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Novel 12-Membered Ring Macrolides with Activity against Erythromycin-resistant Organisms

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A series of novel 12-membered ring macrolides was designed based on available information on structure-activity relationships and macrolide-ribosome interactions. Compounds with the desosamine and the anchor group properly attached to the 12-membered lactone ring exhibited improved activity against erythromycin-resistant organisms.

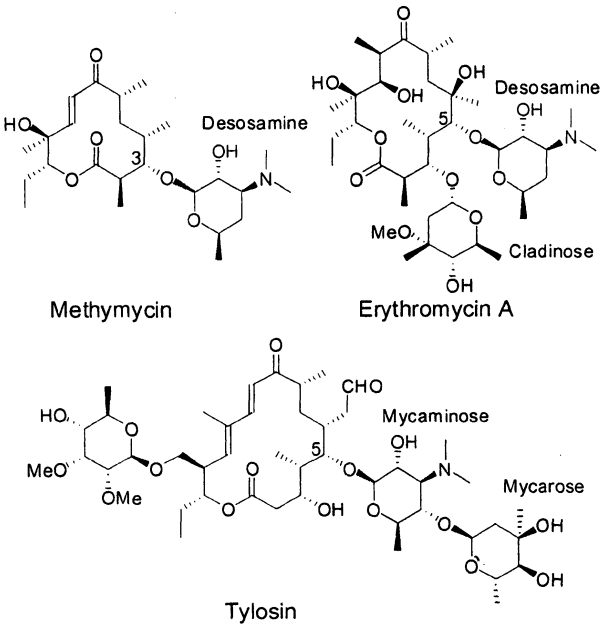
Macrolide antibiotics are natural in origin consisting of 12-, 14- and 16-membered ring systems (Figure 1). Members of the 14- and 16-membered ring macrolides are highly potent against a variety of bacterial species and have been widely used as therapeutic agents. However, the 12-membered ring macrolides are only weakly active and have not shown any therapeutic value.[1)]

Since the discovery of erythromycin in 1952, many semi-synthetic macrolides have been prepared and the structure-activity relationships have been studied in detail.[2)] From these studies, one can easily recognize that the amino-sugar moiety in a macrolide molecule is essential for giving potent antibacterial activities. In contrast, the lactone portion of the molecule is highly variable which can adopt a 14-, 15- or 16-membered ring system with a variety of substitutions. The neutral sugars, cladinose and mycarose, are also found to be dispensable without significant loss of antibacterial activity.[3)]

The stated structure-activity relationships suggest that the amino-sugar group in a macrolide molecule is directly responsible for an effective macrolide-ribosome interaction. The conclusion is fully supported by high-resolution ribosome-macrolide co-crystal structures, which have recently been made available.[4)]

This notion promoted us to explore the possibility of designing some smaller molecules with the key functionalities incorporated. Such smaller molecules could conceivably provide many pharmacological advantages. A smaller, streamlined structure provides the potential for reducing some undesirable interactions, which may be

Fig. 1. Structures of representative 12-, 14- and 16-membered ring macrolides.



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responsible for resistance or toxicity. As an example, cladinose had been found to be responsible for the inducibility of macrolide resistance. Therefore, removal of the cladinose moiety provided compounds with potent activity against inducible resistance.[3] Smaller molecules could also improve drug permeability and improve activity against *H. influenzae*, a key respiratory pathogen.

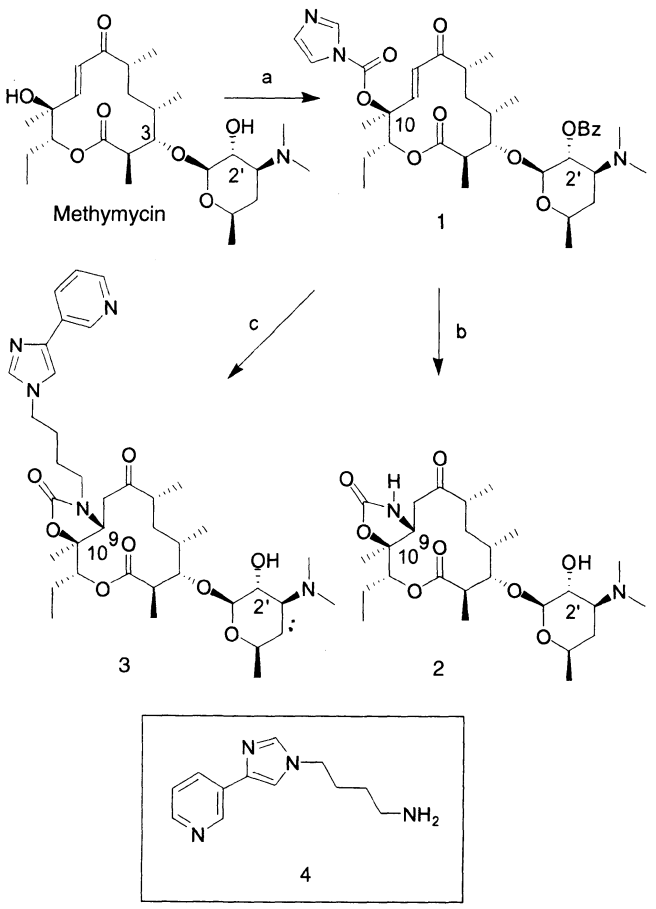
Chemistry

We decided to explore the aforementioned strategy by employing a smaller 12-membered ring macrolide system. Methymycin is a naturally occurring 12-membered ring macrolide with a desosamine attached to the C-3 position. Despite the existence of the key functionality, desosamine in the molecule, the compound is practically inactive against even the most susceptible bacteria.[5] We suspected that the lack of activity of methymycin was due to the conformation of the lactone ring, which positioned the desosamine in a manner that prohibited effective binding. In this context, we first explored to improve methymycin activity by varying the lactone structure and introducing additional functionality to the molecule.

Introduction of a cyclic carbamate group to the 11/12-position of a 14-membered ring macrolide has been used successfully to enhance the molecule's ability to bind to the ribosome.[6] We applied the same strategy to the methymycin system and hoped that introduction of the cyclic carbamate group could vary the conformation enough to allow effective desosamine binding. The γ-hydroxy enone moiety in methymycin was set perfectly for performing such an operation. Scheme 1 illustrated the four-step synthesis of the 9,10-cyclic carbamate derivatives of methymycin. First, the 2′-hydroxy group was selectively protected as its benzoate with the help of neighboring dimethylamino group participation. Introduction of acylimidazole to the C-10 position provided compound **1** in excellent yield. Aminolysis of **1**, followed by spontaneous intramolecular Michael addition, provided the 9,10-cyclic carbamate. The Michael addition process was highly stereoselective and provided a single stereoisomer. Finally, the protecting group was removed by stirring the above carbamate in methanol at room temperature for 3 days to give compound **2**.

Some recent work has indicated that introduction of an anchor group to the carbamate nitrogen could improve the macrolide-ribosome interaction dramatically. Such bifunctional macrolides exhibited significant activity against macrolide-resistant bacteria with methylated binding site.[7] We therefore introduced 4-(3-pyridyl)-

Scheme 1.



Conditions: (a) i. Bz_2O, Et_3N, CH_2Cl_2, rt, 24h, 94%. ii. CDI, NaH, THF, -10 °C to rt, 96%. (b) i. 28% NH_4OH, CH_3CN, rt, 96 h, 64%. ii. MeOH, rt, 72 h, 81%. (c) i. **4**, CH_3CN, rt, 96 h, 86%. ii. MeOH, rt, 72 h, 65%.

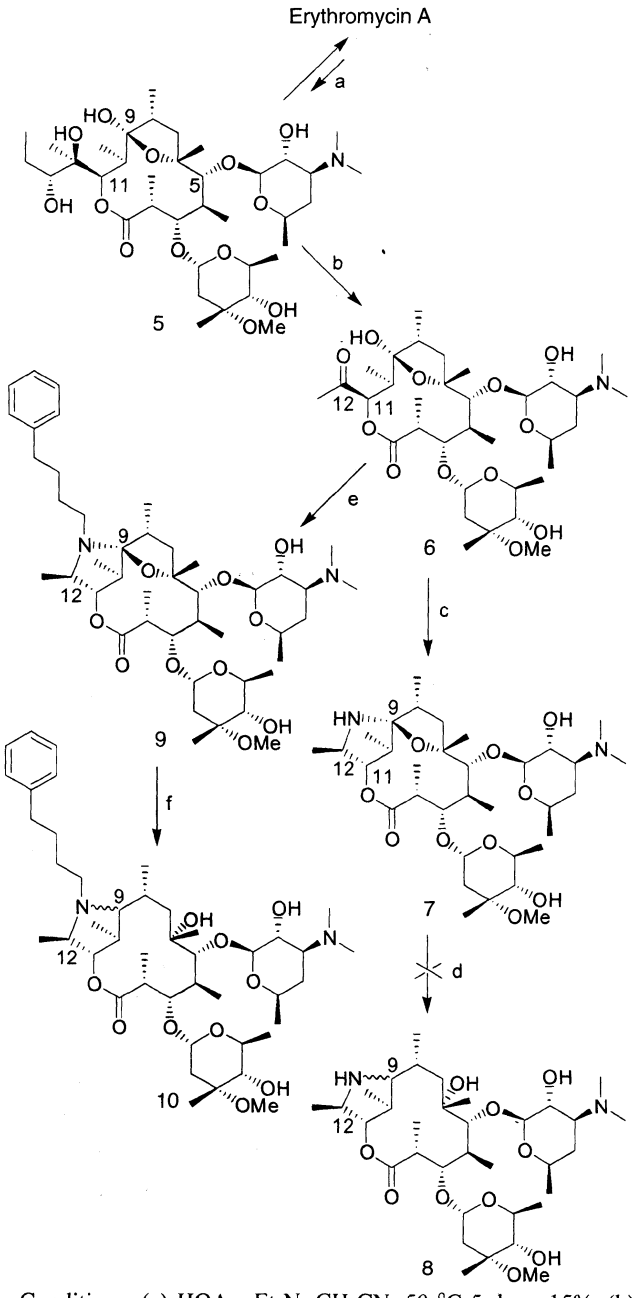
imidazole group to the carbamate nitrogen through a butylene linker. This anchor group had been utilized successfully to improve activity against macrolide resistance.[8] Reaction of acylimidazole **1** with amine **4** followed by deprotection provided the target compound **3** in good yield. The reaction was highly stereoselective and gave the 9-(*R*) isomer as the only isolable product. The stereochemistry of the C-9 center was supported by a strong NOE between H-9 and H-11 observed in a ROESY experiment.

Unfortunately, both compound **2** and **3** were weakly active against various organisms. These results prompted us to explore derivatives with the desosamine group attached to the C-5 position of a 12-membered ring system. The C-5 linkage would in principle place the desosamine in a similar environment as it in a 14- or 16-membered ring macrolide system. We utilized a known translactonization reaction

of erythromycin[9] to prepare the key intermediate **5**, which existed exclusively as the hemiketal form. The translactonization process was reversible and favored the 14-membered ring system. To prevent the retro-translactonization from occurring, we converted **5** to the corresponding 11-acetyl compound **6**. The C-12 carbonyl group in **6** could also serve as an linking point for the anchor group. Scheme 2 illustrated these transformations.

Reductive amination of **6** provided aminal **7** through the 12-amino intermediate. The stereochemistry of the C-11 chiral center was inverted during this process. The structure of **7** was elucidated by various two-dimensional NMR experiments. Strong NOE from H-11 to H-10 and to 12-Me indicated that H-11 is *cis* to H-10 and *trans* to H-12. Strong NOE from H-12 to the 10-Me further confirmed the assignment from C-10 to C-12 region. The stereochemistry of the C-11 center was therefore assigned as *S* and the C-12 center as *R* configurations. Between the two C-9 isomers, only the structure shown was consistent with all the spectroscopy data. Further reduction of aminal **7** to the corresponding cyclic amine **8** under various conditions failed to produce the desired product. In a similar fashion, reductive amination of **6** with phenylbutylamine produced the corresponding aminal **9**. Treatment of **9** with sodium cyanoborohydride in the presence of acetic acid provided the cyclic amine **10**. The stereochemistry of the C-9 center in **10** was not assigned with the available data.

Scheme 2.



Conditions: (a) HOAc, Et_3N, CH_3CN, 50 °C 5 days, 15%. (b) $Pb(OAc)_4$, CH_2Cl_2, rt, 76%. (c) NH_4OAc, molecular sieves, $NaBH_3CN$, MeOH, rt, 24 h, 61%. (d) HOAc, $NaBH_3CN$, MeOH, rt, 24 h, no reaction. (e) 4-phenylbutylamine, molecular sieves, $NaBH_3CN$, MeOH, rt, 25 days, 35%. (f) HOAc, $NaBH_3CN$, MeOH, rt, 24 h, 49%.

Microbiology

The antibacterial activity of the 12-membered ring macrolides and the reference compounds, methymycin and erythromycin, were evaluated against a panel of representative pathogens selected from the Abbott clinical culture collection. Various macrolide-resistant strains were included in these tests in order to evaluate their potential for overcoming macrolide resistance.[10] *Staphylococcus aureus* NCTC10649M, *Streptococcus pyogenes* EES 61 and *Streptococcus pneumoniae* ATCC 6303 are erythromycin-susceptible strains. *S. aureus* A5177 is an inducibly MLS_B resistant strain encoded by an *ermA* gene. *S. aureus* 1775 is a constitutively MLS_B resistant strain also encoded by an *ermA* gene. *S. pyogenes* 930 and *S. pneumoniae* 5979 are MLS_B resistant strains encoded by *ermB* genes. *S. pyogenes* PIU 2548 and *S. pneumoniae* 5649 are efflux resistant strains encoded by *mefA* and *mefE* genes respectively. *Haemophilus influenzae* DILL is an ampicillin-resistant strain with a β-lactamase positive determinant. The *in vitro* antibacterial activities are reported as minimum inhibitory concentrations (MICs), which were determined by the agar dilution method as recommended by the National Committee for Clinical Laboratory Standards. The *in vitro* antibacterial activities of the 12-membered ring macrolides and reference compounds are shown in Table 1.

Table 1. *In vitro* antibacterial activity of 12-membered ring macrolides against selected bacteria.

Organism		MIC (μg/ml)							
		2	**3**	**6**	**7**	**9**	**10**	Methy	Ery
S. aureus NCTC10649M	Ery-S	100	>100	>128	64	128	32	>128	0.5
S. aureus A5177	*erm*A	100	>100	>128	128	128	128	>128	>128
S. aureus 1775	*erm*A	>100	>100	>128	>128	128	64	>128	>128
S. pyogenes EES 61	Ery-S	16	>64	32	>128	8	1	>128	0.015
S. pyogenes 930	*erm*B	>100	>64	>128	>128	>128	64	>128	>128
S. pyogenes PIU 2548	*mef*A	64	>64	>128	>128	64	8	>128	8
S. pneumo ATCC 6303	Ery-S	32	8	32	64	4	0.25	>128	0.015
S. pneumo 5979	*erm*B	>64	>64	>128	>128	>128	128	>128	>128
S. pneumo 5649	*mef*E	64	8	>128	>128	16	4	>128	4
H. influenzae DILL	Amp-R	>64	>64	>128	16	32	8	>128	4

Results and Discussion

Macrolide resistance has become a significant clinical problem during past decade.[11] Two of the most prevalent macrolide-resistant mechanisms involved the modification of macrolide binding site (*erm*) and efflux (*mef*).[12] The *erm* resistance was represented by *S. aureus* A5177, *S. aureus* 1775, *S. pyogenes* 930, and *S. pneumoniae* 5979. The *mef* resistance was represented by *S. pyogenes* PIU 2548 and *S. pneumoniae* 5649. Although erythromycin was highly active against erythromycin-susceptible strains, it was practically inactive against the *erm*-containing strains and only weakly active against the *mef*-containing strains. Methymycin showed no detectable activity against any strains tested, regardless the resistant mechanisms.

Introduction of the 9,10-cyclic carbamate to methymycin, provided a compound (**2**) with weak activity against erythromycin-susceptible and erythromycin-resistant organisms encoded by the *mef* genes. Although compound **2** was cross-resistant with erythromycin against *erm*-containing strains, its activity appeared not to be affected by the efflux pump. Attaching the (3-pyridyl)imidazolyl anchor group to the carbamate nitrogen of structure **2** did not further improve its antibacterial activity, indicating that the anchor group in structure **3** might be improperly placed which prohibited the effective secondary binding with ribosome. However, compound **3** was equally active against the erythromycin-susceptible *S. pneumoniae* ATCC 6303 and efflux resistant *S. pneumoniae* 5649. These results were consistent with what we had observed for structure **2**, suggesting that structures derived from methymycin could avoid the efflux resistance mechanism.

Synthesis of 12-membered ring macrolides from erythromycin was complicated by the hemiketal formation between the 6-hydroxy and the 9-keto groups. The hemiketal form of erythromycin has been known to be significantly less active than erythromycin against bacteria.[13] Therefore it was not surprising to see that the hemiketal **6** was only weakly active. Conversion of the 12-keto groups to the corresponding amine resulted in the formation of aminal **7**. As its hemiketal precursor, aminal **7** was only weakly active. However, compound **7** was significantly more active than **6** against Gram-negative bacterium *H. influenzae*, indicating that structure **7** might penetrate better than **6** into the Gram-negative organisms. Attempts to reduce aminal **7** to the cyclic amine **8** failed to provide the desired product.

Attaching an anchor group to the amine nitrogen gave aminal **9**, which appeared to be more potent than the parent aminal **7**. Interestingly, this compound displayed some weak activity against *erm*-mediated resistance. Further reduction of aminal **9** proceeded successfully to provide cyclic amine **10** with a phenylbutyl group attached. Compound **10** was significantly more active than the corresponding aminal **9** against some bacterial strains. Compound **10** was also significantly more active than erythromycin against the *erm*-containing bacteria. All these data indicated that **10** bound better to resistant ribosomes than erythromycin. However, compound **10** was ineffective against efflux resistance, suggesting that this structure could still be a substrate of the efflux proteins. This finding was consistant with the structure-activity relationships developed for the 14-membered ring system where the C-3 cladinose was proved to be the key structural moiety for efflux pump recognization.[3]

Conclusion

Two series of 12-membered ring macrolides were synthesized and evaluated for antibacterial activity. Compounds derived from methymycin are characterized by having the desosamine group attached to the C-3 position of the lactone ring. They are only weakly active against certain bacterial strains. However, these compound are equally active against susceptible bacteria and resistant bacteria with an efflux mechanism. 12-Membered ring macrolides derived from erythromycin are significantly more potent than erythromycin against *erm*-mediated resistant organisms. However, due to the existence of the C-3 cladinose, these compounds are ineffective against efflux resistance. Further research into the 12-membered ring macrolide system represents a significant opportunity for developing novel macrolides to overcome the existing resistance mechanisms and improve activity against Gram-negative bacteria.

Experimental Section

Methymycin 2′-*O*-Bezoate-10-acylimidazolide (**1**)

Methymycin (0.40 g, 0.90 mmol) was dissolved in CH_2Cl_2 (4 ml) Benzoic anhydride (0.31 g, 1.4 mmol) was introduced to the solution followed by Et_3N (0.19 ml, 1.4 mmol). After stirring at rt for 24 hours, the reaction mixture was washed with 5% Na_2CO_3, dried over Na_2SO_4 and concentrated. The crude mixture was purified *via* column chromatography (silica gel, CH_2Cl_2) to give the desired 2′-*O*-benzoate product (0.49 g, 94%): MS (ESI) *m/z* 575 $(M+H)^+$.

To a solution of above product in THF (10 ml) at −10°C under nitrogen, was added NaH (73 mg 60%, 1.89 mmol) in one portion and stirred at this temperature for 20 minutes. A solution of CDI (0.42 g, 2.7 mmol) in THF (7 ml) was added dropwise and the reaction mixture was slowly warmed up and stirred at rt for 3 hours. The mixture was taken up in EtOAc, washed with 5% Na_2CO_3, dried over Na_2SO_4 and concentrated. The crude mixture was purified *via* column chromatography (silica gel, EtOAc) to give the desired product **1** (0.58 g, 96%): MS (ESI) *m/z* 670 $(M+H)^+$.

Methymycin 9,10-Carbamate (**2**)

To a stirred solution of **1** (50 mg, 0.10 mmol) in CH_3CN (1 ml) was added ammonium hydroxide (28%, 1.0 ml). The mixture was stirred at rt for 4 days and taken up in EtOAc. The resulting mixture was washed with 5% Na_2CO_3, dried over Na_2SO_4 and concentrated. The crude mixture was purified *via* column chromatography (silica gel, 30 : 70 acetone - hexane) to give the cyclized product (30 mg, 64%). The above product was dissolved in MeOH and stirred at rt for 3 days. The solvent was evaporated and the crude product was purified *via* column chromatography (silica gel, 99.5 : 0.5 CH_2Cl_2 - MeOH) to give the desired product **2** (18 mg, 81%): MS (ESI) *m/z* 513 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 5.63 (1H, br s), 5.08 (1H, dd, J=10.8, 3.0 Hz), 4.25 (1H, d, J=7.8 Hz), 3.65 (1H, d, J=10.8 Hz), 3.50 (2H, m), 3.20 (1H, dd, J=10.2, 7.5 Hz), 3.30 (1H, d, J=16.2 Hz), 2.93 (1H, m), 2.59 (1H, m), 2.48 (1H, m), 2.27 (6H, s), 2.09 (1H, dd, J=16.2, 10.2 Hz), 1.82 (1H, m), 1.65 (4H, m), 1.46 (3H, d, J=6.6 Hz), 1.34 (3H, s), 1.25 (2H, m), 1.24 (3H, d, J=6.4 Hz), 1.12 (3H, d, J=6.8 Hz), 1.04 (3H, d, J=6.6 Hz), 0.90 (3H, t, J=6.4 Hz).

Methymycin 9-*N*-(4-(4-(3-Pyridyl)imidazol-1-yl)butyl)-9,10-Carbamate (**3**)

To a stirred solution of **1** (130 mg, 0.20 mmol) in CH_3CN (3 ml) was added **4** (150 mg, 0.70 mmol). The mixture was stirred at rt for 4 days and taken up in EtOAc. The resulting mixture was washed with 5% Na_2CO_3, dried over Na_2SO_4 and concentrated. The crude mixture was purified *via* column chromatography (silica gel, 50 : 50 acetone - hexane) to give the cyclized product (140 mg, 86%). The above product was dissolved in MeOH and stirred at rt for 3 days. The solvent was evaporated and the crude product was purified *via* column chromatography (silica gel, 99.5 : 0.5 CH_2Cl_2 - MeOH) to give the desired product **3** (80 mg, 65%): MS (ESI) *m/z* 712 $(M+H)^+$; ^{13}C NMR ($CDCl_3$) δ 212.8, 174.7, 156.8, 147.6, 146.4, 139.1, 137.8, 131.9, 130.2, 123.5, 115.3, 105.2, 85.0, 81.1, 79.1, 70.2, 69.6, 65.8, 60.4, 46.8, 46.2, 44.7, 42.3, 40.2, 35.6, 34.8, 34.5, 28.3, 28.1, 24.4, 23.1, 21.1, 16.8, 16.7, 16.0, 15.3, 10.1. DQCOSY, HSQC and ROESY results are consistent with the assigned structure.

11-Acetyl Hemiketal **6**

To a stirred solution of the starting material **5** (0.10 g, 0.10 mmol) in dichloromethane (5 ml) was added lead tetraacetate (0.11 g, 0.20 mmol) at room temperature. After 0.5 hours, the precipitate was removed *via* filtration and washed with dichloromethane. The combined filtrates were washed successively with $NaHCO_3$ (sat.) and water, then dried over Na_2SO_4. Filtration and concentration gave the crude product which was purified *via* flash column chromatography (silica gel, 0.5 : 3 : 96.5 NH_4OH - MeOH - dichloromethane) to give the desired product **6** (70 mg,

76%): MS (ESI) *m/z* 674 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 5.99 (1H, d, *J*=2.5 Hz), 4.78 (1H, d, *J*=4.2 Hz), 4.17 (1H, d, *J*=7.8 Hz), 4.06 (1H, m), 3.91 (1H, d, *J*=10.0 Hz), 3.49 (1H, m), 3.48 (1H, d, *J*=10.0 Hz), 3.38 (1H, m), 3.30 (1H, dd, *J*=10.5, 6.9 Hz), 3.24 (3H, s), 3.00 (1H, m), 2.76 (1H, m), 2.30~2.60 (5H, m), 2.29 (6H, s), 2.22 (2H, m), 2.19 (3H, s), 1.65 (1H, m), 1.56 (3H, m), 1.52 (1H, m), 1.37 (3H, s), 1.28 (3H, d, *J*=6.6 Hz), 1.26 (3H, d, *J*=6.6 Hz), 1.23 (3H, s), 1.21 (3H, d, *J*=6.4 Hz), 1.10 (3H, d, *J*=6.8 Hz), 0.98 (3H, d, *J*=6.6 Hz), 0.89 (3H, d, *J*=6.8 Hz). *Anal.* ($C_{34}H_{59}NO_{12}$) calcd. C 60.60, H 8.83, N 2.08; found C 60.44, H 8.72, N 1.98. DQCOSY, HSQC and ROESY results are consistent with the assigned structure.

Aminal **7**

To a stirred solution of the starting material **6** (50 mg, 0.10 mmol) in methanol (3 ml) was added molecular sieves (4 angstrom, powder, 20 mg) and ammonium acetate (57 mg, 1.0 mmol) at room temperature. After stirring for 1 hour, sodium cyanoborohydride (20 mg, 0.30 mmol) was added and the reaction was stirred at ambient temperature over night. The following day the reaction was warmed to 48°C for 5 hours and cooled. The solids were removed *via* filtration and washed with methanol. The combined filtrates were concentrated and purified *via* flash column chromatography (silica gel, 0.5 : 3 : 96.5 NH_4OH-MeOH-dichloromethane) to give aminal **7** (30 mg, 61%): MS (ESI) *m/z* 657 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 5.16 (1H, dd, *J*=9.6, 2.4 Hz), 4.74 (1H, d, *J*=4.2 Hz), 4.17 (1H, d, *J*=7.8 Hz), 4.04 (2H, m), 3.85 (1H, d, *J*=10.0 Hz), 3.49 (1H, m), 3.47 (1H, d, *J*=10.0 Hz), 3.34 (1H, dd, *J*=10.5, 7.0 Hz), 3.26 (1H, m), 3.24 (3H, s), 3.10 (1H, m), 3.00 (1H, d, *J*=9.0 Hz), 2.65 (2H, m), 2.38 (6H, s), 2.20~2.50 (7H, m), 1.72 (1H, m), 1.52 (4H, m), 1.35 (3H, s), 1.28 (3H, d, *J*=6.6 Hz), 1.22 (3H, s), 1.21 (3H, d, *J*=6.6 Hz), 1.16 (3H, d, *J*=6.8 Hz), 1.09 (3H, d, *J*=6.8 Hz), 1.06 (3H, d, *J*=6.6 Hz), 0.95 (3H, d, *J*=6.8 Hz), 0.94 (3H, d, *J*=6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 177.0, 106.6, 105.4, 98.6, 86.3, 84.5, 80.8, 78.2, 77.3, 72.4, 70.4, 69.1, 65.5, 65.0, 49.1, 46.8, 46.7, 41.2, 40.4, 39.4, 38.4, 37.8, 35.2, 30.0, 29.2, 21.5, 21.4, 21.1, 17.5, 13.3, 12.1, 10.6, 9.1. DQCOSY, HSQC and ROESY results are consistent with the assigned structure.

Aminal **9**

To a stirred solution of the starting material **6** (0.10 g, 0.10 mmol) in methanol (6.0 ml) was added 4A sieves, followed by 4-phenylbutylamine (0.24 ml, 1.5 mmol). The resulting mixture was stirred for 1.5 hours after which time the sodium cyanoborohydride (40 mg, 0.60 mmol) was added. The reaction was stirred for 25 days at ambient temperature and concentrated. The remaining residue was diluted with dichloromethane and poured into $NaHCO_3$ (aq.). The layers were separated and the aqueous phase was extracted twice with dichloromethane. The combined organics were dried over $MgSO_4$, filtered and concentrated. Purification *via* flash column chromatography (silica gel, 0.5 : 2.5 : 97 NH_4OH-MeOH-DCM) gave the desired product **9** (30 mg, 35%): MS (ESI) *m/z* 789 $(M+H)^+$; HRMS (ESI) calcd. for $C_{44}H_{73}N_2O_{10}$ *m/z* 789.5260, obs. 789.5258.

Cyclic Amine **10**

To a stirred solution of the starting material **9** (30 mg, 0.038 mmol) in methanol was added glacial acetic acid (2 drops) and sodium cyanoborohydride (10 mg). The mixture was stirred at ambient temperature over night and concentrated. The remaining residue was diluted with ethyl acetate and 2 M NaOH. The layers were separated and the organic phase was concentrated to give the desired product **10** (15 mg, 50%): MS (ESI) *m/z* 791 $(M+H)^+$; HRMS (ESI) calcd. for $C_{44}H_{75}N_2O_{10}$ *m/z* 791.5416, obs. 791.5426; ^1H NMR ($CDCl_3$) δ 7.26 (2H, m), 7.18 (3H, m), 5.08 (1H, d, *J*=6.0 Hz), 4.84 (1H, d, *J*=4.2 Hz), 4.34 (2H, m), 4.08 (1H, m), 3.60 (1H, m), 3.54 (1H, d, *J*=6.0 Hz), 3.49 (2H, m), 3.28 (1H, m), 3.25 (3H, s), 3.20 (1H, m), 3.04 (1H, m), 2.60~2.80 (8H, m), 2.34 (4H, m), 2.28 (6H, s), 2.25 (1H, m), 1.95 (1H, m), 1.50~1.70 (10H, m), 1.35 (3H, d, *J*=6.0 Hz), 1.31(3H, s), 1.25 (3H, d, *J*=6.6 Hz), 1.22 (3H, s), 1.21 (3H, d, *J*=6.6 Hz), 1.16 (3H, d, *J*=6.8 Hz), 1.06 (3H, d, *J*=6.6 Hz), 1.00 (3H, d, *J*=6.6 Hz), 0.95 (3H, d, *J*=6.8 Hz).

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References

1) Bryskier, A.; C. Agouridas & J.-C. Gasc: Classification of Macrolide Antibiotics. *In* Macrolides: Chemistry, Pharmacology and Clinical Uses. *Eds.* Bryskier, A. J., Butzler, J.-P., Neu, H. C., Tulkens, P. M., pp. 5~66, Arnette Blackwell: Paris, 1993
2) (a) Chu, D. T. W.: Recent developments in 14- and 15-membered macrolides. Exp. Opin. Invest. Drugs 4: 65~94, 1995
(b) Bryskier, A.: Novelties in the field of macrolides. Exp. Opin. Invest. Drugs 6: 1697~1709, 1997
3) Ma, Z. & P. A. Nemoto: Discovery and development of ketolides as a new generation of macrolide antimicrobial agents. Curr. Med. Chem. Anti-infective Agents 1:

15～34, 2002

4) SCHLUNZEN, F.; R. ZARIVACH, J. HARMS, A. Bashan, A. TOCILJ, R. ALBRECHT, A. YONATH & F. FRANCESCHI: Structural basis for the interaction of antibiotics with the peptidyl transferase center in eubacteria. Nature 413: 814～821, 2001

5) (a) DONIN, M. N.; J. PAGANO, J. D. DUTCHER & C. M. McKee: Methymycin, a new crystalline antibiotic. Antibiot. Ann. 179～185, 1954
(b) DJERASSI, C. & J. A. ZDERIC: The structure of the antibiotic methymycin. J. Am. Chem. Soc. 78: 6390～6394, 1956

6) BAKER, W. R.; J. D. CLARK, R. L. STEPHENS & K. H. KIM: Modification of macrolide antibiotics. Synthesis of 11-deoxy-11-(carboxyamino)-6-*O*-methylerythromycin A 11,12-(cyclic esters) *via* an intramolecular michael reaction of *O*-carbamates with an α,β-unsaturated Ketone. J. Org. Chem. 53: 2340～2345, 1988

7) MA, Z.; R. F. CLARK, A. BRAZZALE, S. WANG, M. J. RUPP, L. LI, G. GRIESGRABER, S. ZHANG, H. YONG, L. T. PHAN, P. A. NEMOTO, D. T. W. CHU, J. J. PLATTNER, X. ZHANG, P. ZHONG, Z. CAO, A. M. NILIUS; V. D. SHORTRIDGE, R. FLAMM, M. MITTEN, J. MEULBROEK, P. EWING, J. ALDER & Y. S. OR: Novel erythromycin derivatives with aryl groups tethered to the C-6 position are potent protein synthesis inhibitors and active against multidrug-resistant respiratory pathogens. J. Med. Chem. 44: 4137～4156, 2001

8) DENIS, A.; C. AGOURIDAS, J.-M. AUGER, Y. BENEDETTI, A. BONNEFOY, F. BRETIN, J.-F. CHANTOT, A. DUSSARAT, C. FROMENTIN, S. G. D'AMBRIERES, S. LACHAUD, P. LAURIN, O. L. MARTRET, V. LOYAU, N. TESSOT, J.-M. PEJAC & S. PERRON: Synthesis and antibacterial activity of HMR 3647. A new ketolide highly potent against erythromycin-resistant and susceptible pathogens. Bioorg. Med. Chem. Lett. 9: 3075～3080, 1999

9) (a) KIBWAGE, I. O.; R. BUSSON, G. JANSSEN, J. HOOGMARTENS & H. VANDERHAEGHE: Translactonization in erythromycins. J. Org. Chem. 52: 990～996, 1987
(b) KIRST, H. A.; J. A. WIND & J. W. PASCHAL: J. Org. Chem. 52: 4359～4362, 1987

10) (a) SHORTRIDGE, V. D.; G. V. DOERN, A. B. BRUEGGEMANN, J. M. BEYER & R. K. FLAMM: Prevalence of macrolide resistance mechanisms in *Streptococcus pneumoniae* isolates from a multicenter antibiotic resistance surveillance study conducted in the united states in 1994～1995. Clin. Infect. Dis. 29: 1186～1188, 1999
(b) SHORTRIDGE, V. D.; R. K. FLAMM, N. RAMER, J. BEYER & S. K. TANAKA: Novel mechanism of macrolide resistance in *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. 26: 73～78, 1996

11) HOBAN, D. J.; G. V. DOERN, A. C. FLUIT, M. ROUSSEL-DELVALLEZ & R. N. JONES: Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the SENTRY antimicrobial surveillance program, 1997～1999. Clinical Infectious Diseases 32: S81～93, 2001

12) (a) NAKAJIMA, Y.: Mechanism of bacterial resistance to macrolide antibiotics. J. Infec. Chemother. 5: 61～74, 1999
(b) IRIZARRY, L. M.; B. PORTER, R. LENROOT, M. ALEXOV & L. MASSIC: Macrolide-resistant *Streptococcus pneumoniae*: Epidemiology, mechanism of resistance, acquisition and clinical implication. Recent Res. Devel. Antimicrob. Agent Chemother. 3: 511～521, 1999

13) GASC, J.-C. & A. BRYSKIER: Structure-activity relationship of macrolides. *In* Macrolides: Chemistry, Pharmacology and Clinical Uses. *Eds.*, BRYSKIER, A. J., BUTZLER, J.-P., NEU, H. C., TULKENS, P. M., pp. 67～82, Arnette Blackwell. Paris, 1993