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TPU-0037-A, B, C and D, Novel Lydicamycin Congeners with Anti-MRSA Activity from *Streptomyces platensis* TP-A0598

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In screening for anti-MRSA antibiotics, novel lydicamycin congeners, TPU-0037-A, B, C and D, were isolated from a culture broth of an actinomycete strain. The producing strain, TP-A0598, was isolated from a seawater sample collected in Toyama Bay, Japan, and identified as *Streptomyces platensis* based on taxonomic characteristics. TPU-0037-A, B, C and D were purified by HP-20 resin, ODS column chromatographies and preparative HPLC, consecutively, and their structures were determined to be 30-demethyllydicamycin, 14,15-dehydro-8-deoxylydicamycin, 30-demethyl-8-deoxylydicamycin and 8-deoxylydicamycin, respectively, by NMR and MS analyses. The new congeners showed antibiotic activity against Gram-positive bacteria including MRSA with the MIC of 1.56~12.5 μg/ml.

Drug-resistance of pathogenic bacteria is a continuously emerging problem being faced by currently used antibiotics. To overcome this problem, it is important to screen for lead compounds with new chemical structures and/or new mode of action. In the course of screening for new bioactive molecules produced by microorganisms from previously ignored sources, we found several new and additional compounds from actinomycetes isolated from live plants[1~4] and marine environment[5~7]. TPU-0037-A, B, C and D are new congeners of lydicamycin[8,9] produced by *Streptomyces platensis* TP-A0598 isolated from a seawater sample collected in Toyama Bay, Japan. The new antibiotics showed *in vitro* anti-MRSA activity with the MIC of 1.56~12.5 μg/ml.

In this paper, we describe the taxonomy of the producing strain, fermentation, isolation, structure determination and biological properties of TPU-0037-A, B, C and D.

Materials and Methods

Microorganism

The producing strain TP-A0598 was isolated from a seawater sample collected 2,600 meters off the shore and 321 meters in depth at Namerikawa, Toyama, Japan by a membrane filter method. A pure culture of strain TP-A0598 was preserved in 20% glycerol at −80°C. It was also maintained at 10°C for laboratory use as a slant on Bennett's agar.

Taxonomy

Taxonomic characteristics of strain TP-A0316 were determined by cultivation on various media described by SHIRLING and GOTTLIEB[10], WAKSMAN[11] and ARAI[12]. Morphological characteristics were observed after incubation of the culture at 30°C for 14 days on oatmeal agar (ISP med. 3) supplemented with 0.2% yeast extract. Cultural characteristics were determined after growth at 30°C for 20 days. The color names and hue numbers were assigned using the Manual of Color Names (Japan Color

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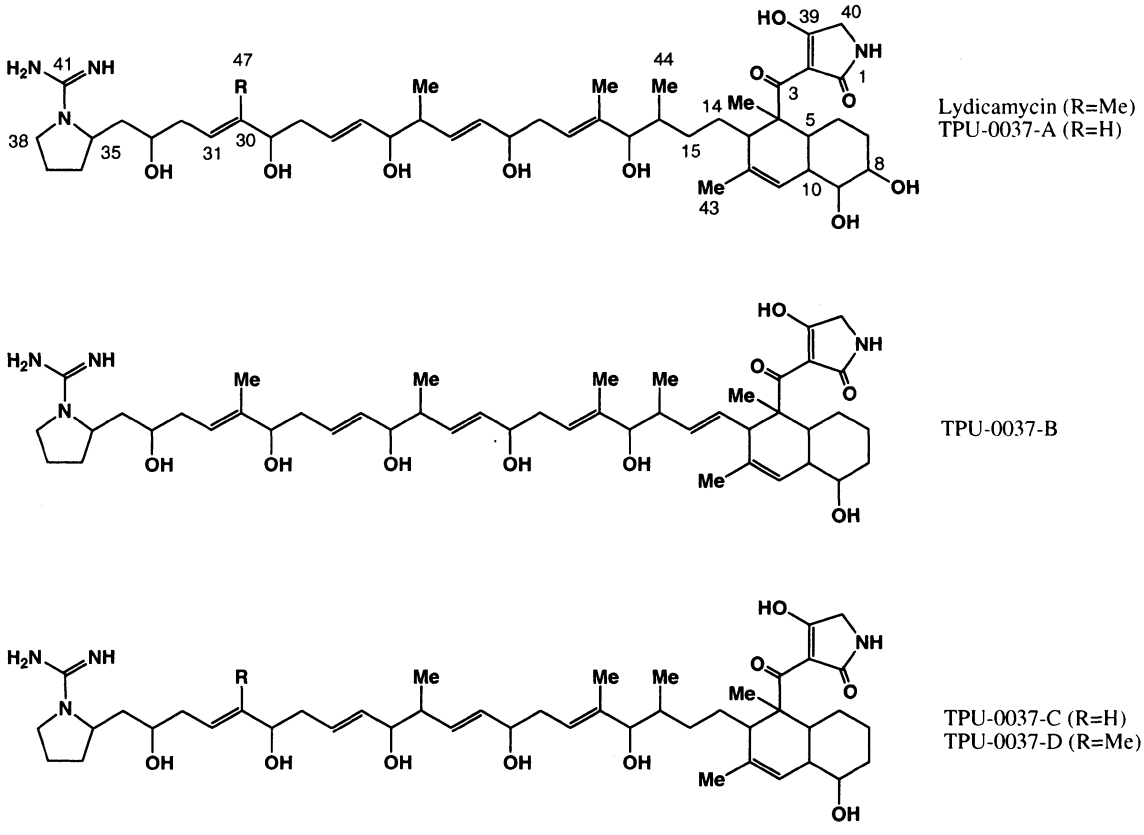
Fig. 1. Structure of lydicamycin congeners produced by *Streptomyces platensis* TP-A0598.



Enterprises Co., Ltd., 1987). The carbon utilization was determined by the method of SHIRLING and GOTTLIEB[10]. Cell wall composition was analyzed by the method of LECHEVALIER *et al.*[13], using thin layer chromatography plates as described by STANECK *et al.*[14]. Fatty acids composition was determined by the method of SUZUKI *et al.*[15].

DNA Sequencing of 16S rDNA

The 16S rDNA fragment was amplified from strain TP-A0598 genomic DNA by using the primers 16S-1 and 16S-2. These PCR primers were prepared on the basis of the consensus sequences of eubacterial 16S rDNA sequences, and the sequences are 16S-1: 5′-GAGAAGCTTAGAGT-TTGATCCTGGCTCAG-3′, and 16S-2: 5′-GAGGAATTC-ACGGCTACCTTGTTACGACT-3′, in which *Eco*RI or *Hin*dIII sites (underline indicated) were created for facilitation of the cloning. PCR was done with PTC-200 DNA Engine (MJ research), and the products were directly used as sequencing templates. Automatic DNA sequencing was carried out with BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems) and primers (16S-1, 16S-2, 16S-4: 5′-GCCTACGAGCTCTTTACGCC-3′, 515F: 5′-GTGCCAGCAGCCGCGGT-3′, 785F: 5′-GGAT-TAGATACCCTGGTAGTC-3′, 1099F: 5′-GCAACGAGC-GCAACCC-3′ and 1115R: 5′-AGGGTTGCGCTCGTTG-3′), and analysed on an ABI PRISM 310 DNA sequencer (Applied Biosystems).

Instrumental Analysis

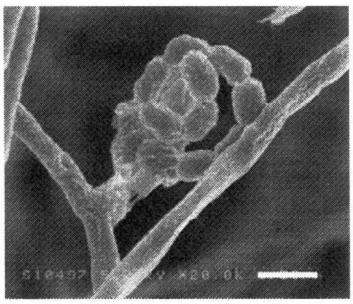
Melting points were determined on a Yanagimoto apparatus and are uncorrected. NMR experiments were performed on a JEOL JNM-LA400 NMR spectrometer in CD_3OD at 30°C. The MS spectra were measured on a JEOL JMS-HX110A spectrometer. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were recorded on a Shimadzu FT IR-300 spectrophotometer. Optical rotations were measured on a Horiba SEPA-300 polarimeter.

Biological Assay

Antibiotic acitivity in fermentation broths and

purification samples were evaluated by the conventional paper disc assay using *Staphylococcus aureus* F597 (MRSA) as an indicator strain. *In vitro* antimicrobial activity was determined by the serial 2-fold agar dilution method using Nutrient Agar (Difco laboratories), pH 7.0. A 5-ml suspension containing 10^5 cells per ml was used as inoculum of the test organisms. The MIC values were determined after the incubation for 18 hours at 32°C.

Fig. 2. Scanning electron micrograph of *Streptomyces platensis* TP-A0598.



Bar represents 1 μm.

Strain TP-A0598 grown on oatmeal agar supplemented with 0.2% yeast extract at 30°C for 10 days.

Results and Discussion

Taxonomy of the Producing Strain

By scanning electron microscope, strain TP-A0597 formed spiral type spore chains and the spores were cylindrical, 0.5×0.9 μm in size, having a warty surface (Fig. 2). The cultural characteristics are summarized in Table 1. The aerial mass color was grayish olive and the color of the reverse side was pale yellow. Diffusible pigments were not formed. Strain TP-A0598 utilized D-glucose, sucrose, inositol, L-rhamnose, D-mannitol, D-raffinose, D-fructose, L-arabinose and D-xylose for growth (Table 2). Whole cell hydrolysates contained L,L-diaminopimelic acid, glycine, ribose and madurose. Fatty acids consisted of 21% 14-methylpentadecanoic acid (*i*-16), 9% 13-methyltetradecanoic acid (*i*-15:0), 8% 12-methyltetradecanoic acid (*a*-15:0) and other minor fatty acids (Table 3). In addition, sequencing analysis revealed that 16S ribosomal DNA from the strain had 99% identity with *Streptomyces platensis* JCM4662^T. Thus the strain was identifeid as *Streptomyces platensis* TP-A0598.

Fermentation

A loopful of a mature slant culture of strain TP-A0598 was inoculated into five 500-ml K-1 flasks containing 100 ml of the seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, tryptone

Table 1. Cultural characteristics of strain TP-A0598.

Medium	Aerial mycelium	Reverse side	Diffusible pigment	Growth
Bennett's agar (Waksman med. 30)	Grayish yellow (156)	Brownish gold (97)	None	Good
Yeast extract - malt extract agar (ISP med. 2)	—	—	—	None
Oatmeal agar (ISP med. 3)	Grayish olive (168)	Pale yellow (127)	None	Good
Inorganic salts - starch agar (ISP med. 4)	Bluish black (431)	Deep orange (81)	None	Good
Glycerol asparagine agar (ISP med. 5)	White (388)	Pale reddish yellow (125)	None	Good
Peptone - yeast extract - iron agar (ISP med. 6)	Pale reddish yellow (126)	Soft reddish yellow (146)	None	Poor
Tyrosine agar (ISP med. 7)	Light reddish yellow (131)	Light reddish yellow (132)	None	Poor
Pridham - Gottlieb agar (ISP med. 9)	Pale yellow (127)	Pale yellow (127)	None	Poor
Yeast starch agar	Grayish yellow (156)	Brownish gold (97)	None	Good

0.5%, K_2HPO_4 0.1%, and $CaCO_3$ 0.3% (pH 7.0). The flask was incubated at 32°C for 5 days on a rotary shaker (200 rpm). Three-ml aliquots of the seed culture were transferred into one hundred 500-ml K-1 flasks each containing 100 ml of the production medium consisting of glycerol 4%, NZ-case 0.5%, pharmamedia 2%, $CaCO_3$ 0.5% and HP-20 1%. The pH of the medium was adjusted to 7.0 before sterilization. Fermentation was carried out at 32°C for 10 days on a rotary shaker (200 rpm).

Table 2. Carbon utilization of strain TP-A0598.

Carbon source	Utilization
D-Glucose	++++
Sucrose	++++
Inositol	++
L-Rammnose	++
D-Mannitol	+++
D-Raffinose	++++
D-Fructose	+++
D-Xylose	+++
L-Arabinose	++
None	–

–: negative, +: positive (32°C, 4 weeks)

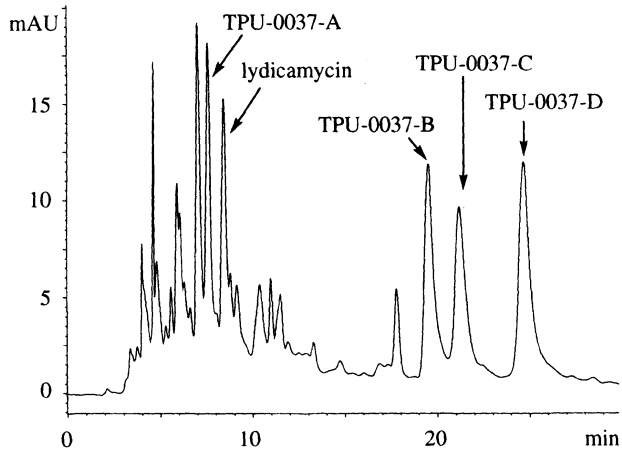
Table 3. Cellular fatty acid composition of strain TP-A0598.

Fatty acid		Composition %
Normal acid	16:0	6
	18:0	2
	$18:1^{9}$	5
	$18:2^{9,12}$	4
Iso acid	15:0	9
	16:0	21
	17:0	3
Anteiso acid	15:0	8

Isolation

The fermented whole broth (10 liters) was centrifuged (8,000 rpm, 10 minutes) to separate the mycelium. The supernatant was discarded and the mycelium was extracted by agitating in 80% acetone (10 liters). The mycelium was removed by centrifugation and the supernatant was applied

Fig. 3. HPLC chromatogram of crude extract.



HPLC conditions: Waters XTerra™ RP18 (250×4.6 mm. i.d.), CH_3CN - 10 mM NH_4HCO_3 (28:72), 0.7 ml/min, UV254 nm

Table 4. Physico-chemical properties of TPU-0037-A (**1**), B (**2**), C (**3**) and D (**4**).

	1	2	3	4
Appearance	Colorless powder	Colorless powder	Colorless powder	Colorless powder
MP	151~153°C	162~164°C	161~163°C	161~163°C
$[\alpha]_D^{28}$	+57.5 (c=1.0, MeOH)	+105.5 (c=1.0, MeOH)	+52.0 (c=0.76, MeOH)	+50.3 (c=1.0, MeOH)
HRFAB-MS				
Found:	841.5322 $[M+H]^+$	837.5376 $[M+H]^+$	825.5377 $[M+H]^+$	839.5527 $[M+H]^+$
Calcd:	841.5326 (for $C_{46}H_{73}N_4O_{10}$)	837.5378 (for $C_{47}H_{73}N_4O_9$)	825.5378 (for $C_{46}H_{73}N_4O_9$)	839.5534 (for $C_{47}H_{75}N_4O_9$)
Molecular formula	$C_{46}H_{72}N_4O_{10}$	$C_{47}H_{72}N_4O_9$	$C_{46}H_{72}N_4O_9$	$C_{47}H_{74}N_4O_9$
UV λ_{max}^{MeOH} nm (log ε)	204 (4.31), 247 (3.95), 282 (3.95)	207 (4.31), 244 (3.95), 283 (3.97)	207 (4.31), 244 (3.96), 281 (3.97)	203 (4.31), 244 (3.95), 282 (3.96)
IR ν_{max} (cm^{-1})	3400, 2930, 1660, 1600	3370, 2930, 1660, 1600	3400, 2930, 1660, 1600	3370, 2930, 1660, 1610
HPLC (Rt)[a]	7.6 min	19.5 min	21.2 min	24.7 min

[a] HPLC conditions: same as shown in Fig. 3.

to a column of HP-20 (1 liter) after removing acetone by evaporation. The column was eluted with a gradient of aqueous acetone and the activity was found in the fractions eluted with 40~80% acetone. The active fractions were combined, evaporated and extracted with *n*-butanol at pH 7. The organic layer was concentrated *in vacuo* to give a crude extract (1.29 g). HPLC analysis of the extract indicated the presence of five components showing a UV spectrum similar to lydicamycin (Fig. 3). The extract (*ca.* 200 mg each time) was then applied to a column of reversed phase silica gel (YMC gel ODS-AM 120-S50; Yamamura Chemical Lab., 0.4 liter) and the column was developed with acetonitrile - 0.15% KH_2PO_4 (pH 3.5) (1 : 4~1 : 1). The active fractions were combined, evaporated, extracted with *n*-butanol at pH 7 and concentrated *in vacuo* to give a brown powder (520 mg). This was further purified by HPLC using an XTerra™ RP_{18} (Waters, 7 μm, 19×300 mm) column with the eluent of acetonitrile - 10 mM

Table 5. 100 MHz ^{13}C NMR data for TPU-0037-A (**1**), B (**2**), C (**3**) and D (**4**) and lydicamycin in CD_3OD.

Position	1	2	3	4	lycicamycin
1	180.94	181.02	180.95	180.92	180.95
2	102.88	102.80	103.27	103.27	102.96
3	203.95	203.76	204.48	204.50	203.93
4	54.36	53.82	55.38	55.29	54.41
5	33.53	34.29	34.63	34.60	33.52
6	23.44	21.65	22.07	22.06	23.38
7	29.57	24.68	25.33	25.29	29.57
8	71.11	30.69	31.58	31.58	71.09
9	75.57	72.88	72.83	72.79	77.60
10	43.75	43.98	43.94	43.98	43.74
11	120.17	122.74	121.48	121.48	120.21
12	141.22	137.71	140.46	140.42	140.14
13	44.97	49.68	44.94	44.93	44.98
14	29.86	133.26	29.80	29.75	29.83
15	37.86	137.62	37.40	37.38	37.84
16	37.86	41.95	37.96	37.92	37.37
17	83.71	83.15	83.81	83.79	83.69
18	139.66	139.22	139.72	139.70	140.40
19	124.06	124.11	124.11	124.12	123.04
20	36.73	36.74	36.77	36.77	36.73
21	73.65	73.64	73.66	73.66	75.54
22	134.32	134.37	134.37	134.38	134.28
23	134.89	134.94	134.85	134.91	134.94
24	43.90	43.98	43.94	43.98	43.95
25	77.53	77.62	77.56	77.63	78.26
26	134.74	134.31	134.76	134.30	134.33
27	129.54	129.87	129.52	129.89	129.87
28	42.13	39.24	42.14	39.23	41.61
29	73.11	78.27	73.12	78.27	73.65
30	136.92	140.43	136.94	140.42	139.64
31	128.02	123.02	128.01	123.04	124.04
32	41.46	37.36	41.49**	37.38	37.84
33	69.86	70.19	69.89	70.19	70.16
34	41.65	41.61	41.66**	41.60	39.23
35	57.52	57.53	57.56	57.53	57.50
36	32.13	32.18	32.16	32.15	32.16
37	24.09	24.11	24.10	24.11	24.11
38	48.19	47.20	48.22	48.21	48.19
39	192.47	192.82	192.94	192.76	192.35
40	50.72	50.69	50.70	50.68	50.69
41	17.84	19.42	17.53	17.53	17.85
42	23.36	23.85	23.36	23.36	23.37
43	17.08	18.49	16.03	17.03	17.09
44	11.94	12.14*	11.86	11.85	11.95
45	16.42	16.61	16.45	16.62	16.37
46	-	11.97*	-	11.96	11.99
47	155.77	155.77	155.79	155.76	155.76

The CD_3OD signal (49.0 ppm) was used as a reference.

*, ** interchangeable

NH_4HCO_3 (25 : 75) at a flow rate of 15.0 ml/minute. With monitoring the UV absorption, five fractions each containing TPU-0037-A, B, C and D and lydicamycin were obtained. TPU-0037-B and C could not be separated when eluted with acidic buffer due to peak broadening. Alkaline buffer was effective to separate two peaks enough with good peak shape. Each fraction was evaporated and the remaining aqueous solution was lyophilized to give TPU-0037-A (5.2 mg), B (33.5 mg), C (12.7 mg) and D (38.1 mg) and lydicamycin (6.7 mg) as colorless powders.

Structure Determination

Physico-chemical properties of TPU-0037-A (**1**), B (**2**), C (**3**) and D (**4**) are summarized in Table 4. Their UV spectra and ^1H and ^{13}C NMR spectra suggested that they were analogs of lydicamycin. Their structures were determined by analyzing the NMR and MS data in comparison with those of lydicamycin.

The molecular formula of **1** was determined as $C_{46}H_{72}N_4O_{10}$ by HRFAB-MS, which gave a $[M+H]^+$ ion at *m/z* 841.5322 (calcd for $C_{46}H_{73}N_4O_{10}$ 841.5326) and NMR data. In the ^1H and ^{13}C NMR and DEPT spectra, a singlet methyl group attaching to an sp^2 carbon was lost and

Table 6. 400 MHz ^1H NMR data for TPU-0037-A (**1**), B (**2**), C (**3**) and D (**4**) and lydicamycin in CD_3OD.

Position	1	2	3	4	lydicamycin*
5	3.06 (1H, m)	3.16 (1H, m)	3.18 (1H, m)	3.17 (1H, m)	3.12
6	1.38, 1.58 (2H, m)	1.38, 1.53 (2H, m)	1.32, 1.45 (2H, m)	1.30, 1.52 (2H, m)	1.42, 1.73
7	1.55, 1.72 (2H, m)	1.45, 1.66 (2H, m)	1.31, 1.60 (2H, m)	1.32, 1.65 (2H, m)	1.62, 1.75
8	3.43 (1H, m)	1.45, 1.53 (2H, m)	1.45, 1.59 (2H, m)	1.46, 1.58 (2H, m)	3.48
9	3.64 (1H, m)	3.73 (1H, m)	3.68 (1H, m)	3.67 (1H, m)	3.69
10	2.48 (1H, m)	2.27 (1H, m)	2.26 (1H, m)	2.27 (1H, m)	2.54
11	4.85 (1H, m)	4.99 (1H, m)	4.96 (1H, m)	4.95 (1H, m)	4.90
13	2.60 (1H, m)	3.48 (1H, m)	2.76 (1H, m)	2.75 (1H, m)	2.66
14	1.10, 1.85 (2H, m)	5.43 (1H, m)	1.11, 1.84 (2H, m)	1.09, 1.83 (2H, m)	1.12, 1.89
15	1.12, 1.86 (2H, m)	5.42 (1H, m)	1.10, 1.74 (2H, m)	1.08, 1.74 (2H, m)	1.19, 1.89
16	1.55 (1H, m)	2.30 (1H, m)	1.52 (1H, m)	1.50 (1H, m)	1.59
17	3.57 (1H, d, 8.3)	3.66 (1H, d, 7.8)	3.55 (1H, d, 8.6)	3.55 (1H, d, 8.3)	3.62
19	5.33 (1H, t, 6.8)	5.37 (1H, m)	5.33 (1H, t, 6.8)	5.33 (1H, t, 6.8)	5.37
20	2.19, 2.36 (2H, m)	2.22, 2.28 (2H, m)	2.20, 2.32 (2H, m)	2.22, 2.30 (2H, m)	2.25, 2.38
21	4.03 (1H, m)	4.03 (1H, m)	4.03 (1H, m)	4.03 (1H, m)	4.08
22	5.48 (1H, m)	5.47 (1H, m)	5.48 (1H, m)	5.43 (1H, m)	5.53
23	5.62 (1H, m)	5.58 (1H, dd, 7.8, 15.6)	5.60 (1H, m)	5.58 (1H, dd, 7.6, 15.4)	5.64
24	2.23 (1H, m)	2.20 (1H, m)	2.26 (1H, m)	2.18 (1H, m)	2.25
25	3.84 (1H, t, 6.6)	3.79 (1H, t, 6.4)	3.84 (1H, t, 6.4)	3.79 (1H, t, 6.3)	3.85
26	5.48 (1H, m)	5.48 (1H, m)	5.48 (1H, m)	5.47 (1H, m)	5.53
27	5.62 (1H, m)	5.52 (1H, m)	5.50 (1H, m)	5.51 (1H, m)	5.57
28	2.21 (1H, m)	2.28 (1H, m)	2.18 (1H, m)	2.29 (1H, m)	2.35
29	4.08 (1H, m)	4.01 (1H, m)	4.08 (1H, m)	4.01 (1H, m)	4.06
30	5.53 (1H, m)	–	5.55 (1H, m)	–	–
31	5.66 (1H, m)	5.42 (1H, m)	5.65 (1H, m)	5.43 (1H, m)	5.47
32	2.20, 2.30 (2H, m)	2.13, 2.27 (2H, m)	2.19, 2.28 (2H, m)	2.14, 2.25 (2H, m)	2.19, 2.33
33	3.66 (1H, m)	3.64 (1H, m)	3.66 (1H, m)	3.65 (1H, m)	3.72
34	1.63, 1.67 (2H, m)	1.63, 1.70 (2H, m)	1.64, 1.67 (2H, m)	1.65, 1.70 (2H, m)	1.68, 1.73
35	4.03 (1H, m)	4.02 (1H, m)	4.02 (1H, m)	4.02 (1H, m)	4.07
36	1.89, 2.08 (2H, m)	1.89, 2.06 (2H, m)	1.89, 2.07 (2H, m)	1.88, 2.06 (2H, m)	1.95, 2.14
37	2.06 (2H, m)	2.06 (2H, m)	2.06 (2H, m)	2.06 (2H, m)	2.09
38	3.36, 3.50 (2H, m)	3.34, 3.50 (2H, m)	3.34, 3.50 (2H, m)	3.34, 3.48 (2H, m)	3.40, 3.53
40	3.52 (2H, m)	3.51 (2H, m)	3.51 (2H, m)	3.51 (2H, m)	3.56
41	1.40 (3H, s)	1.25 (3H, s)	1.32 (3H, s)	1.32 (3H, s)	1.45
42	1.72 (3H, s)	1.65 (3H, s)	1.73 (3H, s)	1.73 (3H, s)	1.77
43	0.76 (3H, d, 6.6)	0.86 (3H, d, 6.6)	0.73 (3H, d, 6.6)	0.73 (3H, d, 6.3)	0.81
44	1.58 (3H, s)	1.60 (3H, s)	1.58 (3H, s)	1.58 (3H, s)	1.63
45	0.95 (3H, d, 6.8)	0.93 (3H, d, 6.8)	0.95 (3H, d, 6.8)	0.92 (3H, d, 6.8)	0.97
46	–	1.62 (3H, s)	–	1.62 (3H, s)	1.67

The CD_3OD signal (3.30 ppm) was used as a reference.

Integral, multiplicities and the coupling constants (Hz) are in parentheses.

* Cited from the data by Hayakawa *et al*[9].

Table 7. *In vitro* antibacterial activities of TPU-0037-A (**1**), B (**2**), C (**3**) and D (**4**) and lydicamycin.

Organism	MIC (μg/ml)				
	1	**2**	**3**	**4**	lydicamycin
Staphylococcus aureus 209P JC-1	3.13	6.25	1.56	3.13	3.13
S. aureus F597 (MRSA)	12.5	12.5	3.13	3.13	6.25
S. aureus A2862 (MRSA)	12.5	12.5	3.13	12.5	6.25
Bacillus subtilis ATCC6633	1.56	6.25	0.39	0.78	0.78
Micrococcus luteus ATCC9341	3.13	12.5	3.13	6.25	3.13
Escherichia coli NIHJ J-2	>50	>50	>50	>50	>50
E. coli RFM795	>50	>50	>50	>50	>50
Proteus mirabillis ATCC2110	>50	>50	>50	>50	>50
P. vulgaris IFO3851	>50	>50	>50	>50	>50
Pseudomonas aeruginosa A3	>50	>50	>50	>50	>50

instead an olefinic proton was shown compared to lydicamycin. HMBC and DQF-COSY confirmed that the methyl group at C-30 was substituted with a hydrogen in **1**. Thus, **1** was determined to be 30-demethyllydicamycin.

The ^{13}C NMR and DEPT spectra indicated that **2** contains two olefinic protons more and one oxygen-bonded methine less, compared to lydicamycin. The HRFAB-MS suggested that the molecular formula of **2** was $C_{47}H_{72}N_4O_9$ ($[M+H]^+$, *m/z* 837.5376, Δ −0.2 mmu). HMBC correlations from 16-Me, H-17 and H-13 to C15 and COSY correlation between H-13 and H-14 established the presence of an olefinic bond between C-14 and C-15. Furthermore, the absence of a hydroxyl group at C-8 was confirmed by COSY correlations between H-9 and H-8. Therefore, **2** was determined to be 14,15-dehydro-8-deoxylydicamycin.

The positive mode FAB-MS of **3** showed $[M+H]^+$ at *m/z* 825, which is 16 mass smaller than that of **1**. The ^{13}C NMR and DEPT spectra suggested that **3** contains one hydroxyl group less compared to **1**, indicating that **3** is a deoxy analog of **1**. 2D NMR analysis revealed that **3** is 30-demethyl-8-deoxylydicamycin.

The HRFAB-MS suggested that the molecular formula of **4** is $C_{47}H_{74}N_4O_9$ ($[M+H]^+$, *m/z* 839.5527, Δ −0.7 mmu), which is 16 mass smaller than that of lydicamycin. The DEPT spectra indicated that **4** contains one hydroxyl group less compared to lydicamycin, and the structure was confirmed to be 8-deoxylydicamycin by 2D NMR data.

Biological Properties

The antimicrobial activities of TPU-0037-A, B, C and D are shown in Table 7 in comparison with lydicamycin. All compounds tested inhibited the growth of Gram-positive bacteria but showed no activities against Gram-negative bacteria and yeast. Compared to lydicamycin, the 30-demethyl analog (**1**) and 14,15-dehydro-8-deoxy analog (**2**) were less potent and the 8-deoxy analog (**4**) was almost equivalent. The 30-demethyl-8-deoxy analog (**3**) showed the most potent anti-MRSA activity among the congeners. Although the influence of 30-methyl and 8-hydroxyl groups on bioactivity is obscure, the presence of C–C double bond between C-14 and C-15 may diminish the activity.

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