THE *IN VITRO* PROFILE OF SELECTED 14-MEMBERED AZALIDES

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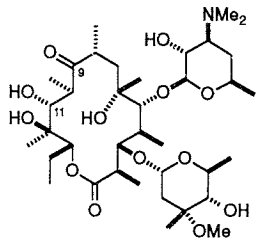
The *in vitro* antimicrobial potency of 10-aza-9-deoxo-11-deoxyerythromycin A, the first member of a new class of macrolide antibiotic, was determined. Several other members of this family of azalide were prepared and similarly screened in order to begin to define the antibiotic potential of the class. The results indicate that the SAR for this structural type parallels that of other macrolides and that it offers no apparent benefit over known 15-membered azalides.

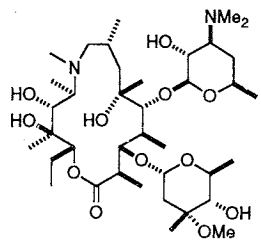
Erythromycin A (**1**) is one of the great survivors in the antibiotic panoply. While it is unlikely to be wholly supplanted in the near future, new semi-synthetic derivatives have emerged recently as somewhat improved versions and have served to rejuvenate the whole area of macrolide research. One of these newcomers, azithromycin (**2**)[1~3]—the prototypical azalide[3]—is of particular interest in that it displays significant potency against Gram-negative pathogens that has not, as yet, been matched by any other macrolide.[4] We recently disclosed the preparation of a new class of macrolide antibiotic, the 14-membered azalides, exemplified by the parent structure (**3**).[5] These agents are a conceptual amalgam of the old (**1**) and the new (**2**). We now wish to report the *in vitro* antibacterial profile of a number of representative members of the class.

Chemistry

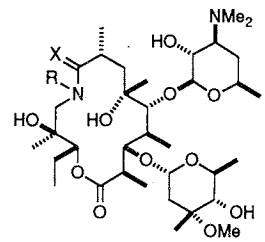
The preparation of 10-*N*-methyl azalide (**3**) and lactam (**4**) has been described.[5] These materials provided our first glimpse of the antimicrobial potential of this class of macrolide. Nonetheless, given that these compounds present a new molecular format for the macrolides it was important to determine whether the established structure-activity relationships for the erythromycins and azithromycin extended to this aglycone. A number of representative derivatives were selected with this in mind. For the most part the chemistry employed reflects existing macrolide technology and need not be discussed in detail. The starting point in many cases was the 3′-*N*-protected synthetic intermediate (**6**).[5] 10-*N*-Substitution was probed by preparation of the cyanoethyl derivative (**9**)[3] (i. acrylonitrile (**6** to **8**), ii. 6% Na/Hg, iii.



Erythromycin A (**1**)

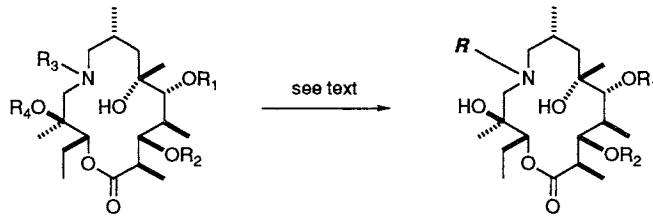


Azithromycin (**2**)



3 X=H,H, R=Me
4 X=O, R=H

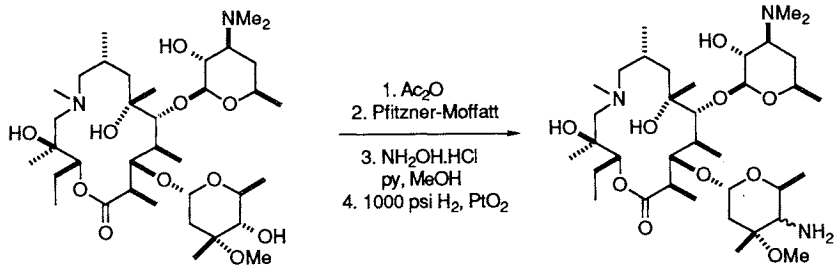
Scheme 1.
R′=desosamine
R_2=cladinose
R″=3′-*N*-demethyl-3′-*N*-phenylsulfonyl desosamine



5 $R_3, R_4 = -CH_2-$, R_1=R′
6 $R_3 = R_4$=H, R_1=R″

7 R=H, R_1=R′
8 R=CH_2CH_2CN R_1=R″
9 R=CH_2CH_2CN R_1=R′
10 R=$CH_2CH_2CH_2NH_2$ R_1=R′
11 R=Ac R_1=R″
12 R=Ac R_1=R′

Scheme 2.



3 **13**

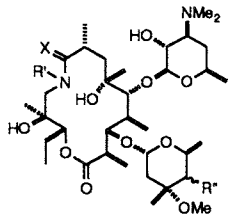
HCHO-HCO_2H (**8** to **9**)), the aminopropyl derivative (**10**)[3] ($NaBH_4$-$CoCl_2$ (**9** to **10**)) and the acetyl derivative (**12**) (i. Ac_2O-py (**6** to **11**), ii. 6% Na/Hg, iii. HCHO-HCO_2H (**11** to **12**)), together with the unsubstituted, secondary amine (**7**) (AcOH-THF-H_2O-ethanolamine (**5**[5] to **7**)). Variation at the 4″-position was confined to preparation of the primary amine (**13**) (Scheme 2), a substitution that has been employed in a number of aglycone systems and generally has a pronounced effect on the *in vitro* performance.[3,6,7] Modifications at other sites of the macrolides/azalides have been less frequently addressed and would consequently be of little value for our current purpose.

Microbiological Activity

Preliminary *in vitro* antimicrobial screening relied on a standard broth microdilution assay for the determination of MIC's. The results are presented in Table 1. The control data (**1**/**2**) reflects the established erythromycin A/azithromycin relationship[3]; the 15-membered azalide carries a slight handicap with regard to the Gram-positive organisms† (although this may not be clinically significant) but reveals its *in vitro* strengths against the Gram-negative strains. The 14-membered azalide (**3**) shows a similar pattern of activity. However the increase in MIC's against the Gram-positive organisms is fractionally more pronounced and the decrease in MIC's against the Gram-negative strains less pronounced.† The

† These differences are consistent and are maintained across an expanded panel of organisms.

Table 1. Comparative *in vitro* antibacterial activities of 14-membered azalides.



	X	R′	R″
3	H,H	Me	(*S*)-OH
4	O	H	(*S*)-OH
7	H,H	H	(*S*)-OH
9	H,H	$-(CH_2)_2CN$	(*S*)-OH
10	H,H	$-(CH_2)_3NH_2$	(*S*)-OH
12	H,H	Ac	(*S*)-OH
13	H,H	Me	(*R*/*S*)-NH_2

Microorganism	MIC (μg/ml)*								
	1†	**2**††	**3**	**4**	**7**	**9**	**10**	**12**	**13**
Enterococcus faecalis MB 5407	1～2	2～4	4	>128	4	8	16	4	4
Staphylococcus aureus MB 2865	0.25	0.5～1	2～4	>128	8	32	4	16	4
S. haemolyticus MB 5412	0.125	0.25	0.5	>128	1	2	1	2	0.5
Streptococcus pyogenes MB 2874	0.015	0.03	0.125	>128	0.25	1	0.25	1	0.125
S. pyogenes MB 5403 (r. con)**	>128	>128	>128	>128	>128	>128	>128	>128	>128
S. pyogenes MB 5406 (r. ind)***	32	16	16	>128	32	32	64	32	16
Enterobacter cloacae CL 4298	32～64	0.5～1	4～8	>128	16	128	128	>128	0.5
Escherichia coli MB 2884	32～64	1～2	4～8	>128	8	>128	64	>128	4
Klebsiella pneumoniae MB 4005	32～64	2	8	>128	8	128	64	128	2
Haemophilus influenzae MB 5363	2～4	0.25～1	2～8	>128	4	32	16	64	1
Pseudomonas stutzeri MB 1231	0.25～1.0	0.03～0.06	0.25	128	0.25	16	2	8	0.5

* Determined by a standard broth microdilution assay.

** r. con: Constitutive macrolide resistance.

*** r. ind: Inducible macrolide resistance.

†Erythromycin A.

††Azithromycin.

10-*N*-substitution group (compounds **3**, **7**, **9**, **10**, **12**) shows variations in activity that are essentially coherent with the SAR in the 15-membered azalide series.[3] The *N*-methyl substitution, as for azithromycin, appears to be appropriate but it is important to note that we cannot claim to have determined the "optimal" substitution from such a limited study. The 4''-amino derivative (**13**) displays the *in vitro* improvements characteristic of this modification—that is decreased MIC's against the majority of Gram-negative organisms while the Gram-positive profile is barely perturbed. Lactam (**4**) is inactive.

Discussion

The SAR for this nucleus clearly correlates with the established macrolide pattern. As a working platform, the framework is inferior to the 15-membered azalides. One might predict that the *in vivo* performance of **3** would emphasize its improvement over erythromycin as a consequence of greater acid stability[8] and changes in pharmacokinetic parameters. However one could not expect advances on azithromycin since both materials would likely display analogous *in vivo* behavior.[9]

It is difficult to draw any definitive conclusions from this data concerning the role of the aglycone amine. The general conception of the amine is that by increasing the overall hydrophilicity of the macrolide it influences bacterial membrane penetration. That these new azalides show a similar pattern of activity even though the amine is positionally altered may support this view of a quiescent, non-specific role. The drop in potency observed for **3** compared to azithromycin may be associated with the lack of the peripheral hydroxy residue at the 11-position. We have reason to believe that the 11-hydroxy group is not a "major" determinant of bioactivity for the azalides (A. B. JONES and C. M. HERBERT, unpublished results) but may play a role commensurate with the observed disparity. Nonetheless we cannot rule out the possibility that the presence of the amine, or subtle conformational changes associated with ring size, make a more specific contribution at the level of ribosomal binding and that the 15-membered format is intrinsically preferable in this regard.

The catastrophic loss of activity in the case of lactam (**4**) cannot be reconciled with a simple change in hydrophilicity but more likely reflects a significant conformational change that totally abrogates ribosomal binding. This is underscored by the far less dramatic drop off observed for the acetyl derivative (**12**).

Experimental

General

Infrared spectra were recorded on a Perkin Elmer 1420 spectrophotometer. ^{1}H NMR were recorded on a Varian XL-400 spectrometer. Mass spectra were recorded on a MAT-731 or JOEL HX-110 mass spectrometer. Microanalyses were performed by Robertson Laboratories, Inc. Erythromycin A was obtained from Aldrich Chemical Co., Inc. All reaction solvents were anhydrous and were obtained from Aldrich Chemical Co., Inc. except for THF which was dried by distillation under nitrogen from sodium benzophenone ketyl. All reactions were performed under dry nitrogen unless otherwise stated. Chromatography was performed on EM Science Silica Gel 60 (230~400 mesh ASTM) or Fisher Scientific Sorbosil C60 40/60H. Thin layer chromatography was performed on Merck Silica Gel 60/Kieselguhr F-254.

10-Aza-10-demethyl-9-deoxo-11-deoxyerythromycin A (7)

Aminal (**5**) (35 mg, 50 μmol) was dissolved in AcOH-THF-H_2O 3:1:1 (1.0 ml, 0.05 N) and ethanolamine hydrochloride (24 mg, 5 equiv) added. After 16 hours the mixture was basified with aq K_2CO_3 and extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and concentrated. The residue, consisting of a mixture of aminal and secondary amine (**7**), was re-subjected to the reaction conditions for a further 16 hours and once again extractively isolated. A third iteration was carried out. The resulting residue was chromatographed (CH_2Cl_2-MeOH-NH_4OH 95:5:1 to 90:10:1) to give the secondary amine (**7**) (9 mg, 26%) as an oil. Lyophilization from benzene produced a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (1H, dd, J=11.0, 1.2 Hz, H-13), 4.77 (1H, d, J=7.2 Hz, H-1''), 4.65 (1H, d, J=2.3 Hz, H-3), 4.52 (1H, d, J=4.8 Hz, H-1'), 3.98~3.90 (2H, m, H-5, H-5''), 3.59 (1H, m, H-5'), 3.35~3.20 (6H, m, including

3.33 (s) OMe), 3.05 (1H, t, J=9.4 Hz, H-4″), 3.02 (1H, d, J=12.4 Hz, H-11), 2.55~2.47 (2H, m, H-2, H-3′), 2.45 (1H, t, J=10.8 Hz), 2.36~2.23 (9H, m, including 2.27 (s) NMe_2), 2.18 (1H, d, J=9.6 Hz), 1.98 (1H, m, H-8), 1.82 (1H, m, H-4), 1.68~1.50 (4H, m, H-7, H-14, H-4′, H-2″), 1.37 (1H, m, H-14), 1.29~1.07 (26H, m) and 0.86 (3H, t, J=7.3 Hz, 3H-15) ppm. HRFAB-MS: Calcd for $C_{35}H_{67}N_2O_{11}$ (MH^+), m/z 691.4743; Found m/z 691.4758.

10-Aza-10-(2-cyanoethyl)-10-demethyl-9-deoxo-11-deoxyerythromycin A (**9**)

Azalide (**6**) (50 mg, 61 μmol) was dissolved in acrylonitrile (0.7 ml, 0.09 N) and heated to 60°C. After 12 hours (TLC CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) the mixture was cooled to room temperature and concentrated. The residue was chromatographed (CH_2Cl_2 - MeOH 95 : 5) to give the cyanoethyl derivative (**8**) (48 mg, 90%) as a glass. Lyophilization from benzene gave a white powder. This material was dissolved in THF - MeOH 1 : 1 (1.0 ml, 0.06 N) and potassium dihydrogen phosphate (263 mg, 35 equiv) added. The mixture was cooled to −20°C and freshly ground 6% sodium amalgam (529 mg, 25 equiv) added. After 30 minutes a further 35 equiv of potassium dihydrogen phosphate and 25 equiv of sodium amalgam were added. After a further 30 minutes (TLC CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) the mixture was decanted into aqueous potassium carbonate solution. The amalgam residue was washed several times with ethyl acetate and decanted each time into the aqueous mixture. The aqueous was extracted with ethyl acetate and the combined organic extracts dried over magnesium sulfate, filtered and concentrated. The residue was dissolved in chloroform (1.0 ml, 0.09 N) and formaldehyde (9 μl of 37% aq solution, 2.0 equiv) added, followed by formic acid (2 μl, 1.0 equiv). The mixture was heated to 60°C. After 40 minutes (TLC CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) it was cooled to room temperature and diluted with potassium carbonate solution. The resulting mixture was extracted with methylene chloride, dried over magnesium sulfate, filtered and concentrated. The residue was chromatographed (CH_2Cl_2 - MeOH 95 : 5 to CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) to give the fully deprotected cyanoethyl azalide (**9**) (28 mg, 68%) as a clear oil. Lyophilization from benzene gave a white powder. ^1H NMR (400 MHz; $CDCl_3$) δ 4.84 (1H, dd, J=10.5, 2.9 Hz, H-13), 4.74 (1H, t, J=4.0 Hz, H-1″), 4.63 (1H, d, J=7.3 Hz, H-1′), 4.50 (1H, br s, OH), 4.38 (1H, br s, H-3), 4.03 (1H, m, H-5″), 3.80 (1H, d, J=3.3 Hz, H-5), 3.63 (1H, m, H-5′), 3.31 (1H, dd, J=10.3, 7.4 Hz, H-2′), 3.28 (3H, s, OMe), 3.18 (1H, br s, OH), 3.06 (1H, d, J=7.7 Hz, H-4″), 2.99~2.81 (2H, m), 2.69 (1H, d, J=13.9 Hz, H-11), 2.63~2.41 (6H, m), 2.29 (6H, s, NMe_2), 2.26~2.20 (2H, m), 1.80 (2H, m, H-4, H-8), 1.68~1.54 (4H, m, H-7, H-14, H-4′, H-2″), 1.36 (1H, m, H-14), 1.27~1.11 (23H, m), 1.07 (3H, d, J=7.0 Hz) and 0.86 (3H, t, J=7.4 Hz, 3H-15) ppm. FAB-MS (Li spike) m/z 751 (M^+ + Li).

Anal Calcd for $C_{38}H_{69}N_3O_{11}$: C 61.35, H 9.35, N 5.65.
Found: C 61.23, H 9.27, N 5.43.

10-(3-Aminopropyl)-10-aza-10-demethyl-9-deoxo-11-deoxyerythromycin A (**10**)

Cyanoethyl azalide (**9**) (15 mg, 20.2 μmol) was dissolved in methanol (1.0 ml, 0.02 N) and cobalt (II) chloride (9.6 mg, 2.0 equiv) added. The mixture was cooled to 0°C and sodium borohydride (7.6 mg, 10.0 equiv) added portionwise. After 30 minutes (TLC CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) the mixture was filtered through Celite, eluting with ethyl acetate. The filtrate was diluted with aqueous potassium carbonate solution and partitioned. The aqueous was re-extracted with ethyl acetate and the combined organic extracts dried over magnesium sulfate, filtered and concentrated. The residue was chromatographed (CH_2Cl_2 - MeOH - NH_4OH 90 : 10 : 1) to give the aminopropyl derivative (**10**) (5.5 mg, 36%) as a white foam. Lyophilization from benzene gave a white powder. ^1H NMR (400 MHz; $CDCl_3$) δ 4.79 (1H, dd, J=4.5, 2.7 Hz, H-1″), 4.76 (1H, dd, J=9.2, 3.3 Hz, H-13), 4.58 (1H, d, J=7.3 Hz, H-1′), 4.24 (1H, br t, J=3.4 Hz, H-3), 4.05 (1H, m, H-5″), 3.83 (1H, d, J=3.3 Hz, H-5), 3.62 (1H, m, H-5′), 3.34 (1H, dd, J=10.3, 7.3 Hz, H-2′), 3.28 (3H, s, OMe), 3.04 (1H, d, J=8.5 Hz, H-4″), 2.86~2.73 (2H, m), 2.70~2.49 (6H, m), 2.40 (1H, d, J=13.7 Hz), 2.30 (6H, s, NMe_2), 2.29~2.17 (2H, m), 2.11 (1H, dd, J=12.5, 4.0 Hz), 1.81 (1H, m), 1.75~1.52 (7H, m), 1.36 (1H, m, H-14), 1.30~1.10 (23H, m), 1.06 (3H, d, J=7.1 Hz) and 0.87 (3H, t, J=7.5 Hz, 3H-15) ppm. HRFAB-MS: Calcd for $C_{38}H_{73}LiN_3O_{11}$ (M^+ + Li) m/z 754.5405; Found m/z 754.5379.

10-Acetyl-10-aza-10-demethyl-9-deoxo-11-deoxyerythromycin A (**12**)

Azalide (**6**) (40.0 mg, 49 μmol) was dissolved in pyridine (0.5 ml, 0.1 N) at room temperature and acetic

anhydride (4.6 μl, 1.0 equiv) added. After 4 hours (TLC EtOAc) the mixture was diluted with aq potassium carbonate solution and extracted with methylene chloride. The combined organics were dried over magnesium sulfate, filtered and concentrated. The residue was submitted to the desulfonylation/remethylation procedure outlined above for compound (**9**). The crude material was chromatographed (CH_2Cl_2 - MeOH 95 : 5 to CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) to give amide (**12**) (11 mg, 31%) as a clear oil. Lyophilization from benzene gave a white powder. ^1H NMR (400 MHz; $CDCl_3$) δ 4.81 (1H, dd, J=10.2, 3.3 Hz, H-13), 4.71 (1H, br d, J=3.3 Hz, H-1″), 4.64 (1H, d, J=7.3 Hz, H-1′), 4.38 (1H, br t, 2.9 Hz, H-3), 4.31 (1H, br s, OH), 4.22 (1H, br s, OH), 4.04 (1H, dq, J=8.9, 6.5 Hz, H-5″), 3.80 (1H, d, J=3.7 Hz, H-5), 3.68 (1H, d, J=14.3 Hz, H-11), 3.61 (1H, m, H-5′), 3.37～3.23 (6H, m, H-9, H-11, H-2′, OMe), 3.10 (1H, dd, J=13.6, 10.3 Hz, H-9), 3.04 (1H, t, J=7.8 Hz, H-4″), 2.61～2.50 (2H, m, H-2, H-3′), 2.35～2.23 (8H, m, H-2″, 4″-OH, NMe_2), 2.15 (1H, br m, H-8), 2.10 (3H, s, Ac), 1.87～1.75 (2H, m, H-4, H-14), 1.67 (1H, br d, J=13.0 Hz, H-4′), 1.61～1.35 (4H, m, 2H-7, H-14, H-2″), 1.30～1.19 (13H, m, H-4′, Me, 2-Me, 5′-Me, 5″-Me), 1.17 (3H, s, Me), 1.15 (3H, s, Me), 1.09 (3H, d, J=7.5 Hz, 4-Me), 1.07 (3H, d, J=6.6 Hz, 8-Me) and 0.86 (3H, t, J=7.4 Hz, 3H-15) ppm. IR (film) cm^{-1} 3450, 2970, 2940, 1730, 1625.

Anal Calcd for $C_{37}H_{68}N_2O_{12}$: C 60.63, H 9.35, N 3.82.
Found: C 60.44, H 9.10, N 3.62.

4″-Amino-10-aza-9-deoxo-11,4″-dideoxyerythromycin A (**13**)

Azalide (**3**) (80 mg, 0.11 mmol) was dissolved in ethyl acetate (2.0 ml, 0.06 N) at room temperature. Acetic anhydride (16 μl, 1.5 equiv) was added. After 12 hours (TLC CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) aq potassium carbonate solution was added and the mixture extracted with ethyl acetate. The combined organic extracts were dried over magnesium sulfate, filtered and concentrated to give the 2′-acetyl derivative (75 mg, 88%) as a white foam that required no further purification. This material was dissolved in methylene chloride (2.0 ml, 0.05 N) at room temperature. Methyl sulfoxide (70 μl, 10.0 equiv) was added, followed by 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (95 mg, 5.0 equiv) and finally pyridinium trifluoroacetate (96 mg, 5.0 equiv). After 3 hours (TLC CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) ethyl acetate was added followed by water. The aqueous was adjusted to pH 10 with 1.0 N sodium hydroxide solution and extracted with ethyl acetate. The organic extracts were dried over magnesium sulfate, filtered and concentrated. Chromatography (CH_2Cl_2 - MeOH 95 : 5 to CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) gave 4″-keto derivative (38 mg, 51%) as a white foam, together with unreacted starting material (18 mg, 24%). The 4″-keto derivative was dissolved in methanol (1.0 ml, 0.05 N) at room temperature. Pyridine (43.5 μl, 10 equiv) was added, followed by hydroxylamine hydrochloride (18.7 mg, 5 equiv). After 24 hours (TLC CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) the mixture was diluted with ethyl acetate and water and the aqueous adjusted to pH 9～10 with 1.0 N sodium hydroxide solution. The aqueous was extracted with ethyl acetate and the combined organics dried over magnesium sulfate, filtered and concentrated. The residue was chromatographed (CH_2Cl_2 - MeOH 95 : 5 to CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) to give partially purified 2′-hydroxy-4″-oximino derivative (33 mg) as a white foam. This was dissolved in acetic acid (1.0 ml, 0.05 N) and platinum oxide (30 mg, ～1 weight equiv) added. The mixture was hydrogenated at room temperature and 70 kg/cm^3 (1000 psi) of hydrogen for 72 hours. The mixture was filtered through Celite, eluting with methylene chloride, and concentrated. The residue was re-concentrated twice from methylene chloride - heptane. Chromatography (CH_2Cl_2 - MeOH - NH_4OH 95 : 5 : 1) gave the amine (**13**) (27 mg, 71% over two steps, 1 : 1 mixture of 4″-stereoisomers) as a white foam. Lyophilization from benzene provided a white powder. ^1H NMR (400 MHz; $CDCl_3$) δ 4.82 (1H, overlapping dd, J=10.7, 2.7 Hz, H-13), 4.71 (1H, m, H-1″), 4.55 (1½H, m, H-1′, H-5″($\frac{1}{2}$)), 4.47 ($\frac{1}{2}$H, br m, H-3), 4.44 ($\frac{1}{2}$H, br m, H-3), 4.00 ($\frac{1}{2}$H, dq, J=8.8, 6.4 Hz, H-5″), 3.71 ($\frac{1}{2}$H, d, J=5.1 Hz, H-5), 3.68 ($\frac{1}{2}$H, d, J=5.1 Hz, H-5), 3.66～3.42 (1H, m, H-5′), 3.34～3.20 (4H, m, H-2′, OMe (singlets at 3.28, 3.27)), 2.81 ($\frac{1}{2}$H, d, J=4.3 Hz, H-11), 2.78 ($\frac{1}{2}$H, d, J=4.3 Hz, H-11), 2.63～2.50 (2H, m, H-2, H-3′), 2.37～2.14 (13½H, m, 2H-9, H-11, H-2″($\frac{1}{2}$), H-4″, NMe NMe_2), 2.00 ($\frac{1}{2}$H, d, J=15.4 Hz, H-2″), 1.98～1.85 (2H, m, H-4, H-8), 1.78 (1H, m, H-7), 1.64 (1H, m, H-4′), 1.59～1.50 (2H, m, H-14, H-2″), 1.38～1.11 (21H, m, H-7, H-14, H-4′, 3 × Me (s), 3 × Me (d)), 1.09 ($\frac{3}{2}$H, d, J=7.3 Hz, Me), 1.08 ($\frac{3}{2}$H, d, J=7.3 Hz, Me), 1.03 ($\frac{3}{2}$H, d, J=6.7 Hz, Me), 1.00 ($\frac{3}{2}$H, d, J=6.6 Hz, Me) and 0.85 (3H, t, J=7.3 Hz, 3H-15) ppm. FAB-MS (Li spike) m/z 710 ($M^+ + Li$).

Anal Calcd for $C_{36}H_{69}N_3O_{10}$: C 61.42, H 9.88, N 5.97.
Found: C 61.13, H 9.75, N 5.76.

In Vitro Antibacterial Activity

MIC's were determined by a liquid turbidimetric microtiter assay. Macrolides were solubilized in ethanol and diluted to 4% ethanol in phosphate buffer. Concentrations in the range 128~0.00015 μg/ml were tested. Stock cultures were stored at −80°C. Organisms were incubated at 35~37°C; *Haemophilus influenzae* and *Streptococcus pneumoniae* were incubated in a 5% CO_2 atmosphere. 10^5 cfu/well were added to microtiter plates containing the diluted macrolides. Plates were incubated at 35~37°C for 22~24 hours. MIC values of >8 μg/ml indicate microorganism resistance, whereas values of 1~4 μg/ml indicate intermediate resistance. Mueller-Hinton broth was used for all strains with the following exceptions: *Haemophilus* were grown in Haemophilus Test Medium and streptococci and enterococci were grown in Mueller-Hinton broth supplemented with cations and 5% lysed horse blood.

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