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Synthesis, Stereochemical Assignment and Biological Activity of a Novel Series of C-4″ Modified Aza-Macrolides

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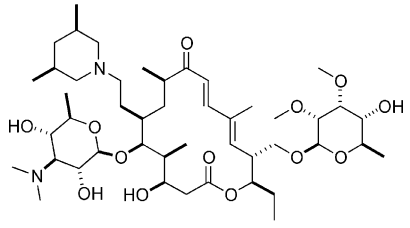
Abstract—Modification of the cladinose C-4″ position via manipulation of the corresponding keto derivatives afforded two stereochemically pure series of compounds. The synthesis and structure determination of these compounds is described within. The in vitro and in vivo biological activity of this novel series of C-4″ modified macrolides is also described.

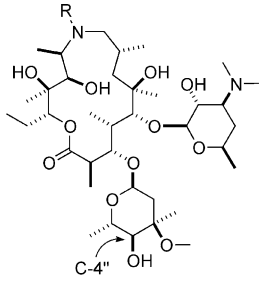
One of the leading causes of mortality and morbidity in North American bovine and swine livestock operations is respiratory disease. While multiple factors can lead to disease, outbreaks are frequently associated with animals that have experienced some form of environmental stress. It is estimated that the global economic losses related to bovine respiratory disease (BRD) and swine respiratory disease (SRD) is in excess of five billion dollars.[1] A wide variety of antibacterial agents are approved for treating livestock respiratory disease, including the 16-membered semisynthetic macrolide tilmicosin (Micotil®, **1**), which is one of the most popular therapeutic agents for BRD based in part on an extended duration of action.[1] Tilmicosin, however, is not useful for the treatment of SRD.

Our initial goal in this area was to design an antibacterial agent that would have improved in vitro potency and spectrum when compared to current therapies such as tilmicosin. Another goal was to discover novel chemotypes that would be safe, long-acting and effective for the treatment of respiratory diseases caused by Gram negative pathogens in both cattle and swine.



Tilmicosin (**1**)

During our efforts to identify compounds with the above-mentioned properties, we became interested in other classes of semi-synthetic macrolides, such as the 15-membered aza-macrolides **2** and **3** previously reported by Pfizer[2] and Merck,[3] respectively. Our interest in these chemotypes was primarily due to their favorable tissue distribution, half-life and broad-spectrum antimicrobial activity.



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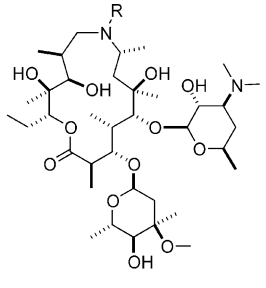

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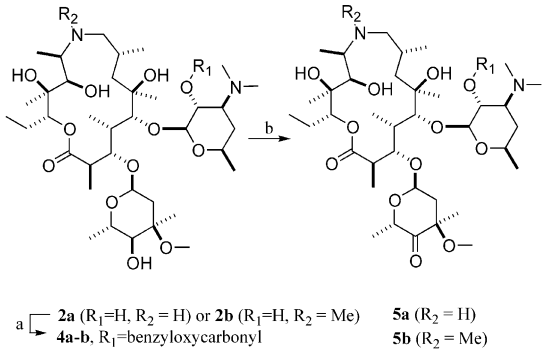
More specifically, we felt that modification of the C-4″ position of the cladinose sugar on these templates could further enhance the potency of our analogues versus the Gram negative pathogens associated with bovine respiratory disease. While there have been previous reports on the modification of this position for both **2** and **3**, including a recent report from this group[4] describing the triamilide class of C-4″ modified aminoalcohols, we were intent on expanding the scope of accessible compounds as well as identifying a synthetic strategy that would provide both efficiency and high levels of stereoselectivity to our analogue program. Our efforts in this area have resulted in the preparation and characterization of a novel series of C-4″ tertiary alcohols that are potent antibacterial agents.

Chemistry

Working from templates **2**, we identified the C-4″ ketones **5** as our key synthetic intermediates. Ketones **5** are readily prepared in two steps from the corresponding alcohols. As described previously,[4] treatment of **2a** (R_1, R_2 = H) with one equivalent of benzyl chloroformate, followed by oxidation of the C-4″ alcohol of **4a** with EDC/PTFA proceeded smoothly to give ketone **5a** in 88% overall yield. Similar results were obtained when preparing the corresponding analogue **5b** (R_2 = Me). Although somewhat unstable in solution, the solid state stability of intermediates **5** was suitable for extended storage and this sequence was routinely conducted on scale in excess of one hundred grams (Scheme 1).

Initial attempts to effect the addition of organometallic reagents to ketones **5** afforded very low yields of the desired compounds. Optimization efforts included pretreatment of the macrolide with TMSCl in THF and modification of the reactivity of the organometallic reagent (RMgX or RLi) via the addition of additives ($ZnCl_2$, $MgBr_2$, $CeCl_3$) in combination with solvent screening (THF, Et_2O, DME). The most significant improvements in the efficiency of the reaction were realized employing excess Grignard reagents with DME as the solvent.[5] Subsequent removal of the C-2′ benzyl carbonate with methanol proceeded smoothly to afford the desired products **6** in moderate to good yields as a single isomer at C-4″ (Scheme 2).[6]

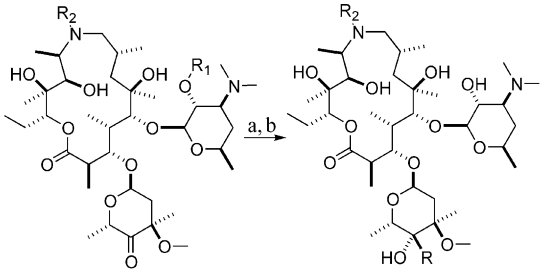


Scheme 1. (a) CBZ-Cl, CH_2Cl_2, 0 °C; (b) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, pyridinium trifluoroacetate, 0–23°C.

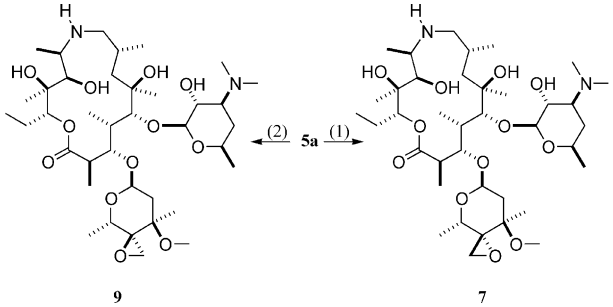
Conversion of the ketone intermediate **5** to the corresponding epoxide was also desirable and, as outlined in Scheme 3 (condition 1), utilization of the stabilized ylide derived from $Me_3S(O)I$ under standard conditions followed by deprotection afforded the desired epoxide **7** in good overall yield. [4]

Initial efforts to complete the analogous transformation with the unstabilized trimethylsulfonium ylide (NaH/DMSO, Me_3SI, THF, 0 °C) were met with failure, instead affording multiple products attributed to decomposition of the starting material. The overall efficiency of the reaction was greatly improved by employing KHMDS as the base and running the reaction in DME at low temperature, although the yield of desired product was variable due to the formation of the corresponding thiomethyl ether **8**. Formation of **8** likely proceeds through attack of the ylide as anticipated, followed by demethylation of the zwitterionic intermediate (Scheme 4). It is postulated that the iodide counter ion introduced via the sulfonium salt is involved in the demethylation reaction. This hypothesis is supported by the fact that the yield of epoxide **9** improved and formation of the thiomethyl ether was suppressed when Me_3SBF_4 was used as the source of the sulfur ylide (Scheme 3, condition 2).

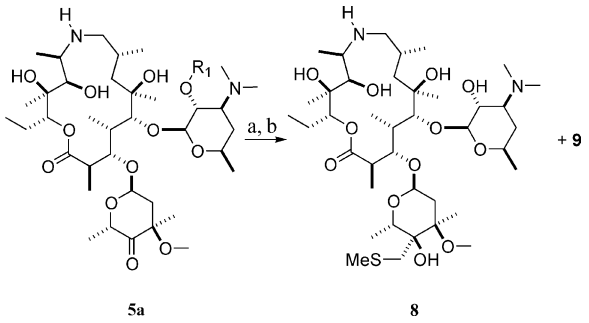
Comparison of both the ^{1}H NMR spectra and HPLC retention times of **7** and **9** indicated that the major product obtained from each epoxidation reaction was unique. In addition, HPLC analysis of the crude reaction mixtures indicated that epoxidation under condition 1 afforded ca. 10% of **9**, while condition 2 afforded less than 5% of **7**. Although significant literature pre-



Scheme 2. (a) RMgX, DME, −78 °C; (b) MeOH.



Scheme 3. (1) (a) $Me_3S(O)I$, NaH, tetrahydrofuran, 100%; (b) MeOH.-*or*- (2) (a) Me_3SBF_4, KHMDS, toluene, tetrahydrofuran, 58%; (b) MeOH.



Scheme 4. (a) Me_3SI, KHMDS, toluene, tetrahydrofuran, −78 °C; (b) MeOH.

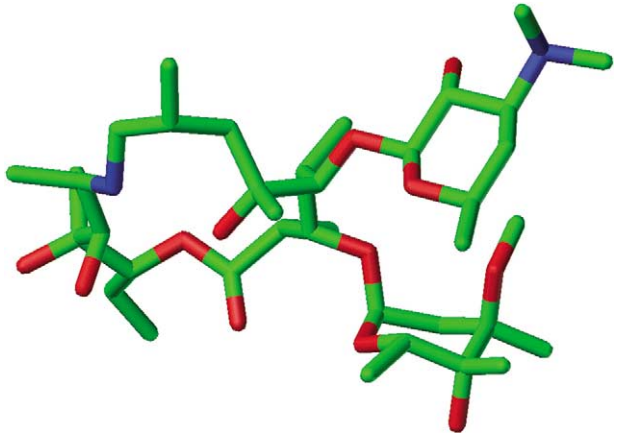


Figure 1. The configuration of **6a** in the solid state. Carbon atoms are in green, oxygen atoms in red and nitrogen atoms in blue.

cedence exists for assigning the stereochemical outcome of these two reactions, our goal was to unambiguously assign the stereochemistry of each of the products. In addition, the stereochemical relationship between the epoxides and the products obtained via direct Grignard addition to the ketone was also unknown at this time.

After numerous attempts, we were successful in obtaining a single crystal X-ray structure of tertiary alcohol **6a** (R = Me, R_2 = H), obtained from the addition of MeMgBr to ketone **5a**. As illustrated in Figure 1, attack of the Grignard reagent affords a single diastereomer that places the alcohol in the axial (unnatural) configuration. The high degree of selectivity observed for this reaction is attributed to chelation of the Grignard reagent to the neighboring methoxy group and subsequent delivery of the Grignard reagent to the same face of the cladinose ring structure.

Conversion of **7** to **6a** using lithium aluminum hydride confirmed the stereochemical assignment of **7**.[7] The stereochemistry of the epoxide **9** was determined by reaction of the epoxide with *n*-propyl amine and subsequent solution of an X-ray structure of the product as described previously.[4] These results confirm our assumption that the stabilized sulfoxonium ylide reacted with the ketone **5a** to form epoxide **7**, the result of equatorial attack on the ketone and that the unstabilized ylide reacted with the ketone to form epoxide **9**, which is the result of axial attack on the ketone.

A wide variety of substituents at the C-4″ position were prepared via addition of the appropriate Grignard reagent to the C-4″ carbonyl compounds **5a** and **5b**. Tables 1 and 2 show the in vitro activity of these compounds against two important livestock pathogens, *Pasteurella multocida* and *Escherichia coli*.[8] The tables also show the activity of many of the analogues in an in vivo mouse *P. multocida* infection model.[9]

Small C-4″ substituents generally gave the best activity versus *P. multocida* (e.g., **6a–e**, **6g–j**), however slightly larger substituents such as *n*-butyl (**6f**), pyridyl (**6m–n**) or phenyl (**6o**) show weaker activity against this pathogen. A similar structure–activity relationship was observed against *E. coli*. Potent in vivo activity was observed with the methyl substituted analogue **6a** and

Table 1. MIC values against *P. multocida* and *E. coli*, and ED_{50} values in a mouse *P. multocida* infection model for compounds **6**, R_2 = H and compounds **7** and **9**

Compd	R^2	*P. mult.* MIC (μg/mL)	*E. coli* MIC (μg/mL)	Mouse ED_{50} (mg/kg)
6a	Me–	<0.1	0.78	28
6b	Et–	0.2	1.56	61
6c	$H_2C=CH$–	0.2	1.56	66
6d	*n*-Pr–	0.2	1.56	81
6e	$H_2C=CHCH_2$–	<0.1	0.78	58
6f	*n*-Bu–	0.78	12.5	n.d.
6g	HCC–	<0.1	1.56/3.13	24
6h	H_3COCH_2CC–	0.2	3.13	20
6i	$(H_3C)_2NCH_2CC$–	0.2	1.56	22
6j	H_3CCC–	0.1/0.2	0.78/1.56	21
6k	2-Thiophenyl–	0.39/0.78	3.13	>80
6l	2-Furanyl–	0.2	1.56	72
6m	2-Pyridyl–	0.78	3.13	>40
6n	3-Pyridyl–	0.39	3.13	>40
6o	Phenyl	1.56/3.13	6.35/12.5	n.d.
6p	2-(NMe)-indole–	0.2	3.13	>40
6q	$H_3CO(CH_2)_3$–	0.1/0.2	0.78	49
6r	$HOCH_2CH_2$–	0.39	6.25	n.d.
6s	H_3CSCH_2–	0.1	3.13	44
7	—	<0.1	1.56	31
9	—	0.1	3.13	n.d.

Table 2. MIC values against *P. multocida* and *E. coli*, and ED_{50} values in a mouse *P. multocida* infection model for compounds **6**, $R_2 = CH_3$

Compd	R^2	*P. mult.* MIC (μg/mL)	*E. coli* MIC (μg/mL)	Mouse ED_{50} (mg/kg)
6t	Me–	0.05/0.1	0.39/0.78	26
6u	H_3COCH_2CC–	0.05	0.39	29
6v	$(H_3C)_2NCH_2CC$–	0.1	0.39	40
6w	HCC–	0.05/0.1	0.39/0.78	33
6x	2-(NMe)-indole–	0.39	6.25	>40
6y	2-(NMe)-imidazole–	0.05	0.39	n.d.
6z	$H_3CO(CH_2)_3$–	0.1	0.39	>80
6aa	H_3CCC–	0.2	1.56	42

the alkynes (**6g–j**). Other substituents including the aryl and heteroaryl substituted tertiary alcohols showed weak in vivo activity. For reference, tilmicosin gave an MIC of 0.78 μg/mL against *P. multocida* and 50 μg/mL against *E. coli* in these same assays. Many of the new compounds reported here are more potent than tilmicosin against *P. multocida* and all of the compounds are significantly more potent than tilmicosin against *E. coli*. Epoxides **7** and **9** were also tested. The stereochemistry of the epoxide had little effect on potency (Table 1).

An analogous set of the *N*-Me azalides were also prepared and characterized. The trends in activity for representative analogues were similar to the trends observed for the *N*-H compounds. Smaller substituents were best and the larger or heteroaromatic analogues were not as potent. In general, the in vitro activity was comparable or slightly better than the *N*-H compounds described previously.

In conclusion, we have unambiguously defined the stereochemical outcome of the addition of nucleophiles to the C-4″ ketones of this class of macrolides and we have utilized this discovery to identify a novel class of C-4″ modified azalide antibiotics with good in vitro potency against *P. multocida* and *E. coli*, two significant veterinary pathogens responsible for BRD. Several members of this class of azalide antibiotics have potent in vivo activity in mice and warrant further in vivo studies in livestock. These studies will be published in due course.

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5. The Grignard reagents were typically employed in 8–10-fold excess. The Grignard acetylides were formed in situ via treatment of the corresponding alkyne with MeMgBr.

6. Compound **6q** was prepared via reduction of **6h** via hydrogenation. Compound **6r** was prepared via ozonolysis of **6e** and subsequent reduction with $NaBH_4$.

7. Epoxides **7** and **9** were treated with excess $LiAlH_4$ in THF and HPLC retention times of the crude product were compared to authentic **6a**. The crude material also contained epoxide starting material and small amounts of products attributed to reduction of the ring lactone.

8. 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 two 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. The minimum inhibitory concentration (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.

9. The experimental parameters for the murine model are as follows. Twenty gram female CF-1 mice were infected intranasally with 50 microliters of a suspension of 2×10^4 *P. multocida* serotype 5A. Compounds were administered subcutaneously 0.5 h post-infection at doses of 5 to 80 mg/kg. The effective dose for 50% of the aminals (i.e., the ED50) was calculated based on the number of surviving mice four days after infection.