SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 20-DEOXO-20-(3,5-DIMETHYLPIPERIDIN-1-YL)DESMYCOSIN (TILMICOSIN, EL-870) AND RELATED CYCLIC AMINO DERIVATIVES

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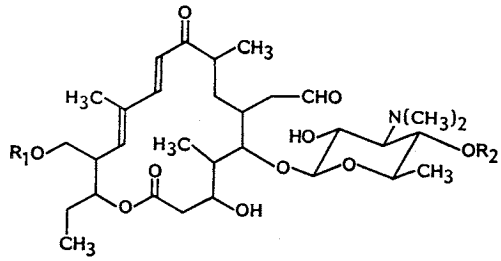
A series of 20-deoxo-20-cyclic (alkylamino) derivatives of tylosin, desmycosin, macrocin and lactenocin was prepared by reductive amination of the C-20 aldehyde group. The majority of the compounds were prepared using metal hydrides (sodium cyanoborohydride or sodium borohydride) as the reducing agents and a suitable cyclic alkylamine. Subsequently, a more convenient procedure was developed using formic acid as a reducing agent. The C-20 amino derivatives prepared from desmycosin exhibited good *in vitro* antimicrobial activity against *Pasteurella haemolytica* and *Pasteurella multocida* (MIC range of 0.78~6.25 μg/ml) as well as *Mycoplasma* species (MIC range of 0.39~6.25 μg/ml). Several derivatives showed excellent oral efficacy against infections caused by *P. multocida* in chicks. One of these derivatives, 20-deoxo-20-(3,5-dimethylpiperidin-1-yl)desmycosin (tilmicosin or EL-870) was selected for development as a therapeutic agent for pasteurellosis in calves and pigs.

The use of tylosin in veterinary medicine for the treatment and prevention of serious respiratory illness among farm animals has been well accepted.[1] The antimicrobial spectrum of this important antibiotic includes Gram-positive bacteria and *Mycoplasma* species.[2] In order to expand this antibiotic spectrum, a program of chemical modification and evaluation of novel derivatives of tylosin was undertaken, using tylosin-related intermediates as starting substrates (see Fig. 1).[3~8]

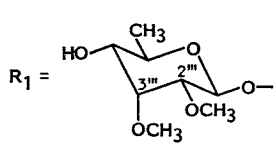
Recent investigations have shown that the C-20 aldehyde group could be radically modified or removed entirely by decarbonylation with retention of antibacterial activity.[9] TANAKA showed that dialkylamino substituents at the C-23 position of 5-*O*-mycaminosyltylonolide increased its antimicrobial activity and expanded its spectrum against some Gram-negative bacteria.[10] ŌMURA reported, however, that 20-deoxo-20-aminotylosin and 20,20″-dideoxo-20,20″-iminoditylosin both had decreased antimicrobial activity.[11] In a subsequent study, MATSUBARA used reductive amination of the C-20 aldehyde group of tylosin-like macrolides to synthesize a series of derivatives having C-20 secondary and tertiary amino functions.[12] Members of this series of C-20-amino macrolides showed no improvement in antimicrobial activity and did not expand the antimicrobial spectrum relative to tylosin against the organisms chosen for study.[12]

In the course of our studies, however, we showed that certain C-20 modified derivatives had good efficacy in chicks against experimental infections due to *Pasteurella multocida* and *Mycoplasma gallisepticum*.[13] One of the first derivatives tested, 20-deoxo-20-(heptamethyleneamino)desmycosin (**4**),

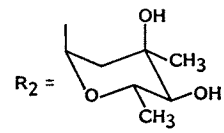
Fig. 1. Structure of tylosin and related 16-membered macrolides.



Tylosin



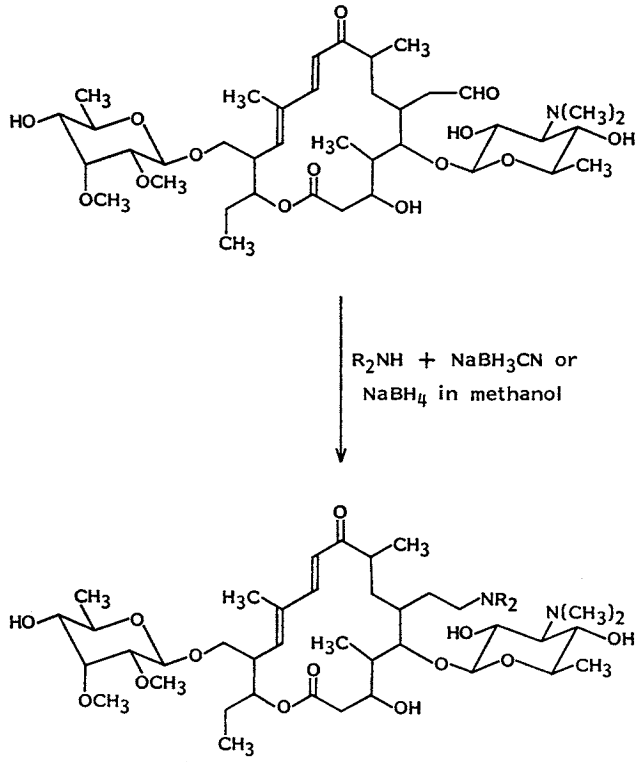
Mycinosyl



Mycarosyl

Desmycosin	R_1=Mycinosyl	R_2=H
Macrocin	R_1=3‴-*O*-Demethylmycinosyl	R_2=Mycarosyl
Lactenocin	R_1=3‴-*O*-Demethylmycinosyl	R_2=H

Fig. 2. Preparation of C-20-amino derivatives of desmycosin by reductive amination.



showed excellent activity against *P. multocida* in chicks both by subcutaneous and oral administration.[13] This compound represented an important advance toward our goal of expanding the antimicrobial spectrum of tylosin and desmycosin to include *P. multocida* and *Pasteurella haemolytica*.

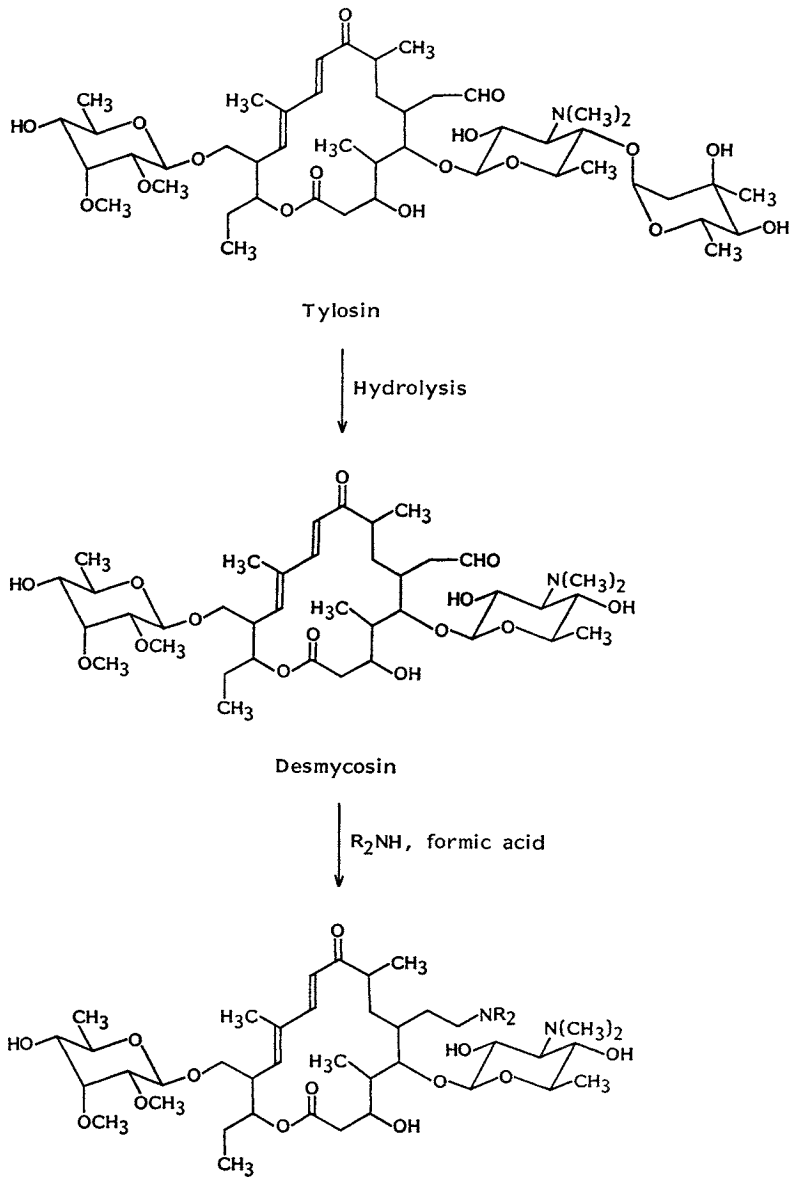
This paper will discuss the synthesis and evaluation of a series of derivatives of desmycosin,

tylosin, macrocin and lactenocin which contain a monocyclic or bicyclic amino function at the C-20 position. These derivatives were evaluated for *in vitro* and *in vivo* antibacterial activity with special emphasis on anti-*Pasteurella* activity. One of these derivatives, tilmicosin (20-deoxo-20-(3,5-dimethyl-piperidin-1-yl)desmycosin, **7**) was selected as a potential candidate for treatment of pasteurellosis in farm animals.[5,14]

Results and Discussion

The majority of the C-20-aminodesmycosin derivatives were synthesized by reductive amination

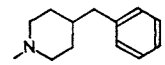
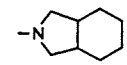
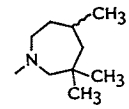
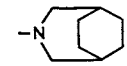
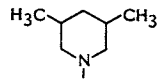
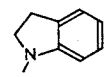
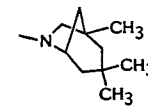
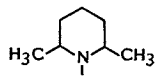
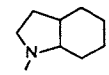
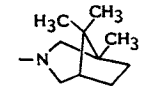
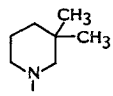
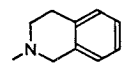
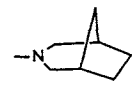
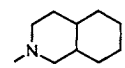
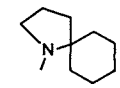
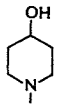
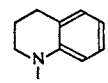
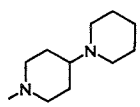
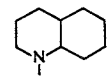
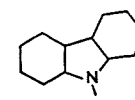
Fig. 3. Preparation of C-20-amino macrolides (Wallach reaction).



of the C-20 aldehyde group with the desired amine and sodium cyanoborohydride using a modification of the method reported by ŌMURA *et al.* (see Fig. 2).[11,12] The products were obtained in high purity by silica gel flash chromatography or extraction at pH 6.5 (0.5 M NaH_2PO_4 buffer). The C-20-amino macrolides were characterized by HPLC, MS, ^1H NMR (loss of the signal for the C-20 aldehyde proton and the presence of signals characteristic for the added amine group) and a new titratable group with a *pKa'* in the range of 7.5~8.0 (C-20 amine). Sodium borohydride could be substituted for sodium cyanoborohydride but was less convenient since the amount of this more reactive reducing agent had to be controlled very carefully to prevent concurrent reduction of the C-9 carbonyl group.

Although the metal hydride approach to reductive amination gave satisfactory results, an alternative method was sought to replace the metal hydrides and be more applicable to large scale operation. The reduction of an enamine with formic acid (Wallach reaction) had been used with simple, chemically stable aldehydes and ketones.[15~17] We found that the Wallach reaction could be applied in a novel manner to macrolides of the tylosin type. The reaction was carried out by dissolving the macrolide

Table 1. Structures of 20-deoxo-20-substituted aminodesmycosin derivatives (-NR_2 in Fig. 2).

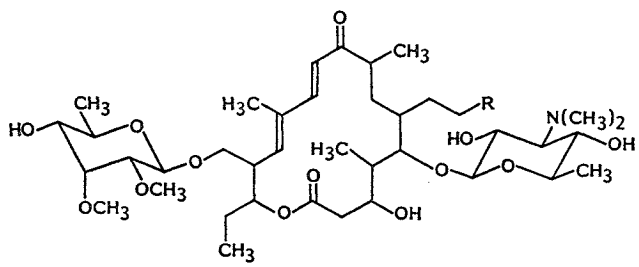
Compound No.	-NR_2	Compound No.	-NR_2	Compound No.	-NR_2
1	-$N(CH_2)_4$	13		21	
2	-$N(CH_2)_5$				
3	-$N(CH_2)_6$				
4	-$N(CH_2)_7$	14		22	
5	-$N(CH_2)_8$				
6	-$N(CH_2)_{12}$				
7		15		23	
8		16		24	
9		17		25	
10		18		26	
11		19		27	
12		20		28	

in formic acid in the presence of the desired amine (70°C, 20 hours). The reaction gave the C-20-amino macrolides in excellent yields in a single step (see Fig. 3). An advantage of this procedure is that it can be simplified to a "one pot reaction" by starting with a solution of tylosin, converting it to desmycosin by hydrolysis, extracting the desmycosin thus formed, and running the Wallach procedure directly on the extract (see Fig. 3). This procedure could be used interchangeably with the metal hydride approach and, on the whole, provided the best yields (85~90%). Since our studies, another group has applied a related reaction (the Clark-Eschweiler reductive methylation) to the synthesis of azahomoerythromycin B.[18]

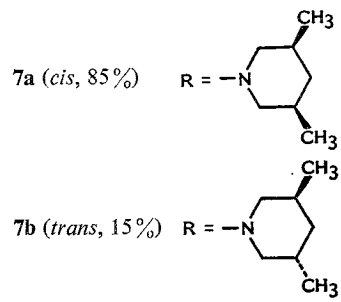
The reductive amination procedures allowed the use of a wide variety of secondary amines. No structural limitations were encountered except with sterically hindered amines such as 2,6-dimethylpiperidine, which gave low yields of the desired derivative. With unhindered amines, yields were generally high (70~80%). The structures of the C-20-aminodesmycosin derivatives which were synthesized are shown in Table 1, and the antimicrobial activity of the desmycosin derivatives is summarized in Tables 2~4. Table 5 lists the antimicrobial activity of the dimethylpiperidine analogs of tylosin (**29**), macrocin (**30**) and lactenocin (**31**).

In the course of these studies, the C-20-(3,5-dimethylpiperidin-1-yl)desmycosin derivative (**7**) assumed increased importance. The synthesis of **7** from commercial sources of 3,5-dimethylpiperidine resulted in a mixture of three closely related compounds. Commercial 3,5-dimethylpiperidine, produced by catalytic hydrogenation of 3,5-dimethylpyridine, is a mixture of *cis* (85%) and *trans* (15%) isomers. Use of this mixture in the reductive amination yielded a mixture of amino macrolides in approximately the same ratio as the amines present in the starting mixture. Furthermore, as the *trans* isomer of 3,5-dimethylpiperidine was itself a mixture of enantiomers, the resulting (*trans*-3,5-dimethylpiperidin-1-yl)desmycosin (**7b**) was a pair of diastereomers, a *threo* and an *erythro* isomer. Pure *cis*-3,5-dimethylpiperidine, obtained from fractional crystallization of the mixture of *cis* and

Fig. 4. Structure of tilmicosin.



Tilmicosin (20-deoxo-20-(3,5-dimethylpiperidin-1-yl)desmycosin, **7**)



trans hydrochloride salts, was used to prepare isomerically pure **7a**; it was shown by HPLC methods to correspond to the major product obtained from reductive amination using the commercial mixture of amines. Comparison of ^{1}H NMR spectra of the product of reductive amination from the pure *cis*-amine with that obtained from the commercial mixture showed that the bulk of the proton signals remained unchanged.

Antibacterial Evaluation of C-20-Amino Macrolides

The above series of C-20 substituted cycloamino 16-membered macrolides was evaluated for antimicrobial activity against a variety of organisms. Table 2 summarized the *in vitro* antibacterial activity of these compounds. As Table 2 shows, they have very similar antibacterial activity against Staphylococci and Streptococci, when compared to desmycosin or tylosin. Although excellent activity against Gram-positive bacteria was observed, activity against *Haemophilus influenzae* was lower (MIC range 8~64 μg/ml), as was activity against enteric Gram-negative organisms (*e.g.*, *Escherichia coli*, *Klebsiella*, *Serratia*, *etc.*; MIC range 64~128 μg/ml). These data show that incorporation of a cycloamino group at C-20 did not alter the antibacterial profile typical of clinically used macrolide

Table 2. *In vitro* antimicrobial activity of C-20-aminodesmycosin derivatives.

Compound No.	MIC (μg/ml)				
	S. a. X1.1	*S. e.* EPI 1	*S. py.* C 203	*S. pn.* Park	*S. f.* X66
1	2	2	1	0.5	32
2	0.5	1.0	0.25	0.25	16
3	0.5	0.5	0.5	0.5	8
4	0.5	0.5	0.125	0.25	4
5	0.5	0.5	0.06	0.5	4
6	0.5	1	0.25	2	8
7	0.25	0.25	0.25	0.25	2
8	8	8	1	1	32
9	0.5	0.5	0.25	2	4
10	1	0.5	0.25	1	8
11	8	8	1	8	128
12	0.5	1	2	2	2
13	1	1	0.25	0.5	2
14	0.5	0.5	0.5	0.5	4
15	0.5	0.5	0.5	0.25	2
16	0.5	0.5	0.5	0.5	8
17	0.5	0.5	0.5	1	4
18	0.5	0.5	0.5	0.5	2
19	0.5	0.5	0.5	2	8
20	0.5	0.5	0.25	0.125	8
21	0.5	1	0.5	1	128
22	0.5	0.5	0.125	1	4
23	1.0	1.0	0.5	0.5	4
24	0.25	0.5	0.25	0.25	4
25	0.5	0.5	0.5	1	16
26	1	1	0.5	0.25	16
27	1	0.5	0.5	2	8
28	1	1	0.5	0.5	4
Desmycosin	1	0.5	0.25	0.5	1
Tylosin	0.5	1	0.25	0.25	1

Abbreviations: *S. a.*, *Staphylococcus aureus*; *S. e.*, *S. epidermidis*; *S. py.*, *Streptococcus pyogenes*; *S. pn.*, *S. pneumoniae*; *S. f.*, *S. faecium*.

Table 3. *In vivo* antimicrobial activity of C-20-aminodesmycosin derivatives against infections experimentally induced by *Streptococcus pyogenes* C 203 in mice.

Compound No.	ED_{50} (mg/kg×2)		Compound No.	ED_{50} (mg/kg×2)	
	po	sc		po	sc
1	>50	1.17	**16**	37.9	0.69
2	50	0.9	**17**	6.25	1.98
3	50	1.3	**18**	10.3	1.56
4	29.3	1.25	**19**	18.86	7.50
5	11.52	1.57	**20**	33.65	1.69
6	68	>10	**22**	9.76	1.02
7	17.68	1.35	**23**	>50	>10
8	>100	2.04	**26**	46.17	2.9
9	10.93	0.88	**27**	8	1.34
10	39.69	0.78	**28**	32.67	0.8
13	16.5	3.8	Desmycosin	80	0.8
14	9.4	1.98	Tylosin	33	0.5
15	25	7.53			

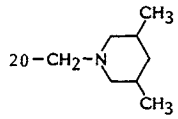
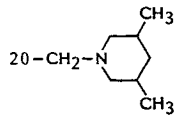
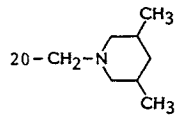
Table 4. *In vitro* activity of C-20-aminodesmycosin derivatives against *Pasteurella* and *Mycoplasma* species.

Compound No.	MIC (μg/ml)			
	P. multocida[a]	*P. haemolytica*[b]	*M. gallisepticum* 38502	*M. hyopneumoniae* S-5972
1	6.25	6.25	12.5	12.5
2	6.25	3.12	3.12	12.5
3	3.12	1.56	1.56	1.56
4	3.12	1.56	0.78	3.12
5	6.25	6.25	0.39	1.56
6	3.12	1.56	0.097	0.78
7	6.25	3.12	1.56	1.56
8	25	50	6.25	3.12
10	12.5	12.5	0.78	1.56
11	50	50	25	6.25
12	12.5	12.5	6.25	3.12
13	6.25	12.5	0.048	0.39
14	6.25	6.25	0.097	0.78
15	25	50	0.097	0.097
16	12.5	12.5	1.56	0.39
17	6.25	3.12	0.39	0.195
18	6.25	6.25	0.195	0.195
19	12.5	25	0.78	1.56
20	6.25	6.25	0.78	0.78
21	6.25	12.5	0.78	1.56
22	3.12	1.56	1.56	0.78
23	3.12	1.56	0.39	1.56
24	6.25	6.25	0.195	0.39
25	6.25	6.25	0.195	3.12
26	3.12	3.12	1.56	3.12
27	6.25	6.25	0.195	0.78
28	12.5	6.25	6.25	0.39
Desmycosin	6.25	25	0.195	0.78
Tylosin	25	50	0.78	0.195

[a] MIC values representative of 5 strains of *P. multocida*.

[b] MIC values representative of 3 strains of *P. haemolytica*.

Table 5. *In vitro* antimicrobial activity of 20-amino-tylosin, -macrocin, and -lactenocin derivatives.

Compound	MIC (μg/ml)			
	P. m.	*P. h.*	*M. g.*	*M. h.*
 -tylosin (**29**)	>50	50	1.56	6.25
 -macrocin (**30**)	>50	>50	6.25	6.25
 -lactenocin (**31**)	6.25	3.12	3.12	0.78
Tylosin	25	50	0.78	0.195
Macrocin	25	25	0.78	0.195
Lactenocin	6.25	25	1.56	0.195

Abbreviations: *P. m.*, *Pasteurella multocida* 17E; *P. h.*, *P. haemolytica* 23C; *M. g.*, *Mycoplasma gallisepticum* 38502; *M. h.*, *M. hyopneumoniae* S-5972.

antibiotics. MATSUBARA, working with a different series of C-20-amino macrolides, reported that, with a few exceptions, the antibacterial activity remained mostly the same or lower than parent macrolide.[12]

The *in vivo* activity of these derivatives against experimental *Streptococcus pyogenes* infections is shown in Table 3. Many of the C-20-cycloamino macrolides showed excellent *in vivo* activity by the subcutaneous route of administration, and several had good ED_{50} values relative to tylosin and desmycosin when given orally. Table 4 shows the results of susceptibility testing of these compounds against *Pasteurella* and *Mycoplasma* species. When compared to desmycosin and tylosin, several of the C-20-cycloamino derivatives had excellent activity against these important pathogens. Most MIC values ranged from 3.12 to 12.5 μg/ml for *Pasteurella*, indicating that the antibacterial spectrum of tylosin had been expanded to include *Pasteurella*. In contrast to the desmycosin derivatives, introduction of the 3,5-dimethylpiperidinyl group into the tylosin and macrocin molecules (**29** and **30**, respectively) did not show as great an analogous expansion of antibacterial spectrum (see Table 5). The corresponding lactenocin derivative (**31**), however, was comparable to tilmicosin (**7**) in activity. Compound **31** showed some improvement against *P. haemolytica* while retaining most of its anti-*Mycoplasma* activity relative to lactenocin.

This expansion of the tylosin spectrum to include potentially useful activity against *Pasteurella* species indicated that the C-20-cycloaminodesmycosin derivatives may be important antibiotics for the treatment of respiratory diseases caused by this organism in farm animals. *In vitro* studies reported by OSE showed that one of these derivatives, **7** (tilmicosin, EL-870), was very effective against 102 strains of *P. multocida* and 155 strains of *P. haemolytica*, with MIC_{90} values of 6.25 and 3.12 μg/ml, respectively.[14] *In vivo* efficacy against *Pasteurella* infections was tested in a chick survival test. In those tests chicks were challenged with *P. multocida* and treated with antibiotic in their drinking water at concentrations of 0.26 and 0.53 g/liter; mortality was assessed for 4 days. The results of such

tests on a selected number of derivatives are shown in Table 6. High survival rates in medicated chicks were achieved for several derivatives at both the higher and lower dose regimens as compared to infected non-medicated controls or medication with desmycosin. These results showed that the anti-*Pasteurella* activity was expressed *in vivo* and that the C-20-cycloamino macrolides studied here are potentially useful for the treatment of *Pasteurella* infections in farm animals.

Table 6. *In vivo* oral activity of C-20-aminodesmycosin derivatives against experimental infections in chicks induced by *Pasteurella multocida* 60A.

Compound No.	Mortality (dead animals/group)		
	Dose group		Infected controls
	0.53 g/liter	0.26 g/liter	
1	9/10	—	15/20
2	5/10	—	18/20
3	2/10	5/10	18/20
4	0/10	3/10	18/20
7	0/10	3/10	17/20
14	3/10	7/10	15/20
16	—	5/5	5/5
17	8/10	—	17/20
18	—	5/5	5/5
20	6/10	8/10	15/20
21	1/10	4/10	14/20
22	0/10	2/10	18/20
23	0/10	4/10	18/20
24	6/10	7/10	18/20
25	3/10	4/10	15/20
28	7/10	—	18/20
Desmycosin	8/10	8/10	19/19

—: Test not run.

These studies have provided a novel series of C-20-cycloaminodesmycosin derivatives which have excellent activity against *Pasteurella* species as well as other macrolide-susceptible Gram-positive organisms. This expansion of the antimicrobial spectrum of tylosin has potential utility in the treatment of serious respiratory infections in veterinary medicine. A number of these derivatives, such as **4**, **7** (tilmicosin) and **22**, showed superior activity in the chick protection test against *Pasteurella* infections. One of these, tilmicosin, is currently undergoing evaluation for efficacy against veterinary respiratory illness.[14]

Additional details of the structure-activity relationships among C-20 reductively aminated derivatives of tylosin-related macrolides will be described in a forthcoming publication.[19]

Experimental

The preparation of desmycosin from tylosin was carried out at the Lilly fermentation facilities at Indianapolis, IN, U.S.A.[20] Reducing agents such as sodium cyanoborohydride, sodium borohydride and formic acid were obtained from chemical sources such as Aldrich Chemical Co., Milwaukee, WI, U.S.A., as were research quantities of 3,5-dimethylpiperidine. Large quantities of the latter amine were obtained in bulk from Reilly Tar and Chemical Co., Indianapolis, IN, U.S.A. This amine was a mixture of *cis*- and *trans*-isomers (85 : 15, respectively).

Physico-chemical determinations were made on the following instruments: ^{1}H NMR—Bruker WH-360 NMR spectrometer using 16K memory and 5 KHz sweep width; field desorption mass spectra (FD-MS)—Varian-MAT 731 spectrometer using carbon dendrite emitters; UV spectra—Cary 219 spectrometer. Analytical HPLC data was collected on an instrument consisting of a Waters No. 6000A pump, a Waters Wisp injector No. 710B, a Schoeffel variable wavelength detector set at 280 nm, and a Hewlett-Packard No. 3390A Integrator. TLC was carried out on E. Merck plates of Silica gel 60 with a fluorescent indicator (F_{254}). Product purification was carried out by chromatography on silica gel, using either flash chromatography techniques with E. Merck silica gel or a Waters 500 Prep LC system, using a flow rate of 250 ml/minute and collecting 250 ml fractions.

Antibiotic susceptibility data were obtained by microtiter (Table 4) and agar dilution (Table 2) procedures. Determination of *in vivo* activity against *Pasteurella* infections was conducted in 1-day-old chicks, in which compounds were administered parenterally or orally after challenge of the chicks with *P. multocida* (0.1 ml of a 10^4-dilution of a 20-hour Tryptose broth culture of an avian *P. multocida*

given subcutaneously). In these tests, unless indicated otherwise, all non-medicated infected chicks died within 24 hours of *Pasteurella* challenge. For parenteral administration, the compounds were administered by subcutaneous injection at the specified dosage, 1 and 4 hours post-challenge of the chicks with *P. multocida*. Testing for oral efficacy was conducted by dissolution of the compounds in the chicks' drinking water, provided *ad libitum*.

20-Deoxo-20-hexamethyleneaminodesmycosin (3)

Desmycosin (10 g, 13 mmol), dissolved in anhydrous MeOH (100 ml), was added rapidly to a solution of $NaBH_3CN$ (3.3 g, 52 mmol) and hexamethyleneimine (6.5 g, 7.5 ml, 65 mmol) in anhydrous MeOH (50 ml) under N_2. The reaction mixture was stirred under N_2 at room temperature for about 3 hours and then was evaporated under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 with just enough EtOAc to aid in dissolving the residue, and this solution was extracted with saturated $NaHCO_3$ solution. The organic layer was separated, dried (Na_2SO_4), and evaporated under reduced pressure to give a light yellow foam. This foam was purified by silica gel flash chromatography, eluting initially with CH_2Cl_2 (1 liter), then stepwise with 500-ml portions of CH_2Cl_2 - MeOH mixture as follows; 98 : 2, 96 : 4, 94 : 6, 92 : 8 and 9 : 1, and finally with CH_2Cl_2 - MeOH - NH_4OH mixtures as follows; 90 : 10 : 0.5 (500 ml) and 75 : 25 : 0.5 (2 liters). Fractions containing the desired product were identified by TLC, combined and evaporated to dryness to give 6.04 g (7.07 mmol) of 20-deoxo-20-hexamethyleneaminodesmycosin as a white foam. Other fractions which contained impure product were combined, redissolved in CH_2Cl_2, extracted again with saturated $NaHCO_3$ solution, and purified as before, to give an additional 1.37 g (1.61 mmol) of product. The total yield of 20-deoxo-20-hexamethyleneaminodesmycosin (**3**) was 7.41 g (8.68 mmol), (67%): FD-MS *m/z* 855 (M+H); UV λ_{max}^{MeOH} nm (ε) 282 (21,500); *pKa'* (66% DMF) 7.9, 9.6.

Anal Calcd for $C_{45}H_{78}N_2O_{13}$: C 63.21, H 9.19, N 3.28.
Found: C 63.41, H 9.09, N 3.46.

The following derivatives were prepared by this method (starting amine shown in parentheses) followed by (compound number from Table 1); UV λ_{max} nm (ε), FD-MS *m/z* and *pKa'* (66% DMF) data:

1: (Pyrrolidine); UV 284 (21,900); FD-MS 827 (M+H); *pKa'* 7.9, 9.5.
2: (Piperidine); UV 283 (24,500); FD-MS 841 (M+H); *pKa'* 7.8, 9.1.
6: (Dodecamethyleneimine); UV 282 (19,500); FD-MS 939 (M+H), 939; *pKa'* 7.8, 9.1.
11: (4-Hydroxypiperidine); UV 282 (20,750); FD-MS 857 (M+H); *pKa'* 7.1, 8.7.
12: (4-Piperidinopiperidine); UV 282 (18,600); FD-MS 924 (M+H); *pKa'* 6.0, 9.2.
20: (Decahydroquinoline); UV 283 (21,500); FD-MS 895 (M+H); *pKa'* 7.9, 9.4 (mixture of isomers).
22: (3-Azabicyclo[3.2.2]nonane); UV 282 (20,500); FD-MS 881 (M+H); *pKa'* 7.9, 9.2.

20-Deoxo-20-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octanyl)desmycosin (23)

Desmycosin (4.0 g, 5.2 mmol) was dissolved in 40 ml of anhydrous MeOH in the presence of 3A Molecular Sieves. 1,3,3-Trimethyl-6-azabicyclo[3.2.1]octane (2.6 ml, 15.6 mmol) was added and stirred for 10 minutes. $NaBH_3CN$ (0.98 g, 15.6 mmol) was added, and the mixture was stirred at room temperature for 18 hours. The reaction was then filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in EtOAc (200 ml) and was washed with H_2O (200 ml). Product was then extracted from EtOAc with pH 6.5, 0.5 M NaH_2PO_4 buffer (200 ml). The aqueous was placed on the rotary evaporator to remove residual EtOAc and was then stirred rapidly while 5 N NaOH was slowly added, yielding a thick white precipitate. (The EtOAc portion still contained product and was reworked as above to improve yield). The white solid was filtered, washed with H_2O, and dried to give 20-deoxo-(1,3,3-trimethyl-6-azabicyclo[3.2.1]octanyl)desmycosin (**23**) (3.2 g, 68% yield): FD-MS 908 (M^+); UV λ_{max}^{EtOH} nm (ε) 282 (21,000); *pKa'* (66% DMF) 8.0, 9.7.

Anal Calcd for $C_{49}H_{84}N_2O_{13}$: C 64.73, H 9.31, N 3.08.
Found: C 65.00, H 9.18, N 2.89.

The derivatives prepared by this procedure and characterizing data are listed below in this sequence: (compound number from Table 1); (starting amine), FD-MS *m/z*, UV λ_{max}^{EtOH} nm (ε), *pKa'* (66% DMF) and relevant ^1H NMR δ data:

5: (Octamethyleneimine); FD-MS 883 (M+H); UV 282 (20,000).

8: (2,6-Dimethylpiperidine); FD-MS 869 (M+H); UV 282 (20,750); *pKa'* 7.9, 9.2.

9: (3,3-Dimethylpiperidine); FD-MS 869 (M+H); UV 283 (18,900); ^{1}H NMR 0.93 (s), 1.0 (s) (3H each, CH_3 groups).

10: (1,2,3,6-Tetrahydropyridine); FD-MS 839 (M+1); UV 283 (21,700); *pKa'* 7.5, 8.7; ^{1}H NMR 5.62, 5.66, 5.80, 5.84 (2H, AB pattern, olefinic protons on new amino function).

Anal Calcd for $C_{44}H_{74}N_2O_{13}$: C 62.98, H 8.89, N 3.34.
Found: C 62.63, H 8.87, N 3.33.

13: (4-Benzylpiperidine); FD-MS 931 (M+H), 931; UV 284 (20,000); ^{1}H NMR 7.20, 7.30 (5H, m, aromatic).

14: (3,3,5-Trimethylhexahydroazepine); FD-MS 897 (M+H); UV 282 (21,130); *pKa'* 7.7, 9.0.

15: (2,3-Dihydroindole); FD-MS 875 (M+H); UV 283 (21,650); *pKa'* 4.2, 8.2; ^{1}H NMR 6.5~6.8, 7.0~7.2 (4H, m, aromatic protons).

Anal Calcd for $C_{47}H_{74}N_2O_{13}$: C 64.51, H 8.52, N 3.20.
Found: C 64.79, H 8.56, N 3.46.

16: (Perhydroindole); FD-MS 881 (M+H); UV 283 (13,600); *pKa'* 8.0, 10.2; ^{1}H NMR aliphatic protons at 1.5~2.5.

Anal Calcd for $C_{47}H_{80}N_2O_{13}$: C 64.07, H 9.15, N 3.18.
Found: C 63.53, H 9.15, N 3.65.

17: (1,2,3,4-Tetrahydroisoquinoline); FD-MS 889 (M+H); UV 283 (21,000); *pKa'* 6.9, 8.5; ^{1}H NMR 6.53, 6.64, 7.07 (4H, m, aromatic).

Anal Calcd for $C_{48}H_{76}N_2O_{13}$: C 64.84, H 8.62, N 3.15.
Found: C 64.59, H 8.33, N 3.14.

18: (Perhydroisoquinoline); FD-MS 895 (M+1); UV 283 (19,950); *pKa'* 7.9, 9.2.

Anal Calcd for $C_{48}H_{82}N_2O_{13}$: C 64.40, H 9.23, N 3.12.
Found: C 64.20, H 8.97, N 3.08.

19: (1,2,3,4-Tetrahydroquinoline); FD-MS 888 (M^+); UV 271 (26,700), 281 (24,500); ^{1}H NMR 6.53, 6.64, 6.92, 7.07 (4H, m, aromatic).

Anal Calcd for $C_{48}H_{76}N_2O_{13}$: C 64.84, H 8.62, N 3.15.
Found: C 64.60, H 8.47, N 3.09.

21: (Perhydroisoindole); FD-MS 881 (M+H); UV 283 (21,600); *pKa'* 7.9, 9.1.

24: (1,8,8-Trimethyl-3-azabicyclo[3.2.1]octane); FD-MS 909 (M+H); UV 281 (21,900); *pKa'* 7.9, 9.2.

25: (3-Azabicyclo[3.2.1]octane); FD-MS 867 (M+H); UV 281 (20,800); *pKa'* 8.1, 9.8.

26: (1-Azaspiro[4.5]decane); FD-MS 895 (M+H); UV 282 (21,500); *pKa'* 7.9, 9.8.

27: (3-Azaspiro[5.5]undecane); FD-MS 909 (M+H); UV 283 (19,140); *pKa'* 7.8, 9.1.

28: (Dodecahydrocarbazole); FD-MS 935 (M+H); UV 283 (22,500); *pKa'* 7.9, 9.9; ^{1}H NMR aliphatic protons 1.2~2.5.

20-Deoxo-20-heptamethyleneaminodesmycosin (4) *via* Reductive Amination with $NaBH_4$

Desmycosin (4.0 g, 5.2 mmol) was dissolved in absolute MeOH (30 ml) and treated with heptamethyleneimine (1.2 g, 1.3 ml, 10.4 mmol) in the presence of 3A Molecular Sieves. After the reaction mixture had stirred for 1 hour at room temperature, a solution of $NaBH_4$ (60 mg, 1.6 mmol) in absolute MeOH (10 ml) was quickly added by pipette. The reaction mixture was stirred for 1.5 hours at room temperature, and then another 30 mg of $NaBH_4$ was added (one portion as the solid). The reaction mixture was stirred for another 75 minutes and then was filtered. The filtrate was evaporated under reduced pressure. The residue was dissolved in EtOAc (150 ml), and this solution was extracted with water (150 ml) and saturated $NaHCO_3$ solution (100 ml). The EtOAc solution was then extracted with pH 6.5, 0.5 M NaH_2PO_4 buffer (150 ml). The aqueous extract was evaporated under vacuum to remove residual EtOAc and then was rapidly stirred while 5 N NaOH was slowly added, yielding a thick white precipitate. The white solid was removed by filtration, washed with a small amount of water, and dried to give 3.55 g of 20-deoxo-20-heptamethyleneaminodesmycosin (**4**, 78.7% yield): FD-MS *m/z* 869 (M+H); UV λ_{max} nm (ε) 282 (21,750); *pKa'* (66% DMF) 7.9, 9.6.

Anal Calcd for $C_{46}H_{80}N_2O_{13}$: C 63.57, H 9.28, N 3.22.
Found: C 63.35, H 9.01, N 2.97.

20-Deoxo-20-(*cis*-3,5-dimethylpiperidinyl)tylosin (**29**)

Tylosin (20 g, 2.2 mmol) was dissolved in absolute MeOH (25 ml) in the presence of 3A Molecular Sieves. To this was added *cis*-3,5-dimethylpiperidine (0.75 g, 6.6 mmol) and the solution was stirred for 5 minutes. $NaBH_3CN$ (0.42 g, 6.6 mmol) was then added and the reaction was stirred at room temperature for 4 hours. Crude product was subjected to silica gel flash chromatography, eluting with $CHCl_3$ (250 ml), a 1.5-liter gradient of 1% MeOH - $CHCl_3$ to 10% MeOH - $CHCl_3$ - 0.5% NH_4OH, and an additional 500 ml of the latter solvent. Fractions containing the desired product were identified by TLC, combined, and evaporated under reduced pressure to give the title compound **29** (590 mg, 26.5% yield): FD-MS *m/z* 1,012 (M^+); UV λ_{max} nm (ε) 284 (21,240); *pKa'* (66% DMF) 7.6, 8.5.

Anal Calcd for $C_{53}H_{92}N_2O_{16}$: C 62.82, H 9.15, N 2.76.
Found: C 62.58, H 9.01, N 2.82.

20-Deoxo-20-(*cis*-3,5-dimethylpiperidinyl)macrocin (**30**)

The following reactants were used to synthesize the title compound: 3.0 g (3.3 mmol) of macrocin, 1.1 g (9.9 mmol) of *cis*-3,5-dimethylpiperidine, 0.62 g (9.9 mmol) of $NaBH_3CN$, 30 ml of absolute MeOH plus 3A Molecular Sieves using the same reaction procedure used to synthesize 20-(*cis*-3,5-dimethylpiperidinyl)tylosin. Reaction time was 18 hours.

The reaction was filtered to remove the sieves and the filtrate was placed on the rotary evaporator to remove the volatiles. The residue was dissolved in EtOAc, washed (H_2O), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give a white foam. This was subjected to silica gel flash column chromatography, eluting with CH_2Cl_2 (250 ml), a 1.5-liter gradient of 1% MeOH - CH_2Cl_2 to 10% MeOH - CH_2Cl_2 - 0.5% NH_4OH, and an additional 1 liter of the latter solvent. Fractions containing product were identified as in 20-deoxo-20-(3,5-dimethylpiperidinyl)tylosin example to give the title compound (2.13 g, 64.7% yield): FD-MS *m/z* 999 (M+H); UV λ_{max} nm (ε) 283 (22,082); *pKa'* (66% DMF) 6.7, 8.6.

Anal Calcd for $C_{52}H_{90}N_2O_{16}$: C 62.50, H 9.08, N 2.80.
Found: C 62.79, H 8.96, N 2.98.

20-Deoxo-20-(*cis*-3,5-dimethylpiperidinyl)lactenocin (**31**)

20-(*cis*-3,5-Dimethylpiperidinyl)macrocin (1.5 g, 1.5 mmol) was dissolved in 1 N H_2SO_4 (50 ml) and stirred at room temperature for 1 hour. The reaction was then neutralized with saturated $NaHCO_3$ solution. Product was extracted from the aqueous with CH_2Cl_2. The CH_2Cl_2 was then dried (Na_2SO_4), filtered and evaporated under reduced pressure to give the title compound as a white foam (1.19 g, 93% yield): FD-MS *m/z* 855 (M+H); UV λ_{max} nm (ε) 284 (22,458); *pKa'* (66% DMF) 7.7, 8.9.

Anal Calcd for $C_{45}H_{78}N_2O_{13}$: C 63.21, H 9.19, N 3.28.
Found: C 63.42, H 9.34, N 3.28.

Purification of *cis*-3,5-Dimethylpiperidine

A 1-liter 3-necked flask was charged with 48 ml (0.36 mol) of commercial grade 3,5-dimethylpiperidine (~85% *cis*) and 600 ml of ether. HCl gas was bubbled through the solution with vigorous stirring until no free amine remained. The product which formed was separated by filtration and air-dried to give 47 g of the hydrochloride salt, mp 160~180°C.

The salt was suspended in acetone (600 ml) and heated to reflux for 1 hour. The reaction mixture was cooled to 50°C and filtered; the separated solid was air-dried to give 25.6 g of product, mp 226~228°C. A portion of this material (18 g) was dissolved in water (100 ml), and the solution was adjusted to pH 10 with NaOH pellets. The free amine was extracted into ethyl ether, dried over $MgSO_4$, filtered and carefully concentrated to yield 8.5 ml of *cis*-3,5-dimethylpiperidine, contaminated with <5% of the *trans*-isomer.

Preparation of 20-Deoxo-20-(*cis*-3,5-dimethylpiperidin-1-yl)desmycosin (**7a**)

a) $NaBH_4$ Method: Desmycosin (100 g, 85% purity, 0.13 mol), and *cis*-3,5-dimethylpiperidine

hydrochloride (21.3 g, 0.142 mol) was dissolved in 130 ml of isobutyl acetate and 250 ml of EtOH (2B grade) containing Na_2CO_3 (15.10 g, 0.142 mol). After stirring for 1 hour, three additions of $NaBH_4$ (3.58 g, 0.097 mol per addition), each in 90 ml of EtOH, were made at 1 hour intervals. The reaction mixture was stirred for 2 hours at ambient temperature following the last addition of reducing agent. The reaction was filtered to remove the base and the solvent was removed under vacuum on a rotary evaporator. The residual solid was dissolved in EtOAc (650 ml), washed with an equal volume of water and extracted with 3.9 liters of 0.5 M NaH_2PO_4 buffer in four portions (pH of the respective extractions were 7.2, 6.9, 6.7 and 6.7). The aqueous layer was retained and residual EtOAc was removed under vacuum. This solution was adjusted to pH 9 with 5 N NaOH (~150 ml) and the mixture was stirred for 15 minutes until the precipitation was complete. After filtering and drying, this solid was dissolved in 650 ml of CH_2Cl_2 and washed with an equal volume of saturated $NaHCO_3$ solution. The organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed under vacuum to give 20-deoxo-20-(*cis*-3,5-dimethylpiperidin-1-yl)desmycosin (91.10 g, 82.3% yield) as a white solid. A portion of this material was purified using a Waters PREP-500 preparative HPLC unit using two silica gel cartridges and an 8-liter gradient of hexane to 25% hexane in EtOAc containing 1% diethylamine. The column was eluted further with 4 liters of 25% hexane in EtOAc containing 1% diethylamine. A second chromatography using the same equipment was used to achieve final purity for analytical purposes. The latter procedure involved loading 30 g of the product from the first chromatography on two cartridges and eluting with 8 liters of a gradient from 100% CH_2Cl_2 to 7.5% MeOH in CH_2Cl_2 followed by 10 liters of 7.5% MeOH in CH_2Cl_2. Analytical HPLC, carried out on a 4.6×250-mm Ultrasphere ODS column with a 22% acetonitrile - 0.01 M aqueous dibutylammonium phosphate solvent system, showed a single peak at 9.88 minutes which accounted for 98.5% of the total sample by integration of the chromatogram. The resulting product was used to obtain the following physical data: FD-MS *m/z* 869 (M+H); UV λ_{max} nm (ε) 283 (22,568); *pKa'* (66% DMF) 7.5, 8.7; $[\alpha]_D^{28}$ 12.92 (*c* 0.01006, 5 cm, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3020, 2968, 2953, 2932, 2875, 1591, 1167, 1141, 1081, 1057 cm^{-1}; ^1H NMR ($CDCl_3$) desmycosin aldehyde proton absent and 3,5-dimethylpiperidine protons present.

Anal Calcd for $C_{46}H_{80}N_2O_{13}$: C 63.57, H 9.28, N 3.22.
Found: C 63.34, H 9.15, N 3.08.

b) $NaBH_3CN$ Method: Desmycosin (10 g, 12.9 mmol) was dissolved in dry MeOH (100 ml), and *cis*-3,5-dimethylpiperidine (4 g, 35 mmol) was added. After the mixture was stirred for 30 minutes at room temperature, $NaBH_3CN$ (0.8 g, 12.9 mmol) was added. The solution was stirred overnight and then was evaporated under reduced pressure. The residue was partitioned between EtOAc and water (150 ml each). The organic layer was then extracted sequentially with pH 6.5 phosphate buffer (100 ml) and pH 4.5 phosphate buffer (100 ml).

The latter solution was then adjusted to pH 10 with 5 N NaOH, and the free amine was reextracted into EtOAc. The solution was dried over magnesium sulfate, filtered and concentrated to yield 6.0 g of **7a**. Analysis of the product by reverse-phase HPLC detected no *trans*-isomer.

Preparation of 20-Deoxo-20-(3,5-dimethylpiperidin-1-yl)desmycosin (Tilmicosin) (7) *via* the Formic Acid Approach (Wallach Reaction)

Tylosin phosphate in water (399 mg/ml, 91.0 ml, 0.04 mol) was slowly heated to 35°C while adjusting the pH of the solution to 1.6 by the addition of H_2SO_4. After being heated for 1 hour, the reaction mixture was cooled to room temperature. Amyl acetate (80 ml) was added to the mixture, and the pH was adjusted to 11 by the addition of 5 N NaOH. The amyl acetate layer was separated. 3,5-Dimethylpiperidine (4.52 g, 0.04 mol) was added to the amyl acetate solution at room temperature, and the reaction mixture was then heated to 70°C. Formic acid (96%, 2.01 g, 0.042 mol) in amyl acetate (20 ml) was added slowly to the amyl acetate solution. After 2 hours, the reaction mixture was cooled to room temperature. Water (100 ml) was added, and the pH of this solution was adjusted to about 4.5 by the addition of concentrated HCl. The aqueous layer was separated and diluted with water (700 ml). This solution was stirred at room temperature as its pH was raised to about 11 by the addition of 5 N NaOH. The resulting precipitate was removed by filtration, washed

with water and vacuum dried at room temperature to give 28.86 g of 20-deoxo-20-(3,5-dimethylpiperidin-1-yl)desmycosin (tilmicosin, EL-870, 7) (87.3%) as a mixture of its *cis*- and *trans*-isomers at the added amine functionality.

A 50-g portion of product produced by the Wallach reaction was chromatographed on a Waters PREP-500 HPLC unit using two silica gel cartridges and an 8-liter gradient of hexane to EtOAc containing 1% triethylamine. This was followed by a second HPLC purification using a single silica gel cartridge and an 8-liter gradient of CH_2Cl_2 to CH_2Cl_2 containing 7.5% MeOH and 1% NH_4OH in order to remove the residual triethylamine. Those fractions which contained the mixture of *cis*- and *trans*-isomers were combined and characterized. Analytical HPLC data showed the presence of the mixture of isomers; *trans*: 2 peaks at 10.41 and 10.76 minutes (mixture of *threo* and *erythro* forms) and *cis*: 1 peak at 12.05 minutes in the ratio of ~6:8:86. This analysis was carried out on a Waters analytical HPLC unit using a 4.6×250-mm Ultrasphere ODS column with a 22% acetonitrile - 0.01 M aqueous dibutylammonium phosphate solvent system. The following physical data was obtained for the product: FD-MS *m/z* 869 (M+H); UV λ_{max} nm (ε) 283 (22,643); *pKa'* (66% DMF) 7.4, 8.5; $[\alpha]_D^{23}$ 12.75 (*c* 0.010004, 5 cm, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3018, 2969, 2953, 2933, 1592, 1167, 1141, 1081, 1058; ^1H NMR ($CDCl_3$) desmycosin aldehyde proton absent and 3,5-dimethylpiperidine protons present.

Anal Calcd for $C_{46}H_{80}N_2O_{13}$: C 63.57, H 9.28, N 3.22.
Found: C 63.36, H 9.34, N 3.35.

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