($E_{1cm}^{1\%}$ 207).

Anal. Calcd. for $C_{50}H_{81}NO_{19}$ (MW 999): C 60.04, H 8.16, N 1.40.
Found: C 59.71, H 8.33, N 1.36.

3,2′-Di-*O*-acetyl-4‴-*O*-monochloroacetyltylosin (**6**)

To a solution of **5** (1.0 g) in a mixture of methylene chloride (20 ml) and pyridine (0.15 g) at −15~−10°C was added chloroacetyl chloride (0.27 g). By the same procedure as described in the preparation of **3**, 3,2′-di-*O*-acetyl-4‴-*O*-monochloroacetyltylosin (**6**) was obtained as a white powder (0.60 g). ^1H NMR, δ 1.79 (3H, s, 12-CH_3), 2.05 (3H, s, 2′-$OCOCH_3$), 2.09 (3H, s, 3-$OCOCH_3$), 2.40 (6H, s, $N(CH_3)_2$), 3.43 (3H, s, OCH_3), 3.50 (3H, s, OCH_3), 4.05 (2H, s, 4‴-$OCOCH_2Cl$), 4.60 (1H, d, $J_{1''',2'''}$=8.5 Hz, H-1‴), 5.90 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.26 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.37 (1H, d, $J_{10,11}$=16 Hz, H-11), 9.63 (1H, s, CHO). λ_{max}^{MeOH} 283 nm ($E_{1cm}^{1\%}$ 204).

Anal. Calcd. for $C_{52}H_{82}NO_{20}Cl$ (MW 1,075): C 58.01, H 7.68, N 1.30.
Found: C 58.38, H 7.90, N 1.25.

3,2′-Di-*O*-acetyl-4‴-*O*-trichloroacetyltylosin (**7**)

This compound was prepared by the same processes of the preparation of **6**, reacting **5** (1.0 g) with trichloroacetyl chloride (0.73 g) instead of chloroacetyl chloride. ^1H NMR, δ 1.82 (3H, s, 12-CH_3), 2.06 (3H, s, 2′-$OCOCH_3$), 2.09 (3H, s, 3-$OCOCH_3$), 2.38 (6H, s, $N(CH_3)_2$), 3.45 (3H, s, OCH_3), 3.51 (3H, s, OCH_3), 4.63 (1H, d, $J_{1''',2'''}$=8 Hz, H-1‴), 5.91 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.26 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.39 (1H, d, $J_{10,11}$=16 Hz, H-11), 9.63 (1H, s, CHO). λ_{max}^{MeOH} 282 nm ($E_{1cm}^{1\%}$ 191).

Anal. Calcd. for $C_{52}H_{80}NO_{20}Cl_3$ (MW 1,145): C 54.52, H 7.04, N 1.22.
Found: C 54.37, H 7.49, N 1.28.

4″-*O*-Hexanoyltylosin (**14**) (General Method a)

To a solution of **3** (200 mg) in pyridine (2 ml) was added hexanoic anhydride (1.96 ml) and the resulting mixture was kept at 5°C for 48 hours. Benzene (100 ml) was added, and after washing twice with a saturated aqueous solution of $NaHCO_3$ and NaCl and drying over anhydrous Na_2SO_4, the benzene extract was concentrated to dryness. The residue was dissolved in a small amount of benzene and applied to a column of silica gel (1.7×15 cm), which was developed with a mixture of benzene - acetone (9: 1). Effluents were collected in 2-g fractions, and evaporation of solvent from fractions 25~30 gave 2′-*O*-acetyl-4‴-*O*-monochloroacetyl-4″-*O*-hexanoyltylosin (85 mg) as a white powder. Selective ester hydrolysis of a derivative was carried out by refluxing in methanol (10 ml) for 22 hours and concentrating to dryness; chromatography over silica gel (1.7×17 cm), developing with benzene - acetone (4: 1) and evaporation to dryness gave 4″-*O*-hexanoyltylosin (**14**) (60 mg). ^1H NMR, δ 1.79 (3H, s, 12-CH_3), 2.52 (6H, s, $N(CH_3)_2$), 3.49 (3H, s, OCH_3), 3.62 (3H, s, OCH_3), 4.57 (1H, d, $J_{1''',2'''}$=8 Hz, H-1‴), 4.59 (1H, d, $J_{4'',5''}$=10 Hz, H-4″), 5.92 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.26 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.35 (1H, d, $J_{10,11}$=16 Hz, H-11), 9.71 (1H, s, CHO). λ_{max}^{MeOH} 283 nm ($E_{1cm}^{1\%}$ 206).

Table 5. Reaction conditions for synthesis of 4″-*O*-substituted tylosin derivatives (General Method a).

Product		Conditions				Yield (%)
No.	Name	Acid anhydride (ml)	Pyridine (ml)	Temperature (°C)	Time (hours)	
12	4″-*O*-(4-Methylvaleryl)tylosin	4-Methylvaleric anhydride (2.0)	2	5	48	35
14	4″-*O*-Hexanoyltylosin	Hexanoic anhydride (1.96)	2	5	48	30
15	4″-*O*-Octanoyltylosin	Octanoic anhydride (2.5)	2	5	48	48
16	4″-*O*-Decanoyltylosin	Decanoic anhydride (3.0)	2	5	50	40
24	4″-*O*-(3-Cyclohexylpropionyl)-tylosin	3-Cyclohexanepropionic anhydride (3.5)	2	5	22	40

Table 6. Reaction conditions for synthesis of 4''-*O*-substituted tylosin derivatives (General Method b).

No.	Product Name	Conditions					Yield (%)
		Carboxylic acid chloride (mg)	Pyridine (mg)	Temperature (°C)	Time (minutes)	Methylene chloride (ml)	
8	4''-*O*-Acetyltylosin	169	265	−15	270	4.5	29
11	4''-*O*-(2-Methoxy)isovaleryltylosin	145	85	−10 ~ −5	30	5	7
13	4''-*O*-(4-Methyl)valeryltylosin	260	293	−5→25	2,460	5 (THF)*	35
17	4''-*O*-Benzoyltylosin	288	753	−10 ~ −5	30	2	33
18	4''-*O*-(2-Furoyl)tylosin	316	287	−10 ~ −5	36	5	74
19	4''-*O*-Phenylacetyltylosin	115	88	−20 ~ −15	60	6	6
20	4''-*O*-(4-Nitrophenylacetyl)tylosin	181	110	0	40	3	29
21	4''-*O*-(α-Naphthyl)acetyltylosin	264	137	−10 ~ −5	20	4	30
22	4''-*O*-(2-Thienyl)acetyltylosin	155	117	0→25	1,080	2.5	44
23	4''-*O*-(3-Phenylpropionyl)tylosin	120	460	−10 ~ −5	30	2	44
25	4''-*O*-Phenoxyacetyltylosin	132	88	−10 ~ −5	60	5	31
26	4''-*O*-(β-Naphthoxy)acetyltylosin	180	88	−10 ~ −5	15	4	45
27	4''-*O*-(Phenylsulfonyl)acetyltylosin	198	108	−5	12	6	33
28	4''-*O*-(Phenylthio)acetyltylosin	204	137	−10 ~ −5	1,200	5	20
31	4''-*O*-(4-Methylphenylthio)acetyltylosin	300	240	−5	120	2.5	8
33	4''-*O*-(Pentachlorophenylthio)-acetyltylosin	491	162	−15 ~ −10	180	2.5	20
34	4''-*O*-(3-Phenylthio)propionyltylosin	204	127	−10 ~ −5	1,200	5	15
36	3-*O*-Acetyl-4''-*O*-(dimethylamino)-acetyltylosin	104	138	5	2,880	9	24
41	3-*O*-Acetyl-4''-*O*-(1,2-dithiolane-3-valeryl)tylosin	245	205	5	1,200	4.5	13
42	3-*O*-Acetyl-4''-*O*-D-(2-hydroxyphenyl-acetyl)tylosin	215	103	−5	90	4.5	56
43	3-*O*-Acetyl-4''-*O*-D-(2-acetoxyphenyl-acetyl)tylosin	185	103	−5	120	4	51

* Tetrahydrofuran (THF) used instead of methylene chloride.

Anal. Calcd. for $C_{52}H_{87}NO_{19}$ (MW 1,013): C 61.58, H 8.65, N 1.38.
Found: C 61.80, H 8.93, N 1.41.

Tylosin derivatives listed in Table 5 were prepared by treatment of 200 mg of **3** or **6** with the corresponding acid anhydride in the presence of pyridine according to the General Method a under the conditions shown in Table 5.

4''-*O*-(3-Phenylpropionyl)tylosin (**23**) (General Method b)

A solution of **3** (200 mg) in a mixture of methylene chloride (2 ml) and pyridine (460 mg) at −10°C was allowed to react with 3-phenylpropionyl chloride (0.1 ml) at −10 ~ −5°C for 30 minutes. By the same procedures of preparation of **14**, 4''-*O*-(3-phenylpropionyl)tylosin (87 mg) was obtained. ^{1}H NMR, δ 1.79 (3H, s, 12-CH_3), 2.49 (6H, s, $N(CH_3)_2$), 3.47 (3H, s, OCH_3), 3.60 (3H, s, OCH_3), 4.55 (1H, d, $J_{4'',5''}$=10 Hz, H-4''), 5.88 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.23 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.10 ~ 7.43 (6H, m, H-11 and aromatic), 9.70 (1H, s, CHO). λ_{max}^{MeOH} 283 nm ($E_{1cm}^{1\%}$ 210).

Anal. Calcd. for $C_{55}H_{85}NO_{13}$ (MW 1,047): C 63.02, H 8.17, N 1.34.
Found: C 63.57, H 8.69, N 1.40.

The carboxylic acid ester derivatives listed in Table 6 were prepared by treatment of 200 mg of **3**, **4**, **6** or **7** with the corresponding carboxylic acid chloride according to the General Method b under the conditions shown in Table 6. When the 2-hydroxy or 3-hydroxy carboxylic acid chlorides were used,

these hydroxyl groups were protected with a suitable blocking substituent group such as monochloroacetyl group, prior to the use for the reaction.

4''-*O*-Phenylthioethanesulfonyltylosin (**49**) (General Method b)

A solution of **4** (250 mg) in methylene chloride (6 ml) was mixed with triethylamine (230 mg) and cooled to −15°C. To the resulting solution was added phenylthioethanesulfonyl chloride (520 mg) and the solution was reacted with stirring at −15°C for 17 hours and poured into benzene (100 ml). By the same procedures described in the preparation of **14**, 4''-*O*-phenylthioethanesulfonyltylosin (58 mg) was obtained. ^{1}H NMR, δ 1.82 (3H, s, 12-CH_3), 2.50 (6H, s, $N(CH_3)_2$), around 3.41 (4H, $SCH_2CH_2SO_2$), 3.51 (3H, s, OCH_3), 3.63 (3H, s, OCH_3), 5.93 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.28 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.23~7.41 (6H, m, H-11 and aromatic), 9.73 (1H, s, CHO). λ_{max}^{MeOH} 283 nm ($E_{1cm}^{1\%}$ 171).

Anal. Calcd. for $C_{54}H_{85}NO_{19}S_2$ (MW 1,115): C 58.09, H 7.67, N 1.25.
Found: C 57.90, H 7.93, N 1.24.

Sulfonic acid ester derivatives listed in Table 7 were prepared by treatment of 250 mg of **3** or **4** with corresponding sulfonic acid chlorides according to the General Method b under the conditions shown in Table 7.

Table 7. Reaction conditions for synthesis of 4''-*O*-substituted tylosin derivatives (General Method b).

Product		Conditions						Yield (%)
No.	Name	Sulfonic acid chloride (mg)	Pyridine (ml)	Triethylamine (mg)	Temperature (°C)	Time (hours)	Methylene chloride (ml)	
44	4''-*O*-(α-Naphthalenesulfonyl)-tylosin	420	3.3	—	−15	18	—	26
45	4''-*O*-Phenylsulfonyltylosin	386	3.0	—	−15	17	—	24
46	4''-*O*-(4-Nitrophenylsulfonyl)-tylosin	504	3.3	—	−15→25	2	—	22
47	4''-*O*-Phenylmethanesulfonyl-tylosin	130	0.1	—	−15→−5	24	5	24
48	4''-*O*-Phenylethanesulfonyltylosin	270	—	150	−15	17	6	16
49	4''-*O*-Phenylthioethanesulfonyl-tylosin	520	—	230	−15	17	6	23

Table 8. Reaction conditions for synthesis of 4''-*O*-substituted tylosin derivatives (General Method c).

Product		Conditions					Yield (%)
No.	Name	Carboxylic acid (mg)	Pyridine (mg)	Temperature (°C)	Time (hours)	Methylene chloride (ml)	
29	4''-*O*-(Cyclohexylthio)acetyltylosin	236	430	−10→ 4	20	2.5	40
30	4''-*O*-(4-Pyridyl)thioacetyltylosin	204	342	−10→ 4	21	8	20
32	4''-*O*-(4-Chlorophenylthio)acetyltylosin	330	516	5	20	5	23
35	3-*O*-Acetyl-4''-*O*-(3-pyridyl)acetyltylosin	153	368	−10→ 4	21	8 (THF)*	33
37	3-*O*-Acetyl-4''-*O*-molpholinoacetyltylosin	500	862	−10→ 4	44	4	72
38	3-*O*-Acetyl-4''-*O*-(4-methylpiperazino-acetyl)tylosin	350	862	−10→ 4	44	4	36
39	3-*O*-Acetyl-4''-*O*-(1-imidazolylpropionyl)-tylosin	169	414	−10→25	48	4	17
40	3-*O*-Acetyl-4''-*O*-(1-triazolylpropionyl)-tylosin	185	414	−10→25	24	4	32

* Tetrahydrofuran (THF) used instead of methylene chloride.

4″-*O*-(Cyclohexylthio)acetyltylosin (**29**) (General Method c)

Cyclohexylthioacetic acid (236 mg) and triethylamine (138 mg) were dissolved in methylene chloride (2.5 ml) and cooled to −10°C. To the solution was added pivaloyl chloride (164 mg) and the solution was reacted for 10 minutes. Subsequently, pyridine (430 mg) and **4** (250 mg) were added to the reaction mixture. The resulting mixture was reacted at 4°C for 20 hours. By the same procedures described in the preparation of **14**, 4″-*O*-(cyclohexylthio)acetyltylosin (100 mg) was obtained. ^{1}H NMR, δ 1.81 (3H, s, 12-CH_3), 2.54 (6H, s, $N(CH_3)_2$), 3.34 (2H, s, $COCH_2S$–C_6H_{11}), 3.51 (3H, s, OCH_3), 3.63 (3H, s, OCH_3), 4.59 (1H, d, $J_{4'',5''}$=10 Hz, H-4″), 5.93 (1H, d, $J_{13,14}$=10.5 Hz, H-13), 6.28 (1H, d, $J_{10,11}$=16 Hz, H-10), 7.36 (1H, d, $J_{10,11}$=16 Hz, H-11), 9.73 (1H, s, CHO). λ_{max}^{MeOH} 283 nm ($E_{1cm}^{1\%}$ 189).

Anal. Calcd. for $C_{54}H_{89}NO_{18}S$ (MW 1,071): C 60.48, H 8.36, N 1.30.
Found: C 59.96, H 8.70, N 1.27.

Tylosin derivatives listed in Table 8 were prepared by treatment of 250 mg of **4** or **7** with the corresponding mixed acid anhydride which was prepared from carboxylic acid by treatment with triethylamine and pivaloyl chloride in methylene chloride under the conditions shown in Table 8.

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