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Synthesis and *In Vitro* Antimicrobial Activity of 3-Keto 16-Membered Macrolides Derived From Tylosin†

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Several series of 14-membered ketolides derived from erythromycin exhibit useful antimicrobial activity against macrolide-resistant bacteria. To determine if 16-membered ketolides may possess analogous activity, 3-keto derivatives of 5-*O*-mycaminosyl-23-*O*-acetyltylonolide and desmycosin were synthesized by protection of susceptible functional groups, oxidation of the 3-hydroxyl group under modified Moffatt-Pfitzner conditions, and subsequent deprotection. The resulting 3-keto products unexpectedly adopted the 2,3-*trans* enol rather than the 3-keto tautomer. The *trans* configuration of the 2,3-double bond in the macrolide chain is most likely the result of hydrogen bond stabilization between the enol hydroxyl and lactone carbonyl, which places these two groups in a *cis* relationship. This preference for the enol tautomer in 16-membered macrolides is not seen with 14-membered ketolides. The *in vitro* antimicrobial activity of the enol derivatives was greatly reduced compared to their unoxidized parent compounds, but the reduced antimicrobial activity of the enol derivatives paralleled results from corresponding 2,3-anhydro derivatives of 16-membered macrolides, which also have 2,3-*trans* stereochemistry. These results are in contrast to those from 14-membered-ring macrolides in which 3-keto and 2,3-anhydro derivatives exhibit greater activity than 3-hydroxy compounds.

The recent success of 3-keto derivatives of 14-membered-ring macrolides (ketolides) against macrolide-resistant bacteria has spawned renewed interest in macrolide antibiotics.[1~3] These ketolides are formally derived from erythromycin by hydrolysis of cladinose at C-3 and subsequent oxidation of the liberated 3-hydroxyl group. We undertook the synthesis of an analogous series of 16-membered-ring ketolides, based on the tylosin (**1**) analogs 5-*O*-mycaminosyltylonolide (**2**) and desmycosin (**3**), to see if new antibiotics could be generated having activity against macrolide-resistant strains (Figure 1).

Results

The first example of this new series of 16-membered-ring ketolides to be synthesized was 2,3-didehydro-5-*O*-mycaminosyl-23-*O*-acetyltylonolide (**7**) (Figure 2). Its synthesis was accomplished by ketalization of the aldehyde in **2** followed by acetylation of the 2′-, 4′-, and 23-hydroxyl groups to give the triacetylated derivative **4** as a mixture along with the 2′,4′-diacetylated compound.[4,5] Oxidation of the 3-hydroxyl group of **4** under modified Moffatt-Pfitzner conditions followed by deprotection gave compound **7**.[6] Attempts to oxidize the 3-hydroxyl group of **4** with pyridinium dichromate led to the *N*-demethyl-*N*-formyl derivative **6**, analogous to oxidation of *N*,*N*-dimethylamino-containing steroids using chromium (VI) oxide in pyridine.[7] Partial deacetylation and deketalization of **4** gave 5-*O*-mycaminosyl-23-*O*-acetyltylonolide (**5**) to use for comparisons of antimicrobial activity.[4,5]

In order to also examine the antimicrobial activity of an

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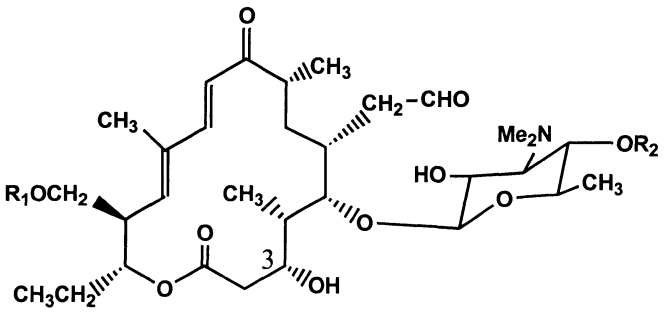
Fig. 1. The structures of tylosin (**1**), 5-*O*-mycaminosyltylonolide (**2**), and desmycosin (**3**).



tylosin (**1**):

R_1 = β-D-mycinosyl

R_2 = α-L-mycarosyl

5-O-mycaminosyltylonolide (**2**):

R_1, R_2 = H

desmycosin (**3**):

R_1 = β-D-mycinosyl

R_2 = H

analog in the desmycosin-related series, 2,3-didehydrodesmycosin (**14**) was synthesized. 2′,4′,4″-Tri-*O*-acetyldesmycosin-20-diethylketal (**8**) was generated from **3** following literature procedures.[8~10] This material was then oxidized under the same conditions that had yielded **7**.[6] In this case, however, the modified Moffatt-Pfitzner conditions gave not only the 3-keto analog **9**, but also the 2-thio-ylide derivative **10** from over-reaction with excess reagent (Figure 3). This ylide formation is similar to thio-ylide formations using the Corey-Kim reagent.[11,12] Careful deacetylation of **9** with methanol and then ammonium hydroxide in methanol followed by deketalization gave **14**. Attempts to remove the acetyl groups using stronger deacylation reagents such as sodium methoxide or ammonia in methanol led to β-elimination of mycaminose. **8** was also deketalized to give **15** to use for comparisons of antimicrobial activity.

The proton NMR spectra of products **7** and **14**, along with their protected intermediates, showed that the compounds exist predominantly as the enol tautomer, with proton signals visible for both the enolic hydroxyl and the enolic vinyl protons (Figure 4). The carbon backbone of

Fig. 2. Protection of 5-*O*-mycaminosyltylonolide (**2**) and subsequent oxidation of the 3-hydroxyl group.

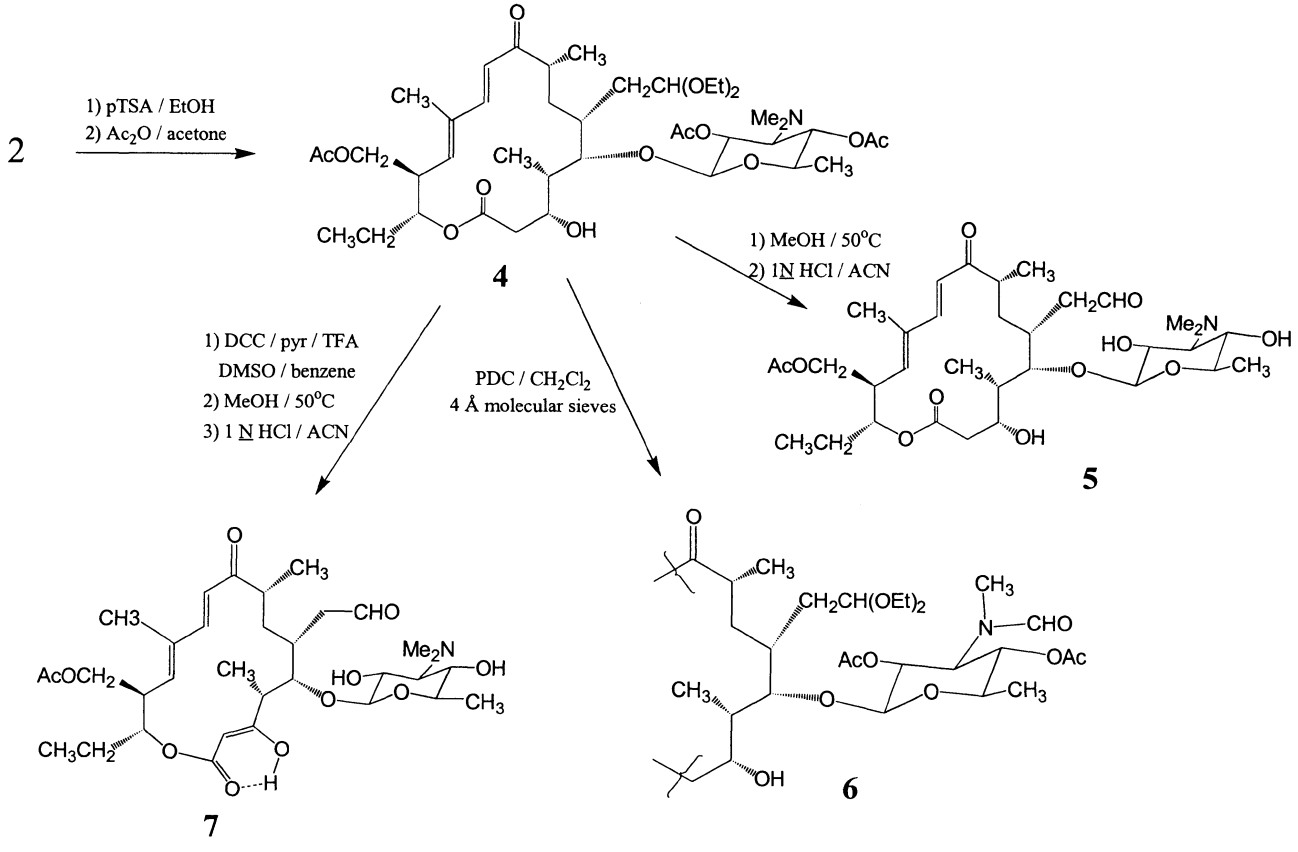


Fig. 3. Protection of desmycosin (**3**) and subsequent oxidation of the 3-hydroxyl group.

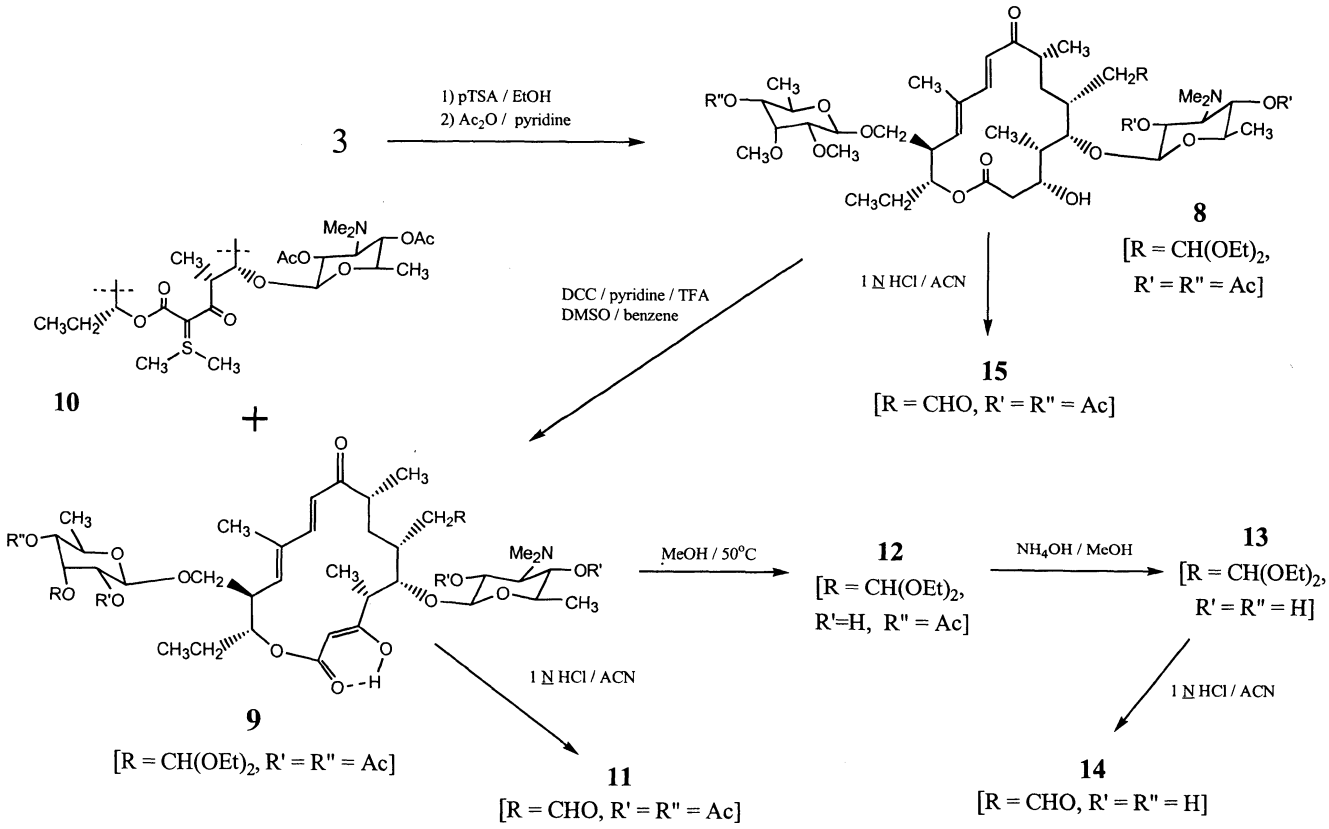


Fig. 4. ^{1}H-NMR of 2,3-didehydrodesmycosin (**14**) in $CDCl_3$ showing *trans* enol tautamer.

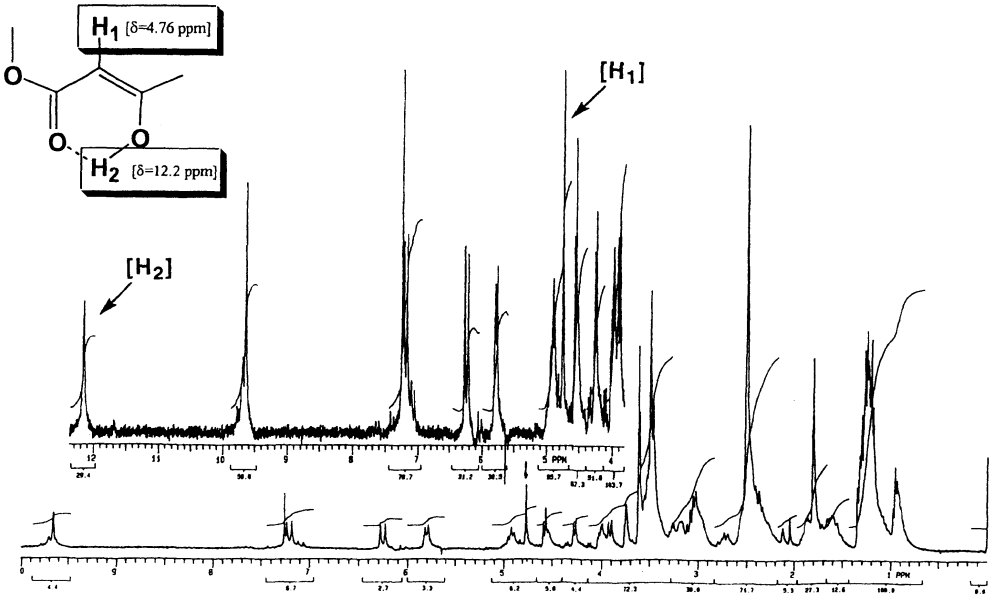
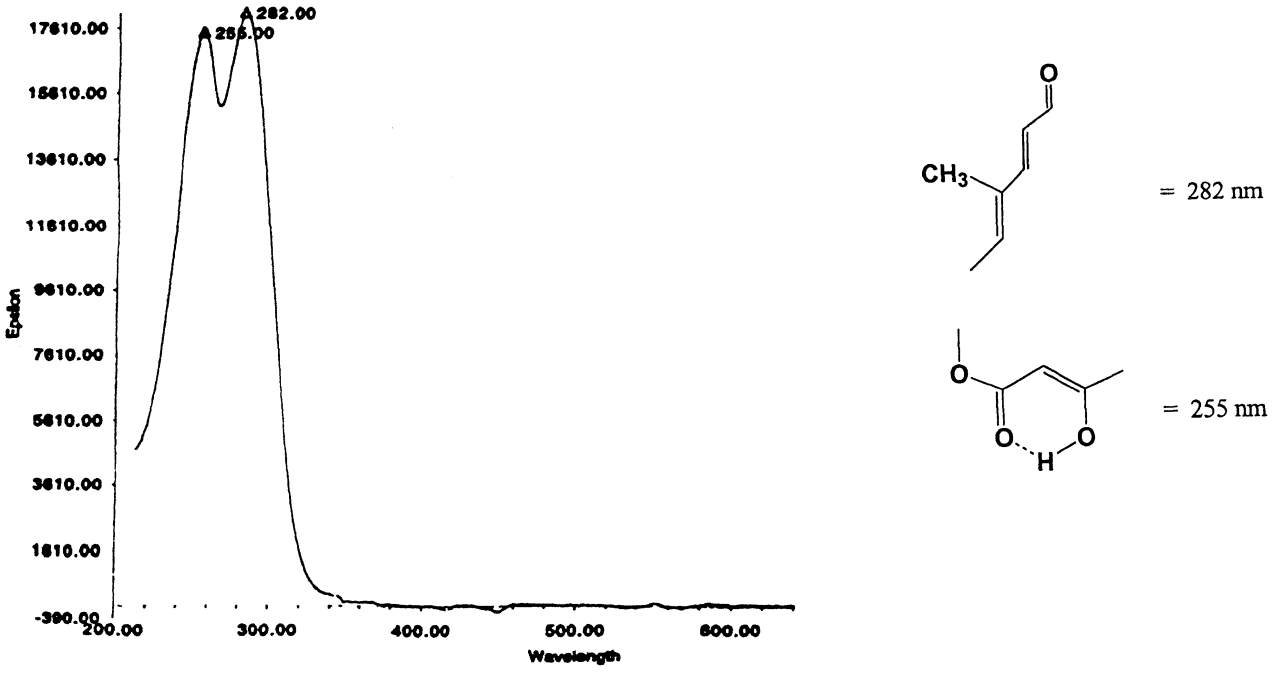


Fig. 5. UV spectrum of 2,3-didehydrodesmycosin (**14**) in EtOH showing *trans* enol tautamer.



the macrolide ring in the enol tautomer has *trans* stereochemistry most likely due to hydrogen bond stabilization between the enol hydroxyl and lactone carbonyl which causes these two groups to adopt a *cis* relationship.[13,14] This tautomeric preference was confirmed by a very strong absorbance at 255 nm in the ultra-violet spectrum, consistent with an enol tautomer (Figure 5).[15,16] The *trans* relationship of the 2,3-double bond in the carbon skeleton of these 16-membered macrolides is also consistent with the stereochemistry of the various 2,3-anhydro compounds discussed below.

The conversion of these 16-membered-ring macrolides to their ketolide analogs resulted in a significant reduction of *in vitro* antimicrobial activity against macrolide-susceptible Gram-positive bacteria. This unexpected loss of activity was shown by the comparison of *in vitro* antimicrobial activity between the parent 3-hydroxy compounds and their corresponding oxidized derivatives (see **5** *vs.* **7**, **15** *vs.* **11**, and **3** *vs.* **14**) (Table 1). Furthermore, no improvement of antibacterial activity was observed from these derivatives against either macrolide-resistant or Gram-negative strains (Table 1). The reduction of activity contrasts with results from the 14-membered ketolides in which oxidation of the 3-hydroxyl group to a 3-keto function improved *in vitro* activity.[17]

To discern if the reduced antimicrobial activity found in these new 16-membered ring analogs might be related to the structural preference of the 3-keto compounds to exist as their enol tautomers, the corresponding 2,3-anhydro derivatives were prepared. 2′,4′,4″-Tri-*O*-acetyl-2,3-anhydrodesmycosin (**17**) and 23-*O*-acetyl-2,3-anhydro-5-*O*-mycaminosyltylonolide (**20**) were synthesized by 3-*O*-mesylation followed by elimination of the 3-*O*-mesylate from their tri-acetyl intermediates **16** and **18**, respectively (Figure 6). The stereochemistry of the resulting 2,3-double bond was readily determined as *trans* from their ^{1}H-NMR spectra. The 18 Hz coupling constant between the two new vinyl protons is consistent with *trans* configuration of the double bond and is not consistent with *cis* stereochemistry.[18] These NMR chemical shifts and coupling constants are in accord with those assigned to other semisynthetic derivatives of 5-*O*-mycaminosyltylonolide having a 2,3-*trans*-double bond.[19] Furthermore, these assignments are also consistent with those made for members of the naturally-occurring series of mycinamicin- and chalcomycin-type macrolide antibiotics that also possess the 2,3-*trans* ring skeleton.[20~22]

Interestingly, the 2,3-anhydro derivatives also showed relatively poor *in vitro* antimicrobial activity compared to their parent macrolides (see **17** *vs.* **15** and **20** *vs.* **5**) (Table

Table 1. The antimicrobial activity (MIC; agar dilution method expressed in μg/ml) of 16-membered ketolides compared to their respective non-oxidized analogs.

Organism	Strain	5	7	20	15	11	17	3	14
Staphylococcus aureus	[X400]	0.5	8	8	2	64	16	2	8
S. aureus	[489]*	>64	>64	>64	>64	>64	>64	>64	>64
Staphylococcus haemolyticus	[415]	0.25	8	4	2	>64	16	1	16
Staphylococcus epidermidis	[270]*	>64	>64	>64	>64	>64	>64	>64	16
Streptococcus pyogenes	[C203]	<.06	2	1	0.25	32	2	<.06	0.5
Streptococcus pneumoniae	[P1]	<.06	1	1	0.5	32	8	<.06	4
Enterococcus faecium	[180-1]	0.25	8	4	2	>64	32	1	16
E. faecium	[180]*	>64	>64	>64	>64	>64	>64	>64	>64
Enterococcus faecalis	[2041]	0.5	16	16	4	>64	64	0.5	16
E. faecalis	[276]*	>64	>64	>64	>64	>64	>64	>64	>64
Moraxella catarrhalis	[M12]	>64	>64	>64	>64	>64	>64	>64	>64
Haemophilus influenzae	[RD]	4	>64	>64	>64	32	>64	16	>64
Escherichia coli	[EC14]	>64	>64	>64	>64	>64	>64	>64	>64

* Constitutively resistant to macrolide antibiotics

1). Furthermore, the *in vitro* activity of the 2,3-anhydro compounds was relatively comparable to that of the corresponding 2,3-didehydro derivatives (see **20** *vs.* **7** and **17** *vs.* **11**) (Table 1). These results again contrast with those found with 14-membered macrolides in which 2,3-anhydro derivatives (anhydrolides) are more active *in vitro* than the corresponding 3-hydroxy compounds.[23]

The isolation of thio-ylide derivative **10** suggested that 3-keto derivatives of 16-membered macrolides could be prepared if the 3-keto structure could be stabilized from tautomerizing. Toward that goal, the α-methylene derivative **21** was successfully synthesized by a Mannich reaction (Figure 7).[24] However, since neither compound **10** nor **21** exhibited any substantial *in vitro* antibacterial activity, they were not pursued further. Initial attempts to synthesize either a 2-fluoro (**9**+Selectfluor/ACN/r.t.) or a 2,2-dimethyl derivative (**9**+NaH/MeI/THF/r.t.) were also not promising. Consequently, additional synthetic effort will be required to prepare appropriately stabilized 3-keto tautomers of 16-membered macrolides for their antimicrobial evaluation.

The reasons for the reduction of *in vitro* antimicrobial activity exhibited by these new 16-membered ring ketolides and 2,3-anhydro derivatives are not known. Removal of the 3-hydroxyl group from 16-membered macrolides is not a deleterious modification since 3-deoxy derivatives are reported to have enhanced *in vitro* antimicrobial activity.[19,25] Deoxygenation at C-3 does not change the sp^3 geometry at these two centers. However, the introduction of the 2,3-double bond into the 16-membered ring may cause

Fig. 6. Formation of 2′,4′,4″-tri-*O*-acetyl-2,3-anhydrodesmycosin (**17**), and 23-*O*-acetyl-2,3-anhydro-5-*O*-mycaminosyltylonolide (**20**).

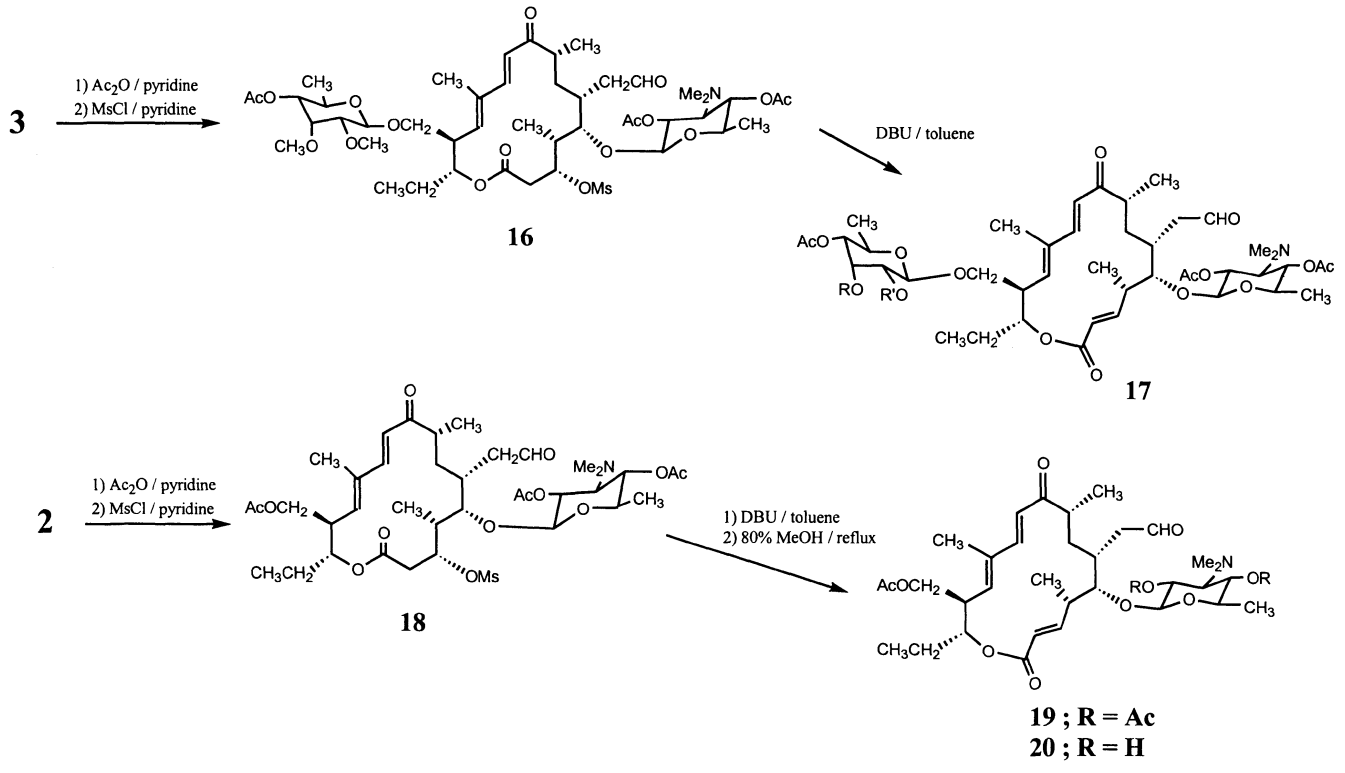
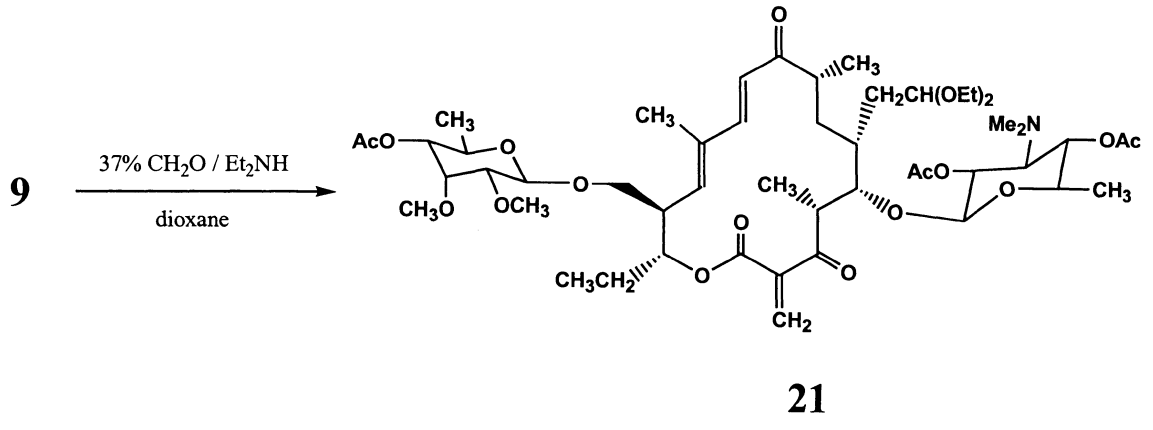


Fig. 7. Synthesis of 2-methylidene-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylketal.



a significant conformational change in the three-dimensional structure that could lead to substantially reduced binding of the macrolide to its antimicrobial target. The parallel reduction of antibacterial activity between the 2,3-enol and 2,3-anhydro derivatives suggests that a common cause may be responsible, which is most likely a conformational change in the molecules due to the change from sp^3 to sp^2 centers at both C-2 and C-3. The antimicrobial effects of these modifications in tylosin-related macrolides appear to differ from the effects

of related transformations of erythromycin-related macrolides.[17,23,26]

It has been well demonstrated that further substitutions at C-6, C-9, C-11, and C-12 of 14-membered ketolides result in substantially improved antimicrobial features, resulting in potent new macrolide antibiotics such as telithromycin and ABT-773.[1~3] It is unknown at the present time whether additional substitutions of 16-membered ketolides and 2,3-anhydro derivatives might similarly enhance their antimicrobial properties.

A recent presentation from another research group has reported very similar results in the synthesis and evaluation of 16-membered ketolide and anhydro derivatives.[27]

Experimental

General Procedures

^{1}H-NMR spectra were measured in $CDCl_3$ solution on either a Varian Gemini-300 or JEOL FX-90 spectrometer and are scaled from internal TMS. IR spectra were obtained on a Nicolet 510P Optical Bench spectrometer. UV spectra were measured in 95% EtOH on a Shimadzu UV-2101 PC spectrophotometer. Field desorption mass spectra (FD-MS) were obtained on a VG ZAB-3F mass spectrometer.

The preparation of desmycosin and 5-*O*-mycaminosyltylonolide were carried out at the Lilly fermentation products research facilities in Indianapolis, IN. All other commercially available reagents were obtained from Aldrich Chemical Co., Milwaukee, WI. Antibiotic susceptibility data were obtained by the broth dilution method.

2′,4′,23-Tri-*O*-acetyl-5-*O*-mycaminosyltylonolide-20-diethylketal (**4**)

5-*O*-Mycaminosyltylonolide-20-diethylketal (9.02 g, 13.4 mmol), produced by literature procedures, was dissolved in acetonitrile (90 ml).[28,29] To this solution was added acetic anhydride (3.2 ml, 33.9 mmol), and the mixture was stirred for 3 days at room temperature. The mixture was then evaporated at room temperature under vacuum. The residue was chromatographed on silica gel, eluting with 75% ethyl acetate in hexane, to give **4** (2.38 g, 22% yield) as a colorless glass. FD-MS *m/z* 798 (M^+); UV λ_{max} nm (ε) 280 (21,465); IR ($CHCl_3$) cm^{-1} 3011, 2976, 2937, 2878, 2791, 1741, 1679, 1456, 1373, 1235, 1169, 1123, 1057, 985; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (6H, s, OAc), 2.09 (3H, s, OAc). 2′,4′-Di-*O*-acetyl-5-*O*-mycaminosyltylonolide-20-diethylketal (4.16 g, 41% yield) was also isolated as a colorless glass.

2′,4′,23-Tri-*O*-acetyl-3′-*N*-demethyl-3′-*N*-formyl-5-*O*-mycaminosyltylonolide-20-diethylketal (**6**)

4 (217.9 mg, 0.27 mmol) was dissolved in dichloromethane (15 ml) along with crushed molecular sieves (0.5 g). To this suspension was added commercially available pyridinium dichromate (228.1 mg, 0.61 mmol) and the mixture was stirred for 1.75 hours at room temperature. The mixture was filtered through Celite and washed with fresh dichloromethane. The combined filtrate was evaporated at room temperature under vacuum. The residue was chromatographed on silica gel, eluting with 75% ethyl acetate in hexane, to give **6** (91.2 mg, 42% yield) as a white solid. FD-MS *m/z* 812 (M^+); UV λ_{max} nm (ε) 279 (21,567); IR ($CHCl_3$) cm^{-1} 2974, 1743, 1674, 1594, 1374, 1231, 1170, 1061, 1043; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (3H, s, OAc), 2.09 (3H, s, OAc), 7.91 (2/3H, s, NCHO), 8.05 (1/3H, s, NCHO).

2,3-Didehydro-5-*O*-mycaminosyl-23-*O*-acetyltylonolide (**7**)

4 (534.3 mg, 0.67 mmol) was dissolved in anhydrous benzene (5 ml). To this solution was added commercially available *N*,*N*-dicyclohexylcarbodiimide (416 mg, 2.0 mmol) and dimethylsulfoxide (5 ml) followed by anhydrous pyridine (54 μl, 0.67 mmol) and then trifluoroacetic acid (26 μl, 0.33 mmol). After stirring at room temperature for 27 hours, ethyl acetate (15 ml) was added and the mixture was filtered. The precipitate was washed with fresh ethyl acetate. The filtrates were combined, washed with water and then brine. The organic solution was dried over anhydrous Na_2SO_4 and evaporated at room temperature under vacuum. The residue was chromatographed on silica gel, eluting with 60% ethyl acetate in hexane, to give 2,3-didehydro-2′,4′,23′-tri-*O*-acetyl-5-*O*-mycaminosyltylonolide-20-diethylketal (302.5 mg, 57% yield).

2,3-Didehydro-2′,4′,23′-tri-*O*-acetyl-5-*O*-mycaminosyltylonolide-20-diethylketal (216.2 mg, 0.27 mmol) was dissolved in anhydrous methanol (20 ml) and heated under nitrogen at 50°C for 18.5 hours. The mixture was then cooled and evaporated at room temperature under vacuum, giving 2,3-didehydro-5-*O*-mycaminosyl-23-*O*-acetyltylonolide-20-diethylketal (162.8 mg, 85% yield).

2,3-Didehydro-5-*O*-mycaminosyl-23-*O*-acetyltylonolide-20-diethylketal (149.3 mg, 0.21 mmol) was suspended in acetonitrile (3 ml) and to this suspension was added 1 N HCl (1 ml). The mixture was stirred at room temperature for 30 minutes and was then poured into saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The organic solution was washed with brine, dried over anhydrous Na_2SO_4 and evaporated at room temperature

under vacuum. The crude material was chromatographed on silica gel, eluting with 25% methanol in dichloromethane, to give **7** (62.9 mg, 47% yield). FD-MS *m/z* 638 $(M+H)^+$, 637 (M^+); UV λ_{max} nm (ε) 277 (16,744), 255 (18,057); IR ($CHCl_3$) cm^{-1} 2975, 1736, 1627, 1595, 1237, 1060, 982; ^1H NMR (300 MHz, $CDCl_3$) δ 2.09 (3H, s, OAc), 4.79 (2/3H, s, enol-H), 9.65 (2/3H, s, CHO), 9.71 (1/3H, s, CHO), 12.15 (2/3H, s, enol-OH).

2′,4′,4″-Tri-*O*-acetyldesmycosin-20-diethylketal (**8**)

Desmycosin-20-diethylketal (4.9 g, 5.79 mmol), produced by literature procedures, was dissolved in anhydrous pyridine (50 ml).[10,30] To this solution was added acetic anhydride (3 ml, 31.8 mmol) and the mixture was stirred at room temperature for 22 hours. The mixture was then evaporated at room temperature under vacuum to give **8** (5.76 g, 100% yield), which was used without further purification.

2,3-Didehydro-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylketal (**9**) and 2-Dimethylthioylide-2,3-didehydro-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylketal (**10**)

8 (3.52 g, 3.6 mmol) was dissolved in anhydrous benzene (35 ml). To this solution was added *N,N′*-dicyclohexylcarbodiimide (3.18 g, 15.3 mmol) dissolved in dimethylsulfoxide (35 ml) followed by anhydrous pyridine (410 μl, 5 mmol) and trifluoroacetic acid (200 μl, 2.6 mmol). The mixture was stirred at room temperature for 19.5 hours and was then diluted with ethyl acetate and washed with water. The organic layer was filtered, washed with brine, dried over anhydrous Na_2SO_4 and evaporated at room temperature under vacuum. The crude product was chromatographed on silica gel, eluting with 50% ethyl acetate in hexane, to give **10** (156.9 mg, 4.2% yield) as the higher Rf material and **9** (1.47 g, 42% yield) as pale yellow solid. For **9**: FD-MS *m/z* 970 (M^+); UV λ_{max} nm (ε) 282 (17,206), 256 (16,442); IR ($CHCl_3$) cm^{-1} 2976, 2936, 1744, 1648, 1373, 1054, 979; ^1H NMR ($CDCl_3$) δ 2.06 (s, 6H, OAc), 2.12 (s, 3H, OAc), 4.83 (s, 2/3H, enol-H), 12.09 (s, 2/3H, enol-OH). For **10**: FD-MS *m/z* 1030 (M^+,100), 968 (40); UV λ_{max} nm (ε) 282 (broad, 10,383); IR (KBr) cm^{-1} 3445 (broad), 2974, 2930, 1750, 1627, 1232, 1088, 1050; ^1H NMR (300 MHz, $CDCl_3$) δ 2.05 (6H, s, SCH_3), 2.14 (6H, s, OAc), 2.19 (3H, s, OAc).

2,3-Didehydro-2′,4′,4″-tri-*O*-acetyldesmycosin (**11**)

9 (221.8 mg, 0.23 mmol) was dissolved in acetonitrile (5 ml). To this solution was added 1 N HCl (1.5 ml) and the mixture was stirred at room temperature for 1 hour. The mixture was then poured in saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic solution was washed with brine, dried over anhydrous Na_2SO_4, and evaporated at room temperature under vacuum to give **11** (201.3 mg, 98% yield) as an off-white solid. FD-MS *m/z* 895 (M^+); UV λ_{max} nm (ε) 284 (16,486), 253 (16,825); IR ($CHCl_3$) cm^{-1} 3020, 1744, 1645, 1595, 1373, 1232, 1054; ^1H NMR (300 MHz, $CDCl_3$) δ 2.05 (6H, s, OAc), 2.12 (3H, s, OAc), 4.78 (1/2H, s, enol-H), 12.12 (1/2H, s, enol-OH).

2,3-Didehydro-4″-*O*-acetyldesmycosin-20-diethylketal (**12**)

9 (3.54 g, 3.6 mmol) was dissolved in methanol (100 ml) and heated at 50°C for 18.5 hours. The solution was then evaporated at room temperature under vacuum to give **12** (3.35 g, 100% yield) as a pale yellow solid which was used without further purification.

2,3-Didehydrodesmycosin-20-diethylketal (**13**)

12 (2.01 g, 2.27 mmol) was dissolved in methanol (250 ml). To this solution was added concentrated ammonium hydroxide (0.5 ml) and the mixture was stirred at room temperature for 3 days. Glacial acetic acid (0.5 ml) was then added and the mixture was evaporated at room temperature under vacuum. The crude product was chromatographed on silica gel, eluting with 15% methanol in acetonitrile, to give **13** (490 mg, 26% yield) as a white solid. FD-MS *m/z* 844 (M^+); UV λ_{max} nm (ε) 278 (18,970), 257 (18,482); IR ($CHCl_3$) cm^{-1} 3018, 2976, 2935, 1652, 1596, 1379, 1237, 1165, 1081, 1060, 1007, 978; ^1H NMR (300 MHz, $CDCl_3$) δ 4.75 (1/3H, s, enol-H), 12.12 (1/3H, s, enol-OH).

2,3-Didehydrodesmycosin (**14**)

This reaction was run as with **11** starting with **13** (460 mg, 0.55 mmol) and gave **14** (375.5 mg, 89% yield) as a pale yellow solid. FD-MS *m/z* 770 (M^+); UV λ_{max} nm (ε) 282 (18,106), 255 (17,507); IR ($CHCl_3$) cm^{-1} 2936, 1723, 1642, 1594, 1165, 1081, 1060; ^1H NMR (300 MHz, $CDCl_3$) δ 4.76 (1H, s, enol-H), 9.65 (1H, s, CHO), 12.15 (1H, s, enol-OH).

2′,4′,4″-Tri-*O*-acetyl-3-*O*-mesyldesmycosin (**16**)

2′,4′,4″-Tri-*O*-acetyldesmycosin (3.0 g; 3.34 mmol) (prepared analogous to literature procedures) was dissolved in distilled pyridine (45 ml).[8~10] To this solution was added methanesulfonyl chloride (1.16 ml, 15 mmol) and the mixture was stirred at room temperature for 24 hours. The mixture was then evaporated under vacuum. The residue was dissolved in dichloromethane and washed with saturated aqueous $NaHCO_3$ solution. The dichloromethane

solution was dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was then dissolved in dioxane and lyophilized to give **16** (3.05 g, 94% yield) which was used without further purification.

2′,4′,4″-Tri-*O*-acetyl-2,3-anhydrodesmycosin (**17**)

16 (3.05 g, 3.13 mmol) was dissolved in dry toluene (40 ml). To this solution was added commercially available 96% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (495 mg, 3.13 mmol) and the mixture was stirred at room temperature for 1 hour. The mixture was then evaporated under vacuum to a small volume and decanted. The residual oil was washed with fresh toluene and decanted again. The toluene solutions were combined and evaporated under vacuum. The crude product was chromatographed on silica gel eluting with 50% ethyl acetate in toluene. The product was dissolved in dioxane and lyophilized to give **17** (2.19 g; 80% yield). FD-MS *m/z* 879 (M^+); UV λ_{max} nm (ε) 285 (19,000), 210 (21,500); IR ($CHCl_3$) cm^{-1} 3010, 2940, 1750, 1600, 1460, 1225, 1175, 1060; ^1H NMR (90 MHz, $CDCl_3$) δ 2.10 (6H, s, OAc), 215 (3H, s, OAc), 5.7 (1H, d, $J_{2,3}$=18 Hz, CO_2CH=CH), 6.83 (1H, dd, $J_{2,3}$=18 Hz, $J_{3,4}$=10.8 Hz, CO_2CH=CH).

2′,4′,23-Tri-*O*-acetyl-3-*O*-mesyl-5-*O*-mycaminosyltylonolide (**18**)

This reaction was run as with **16** starting with 2′,4′,23-tri-*O*-acetyl-*O*-mycamnosyltylonolide (8.6 g, 12 mmol), prepared by the literature procedure, to give **18** (8.07 g, 84% yield).[4~6] This material was used without further purification.

2′,4′,23-Tri-*O*-acetyl-2,3-anhydro-5-*O*-mycaminosyltylonolide (**19**)

This reaction was run as with **17**, starting with **18** (7.3 g, 9.1 mmol), to give **19** (4.17 g, 80% yield). FD-MS *m/z* 705 (M^+); UV λ_{max} nm (ε) 282 (17,500), 210 (21,500); ^1H NMR (90 MHz, $CDCl_3$) δ 2.10 (9H, s, OAc), 5.65 (1H, d, $J_{2,3}$=18 Hz, CO_2CH=CH), 6.82 (1H, dd, $J_{2,3}$=18 Hz, $J_{3,4}$=10.8 Hz, CO_2CH=CH).

23-*O*-Acetyl-2,3-anhydro-5-*O*-mycaminosyltylonolide (**20**)

19 (900 mg, 1.28 mmol) was dissolved in 80% aqueous methanol (60 ml) and the solution was heated to reflux for 1 hour. The mixture was then cooled to room temperature and evaporated under vacuum to near dryness. The residue was dissolved in dichloromethane and dried over anhydrous Na_2SO_4 and evaporated under vacuum. The crude product was chromatographed on silica gel, eluting with a step-wise gradient of 100% ethyl acetate to 15% absolute ethanol in ethyl acetate. The product was dissolved in dioxane and lyophilized to give **20** (514 mg, 65% yield). FD-MS *m/z* 621 (M^+); UV λ_{max} nm (ε) 280 (27,000), 215 (19,000); IR ($CHCl_3$) cm^{-1} 2940, 2900, 1720, 1670, 1640, 1585, 1450, 1360, 1335, 1220, 1170, 1050, 980; ^1H NMR (90 MHz, $CDCl_3$) δ 2.00 (3H, s, OAc), 5.6 (1H, d, $J_{2,3}$=18 Hz, CO_2CH=CH), 6.81 (1H, dd, $J_{2,3}$=18 Hz, $J_{3,4}$=10.8 Hz, CO_2CH=CH).

2-Methylidene-2′,4′,4″-tri-*O*-acetyldesmycosin-20-diethylketal (**21**)

Compound **9** (217.2 mg, 0.22 mmol) was dissolved in dioxane (3 ml). To this solution was added 37% aqueous formaldehyde (50 μl, 1.8 mmol). This addition was followed by diethylamine hydrochloride (29 mg, 0.26 mmol) and then diethylamine (68 μl, 0.33 mmol). This mixture was stirred at room temperature for 22 hours and was then poured into water. The aqueous mixture was extracted with dichloromethane. The dichloromethane was washed with brine, dried over anhydrous Na_2SO_4, and then evaporated at room temperature under vacuum. The crude product was chromatographed on silica gel eluting with 50% ethyl acetate in hexane followed by 75% ethyl acetate in hexane to give **21** (64.7 mg, 30% yield) as a white solid. FD-MS *m/z* 981 (M^+, 50), 980 (100); UV λ_{max} nm (ε) 264 (23,111 with large shoulder); IR (KBR) cm^{-1} 2977, 2937, 1750, 1659, 1374, 1232, 1187, 1168, 1089, 1052; ^1H NMR (300 MHz, $CDCl_3$) δ 6.01 (2H, s (broad with shoulder), $=CH_2$).

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