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Synthesis and Activity of a Novel Class of Tribasic Macrocyclic Antibiotics: The Triamilides

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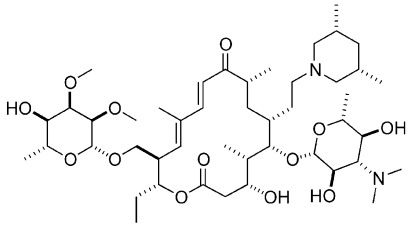
Abstract—The stereoselective synthesis of two novel series of tribasic macrocyclic antibiotics with potent in vitro activity against *Pasteurella multocida* and *Escherichia coli* strains of bacteria is described. The in vitro activity can be significantly influenced by the nature of the substituents on the C-4″ aminoalcohol, with the stereochemistry of the C-4″ alcohol playing a less critical role. The effect of substitution and stereochemistry on the in vivo activity in a murine model of respiratory infection is also described.

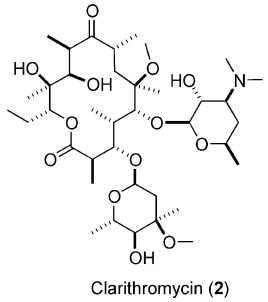
Respiratory disease is the leading cause of morbidity and mortality in North American beef cattle, with outbreaks typically associated with animals that have been recently transported to a feedlot. It has been estimated that bovine respiratory disease (BRD) afflicts up to 30% of cattle in domestic feedlots, with an average mortality rate of 5%.[1] Swine production is also significantly impacted by respiratory disease.[2]

Antibacterial agents continue to play a pivotal role in the responsible management of livestock respiratory disease. Difficulties in handling animals in a safe and stress-free way present an ongoing challenge to providing the best antimicrobial therapy for livestock. Antimicrobial agents that can provide adequate duration of therapy from a single application possess considerable advantage for both the animal and the livestock producer. Although a wide variety of agents are approved for treating BRD, the 16-membered semisynthetic macrolide tilmicosin (Micotil®, **1**) has emerged as one of the most popular therapeutic agents[1] based in part on an extended duration of action.

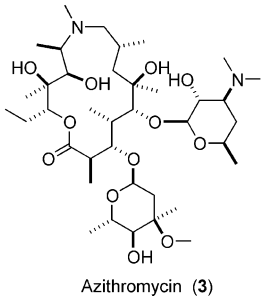
Macrocyclic antibiotics such as clarithromycin (**2**) and azithromycin (**3**) have been used for quite some time for the treatment of human respiratory infections caused by a variety of pathogens.[3] We were particularly interested in investigating the antimicrobial properties of novel antibiotics related to the 15-membered azalides due to their favorable tissue distribution and half-life as well as their broad-spectrum antimicrobial activity.[4]



Tilmicosin (**1**)



Clarithromycin (**2**)



Azithromycin (**3**)

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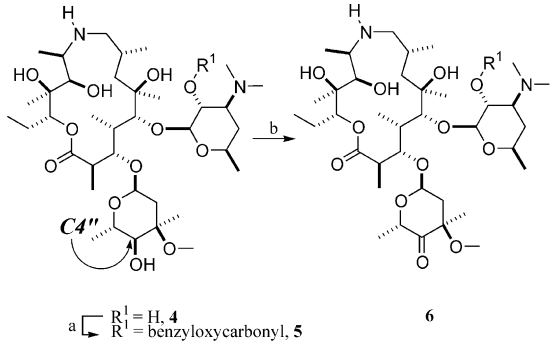
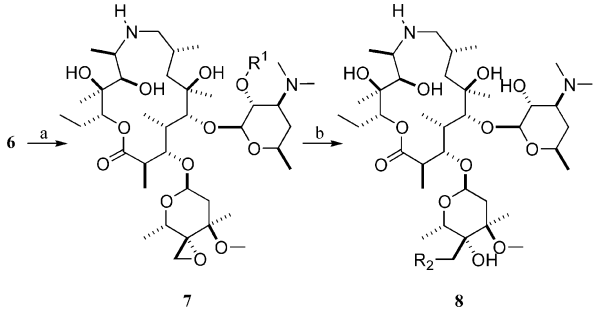


Scheme 1. (a) CBZ-Cl, CH_2Cl_2, 0 °C, 100%; (b) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, pyridinium trifluoroacetate, 0–23 °C, CH_2Cl_2 88%.

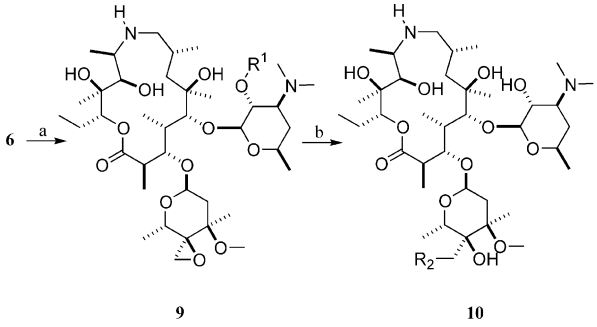
Our efforts in this area included modification of the C-4″ alcohol of the cladinose ring on the 15-membered macrocyclic template via installation of a third basic amine or other heteroatom functionality. The goals of this research were to improve the tissue distribution and to extend elimination half-life of the drug, while maintaining or enhancing potency against *Pasteurella multocida* and *Escherichia coli*, two common pathogens of livestock. We now report the preparation and screening of a novel series of tribasic 15-membered macrocyclic antibiotics that have potent in vitro and in vivo antimicrobial activity. We have named this new class of antibiotics the 'triamilides'.

Aminoalcohols **8** and **10** are readily prepared in four steps from the previously described[5] C-4″ alcohol **4**. Treatment of **4** (Scheme 1) with one equivalent of benzyl chloroformate in dichloromethane afforded the C-2′ protected alcohol **5** in high yield. The initial strategy to protect the reactive functionality on **4** relied on utilization of excess benzyl chloroformate to afford the doubly protected macrolide resulting from reaction at both the C-2′ alcohol and the 9a *N*-H. While this intermediate could be used to prepare **8** and **10**, the sequence required a difficult hydrogenolysis step in order to deprotect the 9a *N*-H. Following the observation that it was possible to efficiently protect only the C-2′ alcohol, we discovered that protection of the nitrogen was not required for the later steps in the sequence. Thus, the reaction of **4** with one equivalent of benzyl chloroformate was found to proceed with excellent selectivity and provide the C-2′ protected alcohol **5** as the sole product that could be used in subsequent steps without the need for further purification. Selective oxidation of the C-4″ alcohol of **5** with EDC/PTFA proceeded smoothly to give ketone **6** in 88% yield.

Scheme 2 depicts the preparation of aminoalcohols **8**. Treatment of ketone **6** with the stabilized ylide formed from the reaction of trimethylsulfoxonium iodide and sodium hydride provided the epoxide **7** in quantitative yield as the major diastereomer (ca. 10:1).[6] Epoxide **7** was converted to the aminoalcohol **8** upon reaction with the appropriate amine in methanol. In all cases, use of excess amine smoothly converted the epoxide to the corresponding aminoalcohol while simultaneously removing the benzyl carbonate at C-2′ to provide the desired macrocycle in modest to good yield.



Scheme 2. (a) $Me_3S(O)I$, NaH, DMSO, tetrahydrofuran, 0–23 °C, 100%; (b) MeOH, KI, 1° or 2° amine, 50 °C.



Scheme 3. (a) Me_3SBF_4, KHMDS, toluene, tetrahydrofuran, −78 °C, 58%; (b) MeOH, KI, 1° or 2° amine, 50 °C.

The preparation of the epimeric aminoalcohol **10** is shown in Scheme 3. In this sequence ketone **6** was treated with the more reactive ylide produced via the reaction of trimethylsulfonium tetrafluoroborate and potassium bis(trimethylsilyl)amide at low temperature to afford epoxide **9** in moderate yield and excellent diastereomeric purity (ca. 20:1). This epoxide was then converted to aminoalcohol **10** upon reaction with the appropriate amine in methanol as described for the preparation of **8**.

Unequivocal proof of the stereochemical outcome of the epoxidation reaction to form **9** was obtained from a single crystal X-ray structure of **10c**. The conformation of crystalline **10c** is shown in Figure 1. The crystal structure reveals several interesting facts concerning the orientation of the macrocyclic ring and the stereochemistry of the modified cladinose ring structure. One of the most notable features is that the oxygen bearing the macrocyclic ring system is in an axial orientation on the cladinose ring. In addition, the aminoalcohol formed from the addition of propyl amine to the epoxide is that derived from axial addition of the sulfur ylide to the ketone. This is the expected epoxide based on literature precedence for unstabilized ylides to form the epoxide via an axial attack on the ketone.

The above procedures efficiently provided the alkyl and aryl aminoalcohols **8a–m**, **8p–q**, and **10a–j**. We were also interested, however, in products resulting from opening

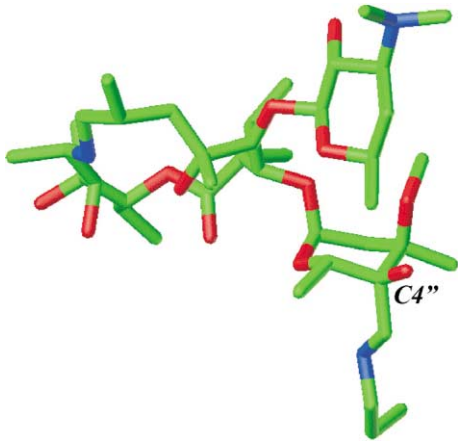
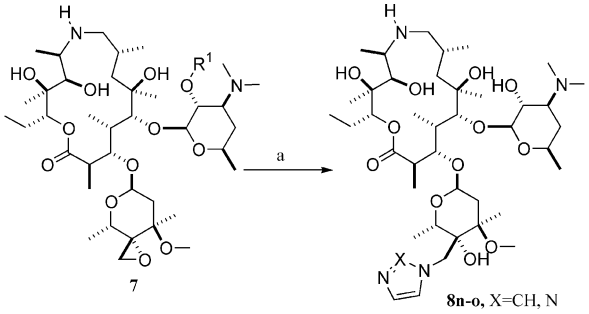


Figure 1. The configuration of **10c** in the solid state. Carbon atoms are in green, oxygen atoms in red and nitrogen atoms in blue.



Scheme 4. (a) Pyridinium-HCl, methanol, 55 °C; imidazole (**8n**), 1,2,3-triazole (**8o**).

epoxides **7** and **9** with other nucleophiles. Although preliminary efforts did not afford conditions to efficiently open the epoxides with oxygen nucleophiles, we were able to produce the alkyl thiols **8r** and **10k** by the reaction of 2-mercaptoethenol and the epoxide in refluxing benzene in the presence of potassium carbonate. In addition, the heteroaryl substituted alcohols **8n** and **8o** were obtained by reaction of **7** with the heterocycle in methanol in the presence of pyridinium-HCl as shown in Scheme 4.

The aminoalcohols **8** and **10** were tested in vitro for their ability to inhibit the growth of *P. multocida* and *E. coli*. The minimum inhibitory concentration (MIC) was determined by the microdilution method described in the NCCLS guidelines.[7] Most of the representative amino-alcohols in Tables 1 and 2 effectively inhibited the growth of *P. multocida* (MIC < 0.39 μg/mL). The least active is the bishydroxyethylamine **8q** (MIC = 3.13 μg/mL). Presumably the increased polarity resulting from introduction of the second hydroxyethyl substituent causes this diminished activity, as both the bis methoxyethylamine **8g** and hydroxyethyl(methyl)amine **8p** retain potency against *P. multocida* (MIC ≤ 0.1 μg/mL). Modest differences in the activity against *P. multocida* are observed within the lipophilic alkyl substituents in both series of aminoalcohols. Thus, the unsubstituted aminoalcohols **8a** and **10a** have MIC's of

Table 1. MIC values against *P. multocida* and *E. coli*, and ED_{50} values in a mouse *P. multocida* infection model for compounds **8**

Compd	R^2	*P. multocida* MIC (Mg/mL)	*E. coli* MIC (μg/mL)	Mouse ED_{50} (mg/kg)
8a	H_2N—	0.39	6.25	30
8b	*n*-BuNH—	< 0.1	0.39	23
8c	*n*-HexylNH—	0.2	0.78	> 80
8d	CyclopropylNH—	0.05	0.2	20
8e	*t*-BuNH—	0.1	0.39	14
8f	F_3CCH_2NH—	< 0.1	1.56	46
8g	$MeO(CH_2)_2NH$—	< 0.1	0.78	9.5
8h	Me_2N—	< 0.1	0.39	9.9
8i	Et(Me)N—	< 0.1	0.78	7.2
8j	Cyclopropyl(Me)N—	0.1	0.78	17
8k	Pyrrolidine	0.05	0.2	7.3
8l	Piperidine	< 0.1	0.39	40
8m	Morpholine	0.2	3.13	49
8n	Imidazole	0.2	3.13	20
8o	1,2,3-Triazole	0.05	0.78	23
8p	$HO(CH_2)_2(Me)N$—	0.1	1.56	12
8q	$(HO(CH_2)_2)_2N$—	3.13	> 50	n.d.
8r	$HO(CH_2)_2S$—	0.2	1.56	51

Table 2. MIC values against *P. multocida* and *E. coli*, and ED_{50} values in a mouse *P. multocida* infection model for compounds **10**

Compd	R^2	*P. multocida* MIC (μg/mL)	*E. coli* MIC (μg/mL)	Mouse ED_{50} (mg/kg)
10a	H_2N—	0.39	6.25	n.d.
10b	*n*-BuNH—	0.1	0.78	17
10c	*n*-PropylNH—	0.1	0.39	12
10d	CyclopropylNH—	0.1	0.78	15
10e	*t*-BuNH—	0.05	0.39	9.6
10f	Me_2N—	0.05	0.39	5.9
10g	Et(Me)N—	0.1	0.39	< 5
10h	$CylcopropylCH_2NH$—	0.1	0.39	5.7
10i	Pyrrolidine	0.05	0.2	< 10
10j	Piperidine	0.05	0.39	n.d.
10k	$HO(CH_2)_2S$—	0.39	25	> 40

0.39 μg/mL while more lipophilic substituents display slightly improved activity. More compact, lipophilic side chains such as trifluoroethyl (**8f**), cyclopropyl (**8d** and **10d**), *t*-butyl (**8e** and **10e**), and pyrrolidine (**8k** and **10i**) were the most active against this pathogen. The polarity of the side chain has a more significant effect on the in vitro activity against *E. coli*. Again, all of the lipophilic substituents improved the *E. coli* activity over the unsubstituted analogues **8a** and **10a**. Introduction of polar substituents at C4″ generally reduced the *E. coli* activity as exemplified with **8q** and **10k**.

Tables 1 and 2 also show the in vivo activity of many of the amino-alcohols as measured utilizing a murine model of respiratory disease.[8] In this model, mice are infected intranasally with *P. multocida*. The drug is subsequently administered subcutaneously. The in vivo activity of aminoalcohols **8** reveals that larger alkyl groups are disfavored (i.e., *n*-hexyl, piperidine and morpholine; **8c**, **8l**, and **8m**) while smaller groups generally show improved activity (i.e., cyclopropyl, dimethyl and ethylmethyl; **8d**, **8h**, and **8i**) although the effects are not dramatic. The isomeric aminoalcohols **10** also show good in vivo activity in this model. As was

observed with the aminoalcohols **8**, the smaller alkyl substituted analogues are the most potent of this group.

In conclusion, we have discovered a new class of antibiotics with very promising in vitro activity against two common veterinary pathogens. In addition, many members of this new series of antimicrobial agents demonstrate excellent in vivo activity against *P. multocida* in a murine infection model indicating that these compounds are promising candidates for the treatment of livestock respiratory disease. Several of the more interesting members of this series of compounds warrant further investigation of pharmacokinetic characteristics and antimicrobial efficacy.

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6. The stereochemical outcome of this reaction was verified by reaction of the epoxide of **7** with lithium aluminum hydride to form the methyl carbinol and comparison to the product resulting from addition of methyl magnesium bromide to the ketone **6**. The stereochemical outcome of the latter reaction was demonstrated through a single crystal X-ray structure, the details of which will be published in due course (manuscript in preparation).
7. The *E. coli* strain 51A0150 (poultry lung origin) and *P. multocida* strain 59A0067 (turkey origin) were used in this assay to test the antibacterial activity. Both strains were grown on Brain Heart Infusion (BHI) plates overnight. Several colonies were suspended into saline and adjusted to $OD_{625nm} = 0.09$ (0.5 McFarland unit). The inoculum solution was made by preparing a 1:100 dilution of 0.5 McFarland saline suspension using BHI broth and 100 μL of this suspension was added to 100 μL of BHI broth containing various concentrations of test antibiotics. The test antibiotic solution was serially diluted 2-fold by automatic pipette in a 96-well microtiter format. After inoculation with both strains (final density was approximately 5×10^5 cfu/mL), the microtiter plates were incubated at 37 °C for 18 h. MIC was determined as the lowest concentration of the test compound in which the absorbency at 600 nm is less than or to equal 0.025. For reference, tilmicosin gave an MIC of 0.78 μg/mL against *P. multocida* and 50 μg/mL against *E. coli* in these assays.
8. The experimental parameters for the murine model are as follows; twenty gram female CF-1 mice were infected intranasally with 50 μL of a suspension of 2×10^4 *P. multocida* serotype 5A. Compounds were administered subcutaneously 0.5 h post-infection at doses of 5–80 mg/kg. The ED_{50} was calculated based on the number of surviving mice 4 days after infection. For reference, Oxytetracycline has an ED_{50} of 17 mg/kg in this assay.