STRUCTURE-ACTIVITY STUDIES OF 20-DEOXO-20-AMINO DERIVATIVES OF TYLOSIN-RELATED MACROLIDES

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Reductive amination of the C-20 aldehyde group of tylosin and related macrolides yielded a large series of derivatives with potentially useful antibiotic properties. Evaluation of these new compounds was conducted on the basis of: 1) Broad antimicrobial spectrum *in vitro*, with particular emphasis on inhibition of *Pasteurella multocida* and *Pasteurella haemolytica*; 2) *in vivo* efficacy, especially when given orally, against *P. multocida* in experimental infections in chicks; and 3) bioavailability after oral administration to laboratory animals. The most useful activity was found within a series of derivatives produced by reductive amination of desmycosin with secondary amines.

Tylosin is an important and well-established antibiotic in veterinary medicine[1]; it is orally effective against infections in poultry and pigs due to Gram-positive bacteria and *Mycoplasma gallisepticum* as well as certain Gram-negative bacteria such as *Fusobacterium necrophorum*, a causative agent of liver abscesses in cattle[2]. A variety of chemical modifications of the C-20 aldehyde group in tylosin and related macrolide antibiotics have recently been reported, which improved efficacy against experimental infections and bioavailability in animals after oral administration[3]. From our extensive evaluation of several series of macrolide derivatives, potentially useful activity was found in a compound obtained by reductive amination of desmycosin, which resulted in an expansion of the effective antimicrobial spectrum of tylosin to include both *Pasteurella multocida* and *Pasteurella haemolytica*[4]. These Gram-negative bacteria are important pathogens responsible for respiratory illness and mortality in both cattle and pigs[5,6]. Since a macrolide antibiotic which could be administered both parenterally and orally for the treatment of infections caused by *Pasteurella* species would be a useful contribution to veterinary medicine, an extensive exploration of the structure-activity relationships among reductively aminated derivatives of macrolides was warranted. In this paper, we report the results of our studies on numerous such derivatives of tylosin-related macrolides.

Results and Discussion

Synthesis of Reductive Amination Derivatives

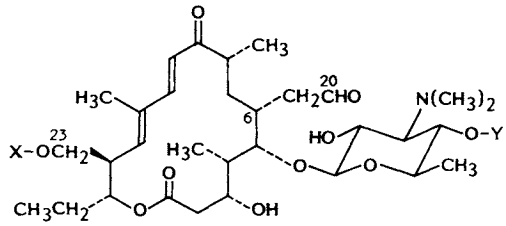
The effects of reductive amination of the aldehyde group in 16-membered macrolide antibiotics have

been only recently investigated[7]. ŌMURA *et al.*, reported that 20-deoxo-20-aminotylosin was a very poor antibiotic, but a dimeric derivative (20,20′-dideoxo-20,20′-iminoditylosin) had good antimicrobial activity[8]. In a subsequent study, reductive amination of tylosin and desmycosin was performed with a variety of primary and secondary amines, yielding a series of secondary and tertiary amino derivatives; several of these compounds demonstrated good antimicrobial activity both *in vitro* and *in vivo* against Gram-positive bacteria[9].

During this period, a new series of derivatives of 5-*O*-mycaminosyltylonolide (OMT) was reported, in which activity against Gram-negative bacteria was significantly increased when the C-23 substituent of OMT was converted into certain tertiary amino groups[10]. Our reductive amination of desmycosin or lactenocin with hexamethyleneimine (one of the C-23 amino substituents which increased activity of OMT)[10] yielded new macrolide derivatives which exhibited activity against *P. multocida* both *in vitro* and *in vivo*[4]. This unexpected and novel result prompted our synthesis of a large number of reductively aminated derivatives in order to define the structure-activity relationships and to determine whether a suitable candidate for use in veterinary medicine could be identified.

Standard reductive amination procedures employing sodium cyanoborohydride proved very suitable for the synthesis of a wide variety of derivatives (Scheme 1)[11]. Selective modification of the aldehyde function was easily accomplished under these mild conditions, with no interference from side reactions such as reduction of the C-9 ketone. Derivatives were prepared from both primary and secondary amines, but the latter were emphasized because of the superior antimicrobial properties of the tertiary amino derivatives (see below). Also, as a result of our initial antimicrobial evaluation, we prepared a greater number of derivatives which possessed aliphatic *N*-alkyl groups in which the total number of carbon atoms of the amino substituents ranged from six to about fourteen. Reductive aminations were conducted using both acyclic and cyclic secondary amines and incorporated both unsaturation and hetero atom substitutions

Scheme 1. Reaction scheme and structures of reductively aminated derivatives of tylosin-related macrolides.



Macrolides used as starting materials for reductive aminations

X	Y=Mycarosyl	Y=H
Mycinosyl	Tylosin	Desmycosin
3-*O*-Demethyl-mycinosyl	Macrocin	Lactenocin
Allosyl	DOMM	DOML
H	DMT	OMT

DMT=Demycinosyltylosin

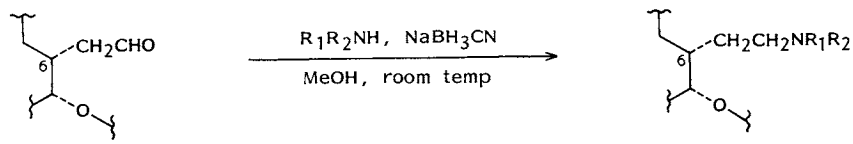


Table 1. Structure and physico-chemical data for derivatives from reductive amination of desmycosin.

Compound	Substituents from Scheme 1		FD-MS[a]	UV λ_{max} nm (ε)	NMR of NR_1R_2[b]
	R_1	R_2			
1	H	H	773	284 (15,200)	Exchangeable H
2	H	Methyl	787	284 (17,900)	2.49 (3H, s, NCH_3)
3	H	*tert*-Butyl	829	284 (22,800)	1.08 (9H, s, *tert*-butyl)
4	H	Neopentyl	843	282 (29,270)	0.96 (9H, s, *tert*-butyl)
5	H	*n*-Hexyl	857	283 (21,840)	0.95 (3H, t, CH_3)
6	H	Benzyl	863	281 (20,900)	7.25~7.35 (5H, m, phenyl)
7	H	2-Ph-ethyl	877	283 (22,900)	7.25~7.40 (5H, m, phenyl)
8	H	1-Adamantyl	907	283 (21,500)	1.5~2.5 (complex multiplets)
9	H	2-Adamantyl	907	282 (20,250)	1.5~2.5 (complex multiplets)
10	H	*n*-Octyl	885	282 (11,000)	0.95 (3H, t, CH_3)
11	Methyl	Methyl	801	283 (19,550)	2.15 (6H, s, NCH_3)
12	Ethyl	Ethyl	829	283 (22,410)	1.05 (6H, t, CH_3)
13	Propyl	Propyl	857	282 (18,370)	0.95 (6H, t, CH_3)
14	*iso*-Propyl	*iso*-Propyl	857	281 (21,730)	1.0 (12H, d, CH_3)
15	*n*-Butyl	*n*-Butyl	885	282 (20,160)	0.95 (6H, t, CH_3)
16	*iso*-Butyl	*iso*-Butyl	885	280 (21,770)	0.90 (12H, m, CH_3)
17	*sec*-Butyl	*sec*-Butyl	885	280 (22,040)	0.9~1.0 (12H, m, CH_3)
18	Ethyl	*sec*-Butyl	857	282 (22,000)	0.9 (9H, m, CH_3)
19	Ethyl	2-Me-butyl	871	282 (23,000)	0.9 (9H, m, CH_3)
20	Ethyl	Neopentyl	871	282 (22,000)	0.86 (9H, s, *tert*-butyl)
21	*n*-Hexyl	*n*-Hexyl	941	283 (22,000)	0.9 (6H, t, CH_3), 1.3 (CH_2)
22	Cyclohexyl	Cyclohexyl	937	282 (16,900)	1.5~1.9 (m, aliphatic)
23	Benzyl	Benzyl	953	281 (20,320)	7.2~7.4 (10H, m, phenyl)
24	*n*-Octyl	Norbornyl	979	281 (21,000)	1.2 (br s, aliphatic)
25	*n*-$C_{12}H_{25}$	Norbornyl	1,035	282 (19,000)	1.3 (br s, aliphatic)
26	*n*-$C_{12}H_{25}$	Cyclooctyl	1,051	282 (20,000)	1.2 (br s, aliphatic)
27	Allyl	Allyl	853	283 (22,000)	5.2 (4H, m), 5.7 (2H, m)
28	Allyl	2-Cl-allyl	887, 888	281 (22,000)	5.0~5.3 (4H, m), 5.7 (1H, m), 3.2 (2H, d)
29	Allyl	2-Br-allyl	930, 932	282 (21,000)	5.0~5.2 (2H, m), 5.55 and 5.77 (1H each, br s), 5.7 (1H, m)
30	*n*-Butyl	Methallyl	882	282 (22,000)	4.7 (2H, d), 2.6 (2H, t)
31	*iso*-Butyl	Allyl	868	281 (22,000)	5.1 (m), 4.7 (m)
32	Propargyl	Propargyl	848	283 (19,000)	2.3 (2H, s, CH)
33	Propargyl	*iso*-Butyl	866	282 (17,000)	0.9 (3H, d, CH_3)
34	1-Me_3Si-propargyl	*n*-Propyl	925	281 (17,000)	0.16 (9H, s, TMS), 0.9 (3H, t, CH_3)
35	Phenyl	Phenyl	925	282 (28,500) 250 (13,200)	6.9~7.35 (10H, m, phenyl)
	NR_1R_2				
36	Morpholinyl		843	284 (20,400)	3.7~3.8 (4H, m, α to oxygen)
37	Thiomorpholinyl		859	283 (20,730)	2.7 (4H, m, α to nitrogen)
38	Piperidinyl		841	283 (24,500)	1.5~1.7 and 2.3 (aliphatic)
39	3,5-Di-Me-morpholinyl		871	282 (20,500)	1.13 and 1.3 (3H each, d, CH_3)
40	3,5-Di-Me-piperidinyl		869	283 (22,570)	0.80 and 1.00 (3H each, CH_3)
41	4-Ph-piperazinyl		917	283 (13,700), 248 (31,200)	6.92~7.35 (5H, m, phenyl)
42	4-Ph-piperidinyl		917	283 (21,700), 205 (14,800)	7.25~7.4 (5H, m, phenyl)
43	4-Ph-1,2,3,6-tetrahydropyridinyl		915	282 (21,000)	6.05 (1H, br s), 7.1~7.4 (5H, m)
44	Thiazolidinyl		845	282 (21,800)	5.3 (2H, s, NCH_2S)

[a] Parent ion in FD-MS.

[b] Chemical shifts in δ(ppm) for characteristic protons of dialkylamino substituent; C-20 aldehyde proton from starting macrolide absent.

Table 2. Structure and physico-chemical data for derivatives from reductive amination of tylosin and related macrolides.

Compound	Substituents from Scheme I		Parent	FD-MS[a]	UV λ_{max} nm(ε)	NMR of NR_1NR_2[b]
	R_1	R_2				
45	H	Methyl	Tylosin	912	283 (19,900)	2.43 (3H, s, NCH_3)
46	H	Benzyl	Tylosin	1,006	283 (20,300)	7.3~7.4 (5H, m, phenyl)
47	H	2-Ph-ethyl	Tylosin	1,021	282 (23,100)	7.25~7.35 (5H, m, phenyl)
48	Methyl	Methyl	Tylosin	945	285 (19,800)	2.47 (6H, s, NCH_3)
49	Benzyl	Benzyl	Tylosin	1,095	283 (17,300)	7.24~7.4 (10H, m, phenyl)
50	Benzyl	Benzyl	DOMM	1,069	281 (21,300)	7.2~7.45 (10H, m, phenyl)
51	Benzyl	Benzyl	DOML	924	282 (20,700)	7.2~7.45 (10H, m, phenyl)
52	H	Methyl	OMT	613	285 (18,900)	2.39 (3H, s, NCH_3)
53	H	Benzyl	OMT	689	285 (17,400)	7.25~7.35 (5H, m, phenyl)
54	H	2-Ph-ethyl	OMT	703	282 (18,800)	7.25~7.35 (5H, m, phenyl)
55	Methyl	Methyl	OMT	627	284 (19,900)	2.36 (6H, s, NCH_3)
56	Phenyl	Phenyl	OMT	750	282 (26,800), 250 (13,300)	6.9~7.4 (10H, m, phenyl)
	NR_1R_2					
57	Heptamethyleneimino		Tylosin	1,013	282 (22,000)	1.6 and 2.5 (aliphatic)
58	3-Azabicyclo[3.2.2]nonanyl		Tylosin	1,025	282 (21,500)	1.6~1.9 and 2.4~2.6 (aliphatic)
59	Morpholinyl		Tylosin	985	283 (20,500)	3.7 (4H, m, α to oxygen)
60	Hexamethyleneimino		Macrocin	985	283 (20,400)	1.6 and 2.5 (aliphatic)
61	Morpholinyl		Macrocin	973	283 (22,700)	3.7 (4H, m, α to oxygen)
62	Heptamethyleneimino		Lactenocin	855	282 (21,500)	1.6 and 2.5 (aliphatic)
63	Morpholinyl		Lactenocin	829	284 (19,800)	3.7 (4H, m, α to oxygen)
64	Hexamethyleneimino		OMT	681	284 (19,700)	1.6 and 2.5 (aliphatic)
65	4-Ph-piperidinyl		OMT	743	284 (19,600)	7.2~7.45 (5H, m, phenyl)
66	3,5-Dimethylpiperidinyl		OMT	695	284 (20,400)	0.80 and 1.0 (3H, d, CH_3)
67	Morpholinyl		OMT	669	283 (19,500)	2.5 (α to N), 3.7 (α to O)
68	3,5-Dimethylmorpholinyl		OMT	696	284 (21,400)	1.15, 1.33 (3H each, CH_3)
69	3-Azabicyclo[3.2.2]nonanyl		OMT	707	282 (19,000)	1.6~2.0 and 2.4~2.6 (aliphatic)
70	Tylosin[c]			915	282 (24,500)	9.68 (1H, s, aldehyde)
71	Desmycosin[c]			771	283 (19,200)	9.69 (1H, s, aldehyde)
72	OMT[c]			597	283 (20,500)	9.70 (1H, s, aldehyde)

[a,b] See footnotes in Table 1.
[c] Reference standard.

within the amino substituents. Approximately one hundred reductive amination derivatives of tylosin-related macrolides have been prepared and evaluated. Additional details of the synthesis and evaluation of one particular group of derivatives, prepared from desmycosin and cyclic secondary amines, have been recently reported[12].

The new compounds were isolated by standard extractive procedures and purified by precipitation from aqueous acidic solution upon addition of aqueous sodium hydroxide; when necessary, further purification was accomplished by chromatographic methods[12]. The new products were fully characterized by their ^1H NMR, IR, UV and MS (Tables 1 and 2).

Evaluation of Reductive Amination Derivatives of Desmycosin: *In Vitro* Activity

All of the derivatives which were prepared by reductive amination of tylosin, desmycosin and related 16-membered macrolides exhibited some antimicrobial activity *in vitro* (Table 3). Representative pathogens from both clinical and veterinary medicine were employed in the initial *in vitro* screening tests. Derivatives with a primary amino group at C-20 (compound **1**) or possessing one or two methyl groups on the amino

Table 3. *In vitro* antimicrobial activity of derivatives from reductive amination of tylosin and related macrolides.

Compound	MIC (μg/ml)[a]									
	S. a.	*S. py.*	*S. pn.*	*H. i.*	*P. m.*	*P. h.*	*M. g.*	*M. s.*	*M. hr.*	*M. hp.*
1	8	32	32	128	>50	>50	6.2	25	>50	25
2	16	4	64	>128	>50	>50	12.5	12.5	>50	25
3	8	2	16	64	50	>50	6.2	3.1	25	3.1
4	1	0.5	4	16	12.5	12.5	3.1	0.4	25	1.6
5	1	0.5	1	8	6.2	12.5	0.4	0.4	50	3.1
6	1	0.2	0.5	8	6.2	6.2	0.8	0.2	50	NG
7	0.5	0.2	1	8	3.1	3.1	0.8	0.4	>50	0.8
8	1	0.2	2	16	6.2	12.5	0.4	0.4	50	6.2
9	0.5	0.2	0.5	8	3.1	6.2	0.1	0.1	50	1.6
10	1	0.1	0.5	16	12.5	6.2	0.4	0.4	25	3.1
11	4	2	4	16	50	50	12.5	6.2	>50	12.5
12	2	0.5	1	16	25	25	6.2	1.6	50	12.5
13	1	0.2	1	16	6.2	6.2	1.6	0.8	12.5	0.8
14	2	1	4	32	25	50	6.2	1.6	>50	12.5
15	0.5	0.2	1	16	12.5	6.2	0.8	0.2	25	0.8
16	1	0.2	1	16	6.2	6.2	0.4	0.2	25	0.8
17	0.5	0.2	0.5	8	12.5	12.5	0.8	0.4	25	0.8
18	2	0.5	2	64	12.5	12.5	0.2	0.2	12.5	0.8
19	1	0.2	0.5	8	12.5	6.2	<0.1	0.4	12.5	0.8
20	1	0.2	0.2	4	12.5	12.5	0.8	0.4	6.2	0.4
21	1	0.2	0.5	8	12.5	12.5	NG	NG	NG	1.6
22	1	0.2	0.2	16	6.2	6.2	0.4	0.2	50	0.4
23	0.5	0.2	0.5	32	12.5	6.2	<0.1	0.2	6.2	1.6
24	1	0.5	1	32	25	25	NG	0.2	6.2	0.1
25	2	1	2	32	12.5	6.2	<0.1	0.8	12.5	1.6
26	4	1	2	64	25	12.5	NG	1.6	6.2	0.2
27	0.5	0.1	0.1	4	12.5	6.2	0.4	0.8	12.5	0.8
28	0.5	0.2	0.5	16	12.5	12.5	0.1	0.8	6.2	0.4
29	0.2	0.2	0.5	8	25	12.5	0.2	0.8	6.2	0.4
30	0.5	0.2	1	16	12.5	6.2	0.1	0.4	12.5	0.8
31	1	0.5	2	16	12.5	6.2	0.2	0.8	12.5	0.8
32	0.5	0.5	1	16	25	50	0.4	1.6	6.2	0.4
33	1	0.5	1	32	12.5	25	NG	0.4	6.2	0.8
34	1	0.5	0.5	16	12.5	25	NG	0.4	6.2	0.1
35	0.5	0.5	1	32	25	50	<0.1	0.8	25	1.6
36	1	0.5	1	32	12.5	12.5	0.2	3.1	50	0.8
37	0.5	0.2	1	8	6.2	3.1	0.1	0.4	12.5	3.1
38	0.5	0.2	0.2	16	6.2	3.1	3.1	0.8	>50	12.5
39	0.5	0.5	1	32	12.5	12.5	0.8	0.4	12.5	1.6
40	0.2	0.2	0.2	4	6.2	3.1	1.6	0.8	12.5	1.6
41	1	0.5	0.5	32	25	25	0.2	0.8	50	1.6
42	0.5	0.2	0.2	8	3.1	6.2	0.4	<0.1	50	0.8
43	0.5	0.2	0.2	32	12.5	12.5	<0.1	0.8	25	0.8
44	0.5	0.5	2	64	25	25	0.2	3.1	25	0.8
45	16	8	64	>128	>50	>50	25	12.5	>50	6.2
46	2	1	8	128	>50	50	1.6	1.6	12.5	3.1
47	1	1	8	64	50	50	1.6	0.4	50	NG
48	8	8	64	>128	>50	50	6.2	12.5	>50	>25
49	1	0.5	0.5	128	>50	50	0.2	0.2	3.1	0.2
50	8	2	2	128	>50	>50	6.2	12.5	NG	6.2
51	0.5	0.5	1	32	6.2	3.1	0.4	3.1	NG	3.1
52	32	128	16	128	50	50	50	25	>50	NG
53	8	4	2	2	3.1	6.2	3.1	12.5	50	NG
54	4	2	1	8	3.1	6.2	1.6	3.1	>50	NG

Table 3. (Continued)

Compound	MIC (μg/ml)[a]									
	S. a.	*S. py.*	*S. pn.*	*H. i.*	*P. m.*	*P. h.*	*M. g.*	*M. s.*	*M. hr.*	*M. hp.*
55	32	32	32	16	25	50	50	50	50	NG
56	4	2	1	32	25	25	1.6	6.2	50	1.6
57	4	2	8	128	>50	50	3.1	0.8	>50	12.5
58	4	4	16	128	50	50	1.6	1.6	>50	25
59	0.5	0.2	0.5	32	25	50	0.4	<0.1	1.6	<0.1
60	4	4	2	128	>50	50	6.2	25	>50	12.5
61	4	4	2	64	>50	>50	3.1	3.1	NG	0.8
62	1	0.5	0.5	32	6.2	6.2	6.2	3.1	50	0.4
63	2	1	2	64	12.5	12.5	6.2	12.5	NG	1.6
64	4	2	2	8	1.6	3.1	12.5	25	50	12.5
65	2	2	0.2	8	3.1	0.8	3.1	12.5	>50	3.1
66	0.2	0.2	<0.1	0.5	1.6	1.6	1.6	NG	NG	0.2
67	4	1	0.5	16	12.5	12.5	3.1	6.2	50	1.6
68	4	8	0.2	16	12.5	6.2	3.1	NG	NG	50
69	1	2	0.2	4	3.1	1.6	6.2	6.2	>50	6.2
70	0.5	0.2	0.2	16	25	50	0.4	0.1	1.6	0.2
71	0.5	0.2	0.2	4	6.2	25	0.8	0.2	3.1	0.2
72	1	0.2	0.2	2	1.6	6.2	0.4	0.8	3.1	0.1

[a] Methodology and organisms: Agar-dilution MIC values for: *S. a.*, *Staphylococcus aureus* X1; *S. py.*, *Streptococcus pyogenes* C 203; *S. pn.*, *Streptococcus pneumoniae* Park 1; *H. i.*, *Haemophilus influenzae* CL. Microtiter MIC values for: *P. m.*, *Pasteurella multocida*, representative MIC of 5 strains; *P. h.*, *Pasteurella haemolytica*, representative MIC of 3 strains; *M. g.*, *Mycoplasma gallisepticum* 29C; *M. s.*, *Mycoplasma synoviae* 40A; *M. hr.*, *Mycoplasma hyorhinis* 29E; *M. hp.*, *Mycoplasma hyopneumoniae* S5972.
NG: Organism did not grow on day of test.

group (compounds **2**, **11**, **45**, **48**, **52** and **55**) were among the least active compounds tested. Activity was improved as the size of the 20-*N*-alkyl substituents increased beyond that of methyl, ethyl and small branched alkyl groups (such as found in compounds **3**, **12**, **14** and **18**). An upper limit to the size of the *N*-alkyl substituents was not very well defined in terms of *in vitro* activity, which was only moderately reduced even with derivatives possessing such large C-20 substituents as (norbornyl)dodecylamino and (cyclooctyl)dodecylamino (compounds **25** and **26**).

The demycarosyl derivatives of tylosin and related macrolides are known to have improved activity *in vitro* against Gram-negative bacteria, including the important veterinary pathogens *P. multocida* and *P. haemolytica*[4,13]. A similar trend was observed with the reductively aminated derivatives; these C-20 amino derivatives of tylosin, macrocin and 3‴-*O*-demethylmacrocin (DOMM) had weak activity against *Pasteurella* species, whereas the analogous demycarosyl derivatives from desmycosin, lactenocin, 3″-*O*-demethyllactenocin (DOML) and OMT possessed good *in vitro* activity against both *Pasteurella* species (Table 3). Consequently, our principal effort was focused upon the demycarosyl derivatives as offering the best chance to significantly expand the useful antimicrobial spectrum of tylosin.

The optimum *in vitro* activity was achieved in those derivatives of desmycosin which possessed C-20 aminoalkyl substituents having approximately six to fourteen carbon atoms. Within this range, the aminoalkyl group could either be cyclic or acyclic, saturated or unsaturated (with double bonds, triple bonds or aromatic rings) or contain additional heteroatoms such as nitrogen, oxygen, sulfur or halogen. However, it was not possible to distinguish much further among the members of this relatively large group on the basis of their *in vitro* activity.

Table 4. *In vivo* antimicrobial efficacy of derivatives from reductive amination of tylosin and related macrolides.

Compound	ED_{50} values against *Streptococcus pyogenes* in mice		Mortality in chicks infected by *Pasteurella multocida*[a]		
	sc[b]	po[b]	sc[c]	po (0.53 g/liter)[d]	po (0.26 g/liter)[d]
1	>10	>100	Nt		
2	4.0	>100	Nt		
3	0.6	44	Nt		
4	<0.6	22	Nt		
5	<0.6	70	Nt	8/10	7/10
6	<1.6	39	0/10	10/10	
7	<1.6	55	2/10	10/10	
8	0.9	62	4/10	6/10	
9	1.1	18	6/10	5/10	10/10
10	3.4	>100	10/10		
11	<0.6	>100	Nt	7/10	8/10
12	1.7	71	Nt	7/10	6/10
13	1.0	41	0/10	1/10	4/10
14	2.0	77	0/10	8/10	
15	1.2	25	1/10	4/10	9/10
16	3.4	10	5/10	6/10	9/10
17	1.1	28	0/10	2/10	4/10
18	1.0	64	0/10	4/10	9/10
19	1.0	43	0/10	1/10	5/10
20	0.8	27	0/10	6/10	7/10
21	7.5	54	10/10	7/10	6/10
22	4.5	31	7/10	4/10	10/10
23	6.6	35	10/10	10/10	
24	10.0	100	10/10		
25	>10	>100	10/10		
26	>10	>100	10/10		
27	0.8	10	1/10	4/10	6/10
28	2.1	20	2/10	9/10	6/10
29	1.7	28	4/10	8/10	9/10
30	1.7	7	5/10	5/10	9/10
31	1.7	8	7/10	3/10	4/10
32	2.0	35	Nt		
33	3.2	18	10/10		
34	2.3	45	8/10		
35	>10	>50	8/10	6/10	
36	2.3	>100	1/10	10/10	10/10
37	1.4	15	6/10	8/10	9/10
38	0.9	50	0/10	5/10	
39	2.0	35	10/10		
40	1.4	18	2/10	0/10	3/10
41	6.5	35	10/10	7/10	
42	6.0	20	9/10	6/10	
43	5.6	13	10/10		
44	8.7	46	6/10	7/10	10/10
45	7.4	>100	Nt		
46	8.2	62	Nt		
47	9.1	76	Nt		
48	10.0	>100	Nt		
49	>10	76	Nt		
50	9.2	>100	Nt		
51	>10	>100	10/10	10/10	
53	16.1	>100	1/10		
54	7.2	>100	1/10	10/10	

Table 4. (Continued)

Compound	ED_{50} values against *Streptococcus pyogenes* in mice		Mortality in chicks infected by *Pasteurella multocida*[a]		
	sc[b]	po[b]	sc[c]	po (0.53 g/liter)[d]	po (0.26 g/liter)[d]
57	>10	44	Nt		
58	>10	30	Nt		
59	1.9	27	Nt		
60	>10	>100	Nt		
61	4.4	>100	Nt		
62	1.8	100	0/10		
63	8.2	>100	0/10	9/10	
64	7.8	>100	0/10	10/10	
65	>10	>100	Nt	10/10	
66	2.9	>100	4/10		
67	>10	>100	Nt		
68	10	>100	Nt		
69	5.9	87	1/10	7/10	
70	1.3	42	8/10		
71	1.4	84	0/10	8/10	8/10
72	3.9	102	0/10	10/10	10/10

[a] Number of chicks which died per number which were medicated.
[b] ED_{50} values given in mg/kg × 2, given 1 and 5 hours post-infection.
[c] Compound administered at 30 mg/kg, given 1 and 4 hours post-infection.
[d] Compound administered at specified concentration in chicks' drinking water.
Nt: Not tested *in vivo*.

In Vivo Activity

Because it was difficult to choose from the many members of this series of derivatives on the basis of their *in vitro* activity, their *in vivo* activity became the critical parameter for selecting the potentially most useful antimicrobial compounds. *In vivo* activity was initially assessed in two different model experimental infections. Efficacy against a typically susceptible Gram-positive bacterium, *Streptococcus pyogenes*, was measured in a traditional mouse infection model, whereas efficacy against *P. multocida* was determined in chicks, due to the greater reproducibility of our results in this experimental infection model[4]. Compounds were generally tested by both the parenteral and oral routes, since antimicrobial efficacy by both routes of administration was being sought.

As reported previously, the C-20 modified derivatives of desmycosin were generally efficacious against infections by Gram-positive bacteria in mice and, in particular, were more efficacious than tylosin or desmycosin when administered orally[3]. A substantial number of the C-20 aminoalkyl derivatives of desmycosin followed this same pattern, exhibiting good efficacy when administered orally against the experimental infection by *S. pyogenes* (Table 4). The C-20 aminoalkyl derivatives possessing either small substituents (compounds **1**, **2**, **11**, **45** and **48**) or large substituents (compounds **10**, **24**, **25** and **26**) were not orally active, which helped define the range of compounds with potentially useful oral efficacy. The C-20 aminoalkyl derivatives of OMT (compounds **52**~**56** and **64**~**69**) were all inactive when given orally, in spite of their good *in vitro* activity and, in many cases, their good *in vivo* activity when given parenterally.

In vivo activity of the derivatives against *Pasteurella* was investigated in a chick infection model to further define the range of effective antibiotic compounds. In this preliminary *in vivo* screen, a dose of 30 mg/kg was used subcutaneously while an oral dose of 0.53 g/liter was administered in the drinking water of the chicks. All compounds with an MIC value against *Pasteurella* of 6.25 μg/ml or less, as

well as some derivatives with higher MIC values, were evaluated *in vivo*. Based upon results from the preliminary *in vivo* tests, dose titration studies were then performed to select the compounds with the best *in vivo* activity. Although an inevitable amount of test-to-test variability was present, this experimental infection provided a suitable basis for selecting the most efficacious compounds.

This chick *Pasteurella* test represented a relatively severe infection and consequently posed a serious challenge to the compounds for demonstrating *in vivo* activity. While a number of the reductive amination derivatives, when given subcutaneously, treated the *Pasteurella* infection quite well at 30 mg/kg or even at lower doses, very few compounds clearly stood out as efficacious when administered orally in the drinking water of the chicks. Among the acyclic aminoalkyl compounds, the better candidates appeared to be those tertiary amino derivatives with alkyl groups of three to five carbon atoms in length, such as the di(*n*-propyl)amino, the di(*sec*-butyl)amino and the ethyl(2-methylbutyl)amino derivatives of desmycosin (compounds **13**, **17** and **19**). In contrast, many of the cyclic aminoalkyl derivatives, exemplified by the 3,5-dimethylpiperidinyl derivative (**40**), appeared to be somewhat more reproducibly efficacious in this model *Pasteurella* infection in chicks and have been reported separately[12].

As previously reported for other aldehyde-modified derivatives of desmycosin[3], the derivatives obtained by reductive amination of desmycosin gave higher blood levels than either desmycosin or tylosin in both rats and chicks after oral administration (Table 5). Furthermore, higher concentrations were achieved more consistently in chicks than in rats. The combination of excellent efficacy in two distinctly different models of experimental infections, utilizing two different animal species and two different bacteria, along with results demonstrating good bioavailability after oral administration, indicated that a useful new oral antibiotic could be selected from this series of reductive amination derivatives of desmycosin.

In order to better distinguish among the number of these derivatives which appeared to exhibit the desired characteristics, a smaller group was selected for further evaluation in the target animals, calves and pigs. Included from the present series as the best candidates were the di(*n*-propyl)amino derivative (compound **13**) and the ethyl(2-methylbutyl)amino derivative (compound **19**) of desmycosin, along with representatives of the cyclic aminoalkyl derivatives, as exemplified by the 3,5-dimethylpiperidinyl derivative

Table 5. Peripheral blood concentrations of reductive amination derivatives at 100 mg/kg orally in rats and chicks.

Compound	Animal	Mean concentration (μg/ml) per sample period					
		0.25 hour	0.5 hour	1.0 hour	2.0 hours	4.0 hours	6.0 hours
Tylosin	Rat	0	0	0	0	0	0
	Chick	0.3	0.7	2.8	1.7	0	0
Desmycosin	Rat	0	0	0	0	0	0
	Chick	0	0	0.1	4.4	—	—
20-Deoxo-20-(hexamethyleneimino)-desmycosin[a]	Rat	0	0	0	0	0	0
	Chick	16.4	19.8	16.3	28.4	13.2	8.8
20-Deoxo-20-(heptamethyleneimino)-desmycosin[a]	Rat	0	0	0	3.3	2.1	1.6
	Chick	3.5	2.4	4.0	3.2	1.8	1.8
20-Deoxo-20-(dodecamethyleneimino)-desmycosin[a]	Rat	1.4	2.2	1.6	3.7	5.0	4.6
	Chick	2.5	5.6	7.8	9.0	11.2	8.2
20-Deoxo-20-(3-azabicyclo[3.2.2]nonanyl)-desmycosin[a]	Rat	0	0	0	2.6	2.5	1.5
	Chick	0.4	9.0	26.2	16.4	9.8	8.9
20-Deoxo-20-(4-phenylpiperidinyl)-desmycosin	Rat	1.0	1.7	2.5	3.5	3.6	0
	Chick	3.8	12.0	9.5	11.1	20.1	14.6

[a] See ref 12.

(compound **40**) of desmycosin. From these more extensive investigations, the latter compound (**40**) was selected as the best candidate for further development. Now generically known as tilmicosin (originally designated as EL-870), it is being investigated for the treatment of pneumonia in cattle and pigs[6,14,15].

Experimental

Materials and Methods

^{1}H NMR spectra were measured in $CDCl_3$ solution on a Bruker WH-360 or Jeol FX90A spectrometer; chemical shifts are given in ppm downfield from internal TMS. Field desorption mass spectra (FD-MS) were obtained on a Varian-MAT 731 spectrometer with carbon dendrite emitters. UV spectra were measured in 95% ethanol solution on a Cary 219 spectrometer. TLC was performed using E. Merck plates of Silica gel 60 with a fluorescent indicator (F-254); visualization was effected by UV light. Product purification was performed by chromatography on silica gel, using either flash chromatography[16] (E. Merck grade 60 silica gel) or a Waters Model 500 Prep LC system.

In vivo evaluations against *S. pyogenes* infections in mice were conducted by treating infected animals 1 and 5 hours post-infection, either subcutaneously or by gavage, with 0.25 ml of a 10% aqueous ethanolic solution of the compound over a range of concentrations; tartaric acid was added when needed to dissolve the compounds. *In vivo* evaluations against *P. multocida* were performed by treating infected 1-day-old chicks with the test compound, either subcutaneously at 1 and 4 hours post-infection or orally by dissolution of the compound in the drinking water of the animals, provided *ad libitum*. Peripheral plasma concentrations were determined by microbiological assay, using *Micrococcus luteus* seeded in Difco Antibiotic Media 1. Concentrations represent an average value of 5 mice per time period or 6 chicks per time period.

20-Deoxo-20-(diisobutylamino)desmycosin (**16**)

Desmycosin (3.0 g, 3.9 mmol) was dissolved in methanol (30 ml) in the presence of 3A molecular sieves. Diisobutylamine (1.5 g, 11.7 mmol) was added, and after 15 minutes, sodium cyanoborohydride (0.74 g, 11.7 mmol) was added. After being stirred at room temperature for 17 hours, the mixture was filtered, and the filtrate was evaporated to dryness. The resultant yellow solid foam was dissolved in ethyl acetate (200 ml) and extracted with water (200 ml); the product was then extracted into aqueous sodium dihydrogen phosphate buffer solution (250 ml, pH 6.5, 0.5 M). The aqueous solution was separated and residual ethyl acetate was evaporated under reduced pressure. The resultant solution was rapidly stirred while aqueous sodium hydroxide (5 N) was added to precipitate the desired product; final pH was approximately 8. The white solid was filtered and dried to give 1.34 g (39%) of 20-deoxo-20-(diisobutylamino)desmycosin: See Table 1 for MS, UV and NMR data; pK_a (66% DMF) 7.6 and 9.0.

20-Deoxo-20-morpholinyldesmycosin (**36**)

Desmycosin (11.6 g, 15 mmol) and morpholine (2.6 ml, 30 mmol) were dissolved in methanol (100 ml) and after 30 minutes, sodium cyanoborohydride (1.25 g, 20 mmol) was added. After being stirred overnight at room temperature, solvent was evaporated under reduced pressure. The residue was partitioned between water (150 ml) and ethyl acetate (150 ml), the aqueous layer was separated, and the organic solution was extracted twice with aqueous sodium phosphate solution (0.5 M, initial pH 6.5, 75 ml each). The combined aqueous layers were evaporated under reduced pressure to remove residual organic solvents and then treated with aqueous sodium hydroxide solution while being vigorously stirred until the pH had been raised to 10. The solid clump of precipitate was extracted with ethyl acetate, and the organic solution was dried, filtered, and evaporated to yield 7.7 g (61%) of the desired compound; pK_a (66% DMF) 6.5 and 8.5.

20-Deoxo-20-neopentylaminodesmycosin (**4**)

The reductive amination was performed as described above, using neopentylamine (3.5 ml, 30 mmol), to yield 8.8 g (70%) of the desired product: pK_a (66% DMF) 7.9 and 9.7. After the extraction with the pH 6.5 phosphate buffer solution, the organic solution was extracted with pH 5.5 aqueous phosphate buffer solution which, upon analogous workup, yielded 1.4 g of a mixture of impurities, indicating the potential for selective purification of the desired products by appropriate extractive procedures.

20-Deoxo-20-(bicyclo[2.2.1]hept-2-yl)(*n*-octyl)aminodesmycosin (**24**)

To a stirred solution of desmycosin (8.0 g, 10.3 mmol) and (bicyclo[2.2.1]hept-2-yl)(*n*-octyl)amine (2.5 g, 11.1 mmol) in dry methanol (50 ml) was added sodium cyanoborohydride (0.7 g, 11 mmol) in portions over a five-minute period. The solution was stirred overnight at room temperature and solvent was then evaporated under reduced pressure. The residue was dissolved in ethyl acetate (100 ml) and extracted first with water (100 ml) and then with 5% aqueous sodium dihydrogen phosphate solution (100 ml). The aqueous acidic extract (pH 4) was adjusted first to pH 6 with 6 N sodium hydroxide and extracted with ethyl acetate, then adjusted to pH 7 and extracted with ethyl acetate, and finally adjusted to pH 8 and extracted as before. The organic extract from the pH 7 aqueous solution was dried (magnesium sulfate) and filtered and the filtrate was evaporated under reduced pressure to yield 4.4 g (45%) of the title compound.

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