Derivatives of Tetrodecamycin

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The derivatives of tetrodecamycin (**1**), being introduced acyl, carbamoyl and alkyl groups at 14-hydroxyl group and modified at exo-methylene group, were synthesized and evaluated on their antibacterial activities. Although 14-O-substituted tetrodecamycins (**3**~**19**) showed weak activity against *Pasteurella piscicida*, they were more active against Gram-positive bacteria than **1**. Among them, **15** showed approximately 10-fold higher activity than **1**. The derivatives (**20**~**23**) modified at 4 or 5 positions had moderate antibacterial activity. The absolute structure of 4(*R*),5-dibromotetrodecamycin (**23**) was determined by X-ray crystallographic analysis.

Novel antimicrobial antibiotics against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *P. piscicida*, tetrodecamycin (**1**) and weakly active dihydrotetrodecamycin (**2**) were isolated from a culture broth of *Streptomyces nashvillensis* MJ885-mF8. (Fig. 1)[1] The elucidation of absolute structure was described in previous paper.[2] In the purpose of obtaining more potent derivative, we examined the introduction of substituents at 14-O position or modifications at exo-methylene moiety. In this paper, we describe the synthesis of derivatives of **1**, the antibacterial activities of the derivatives, and the absolute structure of 4(*R*),5-dibromotetrodecamycin (**23**).

Fig. 1. The structures of tetrodecamycin (**1**) and dihydrotetrodecamycin (**2**).

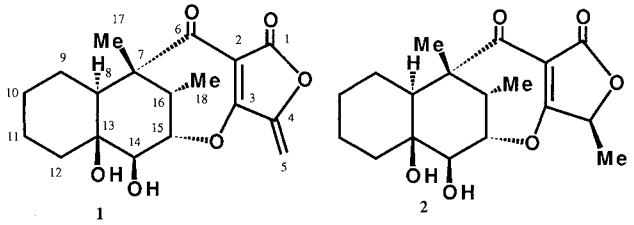
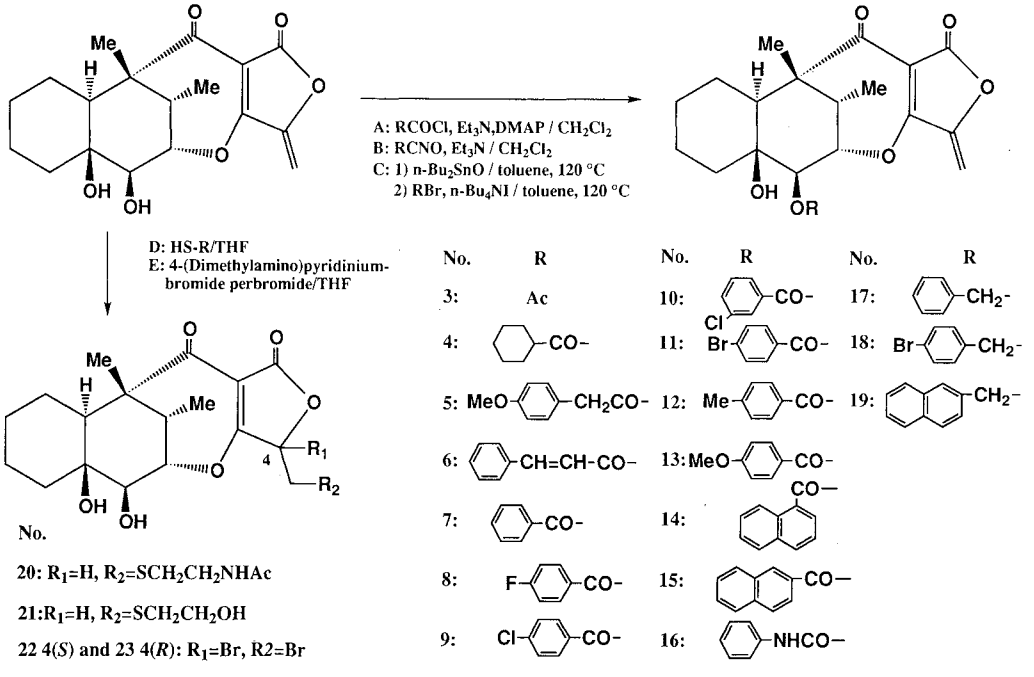


Fig. 2. Synthesis of tetrodecamycin derivatives.



Results

Synthesis

For preparation of tetrodecamycin derivative having more potent antimicrobacterial activity, the structure-activity relationships were investigated by introduction of substituents or modification at possible sites of the molecule, such as the C-14 secondary alcohol and the exo-methylene group. The modification of the 14-O position and the exo-methylene position were carried out as shown in Fig. 2 to obtain 14-O-acyl-, 14-O-carbamoyl-, 14-O-alkyl-, 5-S-alkyl-4-hydro- and 4,5-dibromo-tetrodecamycins.

The 14-O-acyl derivatives (**3**～**15**) were obtained by treatment of **1** with corresponding acyl halide in the presence of triethyl amine and catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2. The 14-O-(N-phenyl)carbamoyltetrodecamycin (**16**) was synthesized

Table 1. Antibacterial activity of tetrodecamycin derivatives. (MIC μg/ml).

Test organism	Derivartive No.										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Staphylococcus aureus FDA209P	6.25	>100	12.5	3.12	3.12	6.25	3.12	6.25	1.56	3.12	1.56
S. aureus Smith	12.5	>100	25	3.12	3.12	6.25	3.12	6.25	3.12	3.12	3.12
S. aureus MS9610	12.5	>100	25	6.25	3.12	12.5	3.12	6.25	3.12	3.12	6.25
S. aureus No. 5 (MRSA)	12.5	>100	25	6.25	6.25	6.25	3.12	6.25	3.12	3.12	3.12
S. aureus No. 17 (MRSA)	12.5	>100	25	6.25	6.25	12.5	6.25	6.25	3.12	3.12	6.25
Micrococcus luteus FDA16	12.5	>100	25	6.25	6.25	12.5	6.25	6.25	6.25	6.25	6.25
M. luteus IFO3333	12.5	>100	25	6.25	6.25	12.5	6.25	6.25	3.12	6.25	3.12
M. luteus PC1001	12.5	>100	25	6.25	6.25	12.5	6.25	6.25	3.12	6.25	3.12
Bacillus anthracis	6.25	>100	12.5	3.12	3.12	6.25	1.56	3.12	0.78	1.56	1.56
B. subtilis NRRL B-558	12.5	>100	12.5	3.12	3.12	12.5	3.12	6.25	3.12	3.12	3.12
B. subtilis PCI219	12.5	>100	12.5	3.12	3.12	6.25	3.12	6.25	3.12	1.56	3.12
B. cereus ATCC10702	6.25	>100	12.5	3.12	3.12	6.25	3.12	3.12	3.12	3.12	3.12
Corynebacterium bovis 1810	25	>100	25	12.5	12.5	25	6.25	12.5	12.5	6.25	6.25
Escherichia coli K-12	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Shigella dysenteriae JSI1910	50	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Salmonella typhi T-63	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Proteus vulgaris OX19	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa A3	>50	>100	>50	>100	>100	>50	>100	>100	>100	>100	>50
Klebsiella pneumoniae PCI602	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Test organism	Derivartive No.										
	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**～**23** (2:1 mixture)
Staphylococcus aureus FDA209P	3.12	1.56	3.12	0.78	12.5	3.12	3.12	1.56	12.5	25	>100
S. aureus Smith	3.12	3.12	3.12	1.56	12.5	3.12	6.25	1.56	25	25	>100
S. aureus MS9610	6.25	3.12	6.25	1.56	12.5	3.12	6.25	3.12	25	50	>100
S. aureus No. 5 (MRSA)	3.12	3.12	6.25	1.56	25	3.12	6.25	3.12	25	50	>100
S. aureus No. 17 (MRSA)	6.25	3.12	6.25	3.12	25	6.25	6.25	3.12	50	100	>100
Micrococcus luteus FDA16	6.25	3.12	3.12	3.12	25	6.25	6.25	1.56	50	100	>100
M. luteus IFO3333	6.25	6.25	6.25	1.56	25	6.25	6.25	3.12	25	100	>100
M. luteus PC1001	6.25	6.25	6.25	3.12	25	6.25	6.25	3.12	25	50	>100
Bacillus anthracis	1.56	0.78	3.12	1.56	12.5	3.12	3.12	0.78	12.5	12.5	>100
B. subtilis NRRL B-558	3.12	3.12	3.12	3.12	12.5	3.12	3.12	1.56	25	25	>100
B. subtilis PCI219	3.12	3.12	3.12	1.56	12.5	3.12	3.12	1.56	50	25	>100
B. cereus ATCC10702	3.12	1.56	3.12	<0.78	12.5	3.12	6.25	1.56	12.5	25	>100
Corynebacterium bovis 1810	12.5	6.25	12.5	6.25	50	12.5	12.5	3.12	100	100	>100
Escherichia coli K-12	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Shigella dysenteriae JSI1910	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Salmonella typhi T-63	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Proteus vulgaris OX19	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa A3	>100	>50	>50	>100	>100	>100	>50	>100	>100	>100	>100
Klebsiella pneumoniae PCI602	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Table 2. Antibacterial activity of tetrodecamycin derivatives against *P. piscicida*. (MIC μg/ml).

Test organism	Derivative No.*									
	1	**2**	**3**	**10**	**11**	**13**	**20**	**21**	**22**	**23**
Pasteurella piscicida sp. 6395	1.56	50	25	>100	100	100	6.25	6.25	12.5	12.5
P. piscicida sp. 6356	1.56	50	50	>100	100	100	6.25	12.5	0.78	3.12

* Derivatives not shown in this Table did not inhibit the growth of *P. piscicida* at 100 μg/ml.

by the reaction of **1** with phenyl isocyanate and triethyl amine in CH_2Cl_2. The 14-O-alkyl derivatives (**17**~**19**) were converted from **1** by the following steps. Dibutyltin oxide and **1** were refluxed in toluene, yielding stannio acetal.[3] The acetal and alkyl halides in the presence of tetrabutylammonium iodide as a catalyst were allowed to react in toluene at reflux temperature, giving the 14-O-alkyl derivatives.

The addition of alkyl mercaptane at C-5 position was carried out by the treatment of **1** with alkyl mercaptanes in THF to provide 5-S-alkyl-4-hydrotetrodecamycins (**20** and **21**) as an epimeric mixture at C-4. **1** was brominated at the exo-methylene group by the reaction with 4-(dimethylamino)pyridinium bromide perbromide in THF to obtain two epimers of 4(*R*,*S*),5-dibromotetrodecamycin, which were separated by HPLC (Senshu-Pak, silica-5251-S, 20×250 mm) using the mixture of hexane and THF (7:3) to afford pure 4(*S*),5- and 4(*R*),5-dibromo-tetrodecamycin (**22** and **23**) as colorless crystals.

Antibacterial Evaluations

The antibacterial activity of the derivatives (**3**~**23**) was summarized in Tables 1 and 2. The antibacterial activity against *P. piscicida* reduced by the introduction of various functional groups to 14-hydroxyl group of tetrodecamycin (**1**). The 14-O-substituted tetrodecamycin however showed higher antibacterial activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). Among the three types of substituent, the degree of activity was acyl group > ether group > carbamoyl group. In the 14-O-acyl-tetrodecamycins, aryl acyl derivatives were more potent than alkyl acyl ones. Among aryl acyl derivatives, **15** was approximately 10-fold more active than **1**.

On the other hand, the modified derivatives at exo-methylene moiety of **1**, 5-S-alkyl-4-hydrotetrodecamycins (**20** and **21**) and 4,5-dibromotetrodecamycin (**22** and **23**) showed weak antibacterial activity.

The dihydrotetrodecamycin (**2**), which was reduced at the exo-methylene group of **1**, was showed no activity against Gram-positive bacteria at 100 μg/ml and weakly active against *P. piscicida*.

This result suggested that the exo-methylene moiety was important for antibacterial activity.

Absolute Configuration

Among the derivatives of tetrodecamycin (**1**), the brominated compound (**23**) was recrystallized from the mixture of CH_2Cl_2 and hexane solution to give colorless prismatic crystals. For confirmation of the absolute configuration of **1** elucidated by modified Mosher's method[2], **23** was studied by X-ray crystallographic analysis. A crystal was chosen for the X-ray analysis and the absolute structure of **23** was determined. The ORTEP drawing of **23** was shown in Fig. 3.[4] The crystal data were summarized in Table 3.

Fig. 3. Molecular structure of 4,5-dibromotetrodecamycin (**23**).

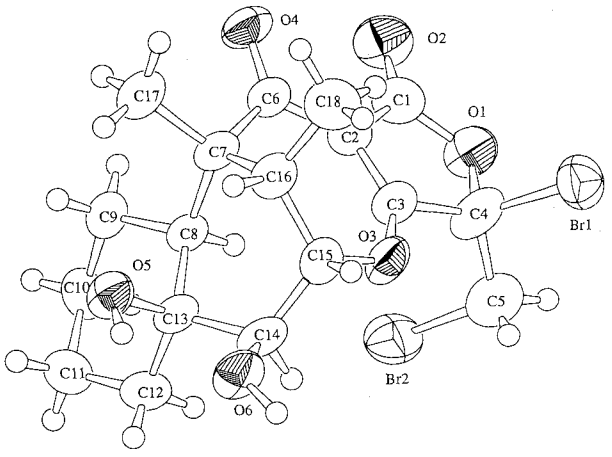


Table 3. Crystal data of **23**.

Emprical formula	$C_{18}H_{22}O_6Br_2$
Formula weight	494.18
Crystal system	Monoclinic
Space group	$P2_1$
Lattice Parameters:	a = 10.471 (2) Å
	b = 8.599 (2) Å
	c = 10.735 (2) Å
	β = 101.17 (1) Å
	V = 948.2 (3) Å^3
Z	2
$D_{calc.}$	1.731 g/cm^3
μ(CuKα)	57.12 cm^{-1}

As a result, it was found that the absolute configuration of **1** agreed with that determined by the modified Mosher's method.

Experimental

General

IR absorption spectra were obtained with a Hitachi 260-30 spectrometer. FAB-MS was obtained on a JEOL JMS-SX102 mass spectrometer. ^{1}H NMR spectra was recorded on JEOL JNM-GX400 spectrometer with TMS as the internal standard. Optical rotations were taken by a Perkin-Elmer 241 polarimeter using a micro-cell (light path 10 cm). MPs were determined on a Yanagimoto micro melting point apparatus.

Measurement of Antibacterial Activity

The minimum inhibitory concentrations (MIC) of

tetrodecamycin (**1**), dihydrotetrodecamycin (**2**) and tetrodecamycin derivatives (**3**~**23**) were examined by serial agar dilution method using Mueller-Hinton agar (Difco) for antibacterial test which was incubated at 37°C for 18 hours and the treble diluted Brain Heart Infusuin agar (Difco) supplemented 2% NaCl for *P. piscicida* test which was incubated at 27°C for 18 hours.

Preparation of 14-O-Acetyltetrodecamycin (**3**)

3 was prepared by treating **1** with acetyl anhydride and pyridine.[2)]

Preparation of 14-O-(2-Naphthoyl)tetrodecamycin (**15**)

A solution of **1** (5 mg, 0.015 mmol) in CH_2Cl_2 (0.5 ml) was cooled to 0°C, and triethyl amine (3.1 μl, 0.022 mmol), catalytic amount of 4-dimethylaminopyridine and 2-naphthoyl chloride (3.7 mg, 0.0195 mmol) were added. This reaction mixture was allowed to stand at room temperature under stirring for 5 hours, and then was diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water and dried over with Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC (Merck, Kiesel gel $60F_{254}$) which was developed with toluene - AcOEt (2 : 1) to give **15** (7 mg, 95%) as white powder: Rf 0.53 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 489 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2950, 1790, 1720, 1680, 1620 sh, 1600; ^1H NMR ($CDCl_3$) δ 1.06 (3H, d, J = 7.32 Hz), 1.16 (1H, m), 1.32 (3H, s), 1.34 (1H, m), 1.45~1.75 (5H, m), 1.81 (1H, m), 1.87 (1H, m), 3.08 (1H, dq, J = 3.90, 7.32 Hz), 4.95 (1H, dd, J = 1.46, 3.90 Hz), 5.07 (1H, d, J = 1.46 Hz), 5.33 (1H, d, J = 2.44 Hz), 5.50 (1H, d, J = 2.44 Hz), 7.60 (1H, br t, J = 8.30 Hz), 7.66 (1H, br t, J = 8.30 Hz), 7.93 (1H, d, J = 8.79 Hz), 7.95 (1H, J = 8.79 Hz), 8.00 (1H, d, J = 8.79 Hz), 8.08 (1H, dd, J = 1.47, 8.30 Hz), 8.66 (1H, s).

The other 14-O-acyltetrodecamycin derivatives (**4**~**14**) were also prepared by the same method.

14-O-Cyclohexanecarbonyltetrodecamycin (**4**):

Rf 0.55 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 445 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2950, 1790, 1740, 1680, 1600; ^1H NMR ($CDCl_3$) δ 1.01 (3H, d, J = 7.32 Hz), 1.1~1.8 (18H, m), 1.26 (3H, s), 1.9~2.0 (2H, m), 2.45 (1H, tt, J = 3.42, 11.72 Hz), 2.90 (1H, dq, J = 3.90, 7.33 Hz), 4.66 (1H, br d, J = 3.90 Hz), 4.69 (1H, br s), 5.28 (1H, d, J = 2.44 Hz), 5.44 (1H, d, J = 2.44 Hz).

14-O-(4-Methoxy)phenylacetyltetrodecamycin (**5**):

Rf 0.43 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 483 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1750, 1680, 1600, 1520; ^1H NMR ($CDCl_3$) δ 0.99 (3H, d, J = 7.33 Hz), 1.1~1.08 (2H, m), 1.24 (3H, s), 1.38~1.65 (6H, m), 1.9 (1H, m), 2.86 (1H, dq, J = 3.90, 7.33 Hz), 3.70 (2H, s), 3.81 (3H, s), 4.66 (1H, dd, J = 0.97, 3.90 Hz), 4.68 (1H, br s), 5.25 (1H, d, J = 2.44 Hz), 5.40 (1H, d, J = 2.44 Hz), 6.90 (2H, d, J = 8.30 Hz), 7.22 (2H, d, J = 8.30 Hz).

14-O-Cinnamoyltetrodecamycin (**6**):

Rf 0.52 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 465 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1720, 1680, 1640, 1600; ^1H NMR ($CDCl_3$) δ 1.03 (3H, d, J = 7.33Hz), 1.1~1.3 (2H, m), 1.29 (3H, s), 1.45~1.85 (7H, m), 2.97 (1H, dq, J = 3.91, 7.33 Hz), 4.82 (1H, dd, J = 1.46, 3.91 Hz), 4.88 (1H, br s), 5.30 (1H, d, J = 2.44 Hz), 5.47 (1H, d, J = 2.44 Hz), 6.54 (1H, d, J = 15.62 Hz), 7.45 (2H, m), 7.56 (2H, m), 7.82 (1H, d, J = 15.62 Hz).

14-O-Benzoyltetrodecamycin (**7**):

Rf 0.53 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 439 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1730, 1680, 1600; ^1H NMR ($CDCl_3$) δ 1.04 (3H, d, J = 7.33 Hz), 1.15~1.35 (2H, m), 1.30 (3H, s), 1.45~1.87 (7H, m), 3.02 (1H, dq, J = 3.91, 7.33 Hz), 4.88 (1H, dd, J = 1.46, 3.91 Hz), 5.00 (1H, d, J = 1.46 Hz), 5.32 (1H, d, J = 2.44 Hz), 5.48 (1H, d, J = 2.44 Hz), 7.52 (2H, m), 7.65 (1H, m), 8.10 (2H, m).

14-O-(4-Fluoro)benzoyltetrodecamycin (**8**):

Rf 0.53 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 457 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1730, 1680, 1600; ^1H NMR ($CDCl_3$) δ 1.04 (3H, d, J = 7.33 Hz), 1.13 (1H, m), 1.29 (3H, s), 1.30 (1H, m), 1.45~1.85 (7H, m), 3.02 (1H, dq, J = 3.91, 7.33 Hz), 4.87 (1H, dd, J = 1.46, 3.91 Hz), 4.98 (1H, d, J = 1.46 Hz), 5.31 (1H, d, J = 2.92 Hz), 5.47 (1H, d, J = 2.92 Hz), 7.19 (2H, d, J = 8.79 Hz), 8.12 (2H, dd, J = 5.37, 8.79 Hz).

14-O-(4-Chloro)benzoyltetrodecamycin (**9**):

Rf 0.57 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 473 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1730, 1680, 1630, 1600; ^1H NMR ($CDCl_3$) δ 1.06 (3H, d, J = 7.33 Hz), 1.23 (1H, m), 1.29 (3H, s), 1.30 (1H, m), 1.40~1.75 (5H, m), 1.70 (2H, m), 3.02 (1H, dq, J = 3.91, 7.33 Hz), 4.95 (1H, d, J = 3.91 Hz), 4.98 (1H, s), 5.31 (1H, d, J = 2.44 Hz), 5.47 (1H, d, J = 2.44 Hz), 7.49 (2H, d, J = 8.30 Hz), 8.03 (2H, d, J = 8.30 Hz).

14-O-(3-Chloro)benzoyltetrodecamycin (**10**):

Rf 0.52 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 473 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1730, 1680, 1600; ^1H NMR ($CDCl_3$) δ 1.05 (3H, d, J = 7.32 Hz), 1.13 (1H, m), 1.30 (3H, s), 1.31 (1H, m), 1.43~1.73 (5H, m), 1.81 (2H, m), 3.04 (1H, dq, J = 3.91, 7.32 Hz), 4.88 (1H, dd, J = 1.46, 3.91 Hz), 4.98 (1H, d, J = 1.46 Hz), 5.32 (1H, d, J = 2.93 Hz), 5.46 (1H, d, J = 2.93 Hz), 7.47 (1H, t, J = 7.82 Hz), 7.63 (1H, ddd, J = 0.98, 1.95, 7.82 Hz), 7.99 (1H, br d, J = 7.82 Hz), 8.06 (1H, br t, J = 1.95 Hz).

14-O-(4-Bromo)benzoyltetrodecamycin (**11**):

Rf 0.61 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 517, 519 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1730, 1680, 1630, 1600; ^1H NMR ($CDCl_3$) δ 1.04 (3H, d, J = 7.32 Hz), 1.05~1.35 (2H, m), 1.29 (3H, s), 1.40~1.82 (7H, m), 3.03 (1H, dq, J = 3.91, 7.32 Hz), 4.87 (1H, dd, J = 1.46, 3.91 Hz), 4.98 (1H, d, J = 1.46 Hz), 5.31 (1H, d, J = 2.93 Hz), 5.47 (1H, d, J = 2.93 Hz), 7.66 (2H, d, J = 8.30 Hz), 8.93 (2H, d, J = 8.30 Hz).

14-O-(4-Methyl)benzoyltetrodecamycin (**12**):

Rf 0.50 (toluene - AcOEt = 2 : 1); FAB-MS (*m/z*) 453 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2950, 1790, 1730, 1680, 1620, 1600; ^1H NMR ($CDCl_3$) δ 1.04 (3H, d, J = 7.82 Hz), 1.1~1.3 (2H, m), 1.30 (3H, s), 1.40~1.9 (7H, m), 2.45 (3H, s), 3.01 (1H, dq, J = 3.91, 7.82 Hz), 4.87 (1H, dd,

J=1.46, 3.91 Hz), 4.98 (1H, d, J=1.46 Hz), 5.31 (1H, d, J=2.45 Hz), 5.47 (1H, d, J=2.45 Hz), 7.31 (2H, d, J=8.30 Hz), 7.98 (2H, d, J=8.30 Hz).

14-O-(4-Methoxy)benzoyltetrodecamycin (**13**):

Rf 0.45 (toluene-acetone=2:1); FAB-MS (m/z) 469 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1720, 1680, 1610, 1600; ^1H NMR ($CDCl_3$) δ 1.04 (3H, d, J=7.82 Hz), 1.13 (1H, m), 1.30 (3H, s), 1.28 (1H, m), 1.42~1.86 (7H, m), 3.01 (1H, dq, J=3.91, 7.82 Hz), 3.90 (3H, s), 4.87 (1H, br d, J=3.90 Hz), 4.97 (1H, br s), 5.31 (1H, d, J=2.45 Hz), 5.48 (1H, d, J=2.45 Hz), 6.98 (2H, d, J=8.78 Hz), 8.04 (2H, d, J=8.78 Hz).

14-O-(2-Naphthoyl)tetrodecamycin (**14**):

Rf 0.53 (toluene-AcOEt=2:1); FAB-MS (m/z) 489 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2950, 1790, 1720, 1680, 1600; ^1H NMR ($CDCl_3$) δ 1.07 (3H, d, J=7.32 Hz), 1.15 (1H, m), 1.31 (3H, s), 1.37 (1H, m), 1.45~1.75 (5H, m), 1.81 (1H, m), 1.93 (1H, m), 3.06 (1H, dq, J=3.90, 7.32 Hz), 4.97 (1H, dd, J=1.46, 3.90 Hz), 5.09 (1H, d, J=1.46 Hz), 5.34 (1H, d, J=2.44 Hz), 5.53 (1H, d, J=2.44 Hz), 7.56 (1H, t, J=7.82 Hz), 7.60 (1H, br t, J=7.82 Hz), 7.69 (1H, m), 7.96 (1H, d, J=7.82 Hz), 8.12 (1H, d, J=7.82 Hz), 8.25 (1H, dd, J=0.98, 7.82 Hz), 8.94 (1H, d, J=7.82 Hz).

Preparation of 14-O-Phenylcarbamoyltetrodecamycin (**16**)

Phenyl isocyanate (2 μl, 0.018 mmol) and triethyl amine (3 μl, 0.021 mmol) were added to a solution of **1** (5 mg, 0.015 mmol) in CH_2Cl_2 (0.5 ml) at room temperature, and this solution was stirred for 16 hours. The reaction mixture was diluted with CH_2Cl_2, and the CH_2Cl_2 solution was washed with water and dried over with Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC (Merck, Kiesel gel $60F_{254}$) which was developed with toluene-AcOEt (2:1) to give **16** (6 mg, 89%): Rf 0.49 (toluene-AcOEt=2:1); FAB-MS (m/z) 454 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 1790, 1740, 1680, 1600; ^1H NMR ($CDCl_3$) δ 1.03 (3H, d, J=7.32 Hz), 1.12 (1H, m), 1.27 (3H, s), 1.32 (1H, br dd, J=4.39, 13.18 Hz), 1.43~1.72 (5H, m), 1.78 (1H, m), 1.91 (1H, m), 2.92 (1H, dq, J=3.91, 7.32 Hz), 4.75 (1H, d, J=1.46 Hz), 4.87 (1H, dd, J=1.46, 3.91 Hz), 5.30 (1H, d, J=2.44 Hz), 5.48 (1H, d, J=2.44 Hz), 6.94 (1H, br s), 7.13 (1H, br t, J=7.32 Hz), 7.36 (2H, d, J=7.32 Hz), 7.43 (2H, br d, J=7.32 Hz).

Preparation of 14-O-Benzyltetrodecamycin (**17**)

A suspension of **1** (12 mg, 0.0359 mmol) and dibutyltin oxide (8.94 mg, 0.0359 mmol) in 1.5 ml of toluene was heated under reflux for 3 hours to give stannylated mixture. The toluene solution of stannylated mixture was cooled to room temperture, and benzyl bromide (5.3 μl, 0.043 mmol) and tetrabutylammonium iodide (13.3 mg, 0.0359 mmol) were added at the same tempreture. The reaction mixture was heated under reflux for 2 hours, and then was diluted with AcOEt. The solution was washed with water and dried over with Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC (Merck, Kiesel gel $60F_{254}$) which was developed with toluene-AcOEt (2:1) to give **17** (7 mg, 53%): Rf 0.57 (toluene-AcOEt=2:1); FAB-MS (m/z) 425 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2950, 1790, 1680, 1600; ^1H NMR ($CDCl_3$) δ 1.01 (3H, d, J=7.33 Hz), 1.0 (1H, m), 1.24 (3H, s), 1.25 (1H, m), 1.6~1.8 (6H, m), 1.97 (1H, m), 2.85 (1H, dq, J=3.42, 7.33 Hz), 3.21 (1H, s), 4.69 (1H, d, J=11.72 Hz), 4.87 (1H, d, J=3.42 Hz), 4.89 (1H, d, J=11.72 Hz), 5.25 (1H, d, J=2.93 Hz), 5.29 (1H, d, J=2.93 Hz), 7.38 (5H, m).

The other 14-O-alkyltetrodecamycin derivatives (**18** and **19**) were obtained the same procedure as described above.

14-O-(4-Bromo)benzyltetrodecamycin (**18**):

Rf 0.49 (toluene-AcOEt=2:1); FAB-MS (m/z) 503, 505 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2950, 1795, 1685, 1600; ^1H NMR ($CDCl_3$) δ 1.01 (3H, d, J=7.81 Hz), 1.02 (1H, m), 1.25 (3H, s), 1.27 (1H, dd, J=3.91, 13.68 Hz), 1.4~1.8 (6H, m), 1.91 (1H, m), 2.86 (1H, dq, J=3.42, 7.81 Hz), 3.19 (1H, s), 4.63 (1H, d, J=11.72 Hz), 4.84 (1H, d, J=3.42 Hz), 4.87 (1H, d, J=11.72 Hz), 5.26 (1H, d, J=2.44 Hz), 5.27 (1H, d, J=2.44 Hz), 7.25 (2H, d, J=8.30 Hz), 7.53 (2H, d, J=8.30 Hz).

14-O-(2-Naphthalene)methyltetrodecamycin (**19**):

Rf 0.28 (toluene-AcOEt=1:1); FAB-MS (m/z) 475 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2940, 1790, 1670, 1595, 1430, 1290; ^1H NMR ($CDCl_3$) δ 1.0 (3H, d, J=7.33 Hz), 0.92~1.01 (2H, m), 1.24 (3H, s), 1.43~1.74 (5H, m), 2.3 (2H, br d, J=12.7 Hz), 2.87 (1H, dq, J=3.42, 7.33 Hz), 3.25 (1H, s), 4.88 (1H, d, J=11.72 Hz), 4.89 (1H, s), 4.90 (1H, d, J=3.42 Hz), 5.03 (1H, d, J=11.72 Hz), 5.14 (1H, d, J=2.45 Hz), 5.17 (1H, d, J=2.45 Hz), 7.46~7.55 (3H, m), 7.79~7.91 (4H, m).

Preparation of 5-(2-Acetamidoethylthio)-4-hydrotetrodecamycin (**20**)

N-Acetylcysteamine (2.5 μl, 0.024 mmol) was added to a solution of **1** (4 mg, 0.012 mmol) in THF (0.5 ml) at room temperature, and this solution was stirred for 2 hours. After removal of the solvent of reaction mixture under reduced pressure, the residue was purified by preparative TLC (Merck, Kiesel gel $60F_{254}$) which was developed with $CHCl_3$-MeOH (10:1) to provide **20** (4.9 mg, 90%): Rf 0.17 ($CHCl_3$-MeOH=10:1); FAB-MS (m/z) 454 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2950, 1770, 1670, 1620, 1430; ^1H NMR ($CDCl_3+CD_3OD$) δ 0.97 and 1.00 (3H, d, J=7.80 Hz), 1.0~1.7 (8H, m), 1.7~1.8 (1H, m), 1.23 and 1.24 (3H, s), 1.97 and 1.98 (3H, s), 1.95~2.05 (1H, m), 2.6~2.8 (4H, m), 2.97~3.23 (2H, m), 3.30~3.40 (1H, m), 3.48 and 3.51 (1H, s), 4.75 (1H, d, J=2.93 Hz), 5.03 (1H, t, J=3.41 Hz).

4-(2-Hydroxyethylthio)-5-hydrotetrodecamycin (**21**)

21 was prepared in the similar manner with **20**: 2:1 epimeric mixture from ^1H NMR; Rf 0.14 ($CHCl_3$-

MeOH = 10 : 1); FAB-MS (*m/z*) 413 $(M+H)^+$; IR ν_{max} ($CHCl_3$) 2950, 1780, 1680, 1620, 1440; ^1H NMR (CD_3OD) δ 0.97 and 1.01 (3H, d, J = 7.81 Hz), 1.05 ~ 1.8 (9H, m), 1.21 (3H, s), 2.06 (1H, br d, J = 13.67 Hz), 2.66 ~ 2.80 (3H, m), 3.04 and 3.15 (1H, dd, J = 3.42, 15.14 Hz), 5-H was overlapping with CD_3OD, 3.53 and 3.55 (1H, s), 3.57 ~ 3.70 (2H, m), 4.80 and 4.81 (1H, d, J = 3.42 Hz), 5.20 and 5.21 (1H, t, J = 3.42 Hz).

Preparation of 4(*S*),5-Dibromotetrodecamycin (**22**) and 4(*R*),5-Dibromotetrodecamycin (**23**)

4-(Dimethylamino)pyridinium bromide perbromide (49 mg, 0.136 mmol) was added to a solution of **1** (41 mg, 0.123 mmol) in THF (2 ml) at room temperature, and this solution was stirred for 18 hours. The reaction mixture was diluted with AcOEt, and the AcOEt solution was washed with waster and dried over with Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography (silica gel 8 ml) which was developed with toluene-acetone (10 : 1 and 5 : 1) to provide the two epimers of 4(*RS*),5-dibromotetrodecamycin (2 : 1 from ^1H NMR) (43 mg, 71%). The epimers were separated by HPLC (Senshu-Pak, silica-5251-S, 20 × 250 mm) using the mixture of hexane and tetrohydrofran (7 : 3) to afford pure 4(*S*),5-dibromotetrodecamycin (**22**) (17.1 mg) and 4(*R*),5-dibromotetrodecamycin (**23**) (3.9 mg) as colorless crystals, respectively.

22: Rf 0.74 (toluene-acetone = 1 : 1); FAB-MS (*m/z*) 493, 495, 497 M^+; mp 164 ~ 167 (dec.); UV λ_{max}^{MeOH} (ε) nm 266 (6000); IR ν_{max} ($CHCl_3$) cm^{-1} 2950, 1800, 1680, 1630, 1430, 1410, 1280; ^1H NMR δ ($CDCl_3$) 1.05 (3H, d, J = 7.32 Hz), 1.10 ~ 1.28 (2H, m), 1.26 (3H, s), 1.36 (1H, dd, J = 3.91, 12.21 Hz), 1.4 ~ 1.5 (2H, m), 1.55 ~ 1.63 (3H, m), 1.79 ~ 1.83 (1H, m), 2.10 (1H, br s), 2.10 ~ 2.14 (1H, m), 2.70 (1H, dq, J = 2.93, 7.32 Hz), 3.21 (1H, br d, J = 5.1 Hz), 3.74 (1H, br d, J = 5.1 Hz), 4.11 (2H, d, J = 11.23 Hz), 4.20 (2H, d, J = 11.23 Hz), 4.93 (1H, d, J = 2.93 Hz); $[\alpha]_D^{24}$ +16.8° (*c* 0.35, MeOH).

23: Rf 0.74 (toluene-acetone 1 : 1); FAB-MS (*m/z*) 493, 495, 497 M^+; mp 204 ~ 207 (dec.); UV λ_{max}^{MeOH} (ε) nm 264 (5600); IR ν_{max} ($CHCl_3$) cm^{-1} 2930, 1800, 1680, 1630, 1420, 1410, 1280; ^1H NMR δ ($CDCl_3$ + 4 drops CD_3OD) 1.03 (3H, d, J = 7.32 Hz), 1.09 ~ 1.18 (2H, m), 1.26 (3H, s), 1.37 ~ 1.65 (6H, m), 1.74 ~ 1.78 (1H, m), 2.00 ~ 2.06 (1H, m), 2.71 (1H, dq, J = 2.93, 7.32 Hz), 3.65 (1H, s), 4.18 (2H, d, J = 11.23 Hz), 4.25 (2H, d, J = 11.23 Hz), 4.91 (1H, d, J = 2.93 Hz); $[\alpha]_D^{24}$ +40.5° (*c* 0.19, MeOH).

X-Ray Crystallography

The single crystals of **23** were obtained from the mixture of CH_2Cl_2 and hexane solution. A colorless prism crystal of $C_{18}H_{22}O_6Br_2$ having approximate dimensions of 0.10 × 0.14 × 0.30 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated CuKα radiation. Crystal data were shown in Table 3. Of the 3347 reflections which were collected, 1730 were unique. No decay correction was applied. An empirical absorption correction using the program DIFABS[5] was applied which resulted in transmission factors ranging from 0.70 to 1.29. The structure was solved by direct method (SHELXS86[6]) and expanded using Fourier techniques (DIRDIF92[7]). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 1677 observed reflections ($I > 2\sigma(I)$) and 235 variable parameters and converged with unweighted and weighted agreement factors of R = 0.038 and Rw = 0.060. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.74 and $-0.48e^-/Å^3$, respectively. Comparing $|Fobs(hkl)|$ // $|Fobs(\bar{h}\bar{k}\bar{l})|$ and $|Fcalc(hkl)|$ // $|Fcalc(\bar{h}\bar{k}\bar{l})|$ for 408 Friedel parirs for which the differences $||Fobs(hkl)| - |Fobs(\bar{h}\bar{k}\bar{l})||$ are greater than 0.5, 383 pairs showed consistently the absolute configuration in Fig. 3. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

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