SYNTHESIS AND ANTIMICROBIAL EVALUATION OF ACYL DERIVATIVES OF 16-MEMBERED MACROLIDE ANTIBIOTICS RELATED TO TYLOSIN

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A large number and wide variety of acyl derivatives of the tylosin-related macrolides 23-demycinosyltylosin (DMT), 23-demycinosyloxytylosin (DMOT) and 5-*O*-mycaminosyltylonolide (OMT) were synthesized and evaluated. This encompassed conversion of the hydroxyl groups at 2',4' and 23 of the appropriate macrolides to the corresponding esters, in which a variety of different substitution patterns were examined. A wide range of acyl substituents was investigated, particularly for 23-*O*-acyl derivatives of OMT, since these were substantially more active *in vitro* than OMT itself. However, the acyl derivatives which were prepared demonstrated no substantial improvement in oral efficacy or bioavailability over the parent macrolides.

The production, isolation and characterization of new 16-membered macrolide antibiotics related to tylosin have recently been reported[1]. Two of these new macrolides, 23-demycinosyltylosin (DMT) and 23-demycinosyloxytylosin (DMOT), lack a saccharide moiety at C-23 (see Fig. 1). Although they would be very difficult to prepare by chemical modification of tylosin, DMT and DMOT are now readily available *via* fermentation of mutant strains of *Streptomyces fradiae*[2,3]. Furthermore, since the terminal neutral sugar (mycarose) is easily hydrolyzed from DMT and DMOT under mildly acidic conditions, 5-*O*-mycaminosyltylonolide (OMT) and 23-deoxy-5-*O*-mycaminosyltylonolide (DOMT) are now conveniently available as well.

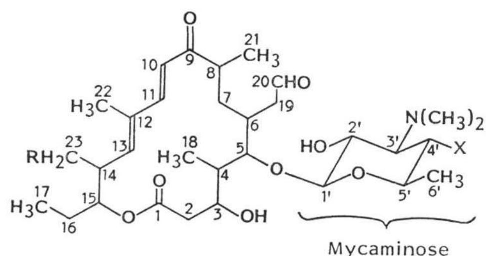
Since acyl derivatives of tylosin have been reported to increase antimicrobial activity against resistant microorganisms and to produce higher blood levels of antibiotic after oral administration[4], the acylation of these newly available 16-membered macrolides was investigated as a means of improving their antimicrobial activity and oral bioavailability. In this paper, we report the synthesis, antimicrobial evaluation and structure-activity relationships of some new acyl derivatives of DMT, DMOT and OMT.

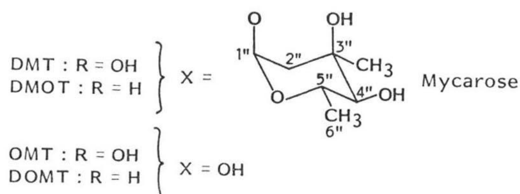
Results and Discussion

Acylation of Hydroxyl Groups of Mycaminose

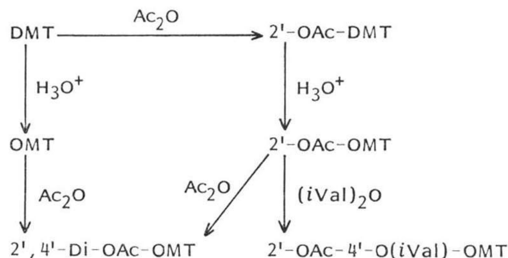
In the absence of an external base, the position of acylation of DMT and DMOT was controlled by the dimethylamino group of mycaminose, which directed the acylation to the hydroxyl group vicinal to it. Thus, in a solvent such as acetone, acylation of these macrolides (in which the 4'-hydroxyl group is substituted by mycarosyl) with anhydrides or reactive esters of carboxylic acids yielded the corresponding 2'-*O*-acyl derivatives in high yield. This acylation was usually readily and selectively achieved, as reported for other macrolides[5]. Acyl chlorides were less preferred as acylating agents

Fig. 1.





Scheme 1. Acylation of 2′- and 4′-hydroxyl groups. *i* Val; Isovaleryl.



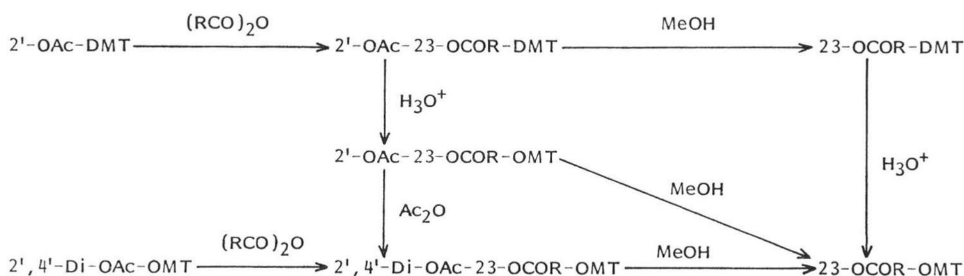
due to their greater reactivity and the liberation of HCl during acylation, which often resulted in some loss of selectivity as well as hydrolysis of the acid-labile mycarose.

Hydrolysis of mycarose from 2′-*O*-acyl derivatives of DMT and DMOT under mildly acidic conditions[5] readily yielded the 2′-*O*-acyl derivatives of OMT and DOMT respectively. Acylation of OMT and DOMT directly in the absence of an external base yielded their 2′,4′-di-*O*-acyl derivatives. No significant selectivity for either 2′- or 4′-*O*-acylation was observed using common reagents such as acetic or propionic anhydride. Diesters of OMT or DOMT with different acyl groups at 2′ and 4′ were easily prepared by treatment of the 2′-*O*-acyl derivative (prepared *supra*) with a second acylating agent in the absence of an external base. The interrelationships of these esters of DMT and OMT are illustrated in Scheme 1; a similar scheme can be drawn for the analogous esters of DMOT and DOMT.

Acylation of C-23 Hydroxyl Group

After the free hydroxyl groups of mycaminose had been protected by acylation, the primary hydroxyl group on C-23 of DMT or OMT was the most reactive hydroxyl group present in these molecules. Selective acylation was thus readily achieved under standard acylation conditions, using an acylating reagent and an external base such as pyridine. As exemplified in the experimental section, acylation of the 23-hydroxyl group was achieved with a variety of acylating reagents, such as acyl halides, anhydrides, reactive esters such as HBT (1-hydroxybenzotriazole) or NOS (*N*-hydroxysuccinimide) esters and acyl imidazole derivatives or by a DCC (dicyclohexylcarbodiimide)-mediated coupling with a carboxylic acid.

Scheme 2. Acylation of 23-hydroxyl group.



After the 23-hydroxyl group had been acylated, the acyl groups at 2′ and 4′ were selectively removed by methanolysis[5], yielding the 23-*O*-acyl derivatives of DMT and OMT. Under these mild conditions, only the esters vicinal to the dimethylamino group (2′ and 4′) were hydrolyzed. By the appropriate combination of acylation, deacylation and hydrolytic procedures, the mono-, di- and triester derivatives shown in Scheme 2 were readily synthesized. A very brief communication has previously been published describing a few similar 23-*O*-acyl derivatives of OMT[6].

We have also found that selective acylation of the 23-hydroxyl group of DMT or OMT can be achieved directly without first protecting the hydroxyl groups on mycaminose. This was done by treating the appropriate macrolide with an acylating agent in the presence of an external base and at low temperature. Under these conditions, the external base eliminated the directing effect of the intramolecular base (*i.e.*, 3′-dimethylamino group) and the primary hydroxyl group at C-23 was selectively acylated. Although the reaction conditions have not yet been optimized, the preparation of 23-*O*-acyl derivatives in a single step without the need for protection and deprotection of the 2′- and 4′-hydroxyl groups of macrolides has been accomplished in reasonable yield.

Elucidation of Structure of Esters

All of the new esters were purified by standard methods of extraction and chromatography to homogeneity by TLC analysis. Their structures were ascertained principally by the combination of mass spectrometry and UV and ^{1}H NMR spectroscopy. Using field desorption mass spectrometry (FD-MS), the parent ion ($m/z = M^+$ or $(M+H)^+$) of each derivative was easily observed, providing confirmation of the molecular weight and a strong suggestion for the composition of the derivative expected from the acylation reaction. The intact presence of the dienone chromophore was confirmed by the UV spectrum. The structures were then firmly established by their ^{1}H NMR spectra. The most indicative proton chemical shifts of a representative sample of ester derivatives are listed in Table 1, which demonstrate the downfield movement of the appropriate proton resonances upon acylation.

In Vitro Activity

Acetylation of the hydroxyl groups on mycaminose of either DMT, DMOT or OMT usually resulted in little or no change of *in vitro* activity (Tables 2 and 3). The MIC values for the 2′-*O*-acetyl or 2′,4′-di-*O*-acetyl derivatives were generally either the same as or only one dilution different from

Table 1. Selected proton chemical shifts for ester derivatives[a].

Compound[b]	2′	4′	23	3	4″
DMT	3.56	3.30	3.77	3.86	2.96
2′-Ac-DMT	5.01	3.28	3.75	3.80	2.95
2′-Ac-23-Pr-DMT	5.01	3.29	4.17	3.80	2.94
2′-Ac-23-PhOAc-DMT	5.01	3.30	4.16	3.80	2.95
DMOT	3.54	3.26	1.08	3.84	2.95
2′-Ac-DMOT	5.01	3.28	1.08	3.80	2.94
OMT	3.47	3.03	3.72	3.83	—
2′,4′-Di-Ac-OMT	4.88	4.73	3.75	3.82	—
2′,4′,23-Tri-Ac-OMT	4.89	4.74	4.15	3.82	—
23-Ac-OMT	3.49	3.03	4.16	3.83	—

[a] Chemical shift values in δ (ppm from internal TMS); deuteriochloroform solvent.
[b] Abbreviations: Ac; Acetyl, Pr; propionyl, PhOAc; phenoxyacetyl.

Table 2. *In vitro* activity of 2′- and/or 23-ester derivatives of DMT and DMOT.

Compound	MIC (μg/ml)				
	S. a. X1	*S. e.* EPI 1	*S. py.* C203	*S. pn.* Park I	*S. f.* X66
DMT	1	1	0.25	0.5	1
2′-Ac-DMT	2	1	0.5	1	2
2′,23-Di-Ac-DMT	0.5	0.5	0.12	0.12	1
2′-Ac-23-Pr-DMT	0.5	1	0.5	0.12	1
2′-Ac-23-Piv-DMT	0.5	0.5	0.25	0.06	0.5
2′-Ac-23-Oct-DMT	2	2	0.5	0.5	2
2′-Ac-23-C18-DMT	64	64	64	16	64
2′-Ac-23-PhAc-DMT	0.5	0.5	0.12	0.015	0.5
2′-Ac-23-PhVal-DMT	1	1	0.5	0.12	2
2′-Ac-23-Naph-DMT	1	2	0.5	0.25	2
2′-Ac-23-PhOAc-DMT	0.25	0.5	0.12	0.06	0.5
2′-Ac-23-Cinnam-DMT	1	1	1	0.25	2
2′-Ac-23-ClPhAc-DMT	0.5	0.5	0.25	0.06	0.5
2′-Ac-23-TCA-DMT	1	1	0.5	0.03	1
2′-Ac-23-Bu(4)COOH-DMT	8	4	2	1	32
2′-Ac-23-BuCONHBn-DMT	1	2	0.25	0.12	1
2′-Ac-23-$COOCH_3$-DMT	0.5	0.5	0.25	0.06	0.5
2′-Ac-23-COIm-DMT	1	2	0.5	0.12	2
23-Ac-DMT	0.5	0.5	0.12	0.06	0.5
23-Pr-DMT	1	1	0.5	0.12	1
23-PhAc-DMT	0.25	0.5	0.12	0.03	0.5
23-COC_6H_{11}-DMT	0.5	0.25	0.25	0.12	0.5
23-$COOCH_3$-DMT	0.25	0.5	0.25	0.06	0.5
23-CONHPrOH-DMT	1	1	0.25	0.12	2
23-$CONHOctNH_2$-DMT	2	2	1	0.25	4
2′-Pr-DMT	1	1	0.5	0.5	1
2′,23-Di-Pr-DMT	0.5	0.5	0.5	0.5	1
2′-PhAc-DMT	0.5	0.5	0.25	0.12	1
2′,23-Di-PhAc-DMT	1	1	0.5	0.12	1
DMOT	0.5	0.5	0.25	0.12	0.5
2′-Ac-DMOT	0.5	0.5	0.25	0.12	1

Organisms: *S.a.*; *Staphylococcus aureus*, *S.e.*; *Staphylococcus epidermidis*, *S.py.*; *Streptococcus pyogenes*, *S.pn.*; *Streptococcus pneumoniae*, *S.f.*; *Streptococcus faecalis*, all organisms were taken from the Lilly culture collection.

Abbreviations: Ac; Acetyl, Pr; propionyl, Piv; pivaloyl, Oct; *n*-octanoyl, C18; *n*-octadecanoyl, PhAc; phenylacetyl, PhVal; 5-phenylvaleryl, Naph; β-naphthoyl, PhOAc; phenoxyacetyl, Cinnam; cinnamoyl, ClPhAc; *p*-chlorophenylacetyl, TCA; trichloroacetyl, Bu(4)COOH; 4-carboxybutyryl, BuCONHBn; 4-(*N*-benzylcarbamyl)butyryl, $COOCH_3$; methoxycarbonyl, COIm; imidazole-1-carbonyl, COC_6H_{11}; cyclohexylcarbonyl, CONHPrOH; *N*-(3-hydroxypropyl)carbamyl, $CONHOctNH_2$; *N*-(8-aminooctyl)-carbamyl.

those of the parent macrolide, and such differences were considered within the experimental error of the test. *In vitro* activity was usually retained with analogous esters of larger carboxylic acids such as valeric, phenylacetic and benzoic acids. The question of whether the antimicrobial activity arose from the esters themselves or only from the parent macrolide regenerated as the result of rapid ester hydrolysis during the test was not studied; this question is relevant since the 2′-esters of this series are at the position analogous to that of the propionate group in propionyl erythromycin[7].

Acylation of the 23-hydroxyl group of DMT and OMT resulted in a significant enhancement of

Table 3. *In vitro* activity of ester derivatives of OMT.

Ester of OMT	MIC (μg/ml)				
	S. a. X1	*S. e.* EPI 1	*S. py.* C203	*S. pn.* Park I	*S. f.* X66
OMT	1	1	0.5	0.25	1
2′-Ac	2	2	0.5	0.5	2
2′,4′-Di-Ac	2	1	0.5	0.25	2
2′,23-Di-Ac	0.25	0.25	0.25	0.12	0.5
2′-Ac-23-Pr	0.5	0.25	0.25	0.12	0.5
2′-Ac-23-PhAc	0.12	0.12	0.12	0.015	0.12
2′,4′,23-Tri-Ac	0.5	0.5	0.5	0.12	1
2′,4′-Di-Ac-23-PhAc	0.25	0.25	0.06	0.03	0.12
23-Ac	0.12	0.12	0.12	0.06	0.25
23-Pr	0.25	0.12	0.12	0.12	0.25
23-*i*Val	0.25	0.25	0.25	0.12	0.5
23-Oct	0.25	0.25	0.5	0.12	0.5
23-Bz	0.25	0.25	0.12	0.06	0.25
23-PhAc	0.06	0.06	0.06	0.015	0.06
23-PhOAc	0.06	0.06	0.06	0.03	0.12
23-PhGly	0.5	0.5	0.12	0.03	0.5
23-Mand	0.25	0.25	0.12	0.06	0.25
23-PyrAc	0.25	0.12	0.015	0.03	0.12
23-(NO_2)PhAc	0.12	0.12	0.06	0.03	0.12
23-Cl_2PhSAc	0.12	0.25	0.06	0.03	0.12
23-Bu(5)COOH	2	2	1	0.25	2
23-$BuCOOCH_3$	0.12	0.25	0.12	0.03	0.25
23-COC_6H_{11}	0.12	0.12	0.06	0.06	0.25
23-COAdamant	1	1	1	0.5	1
23-$COOCH_3$	0.12	0.12	0.12	0.03	0.25
23-CONHBn	0.12	0.12	0.06	0.03	0.25
23-CONHPrOH	2	2	0.5	0.25	2
23-$CONHOctNH_2$	1	2	0.25	0.06	1
23-Phthal	32	16	8	4	32
23-Ts	0.25	0.25	0.12	0.03	0.25
23-$PO(OPh)_2$	0.25	0.25	0.12	0.03	0.25
2′-Pr	2	2	1	4	2
2′-PhPr	1	1	0.5	0.25	1
2′-Ac-4′-*i*Val	1	1	0.25	0.12	1
2′,4′-Di-Pr	2	2	0.5	0.5	2
2′,4′-Di-Bz	4	4	2	1	32

Organisms: See Table 2.

Abbreviations: *i*Val; Isovaleryl, Bz; benzoyl, PhGly; phenylglycyl, Mand; mandelyl, PyrAc; 3-pyridylacetyl, (NO_2)PhAc; *p*-nitrophenylacetyl, Cl_2PhSAc; 3,4-dichlorophenylthioacetyl, Bu(5)COOH; 5-carboxybutyryl, $BuCOOCH_3$; 5-carbomethoxybutyryl, COAdamant; 1-adamantanecarbonyl, CONHBn; *N*-benzylcarbamyl, Phthal; phthaloyl monosodium salt, Ts; *p*-toluenesulfonyl, $PO(OPh)_2$; diphenylphosphoryl, PhPr; 3-phenylpropionyl, and see Table 2.

in vitro antimicrobial activity against Gram-positive bacteria, as illustrated in Tables 2 and 3. The improvement of *in vitro* activity was particularly dramatic for 23-*O*-acyl derivatives of OMT. The increase in antibiotic activity was observed with a very wide variety of ester groups, including carboxylates, carbonates, carbamates, sulfonates and phosphates. The ester groups could be straight-chain, branched or cyclic, saturated or unsaturated, and alkyl, aralkyl, aryl or heteroaryl in composition. Furthermore, a wide variety of substituents could be incorporated into these ester groups,

Table 4. *In vitro* spectrum of antibiotic activity of ester derivatives of OMT and DMT.

	MIC (μg/ml)				
	OMT	23-Ac-OMT	23-PhAc-OMT	DMT	23-PhAc-DMT
Staphylococcus aureus S13E	1	0.12	0.06	1	0.25
Streptococcus pyogenes C203	0.5	0.12	0.06	0.25	0.12
S. pneumoniae Park I	0.25	0.06	0.015	0.5	0.03
Haemophilus influenzae C.L.	4	0.5	0.5	8	2
H. influenzae 76	2	1	0.25	4	2
Escherichia coli EC14	128	32	16	128	128
Klebsiella pneumoniae X68	>128	128	64	>128	>128
Enterobacter aerogenes EB17	>128	128	32	>128	>128
E. cloacae EB5	>128	128	32	>128	>128
Shigella sonnei N9	128	32	16	64	64
Morganella morganii PR15	>128	64	16	>128	128
Providencia rettgeri C24	128	64	32	>128	128
Proteus inconstans PR33	>128	128	64	>128	>128
Salmonella typhi 1335	>128	128	32	128	>128
Pseudomonas aeruginosa X239	64	64	16	>128	>128
Serratia marcescens SE3	>128	128	32	>128	>128
Citrobacter freundii CF17	>128	128	32	>128	>128

Abbreviations: See Tables 2 and 3.

including halo, nitro, amino, amido, hydroxy, alkoxy, acyloxy, carboxy, carboalkoxy or carboxamido groups. The *in vitro* activity of all of these 23-*O*-acyl derivatives was generally good (MIC values $\leqq 1$ μg/ml) and often increased over that of OMT by more than an order of magnitude. A similar trend has been noted in the small group of esters of OMT which has been previously reported[8].

In our larger series of ester derivatives, the activity was reduced when the acyl group contained very polar or charged functional groups, such as primary amino, primary hydroxyl or (especially) carboxyl groups or if the acyl group was excessively bulky, such as octadecanoyl. In contrast, the activity appeared to be greatest when the acyl group was relatively lipophilic in nature and either unsubstituted or substituted with non-polar functional groups. The 23-*O*-phenylacetyl, phenoxyacetyl and similar esters appeared to be the most potent members of these series. In addition, a substantial increase of *in vitro* activity against Gram-negative bacteria was obtained from many 23-*O*-acyl derivatives of OMT (Table 4), although the activity was not enough to render them clinically useful except perhaps against *Haemophilus influenzae*. In contrast, the 23-*O*-acyl derivatives of DMT did not show the corresponding increase of activity against Gram-negative bacteria as they had against Gram-positive bacteria. The excellent *in vitro* spectrum of activity of many of these 23-*O*-acyl derivatives of OMT prompted extensive and detailed evaluation of their *in vivo* activity.

Although some trends could be discerned from the data, it was impossible to establish rules for which there were no exceptions. Furthermore, trends observed when a single acyl group was attached often changed when more than one acyl group had been added. A selective representation of data for antibiotic activity has been assembled in Tables 2~4 to illustrate the structure-activity relationships in our series of ester derivatives.

In Vivo Results

Most of the acyl derivatives were evaluated *in vivo* for treatment of experimentally-induced infections in rodents. Representatives of all of the different structural types of acyl derivatives of DMT,

Table 5. *In vivo* activity of ester derivatives of DMT and DMOT against infections experimentally-induced by *Streptococcus pyogenes* C203 in mice.

Compound	ED_{50} (mg/kg×2)	
	sc	po
DMT	1.9	82
2′-Ac-DMT	>10	193
2′,23-Di-Ac-DMT	>20	117
2′-Ac-23-Pr-DMT	>20	212
23-Pr-DMT	13	300
23-COC_6H_{11}-DMT	5.6	>100
23-PhAc-DMT	>10	>100
23-CONHPrOH-DMT	1.7	>100
DMOT	5.7	78
2′-Ac-DMOT	>10	>100
Josamycin	7.6	35
Erythromycin	0.9	10

Abbreviations: See Table 2.

Table 6. *In vivo* activity of ester derivatives of OMT against infections experimentally-induced by *Streptococcus pyogenes* C203 in mice.

Ester of OMT	ED_{50} (mg/kg×2)	
	sc	po
OMT	2.6	97
2′-Ac	6.8	>150
2′,4′-Di-Ac	>15	139
2′,23-Di-Ac	6.4	137
2′-Ac-23-Pr	8.1	150
2′-Ac-23-PhAc	6.5	155
2′,4′,23-Tri-Ac	>15	100
2′,4′-Di-Ac-23-PhAc	>30	159
23-Ac	3.3	109
23-Pr	6.0	106
23-*i*Val	7.5	>150
23-PhAc	5.0	>100
23-PhOAc	6.0	>200
23-PhGly	2.6	91
23-Mand	11	>150
23-PyrAc	13	150
23-COC_6H_{11}	1.8	>100
23-$COOCH_3$	5.0	>100
23-CONHBn	25	>100
23-Ts	15	146
23-$PO(OPh)_2$	>30	>150
2′-PhAc	2.1	92
2′,4′-Di-Pr	5.7	167
2′-Ac-4′-*i*Val	>30	>100

Abbreviations: See Tables 2 and 3.

DMOT and OMT were tested. Particular emphasis was placed on the 23-*O*-acyl derivatives of OMT since they had such potent *in vitro* activity. Initial testing was done in mice experimentally infected by *Streptococcus pyogenes* C203, which appeared to be the most sensitive organism *in vivo*.

Efficacy of the acyl derivatives of DMT and DMOT in this model infection was relatively poor in most cases, for either subcutaneous or oral administration of the compounds (see Table 5). No suggestion of increased efficacy could be discerned for any of the esters of DMT or DMOT, even for those derivatives which appeared to have increased *in vitro* activity. The parenteral activity of acyl

Table 7. *In vivo* activity of ester derivatives of OMT against experimentally-induced infections in mice.

Compound	ED_{50} (mg/kg×2)			
	S. aureus X1 infection		*S. pneumoniae* Park I infection	
	sc	po	sc	po
OMT	4.4	184	>10	164
23-Ac-OMT	7.1	184	5.5	159
23-PhAc-OMT	6.1	184	>10	>200
23-PhOAc-OMT	9.1	172	8.0	163
23-COC_6H_{11}-OMT	8.5	>200	6.9	119
23-$COOCH_3$-OMT	>10	161	4.4	108
2′,4′-Di-Ac-OMT	12.6	>200	>10	183
DMT	7.9	>200	>10	>200
23-Ac-DMT	7.2	>200	>10	>200
2′-Ac-DMT	14.3	>200	>10	>200
Erythromycin	3.5	22	2.6	25

Abbreviations: See Tables 2 and 3.

Table 8. *In vivo* activity of ester derivatives of OMT against experimentally-induced infections in rats.

Compound	ED_{50} (mg/kg×2)
	S. pyogenes C203 infection (po)
OMT	>200
23-Ac-OMT	>200
23-PhOAc-OMT	>200
23-COC_6H_{11}-OMT	116
23-$COOCH_3$-OMT	126
Erythromycin	15

Abbreviations: See Tables 2 and 3.

derivatives of OMT was somewhat better and occasionally comparable to that of OMT itself, which is relatively effective in treating this infection when given subcutaneously (Table 6). However, for the majority of acyl derivatives of OMT which were investigated, their parenteral efficacy was less than that of OMT despite their substantial improvement of *in vitro* activity. Even more disappointingly, however, none of the acyl derivatives of OMT (nor OMT itself) showed an acceptable degree of oral activity (Table 6), whereas erythromycin showed excellent efficacy by both the subcutaneous and oral routes of administration (Table 5).

Several of the 23-*O*-acyl derivatives of OMT were further investigated in model infections in mice caused by either *Staphylococcus aureus* or *Streptococcus pneumoniae* (Table 7). These acyl derivatives were also tested against an experimental infection in rats induced by *Streptococcus pyogenes* (Table 8). However, treatment did not improve from changing either the infecting organism or the animal species and the conclusions from these experiments were the same as those from the mouse protection experiments using *S. pyogenes*. In all cases, excellent treatment of the infection by erythromycin was observed.

Blood levels for a number of representative derivatives were measured in mice after oral administration of 100 mg/kg. This dose level was selected because the oral ED_{50} values were near or above 100 mg/kg. Even at this relatively high dose, circulating plasma levels were low and of short duration (Table 9). Even though some esters gave slightly improved plasma levels over that of parent macrolide, these increased levels were not sufficient to improve oral efficacy. These results are consistent with data previously reported for esters of tylosin[4].

Conclusion

By use of the procedures outlined in Schemes 1 and 2 and the experimental section, a wide variety

Table 9. Peripheral plasma levels (μg/ml) of ester derivatives of macrolides in mice following a 100 mg/kg oral dose.

Compound	5 minutes	15 minutes	30 minutes	1 hour	2 hours
OMT	0.63	1.68	NZ	NZ	NZ
23-Ac-OMT	0.51	1.31	0.42	NZ	NZ
23-PhAc-OMT	4.54	1.99	NZ	NZ	NZ
2′-Ac-23-Pr-OMT	1.86	1.03	NZ	NZ	NZ
2′,4′-Di-Ac-OMT	2.2	NZ	NZ	NZ	NZ
DMT	NZ	NZ	NZ	NZ	NZ
23-Ac-DMT	NZ	NZ	NZ	NZ	NZ
23-PhAc-DMT	0.32	0.44	NZ	NZ	NZ
2′-Ac-DMT	0.1	0.05	0.04	0.1	0.02
DMOT	0.46	0.34	0.12	0.08	NZ
2′-Ac-DMOT	2.46	1.62	1.3	1.35	NZ

NZ: No measurable level.
Abbreviations: See Tables 2 and 3.

of esters of DMT, DMOT and OMT were prepared. For DMT, representative substitution patterns were 2′- or 23-monoacyl and 2′,23-diacyl. For DMOT, which lacks a hydroxyl group at C-23, the 2′-monoacyl derivative was prepared for this study. For OMT, which lacks mycarose and thus has a hydroxyl group at C-4′, representative substitution patterns were 2′- or 23-monoacyl; 2′,4′- or 2′,23-diacyl and 2′,4′,23-triacyl.

An extensive evaluation was conducted of the *in vitro* and *in vivo* antimicrobial properties of representatives of these ester derivatives of DMT, DMOT and OMT. A substantial increase of *in vitro* activity was observed for many of the derivatives, especially for 23-*O*-acyl derivatives of OMT. However, *in vivo* efficacy in various rodent model infections was not improved and oral efficacy was observed only at relatively high doses. It was concluded that none of the various acyl derivatives of these macrolide antibiotics had any *in vivo* advantage over erythromycin as a potentially new, orally effective antibiotic.

Experimental

Physico-chemical Determinations and Chromatography

^{1}H NMR spectra were measured in $CDCl_3$ solution on a Bruker WH-360 or Jeol FX90Q NMR spectrometer. Field desorption mass spectra were obtained on a Varian-MAT 731 spectrometer using carbon dendrite emitters. UV spectra were measured in 95% EtOH solution on a Cary 219 spectrometer. Thin-layer chromatography (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 carried out by chromatography on silica gel, using either flash chromatography techniques[8] with E. Merck grade 60 Silica gel or a Waters Model 500 Prep LC system, using silica gel columns with a flow rate of 250 ml/minute and collecting 250 ml fractions.

In Vitro and *In Vivo* Evaluation

Antibiotic susceptibility data given in Tables 2~4 were obtained by agar dilution procedures as previously described[9]. Mouse protection experiments were conducted by treating infected animals 1 and 5 hours post-infection with either subcutaneous or oral administration of 0.25 ml of a 10% aq EtOH solution of the antibiotic over a range of concentrations[9]. Peripheral plasma levels were determined by microbiological assay using *Micrococcus luteus*; concentrations represent average values from 5 mice per time period.

2′-*O*-Acetyl-DMT

DMT (10 g, 13.5 mmol) was dissolved in acetone (260 ml) and treated with acetic anhydride (1.6 ml, 15.7 mmol) dropwise with stirring at room temp. After stirring overnight, solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and was extracted with satd $NaHCO_3$ solution. The organic solution was dried (Na_2SO_4), filtered and evaporated. The residue was dissolved in a small volume of EtOAc, loaded on a silica gel column (Prep 500) and eluted with EtOAc (4 liters). Fractions containing the desired product were identified by TLC, combined and evaporated to dryness, yielding 6.5 g (61%) of 2′-*O*-acetyl-DMT: FD-MS 783 (M^+); UV λ_{max} nm (ε) 283 (19,300); ^{1}H NMR δ 2.08 (s, Ac).

2′-*O*-Acetyl-23-*O*-propionyl-DMT

2′-*O*-Acetyl-DMT (6 g, 7.7 mmol) was dissolved in dichloromethane (180 ml) and pyridine (15 ml) and treated with propionic anhydride (1.2 ml, 9.2 mmol) dropwise with stirring at room temp. After being stirred overnight, the solution was diluted with toluene (300 ml) and evaporated to dryness under reduced pressure. The residue was dissolved in toluene and was extracted with satd $NaHCO_3$ solution. The toluene layer was dried (Na_2SO_4), filtered and evaporated. The residue, dissolved in a small volume of toluene, was loaded onto a flash chromatography column of silica gel (300 ml) packed in toluene - EtOAc (1:1). The column was eluted with toluene - EtOAc (1:1, 1 liter). Fractions containing the desired product were identified by TLC, combined and evaporated under reduced pressure

to yield 4.5 g (70%) of 2'-*O*-acetyl-23-*O*-propionyl-DMT: FD-MS 840 $(M+H)^+$; UV λ_{max} nm (ε) 280 (21,500); ^{1}H NMR δ 2.08 (s, Ac), 1.15 (t), 2.35 (q, propionyl).

2'-*O*-Acetyl-23-*O*-(*p*-chlorophenylacetyl)-DMT

p-Chlorophenylacetic acid (4.3 g, 25 mmol) and 1-hydroxybenzotriazole (3.4 g, 25 mmol) were dissolved in THF (150 ml). The solution was cooled in an ice bath and treated with *N,N'*-dicyclohexylcarbodiimide (5.2 g, 25.3 mmol). The reaction mixture was stirred at 0°C for 3 hours and then placed in a refrigerator overnight. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in acetone (75 ml), filtered and treated with 2'-*O*-acetyl-DMT (10 g, 12.8 mmol) and imidazole (0.87 g, 12.8 mmol). Acetone was added to give a solution volume of 125 ml and then triethylamine (1.87 ml, 12.8 mmol) was added. After the reaction had stirred for 20 hours at room temp, the solvent was evaporated under reduced pressure. The residue was loaded on a flash chromatography silica gel column which was eluted with a gradient of toluene - EtOAc (4:1) to EtOAc alone. The desired fractions were combined on the basis of TLC results and evaporated to dryness to give 4.75 g of 2'-*O*-acetyl-23-*O*-(*p*-chlorophenylacetyl)-DMT: FD-MS 936 $(M+H)^+$; UV λ_{max} nm (ε) 280 (19,000), 219 (16,000); ^{1}H NMR δ 2.06 (s, Ac), 3.6 (s, CH_2), 7.2~7.3 (m, Ph).

2'-*O*-Acetyl-23-*O*-(*p*-nitrophenylacetyl)-DMT

2'-*O*-Acetyl-DMT (4.0 g, 5.1 mmol) and *p*-nitrophenylacetic acid (1.4 g, 7.7 mmol) were dissolved in dichloromethane (50 ml) and treated with *N,N'*-dicyclohexylcarbodiimide (1.6 g, 7.7 mmol) and 4-dimethylaminopyridine (0.1 g). The reaction mixture was stirred at room temp for 5 hours and the precipitate which formed was separated by filtration. The filtrate was evaporated to dryness under reduced pressure and the residue thus obtained was dissolved in EtOAc. Insoluble impurities were removed by filtration and the filtrate was evaporated again to dryness. The residue obtained was purified by flash chromatography on silica gel, eluting stepwise with mixtures of toluene - EtOAc (2:1, 400 ml; 1:1, 600 ml; 1:2, 600 ml) and then with EtOAc (1 liter). Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to give 2.2 g of 2'-*O*-acetyl-23-*O*-(*p*-nitrophenylacetyl)-DMT: FD-MS 947 $(M+H)^+$; UV λ_{max} nm (ε) 277 (31,000), end absorption.

2',23-Di-*O*-propionyl-DMT

DMT (3 g, 4.05 mmol) was dissolved in dichloromethane (90 ml) and pyridine (7.8 ml) and treated with propionic anhydride (1.8 ml, 13.8 mmol) dropwise with stirring at room temp. After the reaction had stirred overnight, toluene (15 ml) was added. The resulting solution was evaporated to dryness under reduced pressure. The residue, dissolved in toluene (20 ml), was loaded onto a flash chromatography column which was eluted stepwise with toluene - EtOAc (3:1, 300 ml; 5:4, 300 ml and 1:1, 1,000 ml). Fractions were combined, based on TLC results, and evaporated to dryness to give 2.5 g (72%) of 2',23-di-*O*-propionyl-DMT: FD-MS 854 $(M+H)^+$; UV λ_{max} nm (ε) 279 (20,800); ^{1}H NMR δ 1.15 and 1.2 (t, two propionyl).

23-*O*-Propionyl-DMT

2'-*O*-Acetyl-23-*O*-propionyl-DMT (1.6 g, 1.9 mmol) was dissolved in 95% MeOH (80 ml) and stirred at room temp for 42 hours. The solution was evaporated to dryness under reduced pressure. The residue was dissolved in a small volume of toluene, loaded onto a flash chromatography column and eluted with toluene - EtOAc (1:1, 2 liters). Fractions containing the desired product were identified by TLC, combined and evaporated under reduced pressure to yield 1.2 g (79%) of 23-*O*-propionyl-DMT: FD-MS 798 $(M+H)^+$; UV λ_{max} nm (ε) 280 (17,700); ^{1}H NMR δ 1.14 (t), 2.36 (q, propionyl).

23-*O*-Phenylacetyl-DMT Directly from DMT

DMT (3.0 g, 4.05 mmol) was dissolved in dichloromethane (40 ml) and pyridine (1 ml) under an argon atmosphere. The solution was cooled to −78°C and phenylacetyl chloride (0.65 ml, 1.2 equiv) was added dropwise. After 5~10 minutes, the cooling bath was removed and the reaction mixture was allowed to warm to room temp over a 30-minute period. TLC analysis of an aliquot indicated that acylation of the 23-hydroxyl group was incomplete, so the reaction mixture was again cooled

to −78°C and treated with additional phenylacetyl chloride (0.45 ml). This procedure was repeated once more with addition of further phenylacetyl chloride (0.35 ml) and pyridine (1 ml) to effect complete acylation of the 23-hydroxyl group (TLC analysis). The reaction mixture was worked up as usual and the crude product was purified by flash chromatography, eluting with a linear gradient of dichloromethane (1 liter) and dichloromethane - MeOH (85 : 15, 1 liter). Fractions containing the desired product were located by TLC, combined and evaporated to dryness to give 1.48 g (43%) of 23-*O*-phenylacetyl-DMT: FD-MS 860 $(M+H)^+$; UV λ_{max} nm (ε) 281 (20,600), end absorption; ^{1}H NMR δ 3.61 (s, CH_2), 7.26 (m, Ph).

2′-*O*-Acetyl-OMT

DMT (12.5 g) was dissolved in acetone (250 ml) and treated with acetic anhydride (5.0 ml) at room temp. After stirring overnight, the solution was concentrated under reduced pressure and then was diluted with dichloromethane. This solution was extracted with satd $NaHCO_3$ solution, dried and filtered. The filtrate was evaporated to dryness and the residue was dissolved in 1 N sulfuric acid (225 ml) and stirred for 2 hours at room temp. The solution was slowly and carefully poured into satd $NaHCO_3$ solution, and the product was extracted twice with dichloromethane. The organic layers were combined, extracted with satd $NaHCO_3$ solution, dried and filtered. The filtrate was evaporated to dryness and the residue was triturated with hexane, filtered and dried to yield 10.4 g (96%) of 2′-*O*-acetyl-OMT: FD-MS 639 (M^+); UV λ_{max} nm (ε) 283 (20,500); ^{1}H NMR δ 2.06 (s, Ac).

2′,4′-Di-*O*-acetyl-OMT

OMT (50 g) was dissolved in acetone (900 ml) and treated dropwise with acetic anhydride (25 ml) while stirring at room temp. After 2 hours, solvent was evaporated under reduced pressure; the concentrate was diluted with toluene (200 ml) and re-evaporated. The residue was dissolved in dichloromethane and extracted with satd $NaHCO_3$ solution. The organic layer was separated, dried (Na_2SO_4), filtered and evaporated to dryness. The glassy residue was chromatographed (Prep 500), eluting first with a linear gradient of toluene - EtOAc (3 : 1, 4 liters) and EtOAc (4 liters) and then with EtOAc (2 liters). Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to yield 42.0 g (74%) of 2′,4′-di-*O*-acetyl-OMT: FD-MS 681 (M^+); UV λ_{max} nm (ε) 283 (22,500); ^{1}H NMR δ 2.05 and 2.06 (s, two Ac).

2′-*O*-Acetyl-4′-*O*-isovaleryl-OMT

2′-*O*-Acetyl-OMT (1.0 g, 1.57 mmol) was dissolved in acetone (25 ml). Solid sodium bicarbonate (400 mg) was added and the mixture was treated with isovaleryl chloride (0.25 ml, 2.04 mmol) while stirring at room temp. After 1.5 hours, additional isovaleryl chloride (0.25 ml) was added to consume unreacted starting material. After 3.5 hours, starting material was absent (TLC analysis) and the mixture was poured into satd $NaHCO_3$ solution. The resulting solution was extracted twice with dichloromethane and the extracts were combined, dried (Na_2SO_4) and filtered. The filtrate was evaporated to dryness and the residue was dissolved in a small volume of toluene and purified by flash chromatography, eluting with a linear gradient of toluene - EtOAc (3 : 1, 1 liter) and EtOAc (1 liter). Fractions containing the desired products were located by TLC analysis, combined and evaporated to dryness to yield 735 mg of 2′-*O*-acetyl-4′-*O*-isovaleryl-OMT: FD-MS 723 (M^+); UV λ_{max} nm (ε) 283 (21,000); ^{1}H NMR δ 2.06 (s, Ac), 0.97 (d, $CH(CH_3)_2$); 78 mg of 2′-*O*-acetyl-4′,23-di-*O*-isovaleryl-OMT: FD-MS 807 (M^+); UV λ_{max} nm (ε) 279 (26,000); ^{1}H NMR δ 2.06 (s, Ac), 0.97 (d, two $CH(CH_3)_2$); and 71 mg of 2′,23-di-*O*-acetyl-4′-*O*-isovaleryl-OMT: FD-MS 765 (M^+); UV λ_{max} nm (ε) 280 (15,300); ^{1}H NMR δ 2.07 and 2.08 (s, two Ac), 0.97 (d, $CH(CH_3)_2$).

2′,4′,23-Tri-*O*-acetyl-OMT

OMT (15 g, 25.1 mmol) was dissolved in anhydrous pyridine (300 ml), cooled in an ice bath and treated with acetic anhydride (7.8 ml). The reaction mixture was stirred overnight with exclusion of moisture while warming to room temp. After solvent had been evaporated under reduced pressure, the residue was dissolved in dichloromethane, extracted with satd $NaHCO_3$ solution, dried (Na_2SO_4) and filtered. The filtrate was evaporated under reduced pressure and the residue was chromato-

graphed (Prep 500), eluting with a linear gradient of toluene - EtOAc (1: 1, 4 liters) and EtOAc (4 liters). Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to yield 10.0 g of 2′,4′,23-tri-*O*-acetyl-OMT: FD-MS 723 (M^+); UV λ_{max} nm (ε) 279 (25,700); ^{1}H NMR δ 2.05, 2.06 and 2.07 (s, three Ac).

2′,4′-Di-*O*-acetyl-23-*O*-phenylacetyl-OMT

2′,4′-Di-*O*-acetyl-OMT (6.81 g, 10.0 mmol) was dissolved in dichloromethane (100 ml) and pyridine (5 ml), cooled in an ice bath and treated with a solution of phenylacetyl chloride (1.70 g, 11.0 mmol) in dichloromethane (15 ml) while excluding moisture. Additional amounts of phenylacetyl chloride (0.14 ml, 1 mmol) in dichloromethane (5 ml) were added after 2 and after 3 hours in order to consume unreacted starting material (based on TLC analysis). After 4 hours, the reaction mixture was poured into satd $NaHCO_3$ solution. The organic layer was separated, dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was dissolved in a small volume of toluene and purified by flash chromatography, eluting stepwise with toluene - EtOAc (4 : 1, 400 ml; 3 : 1, 300 ml; 2 : 1, 300 ml; 1: 1, 1,200 ml). Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to give 5.8 g (72%) of 2′,4′-di-*O*-acetyl-23-*O*-phenylacetyl-OMT: FD-MS 799 (M^+); UV λ_{max} nm (ε) 280 (28,000); ^{1}H NMR δ 2.04 (s, two Ac), 3.62 (s, CH_2), 7.2 ~ 7.4 (m, Ph).

2′,4′-Di-*O*-acetyl-23-*O*-benzoyl-OMT

2′,4′-Di-*O*-acetyl-OMT (2.39 g, 3.5 mmol) was dissolved in pyridine (50 ml), cooled in an ice bath and treated with benzoic anhydride (870 mg, 3.8 mmol) in dichloromethane (2 ml). After stirring overnight at room temp, additional benzoic anhydride (870 mg) was added to consume starting material. After stirring for another 5 hours at room temp, the mixture was diluted with toluene and evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane, extracted with satd $NaHCO_3$ solution, dried (Na_2SO_4) and filtered. The filtrate was evaporated to dryness and the residue was dissolved in toluene and purified by flash chromatography, eluting stepwise with mixtures of toluene - EtOAc (4 : 1, 500 ml; 3 : 2, 450 ml; 8 : 7, 450 ml; 1: 1, 450 ml). Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to yield 850 mg of 2′,4′-di-*O*-acetyl-23-*O*-benzoyl-OMT: FD-MS 785 (M^+); ^{1}H NMR δ 2.06 and 2.07 (s, two Ac), 7.3 ~ 8.05 (m, Ph).

2′,4′-Di-*O*-acetyl-23-*O*-phenoxyacetyl-OMT

2′,4′-Di-*O*-acetyl-OMT (5.0 g, 7.35 mmol) was dissolved in dichloromethane (200 ml), treated with *N*-(phenoxyacetyloxy)succinimide (4.5 g, 18 mmol) and pyridine (25 ml) and stirred overnight at room temp, excluding moisture. The mixture was then treated with MeOH (15 ml) for 2 hours to decompose excess acylating agent. The mixture was concentrated under reduced pressure, diluted with toluene and evaporated. The residual oil was dissolved in toluene and chromatographed (Prep 500), eluting with a linear gradient of toluene - EtOAc (3: 1, 4 liters) and EtOAc (4 liters). Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to yield 3.7 g (67%) of 2′,4′-di-*O*-acetyl-23-*O*-phenoxyacetyl-OMT: FD-MS 815 (M^+); UV λ_{max} nm (ε) 276 (21,000), 212 (11,000); ^{1}H NMR δ 2.06 (s, two Ac), 4.66 (s, CH_2).

2′,4′-Di-*O*-acetyl-23-*O*-(*N*-*tert*-butoxycarbonylphenylglycyl)-OMT

D-(−)-*tert*-Butoxycarbonylphenylglycine (6.14 g, 24.5 mmol) and *N*-hydroxysuccinimide (2.8 g, 24.5 mmol) in dichloromethane (300 ml) were treated with *N*,*N*′-dicyclohexylcarbodiimide (5.05 g, 24.5 mmol) for 45 minutes at room temp. 2′,4′-Di-*O*-acetyl-OMT (8.4 g, 12.4 mmol) in dichloromethane (10 ml) and then pyridine (50 ml) were added. After stirring at room temp for 1.5 days, the reaction was quenched by addition to MeOH (20 ml). After stirring for 1.5 hours, the mixture was concentrated under reduced pressure, diluted with toluene, evaporated to near dryness, diluted again with toluene, filtered and concentrated to a smaller volume. This concentrate was chromatographed (Prep 500), eluting with a linear gradient of toluene - EtOAc (3: 1, 4 liters) and EtOAc (4 liters). Fractions containing the desired products were located by TLC analysis, combined and evaporated to

dryness to yield 5.1 g of the title compound: FD-MS 914 (M^+); UV λ_{max} nm (ε) 281 (21,700); ^{1}H NMR δ 2.03 (s, two Ac), 1.41 (s, *tert*-butoxycarbonyl), 7.4 (m, Ph).

2',4'-Di-*O*-acetyl-23-*O*-(3-pyridylacetyl)-OMT

1,1'-Carbonyldiimidazole (3.57 g, 22 mmol) was dissolved in anhydrous tetrahydrofuran (50 ml) and toluene (30 ml) under a N_2 atmosphere and treated with 3-pyridylacetic acid (2.74 g, 20 mmol). After stirring at room temp for 30 minutes, evolution of CO_2 had ceased. An aliquot (44 ml, 1.5 equiv) of this solution was withdrawn *via* syringe and added to a solution of 2',4'-di-*O*-acetyl-OMT (5.0 g, 7.34 mmol) in anhydrous THF (50 ml). This mixture was heated at 85°C for 3.5 hours, treated with another aliquot (10 ml) of the acylimidazole solution and heated for another 3 hours. After stirring overnight at room temp, the solution was concentrated under reduced pressure, diluted with toluene, reconcentrated, diluted with toluene - EtOAc (2 : 1), washed with H_2O, dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by flash chromatography; the column was packed in toluene and eluted first with a linear gradient of toluene - EtOAc (1 : 1, 1 liter) and EtOAc (1 liter) and then with EtOAc. Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to yield 1.8 g (33%) of the title compound: FD-MS 800 (M^+); UV λ_{max} nm (ε) 280 (21,500), 268 (sh, 19,000); ^{1}H NMR δ 7.65, 8.55, 9.7 (m, pyridyl), 2.04 (s, two Ac).

2',4'-Di-*O*-acetyl-23-*O*-(*p*-acetamidobenzenesulfonyl)-OMT

2',4'-Di-*O*-acetyl-OMT (5.0 g, 7.35 mmol) was dissolved in dichloromethane (200 ml) and pyridine (25 ml) and then treated with *p*-acetamidobenzenesulfonyl chloride (1.9 g, 7.35 mmol). After stirring overnight at room temp, additional *p*-acetamidobenzenesulfonyl chloride (1.0 g) was added. The reaction mixture was again stirred overnight at room temp and then was poured into satd $NaHCO_3$ solution; the organic layer was separated, dried (Na_2SO_4) and filtered; the filtrate was evaporated under reduced pressure and the residual gum was dissolved in a small volume of toluene - dichloromethane and purified by flash chromatography, eluting stepwise with mixtures of toluene - EtOAc (4 : 1, 200 ml; 3 : 1, 300 ml; 2 : 1, 400 ml; 1 : 1, 300 ml; 1 : 2, 450 ml) and finally with EtOAc. Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to yield 4.1 g of the title compound: FD-MS 879 $(M+H)^+$; UV λ_{max} nm (ε) 266 (34,000) with 275 (sh), end absorption; ^{1}H NMR δ 2.04 (s, two Ac), 2.20 (s, *N*-Ac), 7.8 (d, Ph), 9.77 (s, NH).

23-*O*-Propionyl-OMT

23-*O*-Propionyl-DMT (678 mg) was dissolved in 95% EtOH (10 ml) and H_2O (10 ml). The solution was adjusted to pH 2.0 with 1 N HCl and stirred overnight at room temp. The solution was concentrated to remove EtOH under reduced pressure, diluted with H_2O (20 ml) and extracted with dichloromethane. The solution was then made basic (pH 8.5) with 1 N sodium hydroxide and extracted twice with dichloromethane (25 ml). The latter extracts were combined, dried (Na_2SO_4) and filtered. The filtrate was evaporated under reduced pressure to yield 331 mg of 23-*O*-propionyl-OMT: FD-MS 653 (M^+); UV λ_{max} nm (ε) 280 (20,400); ^{1}H NMR δ 1.15 (t), 2.35 (q, propionyl).

23-*O*-(*p*-Chlorophenylacetyl)-OMT

2'-*O*-Acetyl-23-*O*-(*p*-chlorophenylacetyl)-OMT (1.88 g, 2.37 mmol), obtained from 2'-*O*-acetyl-23-*O*-(*p*-chlorophenylacetyl)-DMT as described in the preceding paragraph, was dissolved in 80% aq MeOH (113 ml) and heated at 80° for 40 minutes. The solution was cooled, concentrated to remove the MeOH under reduced pressure and diluted with satd $NaHCO_3$ solution. The resulting solution was extracted with dichloromethane and the organic layer was separated, dried (Na_2SO_4) and filtered. The filtrate was evaporated under reduced pressure to yield 1.70 g of 23-*O*-(*p*-chlorophenylacetyl)-OMT: FD-MS 749 (M^+); UV λ_{max} nm (ε) 280 (20,500); ^{1}H NMR δ 3.58 (s, CH_2), 7.16~7.28 (m, Ph).

23-*O*-(D-*tert*-Butoxycarbonylphenylglycyl)-OMT

2',4'-Di-*O*-acetyl-23-*O*-(D-*tert*-butoxycarbonylphenylglycyl)-OMT (3.9 g) was dissolved in 80% aq MeOH (70 ml) and refluxed for 75 minutes. The solution was cooled, concentrated to remove the MeOH, poured into satd $NaHCO_3$ solution and extracted with dichloromethane. The organic layer was separated, dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield 3.0 g (84%)

of 23-*O*-(D-*tert*-butoxycarbonylphenylglycyl)-OMT: FD-MS 830 (M^+); UV λ_{max} nm (ε) 282 (19,200), end absorption; ^1H NMR δ 1.42 (s, *tert*-butoxycarbonyl), 7.4 (m, Ph), 9.8 (s, NH).

23-*O*-(D-Phenylglycyl)-OMT

23-*O*-(D-*tert*-Butoxycarbonylphenylglycyl)-OMT (830 mg) was cooled in an ice bath, dissolved in TFA (5 ml) and stirred for 20 minutes. The solution was diluted with ether. The precipitate which formed was filtered, washed with ether and hexane and air-dried to yield 865 mg (90%) of the bis-trifluoroacetate salt of 23-*O*-(D-phenylglycyl)-OMT: FD-MS 731 $(M+H)^+$; UV λ_{max} nm (ε) 287 (19,300), end absorption; ^1H NMR δ 7.5 (m, Ph), 9.75 (d, NH).

Direct Preparation of 23-*O*-Phenylacetyl-OMT from OMT

OMT (3.0 g, 5.0 mmol) was dissolved in dichloromethane (50 ml) and 2,4,6-collidine (2.5 ml), cooled in an acetone-dry ice bath and treated with phenylacetyl chloride (0.83 ml, 6.3 mmol). The cold bath was removed and the mixture was stirred while allowing it to warm to room temp over a 30-minute period. The mixture was extracted with satd $NaHCO_3$ solution, dried (Na_2SO_4) and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue was dissolved in a small volume of dichloromethane and purified by flash chromatography, eluting with a linear gradient of dichloromethane (1 liter) and 15% MeOH in dichloromethane (1 liter). Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to yield 2.0 g (56%) of 23-*O*-phenylacetyl-OMT: FD-MS 715 (M^+); UV λ_{max} nm (ε) 281 (29,500); ^1H NMR δ 3.64 (s, CH_2), 7.15~7.4 (m, Ph).

23-*O*-Diphenylphosphoryl-OMT

OMT (4.0 g, 6.7 mmol) was dissolved in dichloromethane (10 ml) and pyridine (1 ml), cooled in an acetone-dry ice bath and treated with diphenyl chlorophosphate (3.6 g, 13.4 mmol). The cold bath was removed and the reaction was stirred while warming to room temp over a 30-minute period. Since TLC analysis showed starting material was still present, the mixture was again cooled to −78°C, treated with diphenyl chlorophosphate (1.0 ml) and allowed to warm as before. The mixture was then extracted with satd $NaHCO_3$ solution, dried (Na_2SO_4) and filtered. The filtrate was evaporated under reduced pressure, diluted with dichloromethane - cyclohexane and re-evaporated. The crude product was purified by flash chromatography, eluting with a linear gradient of dichloromethane (1 liter) and 15% MeOH in dichloromethane (1 liter). Fractions containing the desired product were located by TLC analysis, combined and evaporated to dryness to yield 3.2 g (57%) of 23-*O*-diphenylphosphoryl-OMT: FD-MS 830 $(M+H)^+$; UV λ_{max} nm (ε) 280 (19,700), 204 (23,000); ^1H NMR δ 7.1~7.45 (m, Ph).

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