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The Synthesis and Antibacterial Activity of Tetracyclic Macrolides

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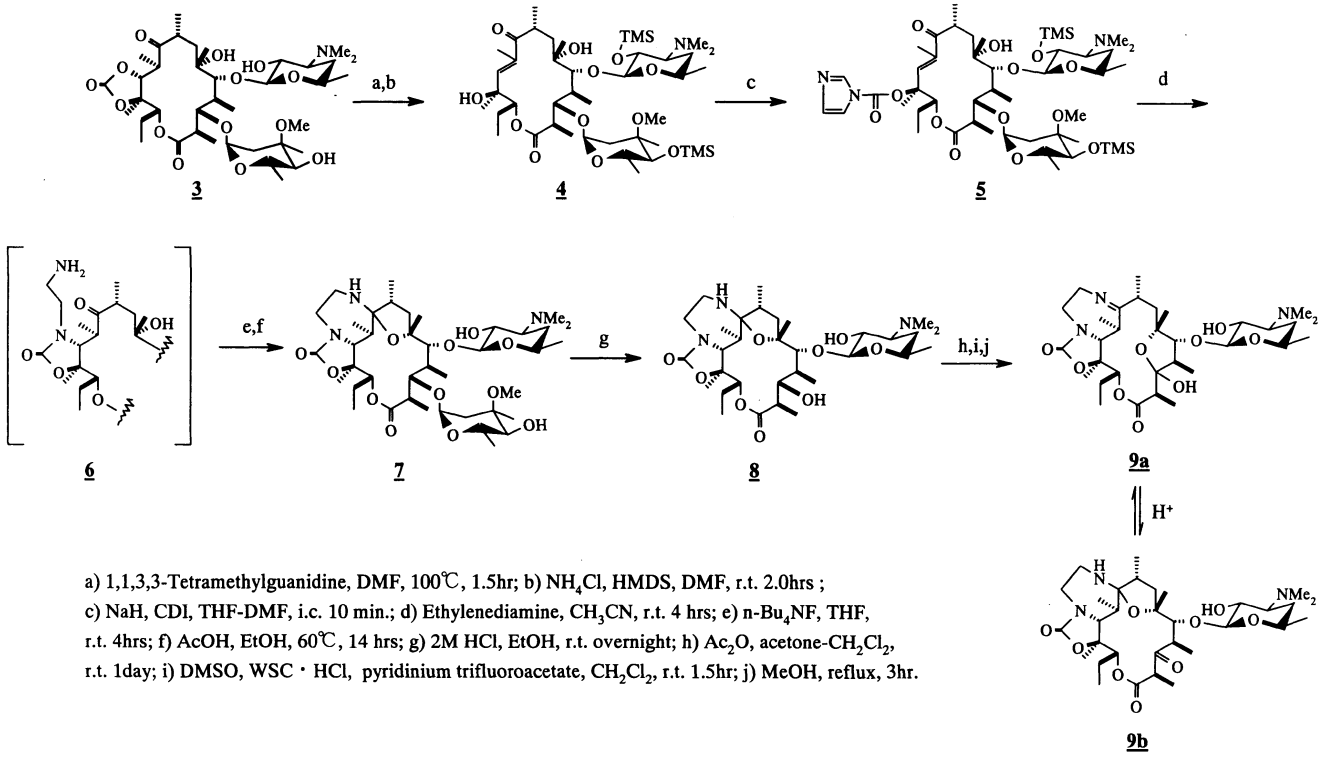
Clarithromycin (**1**)[1] and Azithromycin (**2**)[2], so-called the second-generation macrolides, have greatly expanded the market for macrolide antibiotics worldwide. Following this, the field presently being investigated for the development of new agents[3] is the one which can combat erythromycin resistant pathogens, including the penicillin G and erythromycin A resistant *S. pneumoniae*. We have reported[4] the development of a new ketolide series, which has a unique tricyclic aglycon and excellent antibacterial activity. Owing to its unique skeleton structure and excellent antibacterial activity, the tricyclic ketolide has attracted great attention[5]. Our future plan was to synthesize novel tetracyclic macrolides by using the tricyclic structure as a lead scaffold. We predicted that if tricyclic 6-OH derivatives can be synthesized, they would become tetracyclic macrolides through 6,9-aminal formation. In the present paper, we will describe the preparation and antibacterial activity of the resulting tetracyclic macrolides.

We designed a new synthetic route for tricyclic 6-OH derivatives, as shown in Scheme 1. To avoid the formation of 6,9-hemiacetal, we our plan was to synthesize 10,11-anhydroerythromycin A first. An enone compound **4** was derived from 11,12-cyclic carbonate **3**[6] by means of decarboxylation with 1,1,3,3-tetramethylguanidine (TMG) in DMF, followed by the protection of 2′- and 4″-hydroxyl groups by the trimethylsilyl (TMS) group using 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in DMF. The generated allylic alcohol at a 12-position of **4** was activated with *N*,*N*′-carbonyldiimidazole (CDI), preceded by 11,12-cyclic

Scheme 1. Synthesis of tetracyclic macrolides.



a) 1,1,3,3-Tetramethylguanidine, DMF, 100℃, 1.5hr; b) NH_4Cl, HMDS, DMF, r.t. 2.0hrs ;
c) NaH, CDI, THF-DMF, i.c. 10 min.; d) Ethylenediamine, CH_3CN, r.t. 4 hrs; e) n-Bu_4NF, THF,
r.t. 4hrs; f) AcOH, EtOH, 60℃, 14 hrs; g) 2M HCl, EtOH, r.t. overnight; h) Ac_2O, acetone-CH_2Cl_2,
r.t. 1day; i) DMSO, WSC・HCl, pyridinium trifluoroacetate, CH_2Cl_2, r.t. 1.5hr; j) MeOH, reflux, 3hr.

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carbamation. The obtained 12-*O*-imidazolylcarbonyl compound **5** was converted into tetracyclic carbamate **7** *via* 11,12-cyclic carbamate **6** with ethylenediamine in acetonitrile, followed by a deprotection at 2′- and 4″-positions and the successive cyclization of 9-position by using glacial acetic acid in ethanol. As intermediate **6** was too labile to be isolated, we determined the structure of **7**, the tetracyclic compound. Compound **8** was obtained by the removal of the cladinose of **7** at 3-position. The obtained compound **8** was converted into tetracyclic ketolide **9a** by means of protection at 2′-position as an acetoxy group, followed by modified PFITZNER-MOFFATT oxidation[7] (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC·HCl), DMSO and pyridinium trifluoroacetate (PTA) in dichloromethane (CH_2Cl_2)) and successive deprotection at the 2′-position by means of methanolysis. The 3-hydroxyl compound **8** had three possible positions in the acetylation, 9-NH-, 3-, and 2′-hydroxyl groups. It is well known that the ^1H signal of the base of the hydroxyl group shifts to lowfield by means of the acylation of the hydroxyl group. The chemical shift of 2′-H was 3.27 ppm in **8**, while the chemical shift of acetylated **8** was 4.79 ppm. We were consequently able to determine that the acetylated position was 2′. Moreover, it was revealed that the tetracyclic derivatives **7** and **8** were taking 6,9-aminal formation in $CDCl_3$. That is to say, the chemical shifts of C-9 in **7** and **8** were 99.2 ppm and 98.1 ppm, respectively. These chemical shifts suggested that the compounds were taking the 6,9-aminal formation rather than the 9-imine formation. On the other hand, the chemical shift of C-9 in **9a** was 181.3 ppm and the chemical shift of C-3 was 105.3 ppm. This result showed that the 6,3-hemiacetal formation was more favorable than the 6,9-aminal formation in tetracyclic ketolide **9a**. Interestingly, when a drop of DCl solution was added to the above $CDCl_3$ solution, the signal at 201.3 ppm (C-3) newly appeared and the original signals at 105.3 ppm (C-3) and 181.3 ppm originated in the 9-imine function diasappeared. This fact indicated that **9a** could isomerize into a 6,9-aminal compound (**9b**) and regenerate the 3-keto function in acidic media.

Table 1 shows the *in vitro* activity of tetracyclic macrolides (**7**, **8**, and **9a**) compared to those of **1** against a variety of bacteria. The 3-hydroxyl compound **8** and 6,3-hemiacetal **9a** were less active than **1** against erythromycin-susceptive pathogens, while the activity of compound **7**, which retains cladinose suger at 3-position, was comparable to those of **1** against the same pathogens. We were, however, unable to achieve our final goal of increasing the antibacterial activity against Gram-negative and erythromycin-resistant organisms. Interestingly, **9a** isomerized into **9b** in $CDCl_3$ after the addition of a drop of DCl. This suggests that **9b** could act as a lead compound for the synthesis of promising compounds against some of the pathogens that live in acidic media.

Experimental

IR spectra were recorded using a Paragon 1000 PC FT-IR (Perkin-Elmer). NMR spectra were recorded using a Jeol

Table 1. Antibacteerial activities of tetracyclic macrolides.

Strains	Minimun inhibitory concentration (μg/ml)			
	1	**7**	**8**	**9a**
Micrococcus luteus ATCC9341	0.025	--	1.56	0.78
Bacillus subtillis ATCC6633	0.10	--	12.5	3.13
Staphylococcus aureus 209P-JC	0.10	0.10	25	12.5
S. aureus Smith4	0.20	0.10	25	12.5
S. epidermides IID866	0.10	--	25	12.5
Enterococcus faecalis CSJ1212	0.78	0.78	3.13	1.56
Escherichia coli NIHJ JC-2	100	--	>100	>100
E. coli TM36	6.25	--	100	>100
E. coli K-12	6.25	6.25	--	--
Klebsiella pneumoniae IFO03317	50	25	>100	>100
S. aureus SR138	>100	>100	>100	>100
S. aureus J-109	>100	>100	>100	>100
S. aureus B1	>100	>100	>100	25
S. aureus sp. EMR	>100	>100	>100	>100

Inoculum size : 10^6 cfu/ml.

alpha-500, Jeol lambda-500, or VXR-300 spectrometer. Assignments for proton and carbon signals were based on ^1H-^1H COSY, ^1H-^{13}C COSY, and HMBC experiments. Mass spectra were measured using a Jeol JMS-SX 102. The HRFAB-MS was recorded on a JMS-SX102 (JEOL) mass spectrometer.

2′,4″-Bis-*O*-timethylsilyl-10,11-anhydroerythromycin A(**4**)

1,1,3,3-Tetramethylguanidine (6.6 ml, 52.6 mmol, 2.0 equiv) was added to a solution of **3** (20.0 g, 26.4 mmol) in DMF (100 ml), and the mixture was stirred for 1.5 hours at 100°C. The reaction mixture was poured into H_2O, and extracted with AcOEt (300 ml). The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated. The resulting crude product (18.6 g) was dissolved in DMF (220 ml), and NH_4Cl (1.41 g, 26.4 mmol, 1.0 equiv) and 1,1,1,3,3,3-hexamethyldisilazane (12.0 ml, 57.1 mmol, 2.2 equiv) were added and stirred for 2 hours at 20°C. The reaction mixture was poured into H_2O, and treated in the manner described above. The obtained crude product was purified by silica gel column chromatography (acetone - *n*-hexane - Et_3N (5 : 50 : 1)) to give **4** (17.7 g, 78%) as colorless form. IR (KBr) cm^{-1} 3484, 2973, 2940, 1736, 1672; HRFAB-MS *m/z* 860.5390 ($M+H^+$, calcd for $C_{43}H_{82}NO_{12}Si_2$: *m/z* 860.5376); ^1H NMR (300 MHz, $CDCl_3$) δ 0.09 (9H, s, 2′-OSi$(CH_3)_3$), 0.10 (9H, s, 4″-OSi$(CH_3)_3$), 0.92 (3H, t, J=7.3 Hz, 14-CH_3), 2.03 (3H, d, J=1.1 Hz, 10-CH_3), 2.22 (6H, s, 3′-N$(CH_3)_2$), 3.28 (3H, s, 3″-OCH_3), 4.04 (1H, brs, 12-OH), 4.89 (1H, dd, J=3.3 Hz and 9.9 Hz, 13-H), 6.50 (1H, d, J=1.1 Hz, 11-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.8 (4″-OSi$(CH_3)_3$), 1.0 (2′-OSi$(CH_3)_3$), 40.9 (3′-N$(CH_3)_2$), 49.2 (3″-OCH_3), 138.2 (C-11), 139.2 (C-10), 176.1 (C-1), 210.4 (C-9).

2′,4″-Bis-*O*-timethylsilyl-12-*O*-imidazolylcarbonyl-10,11-anhydroerythromycin A (**5**)

N,*N*′-carbonyldiimidazole (377 mg, 2.33 mmol, 2.0 equiv) and 60% NaH (56 mg, 3.89 mmol, 1.2 equiv) were added to a solution of **4** (1.0 g, 1.16 mmol) in THF-DMF (3 ml-2 ml) and stirred for 10 minutes at 0°C. The reaction mixture was poured into H_2O, and extracted with AcOEt (50 ml). The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated. The resulting crude product was purified by silica gel column chromatography (acetone - *n*-hexane - Et_3N (5 : 50 : 1)) to obtain **5** (680 mg, 61%) as a colorless form. IR (KBr) cm^{-1} 3524, 2974, 2938, 1767, 1727; FAB-MS *m/z* 954 $(M+H)^+$; HRFAB-MS *m/z* 954.5558 ($M+H^+$, calcd for $C_{47}H_{84}N_3O_{13}Si_2$: *m/z* 954.5543); ^1H NMR (300 MHz, $CDCl_3$) δ 0.06 (9H, s, 2′-OSi$(CH_3)_3$), 0.11 (9H, s, 4″-OSi$(CH_3)_3$), 0.92 (3H, t, J=7.2 Hz, 14-CH_3), 1.96 (3H, s, 10-CH_3), 2.21 (6H, s, 3′-N$(CH_3)_2$), 3.23 (3H, s, 3″-OCH_3), 5.46 (1H, dd, J=2.2 Hz and 11.5 Hz, 13-H) 7.03, 7.38, and 8.04 (each 1H, s, imidazole-H), 7.07 (1H, s, 11-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.8 (4″-OSi$(CH_3)_3$), 0.9 (2′-OSi$(CH_3)_3$), 40.9 (3′-N$(CH_3)_2$), 49.0 (3″-OCH_3), 117.5, 130.2, and 137.3 (imidazole-C), 135.7 (C-10), 138.6 (C-11), 146.7 (12-OCO–), 175.9 (C-1), 206.4 (C-9).

9-Deoxo-11-deoxy-9,11-diamino-9-*N*,11-*N*-ethano-6,9-aminalerythromycin A 11,12-cyclic carbamate (**7**)

Ethylenediamine (1.1 g, 17.5 mmol, 10 equiv) was added to a solution of **5** (1.7 g, 1.78 mmol) in CH_3CN (10 ml) and stirred for 4 hours at ambient temperature. The reaction mixture was poured into H_2O, and extracted with AcOEt (50 ml). The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated. The residue was dissolved in THF (15 ml), and *n*-Bu_4NF (0.63 g, 2.41 mmol, 1.4 equiv) was added and stirred for 4 hours at ambient temperature. The reaction mixture was treated in the manner described above. The obtained residue was dissolved in EtOH (11 ml), and glacial acetic acid (0.31 ml, 5.41 mmol, 3.0 equiv) was added and stirred for 14 hours at 60°C. The reaction mixture was poured into H_2O and the pH was adjusted at 10 by 2 N NaOH, and extracted with AcOEt (100 ml). The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated. The obtained crude product was purified by silica gel column chromatography (acetone - *n*-hexane - Et_3N (30 : 50 : 1)) to obtain **7** (0.21 g, 15% from **5**) as a colorless form. IR (KBr) cm^{-1} 3458, 2975, 2941, 1750, 1736; FAB-MS *m/z* 784 $(M+H)^+$; HRFAB-MS *m/z* 784.4948 ($M+H^+$, calcd for $C_{40}H_{70}N_3O_{12}$: *m/z* 784.4960); ^1H NMR (500 MHz, $CDCl_3$) δ 1.51 (3H, s, 6-CH_3), 2.29 (6H, s, 3′-N$(CH_3)_2$), 3.29 (3H, s, 3″-OCH_3), 3.39 (1H, d, J=9.4 Hz, 5-H), 4.25 (1H, d, J=7.0 Hz, 1′-H), 4.27 (1H, s, 11-H), 4.94 (1H, dd, J=21.8 Hz and 10.7 Hz, 13-H), 4.98 (1H, d, J=4.3 Hz, 1″-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 33.0 (6-CH_3), 37.9 (9-NCH_2–), 40.3 (3′-N$(CH_3)_2$), 47.2 (11-NCH_2–), 49.4 (3″-OCH_3), 79.8 (C-3), 84.2 (C-5), 85.0 (C-6), 99.2 (C-9), 156.4 (11-NCOO–), 178.1 (C-1).

9-Deoxo-11-deoxy-5-*O*-desosaminyl-9,11-diamino-9-*N*,11-*N*-ethano-6,9-aminalerythronolide A 11,12-cyclic carbamate (**8**)

2 M HCl solution (2.5 ml) was added to the solution of **7** (0.50 g, 0.64 mmol) in EtOH (2.5 ml) and stirred overnight at ambient temperature. After being neutralized by means of 2 N NaOH solution, the solution was extracted with AcOEt (50 ml), washed with water and brine, dried over

$MgSO_4$, and concentrated. The obtained crude product was purified by silicagel column chromatography ($CHCl_3$-MeOH-28% NH_4OH (100:10:1)) to obtain **8** (0.39 g, 98%) as a colorless form. IR (KBr) cm^{-1} 3396, 2972, 2941, 2880, 1754, 1740; FAB-MS *m/z* 626 $(M+H)^+$; HRFAB-MS *m/z* 626.4019 ($M+H^+$, calcd for $C_{32}H_{56}N_3O_9$: *m/z* 626.4017); ^1H NMR (500 MHz, $CDCl_3$) δ 0.86 (3H, t, J=7.3 Hz, 14-CH_3), 2.26 (6H, s, 3′-N$(CH_3)_2$), 3.66 (1H, s, 5-H), 3.72 (1H, d, J=9.8 Hz, 3-H), 4.31 (1H, s, 11-H), 4.35 (1H, d, J=7.3 Hz, 1′-H), 5.07 (1H, dd, J=2.4 Hz and 11.0 Hz, 13-H); ^{13}C NMR (125 MHz, $CDCl_3$ δ 28.2 (6-CH_3), 39.5 (9-NCH_2–), 40.2 (3′-N$(CH_3)_2$), 47.1 (11-NCH_2–), 80.5 (C-3), 98.1 (C-9), 157.2 (11-NCOO–), 175.6 (C-1).

9-Deoxo-5-*O*-desosaminyl-9,11-diamino-3,11-dideoxy-9-*N*,11-*N*-ethano-3-oxo-6,3-hemiacetalerythronolide A 11,12-cyclic carbamate (**9a**)

Acetic anhydride (0.07 ml, 0.741 mmol, 1.4 equiv) was added to a solution of **8** (0.32 g, 0.512 mmol) in acetone (3 ml) and CH_2Cl_2 (2 ml), and stirred overnight at ambient temperature. The reaction solution was poured into H_2O and the pH was adjusted at **11** by 2 N NaOH, and extracted with AcOEt (30 ml). The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated. The resulting product (0.28 g, 0.420 mmol) was dissolved in CH_2Cl_2 (2 ml), and DMSO (0.30 ml, 4.26 mmol, 10 equiv), WSC·HCl (242 mg, 1.27 mmol, 3.0 equiv), and PTA (243 mg, 1.25 mmol, 3.0 equiv) were added. The mixture was then stirred for 1 hour at ambient temperature. The reaction mixture was treated in the manner described above. The resulting crude product was dissolved in MeOH (5 ml) and refluxed for 3 hours. After the mixture was concentrated again, the obtained crude product was purified by silica gel column chromatography ($CHCl_3$-MeOH-28% NH_4OH (150:10:1)) to give **9a** (0.15 g, 47%) a colorless form. IR (KBr) cm^{-1} 3496, 2974, 2939, 2881, 1760; FAB-MS *m/z* 624 $(M+H)^+$; HRFAB-MS *m/z* 624.3859 ($M+H^+$, calcd for $C_{32}H_{54}N_3O_9$: *m/z* 624.3860); ^1H NMR (500 MHz, $CDCl_3$) δ 0.86 (3H, t, J=7.3 Hz, 14-CH_3), 1.42 (3H, s, 12-CH_3), 1.42 (3H, s, 6-CH_3), 2.29 (6H, s, 3′-N$(CH_3)_2$), 2.69 (1H, q, J=7.3 Hz, 2-H), 3.69 (1H, d, J=4.9 Hz, 5-H), 3.73 (1H, s, 11-H), 4.14 (1H, s, 3-OH), 4.96 (1H, dd, J=1.8 Hz and 11.0 Hz, 13-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.8 (6-CH_3), 40.4 (3′-N$(CH_3)_2$), 42.5 (9-NCH_2–), 50.0 (11-NCH_2–), 95.1 (C-5), 104.6 (C-1′), 105.3 (C-3), 156.0 (11-NCOO–), 176.9 (C-1), 181.3 (C-9).

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