in 10 mL of CH_3OH. After 2 h at room temperature, the suspension was filtered to give 600 mg of the desired salt, mp 203–204 °C.

A solution of 1.5 g (0.4 mmol) of the above fumarate in 14 mL of CH_3OH and 2 mL of H_2O was treated with 0.45 mmol of the requisite phenylsulfonohydrazine in 6 mL of CH_3OH. The mixture was allowed to stand overnight and filtered. The salts were purified by crystallization from aqueous ethanol.

The *p*-tolyl derivative was converted to the free base and crystallized from CH_3OH. The overall yield of the free base was 66% (Table I).

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Registry No. 1, 21738-42-1; **5**, 53503-66-5; **5**·$^1/_2C_4H_4O_4$, 114719-39-0; **7**, 114719-26-5; **8**, 114719-27-6; **8** (acetate), 114719-30-1; **9**, 114719-28-7; **11**, 114719-29-8; **12**, 114719-31-2; **13**, 114719-32-3; **13**·$^1/_2C_4H_4O_4$, 114719-33-4; **14**, 114719-34-5; **15**, 114719-35-6; **15**·$^1/_2C_4H_4O_4$, 114719-36-7; **16**, 114719-37-8; **16**·$^1/_2C_4H_4O_4$, 114719-38-9; 3-nitrobenzenesulfenyl chloride, 7669-54-7; [(2,4,6-trimethylphenyl)sulfonyl]hydrazine, 16182-15-3; (4-tolylsulfonyl)hydrazine, 1576-35-8; [(4-nitrophenyl)sulfonyl]-hydrazine, 2937-05-5; [(4-methoxyphenyl)sulfonyl]hydrazine, 1950-68-1.

Synthesis and Evaluation of Tylosin-Related Macrolides Modified at the Aldehyde Function: A New Series of Orally Effective Antibiotics

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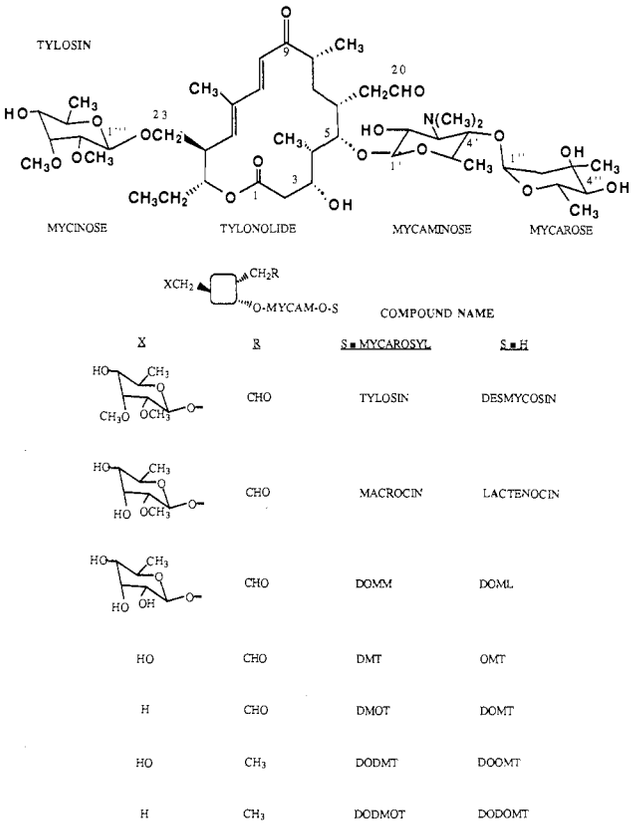
Modification of the aldehyde group in tylosin and related macrolide antibiotics dramatically enhanced the oral efficacy of the derivatives against experimental infections caused by susceptible bacteria in laboratory animals. A large number and wide variety of aldehyde-modified macrolide derivatives were prepared, utilizing the Mitsunobu reaction and other chemical transformations. Evaluation of in vitro and in vivo antimicrobial activity indicated that derivatives of demycarosyltylosin (desmycosin) combined the broadest spectrum of antimicrobial activity with the best efficacy and bioavailability after oral administration.

Biosynthetically blocked mutant strains of the tylosin-producing microorganism *Streptomyces fradiae* have now made available a wide variety of biosynthetic intermediates and shunt metabolites in quantities sufficient for extensive chemical modification studies (Scheme I).[1-4] In this paper, we report the results from one line of investigation that has yielded a new series of semisynthetic antibiotics having increased oral efficacy and bioavailability in laboratory animals.

Initial evaluation of the antimicrobial activity of these tylosin-related biosynthetic intermediates and shunt metabolites suggested that the ratio of oral/subcutaneous efficacy against model experimental infections in mice was substantially lowered for 20-deoxo-5-*O*-mycaminosyltylonolide (DOOMT) compared to 5-*O*-mycaminosyltylonolide (OMT).[5] Prior to our study, the only modification of the aldehyde of tylosin had been reduction to its dihydro derivative, relomycin.[6] Consequently, modification of the aldehyde group was more extensively investigated in both tylosin and this newly available collection of 16-membered macrolides.

Initial Survey. In order to prepare a group of analogues of DOOMT, deformylation of the aldehyde function was investigated for tylosin and related macrolides by utilizing Wilkinson's catalyst [$(Ph_3P)_3RhCl$]. Tylosin, macrocin, desmycosin, 23-demycinosyltylosin (DMT), and OMT were all readily converted to their deformyl derivatives (Scheme II). After our work had been completed, workers in another laboratory independently reported similar results from decarbonylation reactions.[7] A group

Scheme I. Tylosin-Related Macrolides Available as Starting Materials for Chemical Modification



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Scheme II. Decarbonylation Reactions

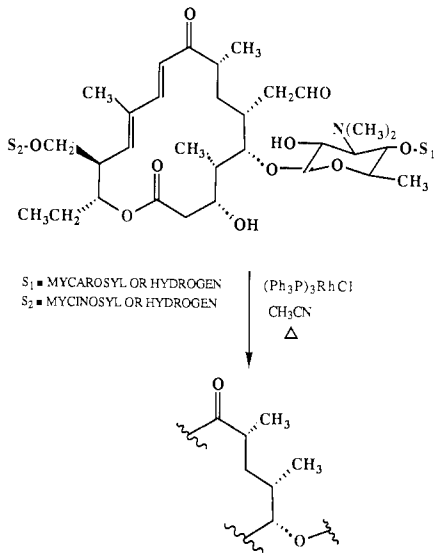


Table I. Antimicrobial Evaluation of Deformyl Derivatives

compd	compd no.	MIC,[a] μg/mL	ED_{50}, mg/kg × 2[a] sc	po
tylosin	**49**	0.25	0.5	33
deformyltylosin	**69**	1.0	2.0	12
macrocin	**74**	0.25	<1.2	>80
deformylmacrocin	**78**	2.0	>10	31
desmycosin	**1**	0.25	0.8	80
deformyldesmycosin	**32**	0.25	1.6	8
DMT	**81**	0.25	1.9	82
deformyl-DMT	**85**	64	NT[b]	NT
OMT	**98**	0.25	3.3	97
deformyl-OMT	**102**	4	>30	121

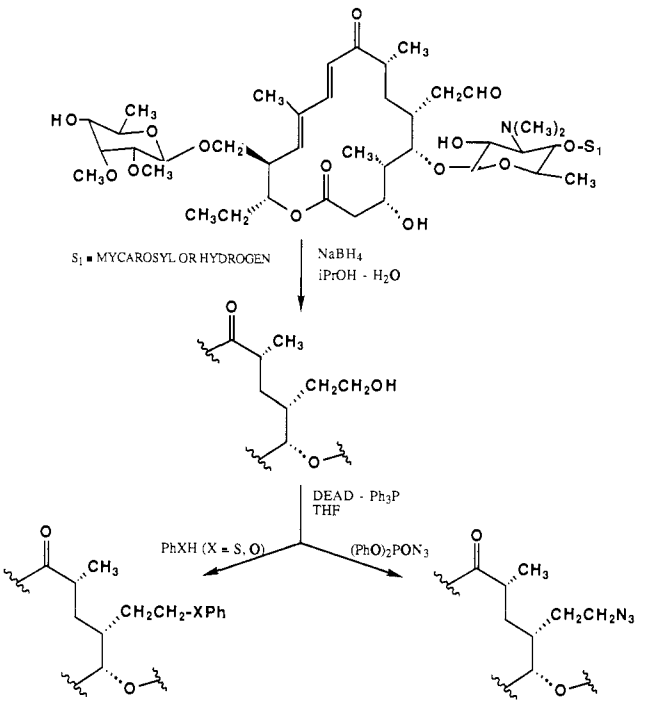
[a] Against *Streptococcus pyogenes* C203. [b] Not tested due to lack of in vitro activity.

of macrolides lacking the aldehyde group has also been isolated from fermentations (mycinamicin family).[8]

Preliminary evaluation of the deformyl derivatives for antibiotic activity in vivo against a sensitive strain of *Streptococcus pyogenes* indicated that oral efficacy in mice was substantially improved for deformyltylosin, deformylmacrocin, and deformyldesmycosin compared to that of the respective parent macrolides (Table I). Since in vitro activity and in vivo efficacy of the deformyl derivatives by parenteral administration was comparable to or slightly less than that of the parent compounds (Table I), the improvement in oral efficacy was most likely due to increased bioavailability of antibiotic after oral administration. In contrast to these three compounds, antibiotic activity was essentially lost with deformyl-DMT, demonstrating that the removal of both mycinose and the aldehyde from tylosin was deleterious to antibiotic activity. As anticipated, deformyl-OMT behaved similarly to DOOMT, having some loss of antimicrobial activity in vitro and no improvement in oral efficacy.

Chemistry. This initial study directed our efforts toward modification of the aldehyde function in those macrolides containing mycinose in our search for a new orally efficacious macrolide. Since macrolide derivatives lacking the saccharide mycarose had better activity against Gram-negative bacteria,[5] our subsequent efforts were focused especially toward the chemical modification of the aldehyde group of desmycosin. The Mitsunobu reaction was found to be particularly applicable for the synthesis of a wide variety of derivatives.[9] Selective reduction of the aldehyde group was achieved with sodium borohydride in slight excess of stoichiometric quantity, and subsequent modification of the 20-hydroxyl group was performed under standard Mitsunobu conditions (Scheme III).[9] A minor byproduct was tentatively identified as the 20-substituted derivative further modified by incorporation of a dicarbethoxyhydrazine moiety at a position as yet undetermined (FD-MS data). Synthesis of 20-dihydro-20-*O*-phenyldesmycosin (**3**) has been successfully accomplished under these conditions on a 100-g scale. Several substituted-phenyl ethers as well as heterocyclic ethers, aryl thioethers, and various other derivatives have also been readily prepared by the Mitsunobu methodology (Tables II and III).

Scheme III. Synthesis of C-20 Derivatives via Mitsunobu Reaction



Scheme IV. Chemical Modification of C-20 Derivatives

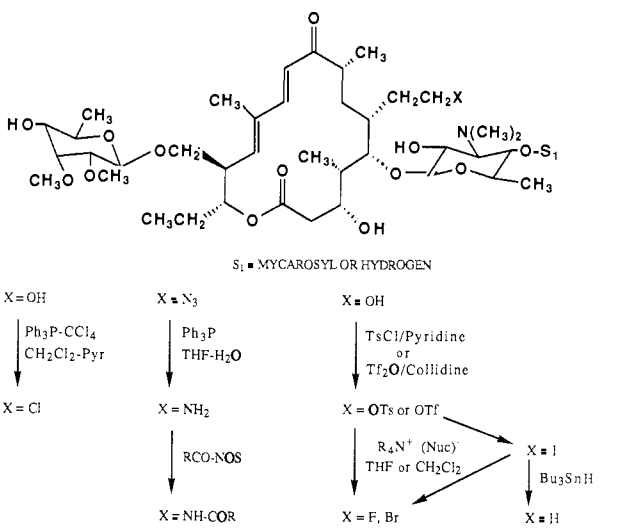


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Table II. In Vitro Antimicrobial Activity of 20-Modified Derivatives of Desmycosin

compd no.	C-19 substituent	MIC values, μg/mL					
		S. aur[a] X1	*S. epi*[b] EPI1	*S. pyog*[c] C203	*S. pneum*[d] Park	*S. faec*[e] X66	*H. flu*[f] CL
1	CHO [desmycosin]	0.5	0.5	0.25	0.25	4	4
2	CH_2OH	2.0	2.0	4.0	4.0	32	32
3	CH_2OPh[g]	0.25	0.25	0.12	1.0	8	32
4	$CH_2OPh(4\text{-}NO_2)$	0.5	0.5	1.0	1.0	4	16
5	$CH_2OPh(4\text{-}OCH_3)$	0.25	0.25	1.0	1.0	4	32
6	$CH_2OPh(3,5\text{-di-Cl})$	0.25	0.25	0.12	1.0	4	32
7	$CH_2OPh(3\text{-}NMe_2)$	0.5	0.5	0.25	1.0	4	32
8	$CH_2OPh(4\text{-CHO})$	0.25	0.25	1.0	1.0	4	16
9	$CH_2OPh(4\text{-}CH_2OH)$	0.5	0.5	0.5	2.0	16	64
10	$CH_2OPh[4\text{-}CH_2N(CH_2)_6]$	0.5	0.5	0.5	1.0	8	32
11	$CH_2OPh(4\text{-COOEt})$	0.5	0.5	0.5	1.0	8	16
12	$CH_2OPh(4\text{-Ph})$	0.5	0.5	0.25	0.25	0.5	16
13	$CH_2OPh(3\text{-OPh})$	0.5	0.5	0.25	0.25	0.5	16
14	$CH_2OPh(4\text{-OPh})$	0.5	0.5	0.25	0.25	0.5	16
15	$CH_2OPh(4\text{-COPh})$	0.25	0.25	0.06	0.06	0.25	16
16	$CH_2O(3\text{-pyridyl})$	0.5	0.5	2.0	2.0	16	16
17	$CH_2O(5,6\text{-di-Ph-}1,2,4\text{-triazin-}3\text{-yl})$	0.5	0.5	0.5	0.5	4	64
18	$CH_2O(4\text{-quinazolinyl})$	0.25	0.25	0.5	0.5	4	16
19	CH_2SPh	0.25	0.25	0.25	2.0	8	32
20	$CH_2S(1\text{-Me-}5\text{-tetrazolyl})$	0.5	0.25	0.25	2.0	16	32
21	$CH_2OCH(CF_3)_2$	0.5	0.5	0.5	4.0	32	64
22	CH_2(phthalimido)	0.12	0.12	0.25	2.0	8	32
23	$CH_2N(COOEt)(NHCOOEt)$	0.5	0.5	1.0	1.0	16	32
24	CH_2N_3	0.12	0.12	0.25	2.0	4	16
25	CH_2NH_2	4.0	8.0	32	32	128	128
26	$CH_2NH(PhOAc)$	0.25	0.25	0.25	0.25	1	8
27	CH_2I	0.5	0.25	0.5	4.0	16	32
28	CH_2Br	0.25	0.25	0.12	2.0	8	16
29	CH_2Cl	0.25	0.12	0.25	1.0	8	16
30	CH_2F	0.25	0.12	0.25	2.0	8	16
31	CH_3	0.25	0.25	0.5	2.0	16	16
32	H	0.25	0.25	0.25	2.0	8	8
33	$CH_2O(PhOAc)$	0.25	0.25	0.12	0.12	0.5	8
34	CH_2OAc	0.5	0.25	0.12	2.0	8	16
35	CH_2OTs	0.5	0.5	1.0	1.0	8	32
36	CH_2OSOPh	0.25	0.25	0.25	NT	4	8
37	$CH_2SO_2Ph(4\text{-}CH_3)$	0.25	0.25	1.0	1.0	8	16
38	CH_2OCH_3	0.5	0.5	0.5	4.0	16	32
39	$CH_2OCH_2CH_2Ph$	0.25	0.25	1.0	0.25	8	32
40	CH_2ONO_2	0.25	0.25	0.25	2.0	8	16
41	desmycaminosyl-5,20-tetrahydrofuranyl	NA	NA	NA	NA	NA	NA
42	$CH_2NHCH_2CH_2Ph$	0.5	0.5	0.25	1.0	8	8
43	$CH_2N(CH_2)_7$	0.5	0.5	0.25	0.25	4	8
44	$CH(OEt)_2$	0.5	1.0	0.5	4.0	8	16
45	CH(1,3-dioxolan-2-yl)	0.5	0.5	2.0	2.0	8	32
46	CH(4-COOEt-1,3-thiazolidin-2-yl)	0.5	0.5	0.5	0.25	4	32
47	CH(N-Bn-1,3-thiazolidin-2-yl)	0.25	0.25	0.25	1.0	4	16
48	CH(benzothiazolidin-2-yl)	0.25	0.25	0.5	0.25	2	16

[a] *Staphylococcus aureus.* [b] *Staphylococcus epidermidis.* [c] *Streptococcus pyogenes.* [d] *Streptococcus pneumoniae.* [e] *Streptococcus faecium* (ATCC 9790, incorrectly described in previous publications as *S. faecalis*). [f] *Haemophilus influenzae.* [g] Ph means phenyl; Me means methyl; Et means ethyl; Bn means benzyl; Ac means acetyl; NA means MIC > 128 μg/mL.

The azidodesmycosin derivative **24** was readily prepared by utilizing diphenyl phosphorazidate as the azide source in the Mitsunobu reaction.[10] Selective reduction of the azido group was accomplished with triphenylphosphine in aqueous THF to give the aminodesmycosin derivative **25**,[11] which was converted to the phenoxyacetamide **26** under standard conditions for acylation of amines (Scheme IV).

Following the synthesis described by Omura,[12] 20-dihydrodesmycosin (**2**) was converted to the 20-iodo derivative **27**, whereas triphenylphosphine and carbon tetrachloride[13] converted **2** to the 20-chloro derivative **29**. Improved procedures for the synthesis of the 20-halo derivatives (**27–30**) have been recently reported by another group.[14] Dehalogenation of the 20-iodo derivative (**27**) with tributyltin hydride produced the 20-methyl derivative (**31**) (Scheme IV); this compound has been previously reported by similar[14] and alternative methods[12,15] of synthesis. The analogous derivative (**101**) of OMT, which we obtained via fermentation, has been synthesized from OMT.[12]

The primary hydroxyl group of **2** was also selectively acylated and sulfonylated by the usual methods, and the 20-*O*–sulfonyl (tosyl, triflyl) and 20-iodo substituents were displaced by a variety of good nucleophiles under standard conditions for S_N2 substitution reactions (Scheme IV).

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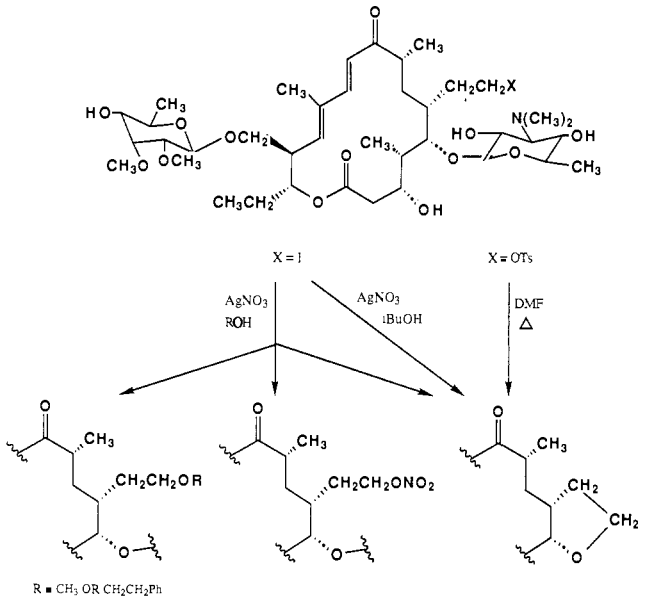
Table III. In Vitro Antimicrobial Activity of 20-Modified Derivatives of Tylosin

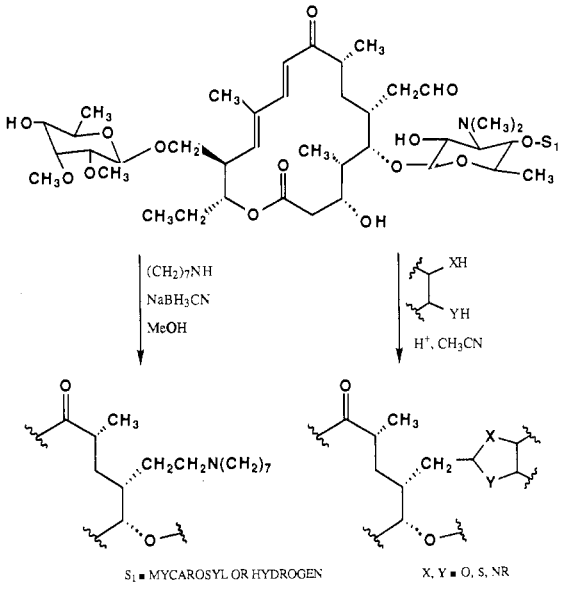
compd no.	C-19 substituent	MIC values, μg/mL					
		S. aur[a] X1	*S. epi*[a] EPI1	*S. pyog*[a] C203	*S. pneum*[a] Park	*S. faec*[a] X66	*H. flu*[a] CL
49	CHO [tylosin]	0.5	1.0	0.25	0.25	2	16
50	CH_2OH	4.0	4.0	2.0	32	64	>128
51	CH_2OPh	1.0	1.0	2.0	32	128	>128
52	$CH_2OPh(4\text{-}NO_2)$	1.0	1.0	2.0	16	128	>128
53	$CH_2OPh(4\text{-}OCH_3)$	0.5	2.0	1.0	8	128	128
54	$CH_2OPh(3,5\text{-di-}Cl)$	1.0	2.0	1.0	16	>128	>128
55	$CH_2OPh(3\text{-}NMe_2)$	1.0	1.0	1.0	8	64	128
56	$CH_2OPh(4\text{-}CHO)$	1.0	1.0	1.0	16	128	>128
57	$CH_2OPh(4\text{-}Ph)$	2.0	2.0	2.0	1	8	>128
58	$CH_2OPh(4\text{-}COPh)$	1.0	2.0	2.0	1	4	>128
59	$CH_2O(4\text{-quinazolinyl})$	0.5	1.0	4.0	4	32	>128
60	CH_2SPh	1.0	2.0	4.0	64	>128	>128
61	$CH_2S(1\text{-Me-}5\text{-tetrazolyl})$	0.5	1.0	1.0	32	128	>128
62	$CH_2OCH(CF_3)_2$	4.0	4.0	8.0	32	>128	>128
63	CH_2(phthalimido)	1.0	1.0	2.0	32	>128	>128
64	$CH_2N(COOEt)(NHCOOEt)$	2.0	4.0	8.0	8	>128	>128
65	CH_2N_3	2.0	1.0	2.0	32	128	>128
66	$CH_2NH(PhAc)$	1.0	0.5	2.0	1	64	128
67	CH_2Cl	1.0	1.0	2.0	32	>128	>128
68	CH_2F	1.0	1.0	4.0	64	>128	>128
69	H	0.5	1.0	1.0	32	64	128
70	$CH_2O(PhOAc)$	0.5	0.5	0.5	0.5	8	>128
71	CH_2OTs	0.25	1.0	4.0	4	128	>128
72	$CH_2NHCH_2CH_2Ph$	1.0	2.0	1.0	8	128	64
73	$CH_2N(CH_2)_7$	4.0	4.0	2.0	8	>128	128

[a] See Table II for definitions of abbreviations.

Scheme V. Solvolysis of C-20 Derivatives of Desmycosin



Scheme VI. Chemical Modification of Aldehyde Function



These procedures were also used to synthesize the analogous derivatives of tylosin (Table III).

Solvolysis of the 20-iodo derivative (**27**) with silver nitrate in methanol yielded four products, one of which was the desired 20-methoxy derivative **38**. Two of the other products were identified as 20-dihydrodesmycosin (**2**) and its nitrate ester (**40**) while the fourth was determined to have the novel tetrahydrofuran structure **41** (Scheme V). The latter product was most likely formed by coordination of the carbonium ion at C-20 (formed in the traditional S_N1 manner) with the glycosidic bond of mycaminose, thereby facilitating its hydrolysis. The same product (**41**) was obtained upon heating the 20-*O*-tosylate (**35**) in DMF, most likely by a similar mechanism. This proposed mechanism is supported by previous studies in which an intramolecular hemiketal was formed between the C-5 hydroxyl group and the C-20 aldehyde group.[16,17]

Reductive amination of the aldehyde group was readily accomplished under standard conditions with sodium cyanoborohydride (Scheme VI).[18] Further development of this series of reductive amination products has yielded compounds useful for the treatment of pneumonia caused by *Pasteurella* species in swine and calves;[19,20] details of

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Table IV. In Vitro Antimicrobial Activity of 20-Modified Macrolide Derivatives

compd no.	C-19 substituent	parent macrolide	MIC values, μg/mL					
			S. aur[a] X1	*S. epi*[a] EPI1	*S. pyog*[a] C203	*S. pneum*[a] Park	*S. faec*[a] X66	*H. flu*[a] CL
74	CHO	macrocin	0.5	1	0.25	1	2	16
75	CH_2OH	macrocin	16	4	4	32	128	>128
76	CH_2OPh	macrocin	2	2	4	32	>128	>128
77	CH_2I	macrocin	2	2	4	16	128	128
78	H	macrocin	2	2	2	64	128	>128
79	CH_2OTs	macrocin	1	2	8	1	128	>128
80	$CH_2N(CH_2)_7$	macrocin	4	8	2	16	64	>128
81	CHO	DMT	1	1	0.25	0.5	1	4
82	CH_2OH	DMT	>128	128	64	64	>128	>128
83	CH_2(phthalimido)	DMT	64	32	128	16	>128	>128
84	CH_3	DMT	128	128	>128	>128	>128	>128
85	H	DMT	64	16	64	64	128	>128
86	$CH_2NHCH_2CH_2Ph$	DMT	16	16	8	4	32	64
87	CHO	DMOT	0.5	0.5	0.25	0.12	0.5	8
88	CH_2OH	DMOT	64	32	32	64	64	>128
89	CH_2(phthalimido)	DMOT	64	64	64	8	128	>128
90	CH_3	DMOT	128	64	128	64	>128	>128
91	$CH_2NHCH_2CH_2Ph$	DMOT	4	4	2	1	8	64
92	CHO	lactenocin	2	2	0.25	0.25	4	8
93	CH_2OH	lactenocin	8	4	2	2	64	32
94	CH_2OPh	lactenocin	0.25	0.25	0.25	1	4	32
95	CH_2I	lactenocin	0.5	0.5	0.5	2	16	32
96	CH_3	lactenocin	0.25	0.12	0.5	4	8	32
97	$CH_2N(CH_2)_7$	lactenocin	1	1	0.5	0.5	16	32
98	CHO	OMT	1	1	0.25	0.25	1	2
99	CH_2OH	OMT	64	32	16	64	64	32
100	CH_2(phthalimido)	OMT	4	4	64	64	16	64
101	CH_3	OMT	4	8	8	8	16	32
102	H	OMT	4	2	4	4	4	4
103	CH_2O(PhAc)	OMT	1	0.5	1	0.5	2	16
104	$CH_2NHCH_2CH_2Ph$	OMT	4	4	2	1	8	8
105	$CH_2N(CH_2)_6$	OMT	4	2	0.5	1	16	16
106	CHO	DOMT	0.25	0.25	0.25	0.12	0.5	1
107	CH_2OH	DOMT	4	16	16	16	32	32
108	CH_2(phthalimido)	DOMT	8	8	64	64	32	128
109	CH_3	DOMT	8	16	8	16	64	64
110	$CH_2N(CH_2)_6$	DOMT	4	2	4	4	8	16

[a] See Table II and Scheme I for definitions of abbreviations.

their synthesis and evaluation will be published separately. A series of reductive amination products of tylosin and desmycosin has also been reported by Omura.[21]

In addition to the reductive procedures described above, several derivatives were prepared in which the oxidation state of the C-20 aldehyde group was maintained (Scheme VI). Although ketal and thioketal derivatives of macrolides are well known as intermediates in chemical syntheses,[22-24] their efficacy as antimicrobial agents has not previously been reported. The C-20 aldehyde has also been recently converted to a series of imine and ketone derivatives.[14]

Antimicrobial Evaluation in Vitro. The most obvious modification of the aldehyde group in tylosin and desmycosin, reduction to the primary alcohol, has long been known to result in a substantial loss of antibiotic activity.[6,25] Analogous results have been reported for all of the newer macrolides obtained from mutant strains of *S. fradiae*.[5] Perhaps due to a generalization of the diminished activity of 20-dihydro derivatives, further work on modification of these aldehyde groups has been minimal until recently.

In contrast to the overall reduction of in vitro activity for 20-dihydrodesmycosin (**2**), almost all other derivatives in Table II were 2–4-fold more active against strains of staphylococci than desmycosin (**1**) itself; the lone exception in our series was the primary amino derivative (**25**). As previously observed with a different series of derivatives of OMT,[26] the present derivatives of 16-membered macrolides were active against MLS-resistant strains that were inducibly resistant to erythromycin, but not those constitutively resistant to erythromycin.[27]

Against streptococci, however, activity was varied, depending on the particular species. The activity of most derivatives of desmycosin was comparable to that of desmycosin itself against *S. pyogenes*, but substantially reduced against *Streptococcus pneumoniae* and *Streptococcus faecium*. The derivatives having the best activity against *S. pneumoniae* and *S. faecium* possessed a phenyl ring at some distance from the C-20 site of substitution (e.g., **12–15**, **26**, **33**, **57**, **58**, **70**). In vitro activity was also substantially lowered for all of the derivatives against *Haemophilus influenzae* and was lacking completely against species of *Enterobacteriaceae* and *Pseudomonas*. In vitro evaluation of several representative compounds against an expanded group of species of staphylococci, streptococci, and *Haemophilus* demonstrated similar

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Table V. In Vitro Antimicrobial Activity of 20-Modified Macrolide Derivatives against an Expanded Spectrum of Aerobic Bacteria

	MIC, μg/mL													
	1	3	12	15	22	24	29	31	48	49	51	81	98	EM[a]
Staph aureus V41	1.0	0.25	0.25	0.25	0.25	0.12	0.12	0.25	0.25	1.0	1.0	1.0	2.0	>128
Staph aureus V135	1.0	0.25	0.25	0.5	0.5	0.25	0.25	0.25	0.25	1.0	1.0	2.0	1.0	0.25
Staph aureus V140	1.0	0.25	0.25	0.5	0.5	0.25	0.25	0.5	0.25	1.0	2.0	2.0	1.0	0.25
Staph aureus X400	1.0	0.25	0.25	0.25	0.5	0.25	0.12	0.5	0.25	1.0	2.0	2.0	2.0	>128
Staph aureus 513E	1.0	0.25	0.25	0.25	0.25	0.12	0.12	0.25	0.25	1.0	1.0	1.0	1.0	0.25
Staph epi 222	0.25	0.12	0.12	0.12	0.12	0.25	0.06	0.12	0.12	0.25	1.0	0.25	0.25	0.06
Staph epi 270	>128	>128	128	>128	>128	>128	>128	>128	128	>128	>128	>128	>128	>128
Strep B 5	0.5	4	2	1	8	8	>128	8	16	1.0	128	0.5	1.0	0.06
Strep B 8	0.5	8	NT	NT	8	8	NT	8	NT	1.0	128	0.25	0.5	0.06
Strep B 14	NT	16	1	1	8	16	64	16	2	NT	128	NT	NT	0.12
Strep C 24	0.5	0.25	NT	NT	0.5	0.5	NT	0.5	NT	0.25	1.0	0.25	0.5	0.12
Strep D 2041	0.5	8	0.5	0.5	8	16	16	8	2	1	>128	0.5	0.5	1.0
Strep D 8043	0.5	2	0.25	0.25	4	4	4	8	2	1	128	0.5	0.5	0.06
Strep D 9960	1.0	8	0.5	0.5	4	8	8	16	4	1	128	1	1	2
Strep G Kruder	0.25	32	NT	NT	32	64	NT	64	NT	0.25	>128	0.25	0.25	0.12
Strep pneum B1-438	>128	>128	32	128	>128	>128	>128	>128	>128	>128	>128	128	>128	64
Strep sanguis MX132	0.5	1	NT	NT	2	1	NT	2	NT	0.5	8	0.25	0.25	0.03
Strep viridans 9943	>128	>128	NT	NT	>128	>128	NT	>128	NT	>128	>128	>128	>128	>128
H. influenzae 76	8	16	16	16	32	4	16	16	16	16	>128	4	2	2
H. influenzae 465	16	16	16	32	NT	NT	16	NT	16	32	NT	8	4	4
H. influenzae MX366	32	16	16	16	32	32	16	16	16	16	128	2	2	4
H. parainfluenzae 9796	8	32	32	32	NT	NT	32	NT	16	16	NT	8	4	4

[a] EM = erythromycin.

Table VI. In Vitro Activity of Macrolide Derivatives against Species of Anaerobic Bacteria and *Chlamydia*

	MIC values, μg/mL															
	1	3	12	15	24	29	31	48	49	50	67	81	98	99	101	EM
Clostridium difficile 2994	0.25	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	1	1	≤0.5	1	4	4	2	≤0.5
Clostridium perfringens 81	0.25	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	2	≤0.5	0.25	>8	≤0.5	0.5	≤0.5	4	2	≤0.5
Clostridium septicum 1128	2	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	0.5	8	≤0.5	0.5	4	8	2	≤0.5
Eubacterium aerofaciens 1235	0.12	2	≤0.5	≤0.5	2	4	2	≤0.5	0.12	1	64	0.12	≤0.5	8	2	≤0.5
Peptococcus asaccharolyticus 1302	8	1	≤0.5	≤0.5	1	2	2	≤0.5	0.06	1	32	≤0.03	2	>8	>8	8
Peptococcus prevoti 1281	4	1	1	2	2	1	2	≤0.5	8	>8	8	8	16	>8	>8	32
Peptostreptococcus anaerobius 1428	4	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	8	>8	4	4	32	>8	>8	1
Peptostreptococcus intermedius 1264	16	2	≤0.5	≤0.5	2	2	4	≤0.5	0.5	>8	64	0.25	1	>8	>8	≤0.5
Propionibacterium acnes 79	2	8	≤0.5	≤0.5	16	16	8	≤0.5	0.5	8	>128	0.25	8	4	2	≤0.5
Bacteroides fragilis 111	8	1	4	2	2	2	4	8	0.5	4	8	2	32	>8	>8	1
Bacteroides fragilis 1877	8	1	2	2	2	2	4	8	1	>8	4	4	64	>8	>8	4
Bacteroides fragilis 1936B	8	1	4	4	2	2	4	8	1	8	4	2	32	>8	>8	2
Bacteroides thetaiotaomicron 1438	2	2	2	4	2	2	4	8	1	4	2	2	128	>8	>8	1
Bacteroides melaninogenicus 1856	8	1	≤0.5	≤0.5	1	1	1	4	0.25	4	2	0.5	8	>8	>8	2
Bacteroides melaninogenicus 2736	16	1	≤0.5	2	2	2	4	8	≤0.03	8	2	2	8	>8	>8	1
Bacteroides vulgatis 1211	8	1	≤0.5	≤0.5	2	1	2	4	1	>8	1	2	32	>8	>8	≤0.5
Bacteroides corrodens 1874	8	1	2	2	2	2	4	8	1	>8	4	2	32	>8	>8	1
Fusobacterium symbiosum 1470	2	1	2	2	2	2	2	8	≤0.03	4	4	0.5	≤0.05	>8	>8	2
Fusobacterium necrophorum 6054A	1	8	4	4	16	8	8	8	0.5	1	>128	0.12	≤0.5	4	8	2
Chlamydia trachomatis	0.5	0.25	>2	>2	0.5	0.25	0.5	0.5	0.25	>1	1	1	0.5	>1	0.5	0.06

trends against several different species and strains (Table V). The derivatives of desmycosin were unexpectedly more active than the parent against both Gram-positive and Gram-negative anaerobic bacteria (Table VI). However, since a more dramatic improvement of in vitro activity had not occurred with these derivatives, it became especially important to look for pharmacokinetic advantages (see below).

Modifications of the aldehyde group of tylosin and the tylosin-related macrolides other than desmycosin resulted in a general loss of in vitro antimicrobial activity against all species of bacteria tested (Tables III and IV). The only exceptions were the derivatives of lactenocin (**92**), whose structure differs from that of desmycosin only in a 3″-O-demethylation (Scheme I); the 20-modified derivatives of lactenocin had a profile of antimicrobial activity similar to that of their corresponding desmycosin analogues. These results indicate that the exact pattern of substitution with the saccharides mycarose and mycinose is very important for activity and that the broadest spectrum of in vitro activity is found with mycinose present and mycarose absent (desmycosin series).

Antimicrobial Evaluation in Vivo. Initial evaluation of in vivo antimicrobial activity was performed against experimental infections caused by *S. pyogenes* in mice. When administered parenterally, none of the 20-modified derivatives were more efficacious than desmycosin or tylosin, and many were considerably less active. In contrast, a substantial number of derivatives of desmycosin and tylosin were efficacious when administered orally (Table VII). The wide variety of substituents at C-20 in derivatives exhibiting oral efficacy indicated that improved oral bioavailability was a characteristic of this series of semisynthetic macrolides. This increased bioavailability could result from either increased absorption or decreased first-pass metabolism in the liver; in either event, a larger amount of antibiotic would pass through the liver and into circulation in the animal. In the latter event, the aldehyde function would be a site of rapid metabolism, and thus its modification to a less rapidly metabolized substituent would result in greater bioavailability after oral administration.

Although several 20-modified derivatives of desmycosin also treated infections of *Staphylococcus aureus* in mice,

Table VII. In Vivo Activity of Macrolide Derivatives against Experimental Infections in Rodents Caused by *S. pyogenes* C203

	ED_{50}, mg/kg × 2			ED_{50}, mg/kg × 2	
compd no.	subcut.	oral	compd no.	subcut.	oral
1	1.4	84	**37**	4.2	>50
2	2.1	>100	**38**	>6.3	25
3	5.0	9	**42**	<1.6	55
4	1.1	5	**43**	0.9	20
5	1.6	11	**44**	3.4	35
6	>10	31	**45**	3.3	>25
7	6.7	19	**46**	4.8	17
8	2.2	19	**47**	>10	19
9	5.0	44	**48**	1.7	31
10	1.9	27	**49**	1.3	42
11	>10	35	**50**	15.4	>100
12	>10	25	**51**	>6.3	30
13	>10	35	**61**	>6.3	56
14	>10	29	**63**	>6.3	23
15	7.2	18	**65**	6.3	18
16	3.9	50	**67**	>6.3	11
17	>10	>50	**69**	2.0	12
18	5.4	>50	**72**	9.1	76
19	10	17	**73**	>10	44
20	2.4	50	**74**	1.9	71
21	>10	25	**75**	>10	>100
22	2.7	15	**76**	>10	91
23	2.1	>50	**78**	>10	31
24	3.8	13	**92**	1.8	81
25	>10	>100	**93**	8.9	>100
26	3.8	21	**94**	3.2	50
27	4.3	19	**95**	5.2	79
28	4.5	19	**96**	3.9	46
29	0.7	5	**97**	1.8	100
30	5.0	8	**98**	3.9	102
31	4.3	7	**99**	>10	>100
32	1.6	8	**101**	>30	75
33	2.4	>25	**102**	>30	121
34	3.4	36	**103**	>30	>100
35	3.1	>25	**104**	7.2	>100
36	5.0	45	**105**	7.8	>100

Table VIII. In Vivo Activity of Macrolide Derivatives against Experimental Infections in Mice

	ED_{50}, mg/kg × 2			
	Strep. pneumoniae Park		*Staph. aureus* X1	
compd no.	subcut.	oral	subcut.	oral
1	>10	>100	3.9	>100
3	>20	>50	4.2	17
12	>20	37	NT	NT
13	>20	46	NT	NT
14	>20	38	NT	NT
15	>20	>50	NT	NT
24	>20	>50	2.8	18
29	>20	>50	1.8	9
31	>20	>50	2.2	10
43	>20	>50	2.0	25

they failed to treat *S. pneumoniae* infections at reasonable doses by either subcutaneous or oral routes of administration (Table VIII). Although the lack of in vivo efficacy against *S. pneumoniae* parallels the general decline of in vitro activity against this bacterium, even those derivatives that had retained in vitro activity failed to treat in vivo (**13–15**). As a result of poor in vivo activity against such critical pathogens as *S. pneumoniae* and the failure to increase in vitro activity against bacteria such as *H. influenzae*, development of this series for human medicine did not appear appropriate in spite of the improvement in oral bioavailability.

The increased oral bioavailability of compounds in this series was further demonstrated by measurement of peripheral plasma levels in both mice and rats (Tables IX and X). In contrast to tylosin and desmycosin, which gave no detectable blood levels in either species after 100 mg/kg oral doses, many of the 20-modified derivatives gave blood levels that were sustained beyond 4 h after oral administration. Several derivatives also gave measurable blood levels in mice at a 50 mg/kg dose. These results correlated well with the increased oral efficacy observed for this series. Since the 20-dihydro-20-*O*-phenyl (**3**), 20-dihydro-20-deoxy-20-chloro (**29**), and 20-dihydro-20-deoxy (**31**) derivatives of desmycosin gave the highest blood levels in both species, further chemical modification of these compounds to expand their spectrum of antimicrobial activity appears warranted.

2′,4′-Ester Derivatives. Since 2′-esters of erythromycin have been used to improve the oral bioavailability of erythromycin,[28] a series of 2′- and 4′-esters of 20-modified derivatives of desmycosin and tylosin were prepared to determine if such derivatives would further enhance oral antimicrobial efficacy. Since short-chain acyl esters yielded the best results in the erythromycin series,[29] acetyl and propionyl esters were selected for testing with the present series.

2′-Monoesters of tylosin and desmycosin derivatives and 2′,4′-diesters of desmycosin derivatives were prepared by standard procedures. Esterification of each hydroxyl group lowered the pK_a of the dimethylamino group by approximately 2 units. Thus, the pK_a of 7.2 for 20-dihydro-20-*O*-phenyltylosin (**51**) was lowered to 5.0 in its 2′-*O*-acetyl derivative (**116**) and the pK_a of 8.2 for 20-dihydro-20-*O*-phenyldesmycosin (**3**) was lowered to 6.3 in its 2′-*O*-acetyl derivative (**111**) and to 4.3 in its 2′,4′-di-*O*-acetyl derivative (**112**). However, the antimicrobial activity of the ester derivatives was generally reduced from that of parent compound both in vitro and in vivo (Table XI).

Summary

Modification of the aldehyde function of tylosin and especially desmycosin has yielded a series of macrolide derivatives with substantially increased oral efficacy and bioavailability. The aldehyde-modified derivatives of desmycosin possessed greater in vitro activity against staphylococci and some streptococci, but did not sufficiently inhibit enough species of clinically important pathogens to warrant their development for human medicine. This series has been further developed to yield compounds with potentially useful antimicrobial activity against bacterial pathogens responsible for pneumonia in swine and calves.

Experimental Section

Physicochemical Determinations and Chromatography. Proton NMR spectra were measured in $CDCl_3$ solution on a Bruker WH-360 or JEOL FX90A NMR spectrometer; chemical shifts are given in ppm from internal TMS. Field desorption mass spectra were obtained on a Varian-MAT 731 spectrometer with carbon dendrite emitters. Ultraviolet spectra were measured in 95% ethanol solution on a Cary 219 spectrometer. Infrared spectra were recorded in chloroform solution on a Nicolet MX-1 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. pK_a values were determined in 66% aqueous DMF. Melting points were taken on a Mel-temp apparatus and are uncorrected; melting points were generally not sharp and occurred after a period of gradual softening. Thin-layer chromatography (TLC) was performed with use of E. Merck plates of silica gel 60 with a fluorescent indicator (F-254); visualization

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Table IX. Peripheral Plasma Levels of Macrolide Derivatives in Mice after Oral Administration

compd no.	dose, mg/kg	concentration, μg/mL				
		0.25 h	0.5 h	1 h	2 h	4 h
1 (desmycosin)	50	–[a]	–	–	–	–
1	100	–	–	–	–	–
3	50	3.2	2.7	2.4	1.2	0.2
3	100	8.9	11.5	13.1	6.5	1.2
15	50	0.1	0.4	0.1	1.8	0.8
15	100	1.3	1.1	2.3	2.0	1.2
22	50	–	–	0.8	–	–
22	100	<0.1	<0.1	2.1	0.9	0.3
29	50	0.3	0.4	0.3	0.4	<0.1
29	100	8.4	6.4	2.8	1.5	0.3
30	50	<0.1	<0.1	0.2	0.4	0.1
31	50	0.9	0.5	2.0	2.2	0.9
31	100	2.6	4.5	3.4	2.3	0.8
43	50	–	–	–	–	–
43	100	–	0.1	0.1	0.1	–
49 (tylosin)	50	–	–	–	–	–
49	100	–	–	–	–	–
51	50	1.6	2.0	2.3	1.2	–
51	100	7.9	7.4	6.0	4.0	3.5
63	50	2.4	2.2	1.6	1.3	–
63	100	13.1	6.0	6.9	–	–
67	50	1.9	1.9	0.9	0.5	–
67	100	10.0	8.8	3.0	1.3	0.4
98 (OMT)	50	–	–	–	–	–
98	100	–	–	–	–	–
101	50	–	–	–	–	–
101	100	–	–	–	–	–
EM	50	–	–	–	–	–
EM	100	2.5	5.6	2.7	0.7	–

[a] No detectable concentration of antibiotic.

Table X. Peripheral Plasma Levels of Macrolide Derivatives in Rats after Oral Administration of 100 mg/kg

compd no.	concentration, μg/mL				
	0.25 h	0.5 h	1 h	2 h	4 h
1 (desmycosin)	–	–	–	–	–
3	2.6	3.9	3.4	4.8	5.6
12	–	–	–	–	–
15	–	–	–	–	–
19	0.5	1.3	2.5	2.9	4.2
29	5.4	8.8	9.4	12.7	7.7
30	0.3	0.5	0.1	1.9	1.5
31	4.6	7.4	7.2	8.7	5.1
43	0.3	0.4	0.2	1.1	0.2
49 (tylosin)	–	–	–	–	–
51	0.2	0.6	2.0	2.7	2.6
63	0.5	1.6	2.1	1.4	1.4
98 (OMT)	–	–	–	–	–
101	–	–	–	–	–
EM	0.1	0.1	0.1	0.2	0.4

was effected by ultraviolet light. Product purification was carried out by chromatography on silica gel, with either flash chromatography techniques[30] (E. Merck grade 60 silica gel) or a Waters Model 500 Prep LC system.

In Vitro and in Vivo Evaluation. Antibiotic susceptibility data in Tables I–VI were obtained by agar dilution methods. In vitro testing vs *Chlamydia* was performed in cell culture. Mouse protection experiments were conducted by treating infected animals 1 and 5 h postinfection, either subcutaneously or orally, with 0.25 mL of a 10% aqueous ethanol solution of the antibiotic over a range of concentrations; tartaric acid was added if needed to dissolve the compounds. Peripheral plasma levels were determined by microbiological assay using *Micrococcus luteus* seeded in Difco Antibiotic Media 1. Zone sizes were measured with a Fisher Zone Reader, and antibiotic concentrations were calculated from the standard curve for the appropriate compound. Concentrations in Table IX represent an average value from five mice per time period, and in Table X, three rats per time period.

(30) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

20-Dihydrotylosin and 20-Dihydrodesmycosin. A solution of tylosin base (30.0 g, 32.8 mmol) in 2-propanol (300 mL) and water (200 mL) was treated with sodium borohydride (315 mg, 8.2 mmol), in portions, over a 5-min period. Thirty minutes after addition had been completed, the pH of the reaction was adjusted to 7.0 by addition of 1 N sulfuric acid. The neutralized solution was evaporated to aqueous and then treated with saturated sodium bicarbonate (500 mL). The mixture was extracted with dichloromethane (3 × 300 mL), and the combined extracts were extracted with saturated sodium chloride (200 mL) and dried (sodium sulfate). Filtration followed by evaporation gave a glass, which was triturated with *n*-hexane, collected on a filter, and air-dried to yield 28.5 g (95%) of 20-dihydrotylosin: mp 162–166 °C; $[\alpha]^{25}_D$ −47.8° (*c* 1.0, MeOH); IR ($CHCl_3$) 1712, 1674 cm^{-1}; UV λ_{max} (EtOH) 283 nm (ϵ 21 200); FDMS, *m/e* 917 (M^+). Anal. Calcd for $C_{46}H_{79}NO_{17}$: C, 60.17; H, 8.67; N, 1.53. Found: C, 59.98; H, 8.60; N, 1.67.

In a similar manner, reduction of desmycosin (10 g, 13 mmol) in 2-propanol–water (1:1, 175 mL) with sodium borohydride (125 mg, 3.3 mmol) yielded 9.65 g (96%) of 20-dihydrodesmycosin: mp 174–176 °C; $[\alpha]^{25}_D$ −18.8° (*c* 1.0, MeOH); IR ($CHCl_3$) 1712, 1676 cm^{-1}; UV λ_{max} (EtOH) 284 nm (ϵ 17 700); FDMS, *m/e* 774 (MH^+). Anal. Calcd for $C_{39}H_{67}NO_{14}$: C, 60.52; H, 8.73; N, 1.81. Found: C, 60.82; H, 8.96; N, 2.02.

20-Dihydro-20-*O*-phenyltylosin. Tylosin (100 g, 109.3 mmol) was reduced with sodium borohydride (1.04 g, 27.3 mmol) as described above, and the product was dissolved in toluene–dichloromethane–tetrahydrofuran (1600:200:400 mL) and treated sequentially with phenol (15.1 g, 164 mmol), triphenylphosphine (43 g, 164 mmol) and diethyl azodicarboxylate (28.5 g, 164 mmol). After 0.5 h at room temperature, methanol (10 mL) was added to quench the reaction. After being stirred for 5 min, the solution volume was reduced in vacuo to about 600 mL and the precipitate of triphenylphosphine oxide was removed by filtration and washed with toluene. The combined filtrates were extracted with saturated sodium bicarbonate solution, dried (sodium sulfate), filtered, and evaporated under reduced pressure. The residue was divided into two portions and each was purified by chromatography on silica gel, eluting with a gradient of dichloromethane to methanol–dichloromethane (final percentage of 5–7.5% methanol). Suitable fractions were combined, and those containing impurities were rechromatographed to produce 56.4 g (52%) of 20-di-

Table XI. Antimicrobial Evaluation of 2'- and 4'-Ester Derivatives

compd no.	substituents 2'	4'	20	parent macrolide	MIC,[a] μg/mL	ED_{50}, mg/kg × 2[a] sc	po
1	OH	OH	CHO	desmycosin	0.25	0.5	67
3	OH	OH	CH_2OPh	desmycosin	0.12	1.2	9
111	OAc	OH	CH_2OPh	desmycosin	1.0	1.0	9
112	OAc	OAc	CH_2OPh	desmycosin	0.5	2.9	16
22	OH	OH	CH_2NFt	desmycosin	0.25	1.6	16
113	OPr	OH	CH_2NFt	desmycosin	0.5	1.7	20
114	OPr	OPr	CH_2NFt	desmycosin	2.0	>6.3	31
29	OH	OH	CH_2Cl	desmycosin	0.25	0.7	6
115	OPr	OH	CH_2Cl	desmycosin	2.0	3.3	27
49	OH	OMyc	CHO	tylosin	0.25	0.5	42
51	OH	OMyc	CH_2OPh	tylosin	2.0	>6.3	30
116	OAc	OMyc	CH_2OPh	tylosin	4.0	>10.0	76.5
63	OH	OMyc	CH_2NFt	tylosin	2.0	>6.3	17
117	OPr	OMyc	CH_2NFt	tylosin	2.0	>6.3	66
67	OH	OMyc	CH_2Cl	tylosin	2.0	>6.3	11
118	OPr	OMyc	CH_2Cl	tylosin	4.0	>6.3	22

[a] Against *Strep. pyogenes* C203. Ft = phthalimido; Myc = mycarosyl.

hydro-20-*O*-phenyltylosin: mp 132–135 °C; $[\alpha]^{25}_D$ −76.8° (*c* 1.0, MeOH); IR ($CHCl_3$) 1715, 1677 cm^{-1}; UV λ_{max} (EtOH) 220 nm (ε 13 500), 278 (25 000); ^1H NMR ($CDCl_3$) δ 6.84–7.0 (m, 2 H, ortho phenyl), 7.2–7.4 (m, 4 H, meta and para phenyl and H-11); FDMS, *m/e* 993 (M^+). Anal. Calcd for $C_{52}H_{83}NO_{17}$: C, 62.82; H, 8.41; N, 1.41. Found: C, 63.08; H, 8.66; N, 1.65.

20-Dihydro-20-*O*-phenyldesmycosin. 20-Dihydro-20-*O*-phenyltylosin (43.4 g, 43.7 mmol) was dissolved in 1 N sulfuric acid (1.5 L), diluted with water (1 L), and stirred at room temperature for 2 h. The solution was then mixed with dichloromethane (500 mL) and treated carefully with solid sodium bicarbonate until basic. The aqueous layer was separated and extracted twice more with dichloromethane. The combined organic extracts were dried and evaporated, and the residual solid was triturated with hexane and dried in vacuo to yield 36.7 g (99%) of 20-dihydro-20-*O*-phenyldesmycosin: mp 122–128 °C; $[\alpha]^{25}_D$ −52.4° (*c* 1.0, MeOH); IR ($CHCl_3$) 1714, 1677 cm^{-1}; UV λ_{max} (EtOH) 220 nm (ε 14 000), 278 (26 000); ^1H NMR ($CDCl_3$) δ 6.84–7.0 (m, 2 H, ortho phenyl), 7.18–7.40 (m, 4H, meta and para phenyl and H-11); FDMS, *m/e* 849 (M^+). Anal. Calcd for $C_{45}H_{71}NO_{14}$: C, 63.58; H, 8.42; N, 1.65. Found: C, 63.77; H, 8.32; N, 1.75.

20-Dihydro-20-deoxy-20-(phenylthio)tylosin. 20-Dihydrotylosin (10.0 g, 10.9 mmol) was dissolved in toluene (350 mL) under argon and treated sequentially at room temperature with triphenylphosphine (2.86 g, 10.9 mmol), diethyl azodicarboxylate (1.8 mL, 10.9 mmol), and thiophenol (1.1 mL, 10.9 mmol). After 30 min, the reaction was incomplete, so an additional amount (10.9 mmol) of each reagent was added as before. After a further 30 min, methanol (15 mL) was added and the reaction mixture was stirred for 15 min. Solvent was evaporated to one-fourth volume, the precipitate was filtered, and the filtrate was evaporated. The residue was purified by chromatography on silica gel, eluting with dichloromethane (2 L) followed by a linear gradient of dichloromethane (4 L) and dichloromethane–methanol (95:5, 4 L) and an additional 2 L of the latter. Appropriate fractions were located by TLC, pooled and evaporated to yield 5.1 g (56%) of 20-dihydro-20-deoxy-20-(phenylthio)tylosin: mp 145–148 °C; $[\alpha]^{25}_D$ −78.8° (*c* 1.0, MeOH); IR ($CHCl_3$) 1713, 1677 cm^{-1}; UV λ_{max} (EtOH) 204 nm (ε 14 500), 258 sh (17 500), 281 (23 500); ^1H NMR ($CDCl_3$) δ 7.06–7.50 (m, 6 H, phenyl and H-11); FDMS, *m/e* 1010 (MH^+). Anal. Calcd for $C_{52}H_{83}NO_{16}S$: C, 61.82; H, 8.28; N, 1.39; S, 3.17. Found: C, 62.05; H, 8.35; N, 1.64; S, 3.32.

20-Dihydro-20-deoxy-20-(phenylthio)desmycosin. 20-Dihydro-20-deoxy-20-(phenylthio)tylosin (4.0 g) was hydrolyzed in 0.5 N sulfuric acid (400 mL) for 1 h at room temperature, and the reaction was worked up as described above to yield 3.4 g (99%) of 20-dihydro-20-deoxy-20-(phenylthio)desmycosin: mp 104–107 °C; $[\alpha]^{25}_D$ −59.6° (*c* 1.0, MeOH); IR ($CHCl_3$) 1715, 1677 cm^{-1}; UV λ_{max} (EtOH) 203 nm (ε 15 700), 260 sh (17 800), 281 (23 300); ^1H NMR ($CDCl_3$) δ 7.1–7.4 (m, 6 H, phenyl and H-11); FDMS, *m/e* 865 (M^+). Anal. Calcd for $C_{45}H_{71}NO_{13}S$: C, 62.40; H, 8.26; N, 1.62; S, 3.70. Found: C, 62.17; H, 8.17; N, 1.46; S, 3.64.

20-Dihydro-20-deoxy-20-[(1-methyl-5-tetrazolyl)thio]desmycosin. Via the procedures described above, 20-dihydrotylosin (3.0 g) was treated with 2 equiv each of triphenylphosphine, diethyl azodicarboxylate and 1-methyltetrazol-5-thiol in THF to give 2.24 g of 20-dihydro-20-deoxy-20-[(1-methyl-5-tetrazolyl)thio]tylosin, of which 1.2 g was hydrolyzed to produce 0.92 g of 20-dihydro-20-deoxy-20-[(1-methyl-5-tetrazolyl)thio]-desmycosin: mp 116–121 °C; $[\alpha]^{25}_D$ −68.1° (*c* 1.0, MeOH); IR ($CHCl_3$) 1715, 1678 cm^{-1}; UV λ_{max} (EtOH) 232 nm (ε 5700), 282 (22 000); ^1H NMR ($CDCl_3$) δ 3.98 (s, 3 H, tetrazole NCH_3); FDMS, *m/e* 872 (MH^+). Anal. Calcd for $C_{41}H_{69}N_5O_{13}S$: C, 56.47; H, 7.98; N, 8.03; S, 3.68. Found: C, 56.45; H, 7.88; N, 7.90; S, 3.51.

20-Dihydro-20-*O*-(hexafluoroisopropyl)desmycosin. Via the procedures described above, 20-dihydrotylosin (4.0 g) was treated with 3 equiv each (in 3 portions each) of triphenylphosphine, diethyl azodicarboxylate, and 1,1,1,3,3,3-hexafluoro-2-propanol in dichloromethane–toluene to give 1.0 g (22%) of 20-dihydro-20-(hexafluoroisopropyl)tylosin, from which mycarose was hydrolyzed in 0.5 N sulfuric acid to yield 20-dihydro-20-(hexafluoroisopropyl)desmycosin: mp 104–109 °C; $[\alpha]^{25}_D$ −19.5° (*c* 1.0, MeOH); IR ($CHCl_3$) 1717, 1680 cm^{-1}; UV λ_{max} (EtOH) 282 nm (ε 20 500); FDMS, *m/e* 924 (MH^+). Anal. Calcd for $C_{42}H_{67}NO_{14}F_6$: C, 54.60; H, 7.31; N, 1.52; F, 12.34. Found: C, 54.35; H, 7.09; N, 1.67; F, 12.08.

20-Dihydro-20-deoxy-20-azidodesmycosin. 20-Dihydrotylosin (16.0 g, 17.4 mmol) in THF (350 mL) under argon at 0 °C was sequentially treated with triphenylphosphine (9.14 g, 35 mmol) and diethyl azodicarboxylate (5.8 mL, 36.7 mmol) over a 0.5-min period and after 4 min diphenyl phosphorazidate (7.5 mL, 35 mmol) over a 4-min period. After 40 min, the reaction was quenched with methanol (10 mL) and stirred for an additional 40 min. Solvent was carefully evaporated in vacuo (Care! volatile methyl azide may be present), and the residue was partitioned between toluene and saturated sodium bicarbonate solution. The organic phase was separated, dried, and evaporated, and the residue was purified by chromatography on silica gel, eluting with dichloromethane (2 L), a linear gradient of dichloromethane (4 L) and dichloromethane–methanol (95:5, 4 L), and finally with 2 L of the latter solvent. Appropriate fractions were located by TLC, combined, and evaporated to yield 9.5 g (55%) of 20-dihydro-20-deoxy-20-azidotylosin: mp 139–144 °C; $[\alpha]^{25}_D$ −73.8° (*c* 1.0, MeOH); IR ($CHCl_3$) 2096 (azide), 1713, 1677 cm^{-1}; UV λ_{max} (EtOH) 282 nm (ε 25 300); FDMS, *m/e* 943 (MH^+). Anal. Calcd for $C_{46}H_{78}N_4O_{16}$: C, 58.58; H, 8.34; N, 5.94. Found: C, 58.37; H, 8.10; N, 5.95.

Hydrolysis of the tylosin derivative as described above gave a quantitative yield of 20-dihydro-20-deoxy-20-azidodesmycosin: mp 135–141 °C; $[\alpha]^{25}_D$ −42.2° (*c* 1.0, MeOH); IR ($CHCl_3$) 2097 (azide), 1713, 1677 cm^{-1}; UV λ_{max} (EtOH) 282 nm (E 24 000); FDMS, *m/e* 798 (M^+). Anal. Calcd for $C_{39}H_{66}N_4O_{13}$: C, 58.63; H, 8.33; N, 7.01. Found: C, 58.50; H, 8.23; N, 6.86.

20-Dihydro-20-deoxy-20-aminodesmycosin. A solution of 20-dihydro-20-deoxy-20-azidodesmycosin (2.0 g, 2.51 mmol) and triphenylphosphine (690 mg, 2.65 mmol) in tetrahydrofuran (40

mL) under argon was refluxed for 15 h; water (0.2 mL) was then added, and refluxing was continued for 4 h. The reaction mixture was evaporated, treated with 0.1 M acetic acid (75 mL), and stirred vigorously for 2 h. The resulting mixture was filtered, and the filtrate was lyophilized. The residue was dissolved in a minimum amount of water and filtered, and the filtrate was lyophilized to give 2.0 g (89%) of 20-dihydro-20-deoxy-20-aminodesmycosin as a tris(acetic acid) salt (best fit of elemental analysis): mp 89–95 °C: $[\alpha]^{25}_D$ -26.8° (c 1.0, MeOH); IR ($CHCl_3$) 3420 (br, NH_2 and OH), 1720, 1670 cm^{-1}; UV λ_{max} (EtOH) 284 nm (ϵ 15 200); FDMS, m/e 773 (MH^+). Anal. Calcd for $C_{45}H_{80}N_2O_{19}$: C, 56.70; H, 8.46; N, 2.94. Found: C, 56.51; H, 8.42; N, 3.09.

20-Dihydro-20-deoxy-20-(phenoxyacetamido)desmycosin. 20-Dihydro-20-deoxy-20-aminodesmycosin tris(acetic acid) salt (1.44 g, 1.51 mmol) was dissolved in acetone (25 mL)–water (10 mL) and treated with potassium carbonate (234 mg, 1.70 mmol) and then with *N*-[(phenoxyacetyl)oxy]succinimide (425 mg, 1.7 mmol). After 1 h, methanol (1 mL) was added, and 10 min later, the reaction mixture was evaporated to aqueous, diluted with saturated sodium bicarbonate solution (25 mL) and extracted with dichloromethane. The extract was dried, filtered, and evaporated. The residue was purified by silica gel chromatography, eluting with a linear gradient of dichloromethane (1 L) and methanol–dichloromethane (1:9, 1 L) to give 885 mg (65%) of 20-dihydro-20-deoxy-20-(phenoxyacetamido)desmycosin: mp 126–131 °C; $[\alpha]^{25}_D$ -33.6° (c 1.0, MeOH); IR ($CHCl_3$) 3400 (br, NH), 1730, 1715 (sh), 1672 cm^{-1}; UV λ_{max} (EtOH) 216 nm (ϵ 10 500), 283 (19 600); FDMS, m/e 907 (MH^+). Anal. Calcd for $C_{47}H_{74}N_2O_{15}$: C, 62.23; N, 8.22; N, 3.09. Found: C, 62.43; H, 8.00; N, 3.07.

20-Dihydro-20-deoxy-20-chlorodesmycosin. A solution of 20-dihydrotylosin (3.0 g, 3.27 mmol), triphenylphosphine (2.57 g, 9.8 mmol), and carbon tetrachloride (0.48 mL, 4.9 mmol) in dichloromethane (60 mL) and pyridine (6 mL) was stirred at room temperature under argon for 64 h. The reaction was treated with methanol (1 mL) and stirred for 30 min. Solvent was evaporated (repetitive evaporation with cyclohexane to remove pyridine), and the residue was purified by silica gel chromatography, eluting with dichloromethane (300 mL) followed by a gradient of dichloromethane (1 L) and methanol–dichloromethane (7:93, 1 L) to yield 2.1 g (67%) of 20-dihydro-20-deoxy-20-chlorotylosin. A portion of this material (935 mg, 1.0 mmol) was hydrolyzed in 1 N sulfuric acid (50 mL) as described above to produce 790 mg (100%) of 20-dihydro-20-deoxy-20-chlorodesmycosin: mp 115–121 °C; $[\alpha]^{25}_D$ -27.8° (c 1.0, MeOH); IR ($CHCl_3$) 1717, 1680 cm^{-1}; UV λ_{max} (EtOH) 282 nm (ϵ 22 700); FDMS, m/e 791 (M^+). Anal. Calcd for $C_{39}H_{66}NO_{13}Cl$: C, 59.11; H, 8.40; N, 1.77; Cl, 4.47. Found: C, 58.89; H, 8.14; N, 1.48; Cl, 4.72.

20-Dihydro-20-deoxy-20-iododesmycosin. A solution of 20-dihydrodesmycosin (30.0 g, 38.8 mmol) and triphenylphosphine (20.4 g, 77.6 mmol) in DMF (100 mL) under argon was treated dropwise at room temperature with a solution of iodine (19.7 g, 77.6 mmol) in DMF (30 mL) over a 45-min period. After being stirred for 1.5 h, the reaction mixture was poured into saturated sodium bicarbonate (500 mL) and extracted three times with dichloromethane. The combined extracts were shaken with 0.1 M sodium thiosulfate, dried, and evaporated. The residue was purified by chromatography on silica gel, eluting with a linear gradient of dichloromethane (4 L) and dichloromethane–methanol (95:5, 4 L) followed by an additional 3 L of the latter. Appropriate fractions were combined and evaporated to yield 12.4 g (36%) of 20-dihydro-20-deoxy-20-iododesmycosin: mp 122–125 °C; $[\alpha]^{25}_D$ -41.7° (c 1.0, MeOH); IR ($CHCl_3$) 1718, 1675 cm^{-1}; UV λ_{max} (EtOH) 280 nm (ϵ 22 600); FDMS, m/e 882 (M^+). Anal. Calcd for $C_{39}H_{66}NO_{13}I$: C, 53.00; H, 7.53; N, 1.58; I, 14.36. Found: C, 53.22; H, 7.51; N, 1.71; I, 14.44.

20-Dihydro-20-deoxydesmycosin. A solution of 20-dihydro-20-deoxy-20-iododesmycosin (7.96 g, 9.01 mmol) in toluene (200 mL) at 80 °C under argon was treated with tributyltin hydride (2.56 mL, 9.65 mmol) and AIBN (50 mg). Additional AIBN was added after 30 and 60 min. After 1.75 h at 75–80 °C, the reaction mixture was cooled and solvent was evaporated. The residue was dissolved in ethyl acetate and mixed with an aqueous solution of potassium fluoride (16 mmol in 75 mL of water). The resultant precipitate was filtered, and the organic layer was separated from the filtrate and then combined with an ethyl acetate extract of the aqueous layer. The organic layers were dried and evaporated, and the residue was dissolved in acetonitrile and extracted twice with pentane. The acetonitrile phase was again evaporated, and the residue was purified by chromatography on silica gel, eluting with a linear gradient of dichloromethane (4 L) and dichloromethane–methanol (95:5, 4 L) followed by an additional 2 L of the latter. Appropriate fractions were combined and evaporated to yield 2.4 g (35%) of 20-dihydro-20-deoxydesmycosin: mp 112–115 °C; $[\alpha]^{25}_D$ -15.3° (c 1.0, MeOH); IR ($CHCl_3$) 1716, 1676 cm^{-1}; UV λ_{max} (EtOH) 282 nm (ϵ 21 000); FDMS, m/e 757 (M^+). Anal. Calcd for $C_{39}H_{67}NO_{13}$: C, 61.80; H, 8.91; N, 1.85. Found: C, 61.54; H, 8.80; N, 1.93.

Deformyltylosin. A mixture of tylosin (1.83 g, 2.0 mmol) and tris(triphenylphosphine)rhodium chloride (1.85 g, 2.0 mmol) in acetonitrile (70 mL) was refluxed for 4 h under argon. The mixture was cooled and filtered, and the filtrate was evaporated. The residue was purified by chromatography on silica gel to yield 644 mg (36%) of deformyltylosin: mp 119–122 °C; $[\alpha]^{25}_D$ -32.9° (c 0.3, MeOH); IR ($CHCl_3$) 1713, 1679 cm^{-1}; UV λ_{max} (EtOH) 282 nm (ϵ 21 000); FDMS, m/e 888 (MH^+). Anal. Calcd for $C_{45}H_{77}NO_{16}$: C, 60.86; H, 8.74; N, 1.58. Found: C, 60.58; H, 8.44; N, 1.29.

20-Dihydro-20-*O*-tosyltylosin. A solution of 20-dihydrotylosin (10.0 g, 10.9 mmol) and 4-(dimethylamino)pyridine (24 mg, 0.2 mmol) in dichloromethane (100 mL) and pyridine (10 mL) was treated with *p*-toluenesulfonyl chloride (2.08 g, 10.9 mmol). The resulting solution was stirred at room temperature with the exclusion of moisture. Additional *p*-toluenesulfonyl chloride was added after 3 h, (1.0 g, 5.2 mmol) and after 22 h (240 mg, 1.3 mmol). After 27 h, methanol (0.8 mL) was added, and the solution was evaporated. The residue was partitioned between dichloromethane and saturated sodium bicarbonate solution, and the organic layer was dried, filtered, and then evaporated. The residue was purified by silica gel chromatography, eluting with a gradient of dichloromethane (1 L) and dichloromethane–methanol (96.5:3.5, 1 L) followed by 2 L of the latter. Appropriate fractions were combined and evaporated to yield 5.4 g (46%) of 20-dihydro-20-*O*-tosyltylosin along with 2.3 g of slightly impure product: mp 148–154 °C; $[\alpha]^{25}_D$ -57.2° (c 1.0, MeOH); IR ($CHCl_3$) 1710, 1670 cm^{-1}; UV λ_{max} (EtOH) 223 nm (ϵ 13 900), 281 (22 100); FDMS, m/e 1072 (MH^+). Anal. Calcd for $C_{53}H_{85}NO_{19}S$: C, 59.36; H, 7.99; N, 1.31; S, 2.99. Found: C, 59.10; H, 8.00; N, 1.31; S, 3.01.

20-Dihydro-20-deoxy-20-fluorodesmycosin. A mixture of 20-dihydro-20-*O*-tosyltylosin (2.1 g, 1.98 mmol) and tetrabutylammonium fluoride dihydrate (450 mg, 2.4 mmol) in THF (80 mL) was refluxed under argon. After 1 h, additional TBAF (100 mg) was added, and reflux was continued for 3 h. The mixture was cooled and filtered, and the filtrate was evaporated. The residue was purified by chromatography on silica gel, eluting with a linear gradient of dichloromethane (1 L) and dichloromethane–methanol (90:10, 1 L) to yield 1.52 g (83%) of 20-dihydro-20-deoxy-20-fluorotylosin. A portion of this material (1.25 g, 1.36 mmol) was hydrolyzed in 0.5 N sulfuric acid (100 mL) as described above to produce 1.0 g (95%) of 20-dihydro-20-deoxy-20-fluorodesmycosin: mp 120–125 °C; $[\alpha]^{25}_D$ -10.7° (c 0.3, MeOH); IR ($CHCl_3$) 1714, 1677 cm^{-1}; UV λ_{max} (EtOH) 282 nm (ϵ 22 500); FDMS, m/e 775 (M^+). Anal. Calcd for $C_{39}H_{66}NO_{13}F$: C, 60.37; H, 8.57; N, 1.81; F, 2.45. Found: C, 58.79; H, 8.25; N, 1.85; F, 2.11.

Preparation of Desmycaminosyltetrahydrofuranyl Compound 41. 20-Dihydro-20-*O*-tosyltylosin (1.5 g, 1.4 mmol) was heated with sodium fluoride (500 mg) and 4A molecular sieves (1.5 g) in DMF at 90–100 °C under argon. Additional sodium fluoride was added after 48 and 72 h. After 4 days, the reaction was cooled and solvent was evaporated. The residual oil was partitioned between dichloromethane and sodium bicarbonate solution, and the organic layer was dried and evaporated. The residue was chromatographed on silica gel, eluting with a linear gradient of dichloromethane (1 L) and dichloromethane–methanol (95:5, 1 L). Appropriate fractions were combined and evaporated to yield 400 mg (49%) of compound 41: mp 154–157 °C; $[\alpha]^{25}_D$ -30.9° (c 1.0, MeOH) IR ($CHCl_3$) 1710, 1677 cm^{-1}; UV λ_{max} (EtOH) 282 nm (ϵ 21 200); FDMS, m/e 582 (M^+). Anal. Calcd for $C_{31}H_{50}O_{10}$: C, 63.89; H, 8.65. Found: C, 63.63; H, 8.55.

Solvolysis of 20-Dihydro-20-deoxy-20-iododesmycosin. 20-Dihydro-20-deoxy-20-iododesmycosin (725 mg, 0.82 mmol) was

dissolved in methanol (40 mL) and treated with silver nitrate (375 mg). After the mixture was stirred for 4.5 h at room temperature, solvent was evaporated and the residue was partitioned between dichloromethane and aqueous sodium bicarbonate solution. The organic layer was separated, dried, and evaporated, and the residue was purified by chromatography on silica gel, eluting with a linear gradient of ethyl acetate (1 L) and ethyl acetate–methanol–concentrated ammonium hydroxide (94:4:2, 1 L), to give 690 mg of partially purified products along with 180 mg (38%) of compound 41. The 690-mg fraction was rechromatographed, eluting with a linear gradient of ethyl acetate (1 L) and ethyl acetate–methanol–concentrated ammonium hydroxide (85:10:5, 1 L) to yield 20 mg (3%) of 20-dihydrodesmycosin, 205 mg (32%) of 20-dihydro-20-*O*-methyldesmycosin (**38**), and 128 mg (19%) of the nitrate ester of 20-dihydrodesmycosin (**40**). For **38**: mp 86–93 °C; UV λ_{max} (EtOH) 282 nm (ϵ 19 200); ^{1}H NMR ($CDCl_3$) δ 3.30 (s, 3 H, OCH_3); FDMS, *m/e* 787 (M^+). For **40**: mp 108–114 °C; UV λ_{max} (EtOH) 282 nm (21 800); FDMS, *m/e* 819 (MH^+). Anal. Calcd for $C_{39}H_{66}N_2O_{16}$: C, 57.19; H, 8.12; N, 3.42. Found: C, 57.16; H, 7.95; N, 3.17.

Deformyl-19-(4-Carbethoxy-2-thiazolidinyl)desmycosin. A solution of desmycosin (2.0 g, 2.6 mmol) and L-cysteine ethyl ester hydrochloride (482 mg, 2.6 mmol) in acetonitrile (30 mL) was stirred at room temperature for 30 min. Solvent was evaporated, and the residue was dissolved in dichloromethane, extracted with saturated sodium bicarbonate solution, dried, and evaporated. The residue was purified by silica gel chromatography, eluting with a linear gradient of dichloromethane (1 L) and methanol–dichloromethane (1:9, 1 L) to give deformyl-19-(4-carbethoxy-2-thiazolidinyl)desmycosin: mp 124–130 °C; $[\alpha]^{25}_D$ −70.7° (*c* 1.0, MeOH); IR ($CHCl_3$) 1736, 1715 sh, 1677 cm^{-1}; UV λ_{max} (EtOH) 283 nm (ϵ 21 600), FDMS, *m/e* 903 (M^+). Anal. Calcd for $C_{44}H_{74}N_2O_{15}S$: C, 58.51; H, 8.26; N, 3.10; S, 3.55. Found: C, 58.78; H, 8.27; N, 3.10; S, 3.49.

20-Dihydro-20-deoxy-20-phthalimido-DMOT. 20-Dihydro-DMOT (3.64 g, 5 mmol), triphenylphosphine (2.62 g, 10 mmol), and phthalimide (1.47 g, 10 mmol) were dissolved in tetrahydrofuran (40 mL) under a nitrogen atmosphere. The solution was treated dropwise with diethyl azodicarboxylate (1.58 mL, 10 mmol) and then stirred for 1 h at room temperature. The excess reagent was quenched with methanol (25 mL), and the solution was evaporated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with dichloromethane (1 L) followed by a linear gradient of dichloromethane (1 L) and 5% methanol in dichloromethane (1 L). Fractions containing the desired product were located by TLC analysis, combined, and evaporated to dryness to yield 2.44 g (57%) of 20-dihydro-20-deoxy-20-phthalimido-DMOT: mp 128–134 °C; $[\alpha]^{25}_D$ −109.7° (*c* 1.0, MeOH); IR ($CHCl_3$) 1765, 1710, 1678 cm^{-1}; UV λ_{max} (EtOH) 219 nm (ϵ 44 500), 232 sh, 241 (12 500), 282 (20 200); FDMS, *m/e* 857 (MH^+). Anal. Calcd for $C_{46}H_{68}N_2O_{13}$: C, 64.46; H, 8.00; N, 3.27. Found: C, 64.41; H, 7.76; N, 3.27. A sample (1 g) was hydrolyzed in 1 N H_2SO_4 for 1 h and isolated as described previously to yield 502 mg (60%) of 20-dihydro-20-deoxy-20-phthalimido-DOMT: mp 121–124 °C; $[\alpha]^{25}_D$ −86.8° (*c* 1.0, MeOH); IR ($CHCl_3$) 1770, 1710, 1678 cm^{-1}; UV λ_{max} (EtOH) 219 nm (ϵ 43 300), 232 sh, 241 (12 400), 282 (20 000); FDMS, *m/e* 712 (M^+). Anal. Calcd for $C_{39}H_{56}N_2O_{10}$: C, 65.71; H, 7.92; N, 3.93. Found: C, 65.73; H, 7.83; N, 3.88.

20-Dihydro-20-*O*-phenyl-2′-*O*-acetyltylosin. A solution of 20-dihydro-20-*O*-phenyltylosin (5.0 g, 5.04 mmol) in acetone (90 mL) was treated with acetic anhydride (2.0 mL, 21.2 mmol) and stirred at room temperature with the exclusion of moisture for 16 h. The reaction mixture was evaporated to half volume and poured into a saturated sodium bicarbonate solution (200 mL). The resulting mixture was extracted three times with dichloromethane. The extracts were combined, dried, and evaporated to give a quantitative yield of 20-dihydro-20-*O*-phenyl-2′-*O*-acetyltylosin: mp 96–100 °C; $[\alpha]^{25}_D$ −82.0° (*c* 1.0, MeOH); IR ($CHCl_3$) 1743, 1717, 1676 cm^{-1}; UV λ_{max} (EtOH) 219 nm (ϵ 14 100), 279 (24 300); ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H, acetyl); FDMS, *m/e* 1036 (MH^+). Anal. Calcd for $C_{54}H_{85}NO_{18}$: C, 62.59; H, 8.27; N, 1.35. Found : C, 62.33; H, 8.03; N, 1.51.

20-Dihydro-20-*O*-phenyl-2′-*O*-acetyldesmycosin. 20-Dihydro-20-*O*-phenyl-2′-*O*-acetyltylosin (4.5 g, 4.35 mmol) was hydrolyzed in a solution of dioxane (30 mL) and 1 N sulfuric acid (175 mL) for 1.5 h at room temperature. After usual workup, the product was purified by chromatography on silica gel, eluting with a linear gradient of dichloromethane (1 L) and dichloromethane–methanol (95:5, 1 L) to give 2.9 g (75%) of 20-dihydro-20-*O*-phenyl-2′-*O*-acetyldesmycosin: mp 102–106 °C; $[\alpha]^{25}_D$ −54.0° (*c* 1.0, MeOH); IR ($CHCl_3$) 1742, 1717, 1679 cm^{-1}; UV λ_{max} (EtOH) 220 nm (ϵ 12 600), 279 (24 500); ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3 H, acetyl); FDMS, *m/e* 891 (M^+). Anal. Calcd for $C_{47}H_{73}NO_{15}$: C, 63.28; H, 8.25; N, 1.57. Found: C, 63.33; H, 8.31; N, 1.58.

20-Dihydro-20-*O*-phenyl-2′,4′-di-*O*-acetyldesmycosin. 20-Dihydro-20-*O*-phenyldesmycosin (3.0 g, 3.53 mmol) was dissolved in acetone (40 mL) and treated with acetic anhydride (1.0 mL, 10.6 mmol) for 15 h at room temperature. The reaction was then carefully poured into saturated sodium bicarbonate (300 mL), and the product was extracted into dichloromethane (3 × 75 mL). The combined extracts were dried and evaporated to give 3.2 g (97%) of 20-dihydro-20-*O*-phenyl-2′,4′-di-*O*-acetyldesmycosin: mp 85–88 °C; $[\alpha]^{25}_D$ −55.2° (*c* 1.0, MeOH); IR ($CHCl_3$) 1743, 1715 (sh), 1677 cm^{-1}; UV λ_{max} (EtOH) 219 nm (ϵ 12 200), 278 (22 500); ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 6 H, acetyl); FDMS, *m/e* 933 (M^+). Anal. Calcd for $C_{49}H_{75}NO_{16}$: C, 63.00; H, 8.09; N, 1.50. Found: C, 62.75; H, 7.87; N, 1.47.

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