SYNTHESIS, ANTIMICROBIAL EVALUATION AND STRUCTURE-ACTIVITY RELATIONSHIPS WITHIN 23-MODIFIED DERIVATIVES OF 5-*O*-MYCAMINOSYLTYLONOLIDE

H. A. KIRST, J. E. TOTH, J. A. WIND, M. DEBONO, K. E. WILLARD, R. M. MOLLOY, J. W. PASCHAL, J. L. OTT, A. M. FELTY-DUCKWORTH and F. T. COUNTER

Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, U.S.A.

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A large series of C-23-modified derivatives of 5-*O*-mycaminosyltylonolide were synthesized, in which the C-23 hydroxyl group was replaced by halo, aryl ether or thioether, azido, amino or dialkylamino substituents *via* S_N2 displacement reactions. The majority of derivatives possessed excellent *in vitro* activity against a variety of aerobic and anaerobic bacteria. While some of the compounds treated experimental infections in rodents by parenteral administration, none showed any significant efficacy or bioavailability after oral dosing. Novel rearrangement products were obtained from some of the reactions; these were identified as 13,23-cyclopropyl-12,22-exomethylene and 13,23-cyclopropyl-12-alkoxy derivatives.

Chemical and biochemical modifications of tylosin, an important veterinary macrolide antibiotic, have been actively pursued in several laboratories and a comprehensive review has been recently published.[1] One approach to the synthesis of new derivatives of tylosin utilizes 5-*O*-mycaminosyltylonolide (OMT) as starting material. It was originally prepared by carefully-controlled acidic hydrolysis of tylosin,[2] but is now more conveniently obtained *via* biosynthetically-blocked mutant strains of *Streptomyces fradiae* (Fig. 1).[3,4] Replacement of the C-23 hydroxymethyl group by a methyl or halomethyl group was found to result in a significant increase of *in vitro* antimicrobial activity.[5~7] Consequently, a thorough investigation of the structure-activity relationships for 23-modified derivatives of OMT was warranted.

Esterification of the 23-hydroxyl group of OMT has already been reported to yield derivatives having substantially greater *in vitro* antimicrobial activity than OMT itself.[7,8] Unfortunately, this increase of *in vitro* activity did not generally lead to a corresponding increase of *in vivo* activity.[8] Despite these discouraging results, the dramatic improvement of *in vitro* activity from compounds in the initial series justified the synthesis and evaluation of additional derivatives of OMT. Derivatives which had been modified at C-23 by substituents other than acyloxy groups were of particular interest, since these could not be hydrolyzed back to OMT. In this paper, we report our syntheses, biological evaluation and structure-activity relationships of C-23-modified derivatives of OMT.

Results and Discussion

Synthesis of C-23-Derivatives

The C-23 hydroxyl group of OMT was readily converted into a variety of *O*-sulfonate groups which were potentially useful leaving groups for S_N2 displacement reactions. After protecting the hydroxyl

Fig. 1. Preparation of 5-*O*-mycaminosyltylonolide.

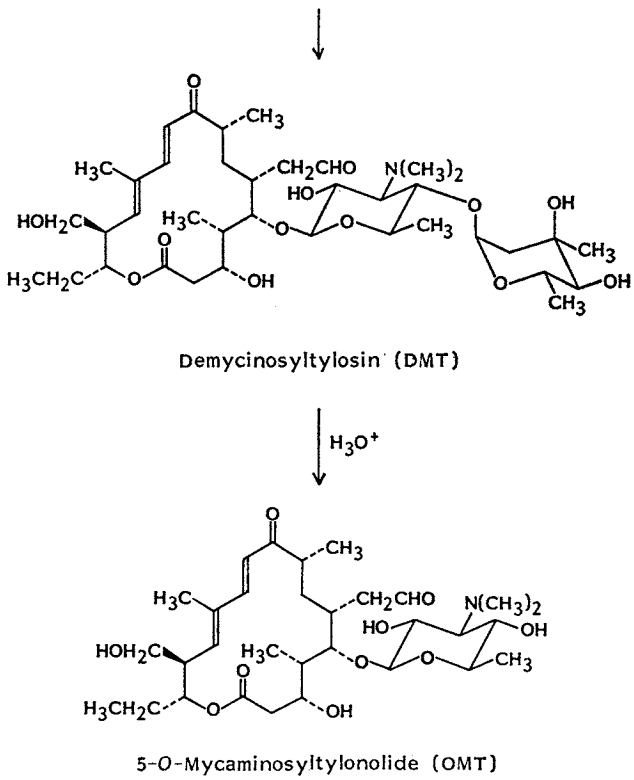


Table 1. 23-*O*-Sulfonate derivatives and products of displacement of 23-*O*-Tf of OMT.

Compound number	Substituent on C-23	FD-MS (*m/z*)	UV λ_{max} (ε)	^{1}H NMR characteristic chemical shifts (δ) of C-23 substituent
3	*p*-Toluenesulfonyloxy[6]	751	280 (20,700)	2.06 (s, CH_3) and 7.36~7.8 (m, phenyl)
4	*p*-(Acetamido)benzenesulfonyloxy[8]	794	265 (32,500)	2.24 (s, Ac), 8.34 (s, NH), 7.8 (m, phenyl)
5	Methanesulfonyloxy[a]	759	279 (21,000)	3.04 (s, CH_3SO_3)
6	Pyridinium[b]	851, 701	276 (22,000)	8.2~9.2 (m, pyridinium)
7	Fluoro	599	276 (14,700)	None for fluoro
8	Bromo[7]	659, 661	280 (20,500)	None for bromo
9	Iodo[6,7]	707	282 (19,800)	None for iodo
10	Phenylthio	689	285 (18,000)	7.3 (m, S-phenyl)
11	5-Methyl-1,3,4-thiadiazolyl-2-thio	712	282 (26,000)	2.72 (s, 5-methyl)
12	5-Methyl-1,3,4-oxadiazolyl-2-thio	696	282 (22,000)	2.55 (s, 5-methyl)
13	1-Methyl-1,2,3,4-tetrazolyl-5-thio	696	285 (22,500)	3.90 (s, N-methyl)
14	4,5-Dihydro-4-methyl-5-oxo-6-hydroxy-1,2,4-triazinyl-3-thio	739	285 (21,000)	3.30 (s, N-methyl)
15	Acetylthio	655	282 (20,000)	2.32 (s, S-acetyl)
16	Azido	622	282 (20,300)	(IR ν 2100 cm^{-1})

[a] 2′,4′-Di-*O*-acetyl derivative.
[b] 2′-*O*-Acetyl derivative, Tf salt.

Fig. 2. Synthesis of displacement products at C-23.

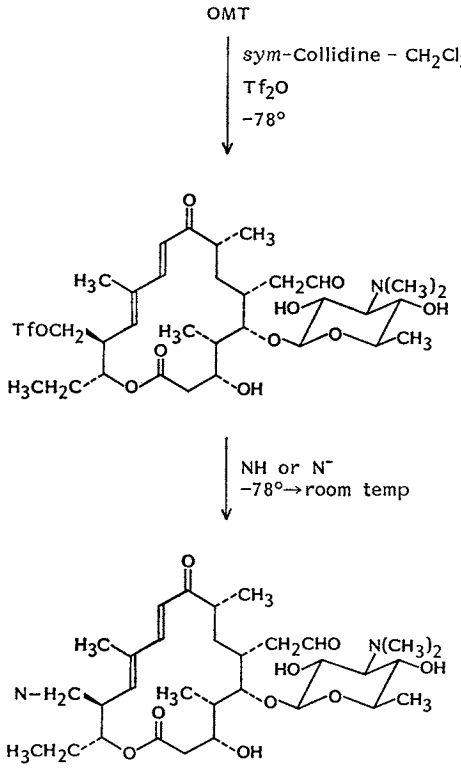
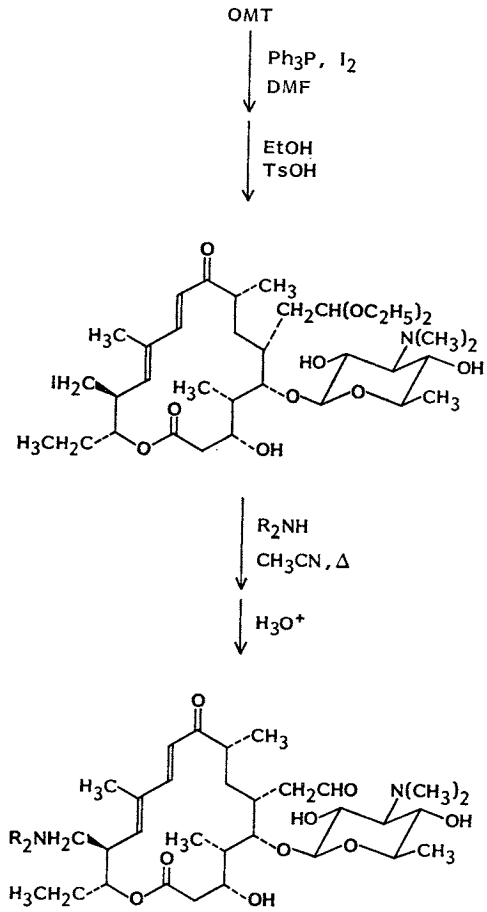


Fig. 3. Synthesis of C-23-amino derivatives.



groups at 2′ and 4′ as their acetyl esters, sulfonylation of the 23-hydroxyl group was achieved using various benzenesulfonyl chlorides or methanesulfonyl chloride and pyridine; no displacement of sulfonate by pyridine was observed in these cases. The 23-*O*-benzenesulfonates were sufficiently stable to permit removal of the 2′- and 4′-*O*-acetyl groups by methanolysis, but the 23-*O*-mesylate was not stable under these conditions.

The more reactive 23-*O*-triflate (Tf) of OMT was readily prepared with triflic anhydride in an analogous manner, but was not isolable due to displacement by pyridine at 0°C to yield the 23-deoxy-23-pyridinium derivative. A more advantageous synthesis was subsequently developed, using *sym*-collidine as base and a reaction temperature of −78°C, to convert OMT directly to its 23-*O*-Tf without any protection of other hydroxyl or carbonyl groups. Addition of good nucleophiles such as thiols, halides or azide to this pre-formed Tf intermediate and subsequent warming to 0°C successfully produced the expected S_N2 displacement products[9] (Fig. 2 and Table 1). By this procedure, activation and replacement of the 23-hydroxyl group was achieved in a single vessel without the need for any protection and deprotection of other functional groups in OMT.

23-Amino-23-deoxy-OMT was readily prepared by reduction of the 23-azide with triphenylphosphine in aqueous THF[10] (preferably) or with chromous chloride;[11] the dienone and formyl groups were not reduced under these mild conditions. Selective acylation of the primary amino group was easily performed using standard acylation reactions to produce a short series of amide derivatives. 23-Alkylamino and 23-dialkylamino derivatives were obtained *via* S_N2 displacement reactions by the

Table 2. 23-Amino-23-deoxy derivatives of OMT.

Compound number	Substituent on C-23	FD-MS (*m/z*)	UV λ_{max} (ε)	^{1}H NMR characteristic chemical shifts (δ) of C-23 substituent
17	Amino	579	283 (17,700)	None
18	Phenylacetylamino	714	282 (23,000)	7.3 (m, phenyl)
19	D-Phenylglycylamino[a]	NA	290 (16,000)	7.7 (m, phenyl)
20	(*N-tert*-BOC-phenylglycyl)amino	830	283 (19,600)	7.3 (m, phenyl), 5.8 (s, NH), 1.4 (s, *tert*-BOC)
21	D-Alanylglycylamino[a]	707	280 (22,000)	NA
22	(*N-tert*-BOC-alanylglycyl)amino	825	282 (20,000)	3.80 (CH, alanine), 1.42 (s, *tert*-BOC)
23	Diethylamino[12]	652	284 (21,200)	2.4~2.6 (NCH_2)
24	Dicyclohexylamino	760	283 (22,400)	2.4~2.6 (NCH)
25	*N*-Allyl-*N*-cyclohexylamino	718	284 (20,300)	2.9 (allyl CH_2N), 5.2 and 5.7 (olefin)
26	*N*-Benzyl-*N*-norbornylamino	781	284 (19,000)	3.6 (CH_2Ph), 7.3 (phenyl)
27	1-Adamantylamino	731	281 (20,800)	2.5~2.8 (NCH)
28	Octahydroazocinyl[12]	692	284 (20,000)	2.5~2.6 (NCH_2)
29	3,3,5-Trimethylhexahydroazepinyl	720	284 (19,800)	2.4~2.7 (NCH_2), 0.8~1.0 ($3CH_3$)
30	Piperidinyl[12]	664	285 (30,200)	2.3~2.6 (NCH_2)
31	1,2,3,6-Tetrahydropyridinyl	662	285 (20,400)	5.6 (olefin)
32	4-*N*-(Piperonyl)piperazinyl	800	284 (25,000)	2.4~2.6 (NCH_2), 3.4 (benzylic)
33	Imidazolyl	648	281 (18,800)	6.84, 7.01, 7.42 (3×s, imidazole)
34	1-Aza[4.5]spirodecyl	719	285 (18,000)	2.4~2.7 (NCH_2)
35	3-Aza[5.5]spiroundecyl	732	285 (21,500)	2.3~2.6 (NCH_2)
36	Octahydroindolyl	704	284 (17,700)	2.2, 2.5 (NCH_2)
37	1,3,3a,4,7,7a-Hexahydroisoindolyl	702	284 (21,100)	2.2~2.6 (NCH_2), 5.75 (olefin)
38	Decahydroquinolinyl	719	284 (18,700)	2.3~2.6 (NCH_2)
39	Decahydroisoquinolinyl	719	285 (21,100)	2.4~2.6 (NCH_2)
40	1,2,3,4-Tetrahydroisoquinolinyl	712	284 (20,600)	2.5~2.7 (NCH_2), 7.0~7.3 (aromatic)
41	Octahydro-4,5-pyrrolyl-1,4-diazepinyl	720	285 (19,300)	2.5~2.7 (NCH_2)
42	3-Aza[3.2.2]bicyclononyl	705	284 (20,000)	2.4~2.6 (NCH_2)
43	1,3,3-Trimethyl-6-aza[3.2.1]-bicyclooctyl	732	284 (21,000)	2.5~2.8 (NCH_2), 0.8~1.0 ($3CH_3$)
44	Dodecahydrocarbazolyl	758	285 (19,400)	2.4~2.8 (NCH)

[a] Bis-TFA salt.
NA: Not assigned.

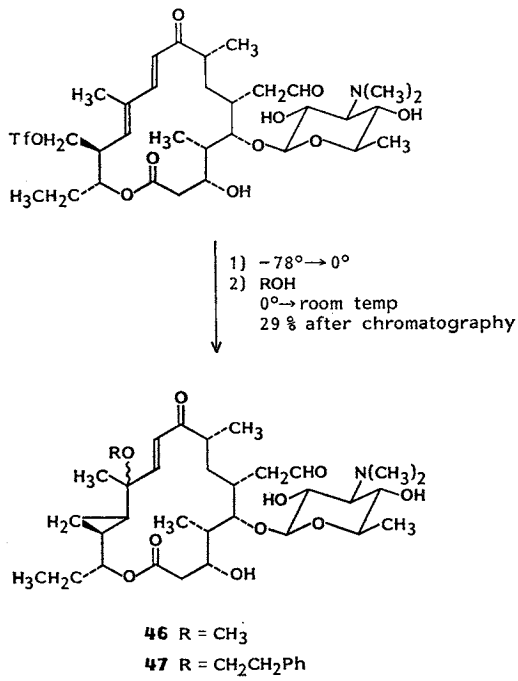
appropriate amine on the 23-iodo derivative of OMT in refluxing acetonitrile (Fig. 3); protection of the aldehyde group as a diethyl ketal was necessary to achieve good yields, as implied in an earlier report, but performing the reaction in a sealed tube was unnecessary.[12] Because of the excellent *in vitro* and parenteral *in vivo* antibiotic activity of this group of derivatives, a substantial number of 23-dialkylamino derivatives were prepared (Table 2). In contrast to earlier reports,[12~14] our series concentrated on monocyclic- and bicyclic-amino substituents. Large-scale synthesis of the starting material for this series, 23-iodo-23-deoxy-OMT, was best achieved directly from OMT, using iodine

and triphenylphosphine in DMF.[6]

Attempts to perform S_N2 displacement reactions with relatively weaker nucleophiles, such as alcohols or phenols, were unsuccessful, due to a competing rearrangement reaction. Addition of alcohols such as methanol or 2-phenylethanol to the 23-*O*-Tf of OMT gave moderate quantities of new products that were isomeric with the desired 23-*O*-alkyl derivatives of OMT (Fig. 4). In contrast, addition of *p*-nitrophenol or potassium cyanide to the 23-*O*-Tf of OMT yielded a different compound in which the elements of TfOH had been eliminated from the starting material (Fig. 5). This elimination-rearrangement product (**45**) was subsequently found as a minor product in many reactions involving the 23-*O*-Tf of OMT and was prepared in highest yield by treatment of the 23-*O*-Tf with diazabicycloundecene (DBU) in dichloromethane. Somewhat surprisingly, however, this same product (**45**) was obtained when the 23-iodo derivative was mixed with silver nitrate in methanol. Although formation of a triene from treatment of 23-iodo-23-deoxy-OMT with silver fluoride has been reported,[15] physical-chemical data immediately ruled out this possibility in our case. The structure which best fit the NMR data (Table 3) was a cyclopropyl-dienone (**45**) in which the γ,δ-double bond had migrated to an exo-methylene position. The products of the 23-*O*-Tf with alcohols were proposed as cyclopropyl-ether-enones (**46** and **47**), in which addition of alcohol has formally occurred across the exo-methylene function of **45**. These alcohol-addition products appeared to be single isomers at C-12, but their stereochemical configuration has not been determined.

The ^{13}C NMR spectrum of **45** in deuteriochloroform solution has an olefinic methylene moiety at δ 119.35 as shown by gated decoupling. The olefinic methyl at C-22 is not present. There is a

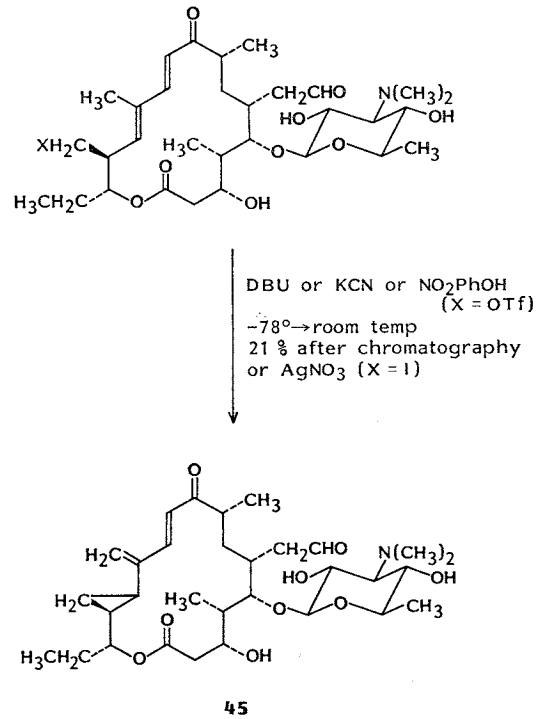
Fig. 4. Synthesis of 12-*O*-alkyl rearrangement products.



46 R = CH_3

47 R = CH_2CH_2Ph

Fig. 5. Synthesis of exo-methylene rearrangement product.



45

DBU : Diazabicycloundecene

Table 3. ^{1}H and ^{13}C NMR data of rearrangement products.

Position	45[a]		46[b]		OMT[a]	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	—	173.02	—	174.57	—	173.89
2	2.57	40.00	2.42/2.30	40.21	2.48/1.92	39.51
3	4.24	71.19	3.80	69.20	3.83	67.80
4	NA	38.35	1.80	40.77	1.62	40.93
5	3.89	83.01	3.57	83.28	3.73	81.12
6	2.75	34.03	NA	34.89	2.15	31.95
7	NA/1.64	35.08	NA	34.56	~1.6	32.69
8	2.84	42.82	2.91	45.33	2.60	44.54
9	—	203.84	—	204.50	—	203.20
10	7.28	125.47	6.41	125.61	6.26	118.75
11	6.54	145.90	6.86	152.98	7.29	148.13
12	—	145.23	—	76.61	—	135.66
13	~1.70	18.70	0.92	23.77	5.84	142.04
14	0.95	28.17	~0.9	18.21	2.88	47.07
15	4.18	80.84	4.14	80.64	4.94	75.09
16	~1.7	27.59	1.72	28.09	1.84/~1.62	25.45
17	0.95	9.79	0.93	10.10	0.91	9.87
18	0.95	8.09	1.05	8.95	0.97	8.58
19	2.20/3.00	45.82	2.87/2.03	47.71	2.8/~2.4	43.91
20	9.69	202.51	9.72	203.53	9.69	203.68
21	1.17	16.06	1.00	19.01	1.18	17.25
22	5.04/5.27	119.35	0.90	16.92	1.80	12.97
23	0.74/1.09	10.47	0.98/0.74	8.80	3.72	62.91
1′	4.36	105.09	4.26	105.08	4.25	103.99
2′	3.49	70.78[c]	3.44	71.15	3.47	70.85
3′	2.40	70.37[c]	2.44	71.62	2.35	70.12
4′	3.07	70.37[c]	3.07	71.50	3.03	70.85
5′	3.32	73.54	3.25	73.79	3.25	73.72
6′	1.28	17.97	1.23	16.92	1.24	17.72
$N(CH_3)_2$	2.50	41.79	2.54	42.03	2.48	41.63
OCH_3	—	—	3.26	50.35	—	—

[a] $CDCl_3$ solvent.
[b] Acetone-d_6 solvent.
[c] May be interchanged.
NA: Not assigned.

methylene with a chemical shift of δ 10.47 and a one-bond coupling constant of 160 Hz, which is consistent with a strained ring such as cyclopropyl. The remaining resonances were assigned using proton decoupling and comparisons with OMT and tylosin.

The ^{1}H NMR spectrum in the same solvent shows, through homonuclear decoupling experiments, that 14-H is at δ 0.95 and is coupled to resonances at δ 0.74, 1.09 and 1.70. These three latter resonances are coupled to each other, and along with 14-H, constitute the cyclopropyl ring. The exocyclic methylene has resonances at δ 5.04 and 5.27. The δ 5.27 resonance shows nuclear Overhauser effects (NOEs)' to 10-H at δ 7.28 and to its geminal proton. Saturation of 11-H produces an NOE at 13-H (δ 1.70). The remaining resonances were assigned by comparison to OMT (see Table 3).

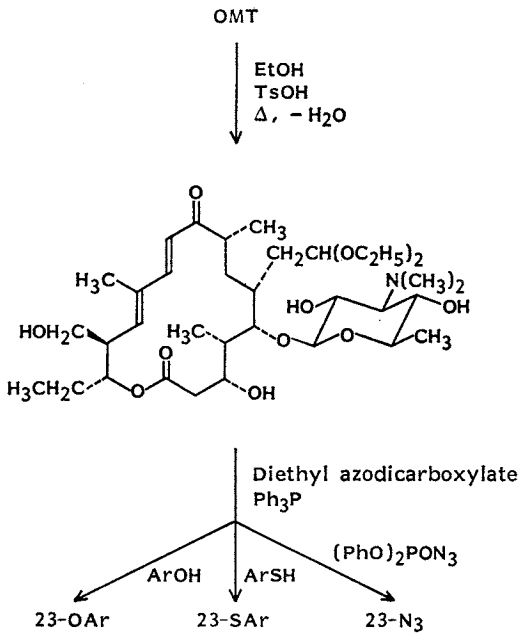
The ^{13}C NMR spectrum of **46** in acetone-d_6 shows that the exo-methylene is not present. A new methyl at δ 16.92 and a quaternary carbon at δ 76.61 are now observed. The ^{1}H NMR spectrum shows that the new methyl is a singlet (from heteronuclear correlation). The shift of the quaternary carbon

resonance indicates that there is an oxygen attached. Therefore, the hydroxyl group has added across the double bond as depicted. The ^{1}H and ^{13}C NMR data for these unusual rearrangement products is summarized in Table 3.

Although the mechanism(s) of these reactions has not been established, the structures of the products suggest that the 12,13-double bond reacted as an isolated double bond and that the conjugation of the dienone system is readily disrupted. This is in accord with results from Michael addition reactions with tylosin, in which addition occurred across the 10,11-double bond exclusively.[16]

A series of 23-*O*-aryl derivatives was successfully synthesized by application of the MITSUNOBU reaction.[17] Although the 23-*O*-aryl derivatives could be prepared directly from OMT, the yields were low and complex reaction mixtures were obtained. However, when the aldehyde was first protected as its diethyl ketal, the MITSUNOBU reaction was performed quite cleanly and selectively at the C-23 hydroxyl group (Fig. 6). In order to investigate the effects of different functional groups on antimicrobial activity, a series of aryl ethers was prepared *via* the MITSUNOBU reaction, using a wide variety of substituted phenols (Table 4).

Fig. 6. Synthesis of C-23 coupling products.



In Vitro Antimicrobial Activity

Initial evaluation of antimicrobial activity involved determination of the minimum inhibitory concentration (MIC) against a variety of Gram-positive and Gram-negative bacteria (Table 5). Most of the derivatives which were evaluated possessed excellent *in vitro* activity (MIC≦1 μg/ml) against

Table 4. 23-*O*-Aryl derivatives of OMT.

Compound number	Substituent on C-23-hydroxyl	FD-MS (*m/z*)	UV λ_{max} (ε)	^{1}H NMR characteristic chemical shifts (δ) of C-23 substituent
48	Phenyl	673	278 (19,800)	6.88, 6.96, 7.3 (aromatic)
49	4-Methoxyphenyl	703	282 (21,000)	3.79 (OCH_3); 6.86, 7.42, 7.82 (aromatic)
50	2,3-Dimethoxyphenyl	733	282 (18,900)	3.86, 3.90 ($2OCH_3$); 6.56, 6.98 (aromatic)
51	3,4,5-Trimethoxyphenyl	763	280 (20,800)	3.77, 3.83 ($3OCH_3$); 6.14 (aromatic)
52	4-Phenoxyphenyl	765	280 (32,000)	6.90, 6.98, 7.30, 7.80 (aromatic)
53	4-Nitrophenyl	718	286 (24,500)	6.96, 8.23 (aromatic)
54	3-Dimethylaminophenyl	716	284 (22,500)	2.62, 2.94 (NCH_3); 6.21, 6.38, 7.13 (aromatic)
55	4-Benzoylphenyl	777	285 (32,500)	6.92, 7.48, 7.56, 7.76, 7.83 (aromatic)
56	4-Formylphenyl	701	282 (39,000)	9.93 (CHO); 6.98, 7.90 (aromatic)
57	4-(Hexamethylene-aminomethyl)phenyl	785	280 (50,000)	3.57 (CH_2Ph), 6.83, 7.26 (aromatic)
58	2-Pyridinyl	674	290 (22,200)	6.72, 6.87, 7.56, 8.13 (aromatic)
59	2-Quinolinyl	726	280 (20,800)	6.91, 7.38, 7.61, 7.75, 8.02 (aromatic)

Table 5. *In vitro* antimicrobial activity of 23-modified derivatives of OMT.

Compound number	MIC values (μg/ml)								
	S. a. X1	*S. a.* V41	*S. e.* EPI 1	*S. py.* C203	*S. pn.* Park I	*S. f.* X66	*H. i.* C.L.	*E. c.* EC14	*P. a.* X239
1	1	2	1	0.5	0.25	1	4	128	64
2	0.25	0.25	0.25	0.25	0.25	0.5	1	32	32
3	0.25	0.25	0.25	0.12	0.03	0.25	1	32	64
4	1	2	0.5	0.12	0.06	1	2	64	128
6	32	64	32	8	4	64	16	>128	>128
7	0.25	0.25	0.25	0.06	0.12	0.25	1	16	16
8	0.25	0.5	0.25	0.25	0.06	0.25	1	32	64
9	1	1	1	0.5	0.5	1	4	128	>128
10	0.12	0.12	0.12	0.03	0.06	0.12	1	32	64
11	0.25	0.25	0.12	0.12	0.06	0.25	2	64	>128
12	0.25	0.5	0.25	0.25	0.06	0.5	4	128	>128
13	0.25	0.25	0.25	0.12	0.12	0.5	2	64	64
14	2	4	4	1	0.5	4	8	>128	>128
15	0.25	0.25	0.12	0.25	0.06	0.5	1	64	128
16	0.12	0.25	0.12	0.12	0.06	0.25	1	32	32
17	2	2	8	1	0.5	2	1	>128	>128
18	0.5	1	0.25	0.25	0.25	0.5	2	128	128
19	4	8	4	0.5	0.5	8	16	>128	>128
20	1	2	1	0.5	0.25	2	8	>128	>128
21	64	64	64	16	16	64	>128	>128	>128
22	16	32	16	4	4	32	64	>128	>128
23	0.5	0.5	0.5	0.25	0.06	0.5	1	16	>128
24	0.25	0.25	0.25	0.25	0.06	0.25	2	32	64
25	0.25	0.25	0.25	0.12	0.12	0.25	2	16	128
26	0.25	0.25	0.25	0.06	0.12	0.25	1	32	128
27	0.5	0.5	0.5	0.12	0.06	0.5	2	16	>128
28	0.25	0.25	0.12	0.03	0.03	0.12	0.5	8	32
29	0.25	0.25	0.25	0.06	0.03	0.25	1	16	128
30	0.5	0.5	0.25	0.06	0.06	0.5	1	8	128
31	0.25	0.25	0.25	0.12	0.01	0.25	1	8	32
32	0.25	0.25	0.25	0.12	0.06	0.25	4	64	>128
33	8	8	2	0.5	0.25	4	16	>128	>128
34	0.25	0.25	0.25	0.12	0.03	0.25	1	16	128
35	0.5	0.5	0.5	0.25	0.06	0.25	2	32	>128
36	0.5	0.5	0.5	0.03	0.06	0.5	0.5	16	128
37	0.25	0.25	0.25	0.03	0.06	0.12	0.5	8	64
38	0.25	0.25	0.25	0.12	0.03	0.25	1	16	>128
39	0.5	0.5	0.5	0.03	0.06	0.5	0.5	16	128
40	0.25	0.25	0.12	0.06	0.03	0.12	1	32	64
41	4	4	4	0.5	1	4	16	>128	>128
42	0.25	0.25	0.25	0.12	0.06	0.25	2	16	>128
43	0.5	0.5	0.5	0.25	0.06	0.5	2	16	>128
44	0.5	0.5	0.5	0.25	0.12	0.5	4	64	>128
45	2	2	4	1	1	2	8	>128	>128
46	2	2	2	1	0.25	2	8	>128	>128
47	1	2	1	1	0.25	1	8	>128	>128
48	0.12	0.12	0.12	0.12	0.06	0.12	1	32	64
49	0.5	0.5	0.5	0.25	0.06	0.25	4	64	128
50	0.25	0.25	0.25	0.25	0.06	0.25	2	64	128
51	0.5	0.5	0.5	0.12	0.25	0.5	8	128	>128
52	0.5	0.5	1	0.25	0.06	0.5	4	128	>128

Table 5. (Continued)

Compound number	MIC values (μg/ml)								
	S. a. X1	*S. a.* V41	*S. e.* EPI 1	*S. py.* C203	*S. pn.* Park I	*S. f.* X66	*H. i.* C.L.	*E. c.* EC14	*P. a.* X239
53	0.12	0.12	0.12	0.25	0.06	0.25	2	64	>128
54	0.25	0.25	0.25	0.25	0.06	0.25	2	128	128
55	1	1	1	0.25	0.25	0.5	8	128	>128
56	0.25	0.25	0.25	0.12	0.12	0.25	2	64	>128
57	1	1	1	0.5	0.5	2	8	128	>128
58	0.25	0.25	0.12	0.06	0.06	0.12	2	32	16
59	0.5	0.5	0.5	0.25	0.12	0.25	4	64	>128
60	0.12	0.12	0.12	0.12	0.06	0.25	0.5	32	64
61	0.06	0.06	0.06	0.06	0.03	0.12	0.5	32	32
62	0.12	0.12	0.12	0.06	0.06	0.25	1	32	32
63	0.12	0.12	0.12	0.12	0.03	0.25	2	32	32
Ros	0.25	0.25	0.25	0.12	0.25	0.5	1	16	32
Rep	0.12	0.25	0.25	0.12	0.25	0.5	0.5	16	16
Cir	0.5	0.5	0.25	0.25	1	0.5	2	32	64
EM	0.25	>128	>128	0.06	0.03	0.12	2	64	128

Organism abbreviations: *S. a.*; *Staphylococcus aureus*, *S. e.*; *Staphylococcus epidermidis*, *S. py.*; *Streptococcus pyogenes*, *S. pn.*; *Streptococcus pneumoniae*, *S. f.*; *Streptococcus faecalis*, *H. i.*; *Haemophilus influenzae*, *E. c.*; *Escherichia coli*, *P. a.*; *Pseudomonas aeruginosa*.

1: OMT, **2**: 23-deoxy-5-*O*-mycaminosyltylonolide (DOMT), **60**: 23-*O*-acetyl-OMT, **61**: 23-*O*-phenoxyacetyl-OMT, **62**: 23-*O*-(cyclohexylcarbonyl)-OMT, **63**: 23-*O*-(methoxycarbonyl)-OMT (see ref 8), Ros: rosaramicin, Rep: repromicin (des-epoxyrosaramicin), Cir: cirramycin A, EM: erythromycin.

strains of Staphylococci and Streptococci, including strains of MLS-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* which were inducibly-resistant to erythromycin. However, strains of MLS-resistant Staphylococci which were constitutively-resistant to erythromycin were also resistant to these derivatives of OMT. Thus, these derivatives of OMT show the same pattern of resistance as does tylosin.[18]

Against sensitive strains, the *in vitro* activity of most of these derivatives of OMT compared favorably with that of erythromycin, the most commonly used macrolide, and rosaramicin, a macrolide which has been clinically investigated and which is structurally related to OMT.[19] The majority of 23-modified derivatives of OMT which were synthesized and tested were 2~8-fold more active than OMT itself against strains of Staphylococci and Streptococci. The comparable increase in activity of some derivatives against *Haemophilus influenzae* was encouraging as being potentially useful for improving therapy with macrolide antibiotics against this pathogen. However, the increased activity against Enterobacteriaceae and *Pseudomonas* species was insufficient to render them clinically useful. The modifications of OMT at C-23 also resulted in increased *in vitro* activity against strains of anaerobic bacteria (Table 6) and excellent activity against *Chlamydia trachomatis* (Table 7).

Examination of the data in Table 5 revealed several interesting trends in the relationship between structure and activity. A substantial increase of *in vitro* activity over that of OMT resulted from modification of the 23-hydroxyl group into a more lipophilic substituent. This was well exemplified for ethers (*e.g.* **48~59**), thioethers (**10~13**), azido (**16**), deoxy (**2**) and halo derivatives (**7~9**) as well as acyl derivatives such as esters (**60~63**), thioesters (**15**) and sulfonates (**3** and **4**). In contrast, *in vitro* activity was substantially diminished compared to OMT for the positively charged pyridinium derivative (**6**), suggesting that a permanently ionized substituent at C-23 was deleterious for activity. Al-

Table 6. *In vitro* antimicrobial activity of 23-modified derivatives of OMT against anaerobic bacteria.

	OMT	DOMT	7	10	13	16	17	18	19
Clostridium difficile 2994	2	1	1	0.5	0.5	1	16	1	16
C. perfringens 81	0.12	0.12	0.25	≦0.06	0.12	0.25	4	0.12	8
C. septicum 1128	2	1	1	0.25	0.5	1	16	1	16
Eubacterium aerofaciens 1235	0.12	0.25	0.12	≦0.06	0.25	0.25	2	0.25	4
Peptococcus prevotii 1281	2	0.5	1	0.25	0.5	1	16	1	32
P. asaccharolyticus 1302	16	16	16	8	32	8	>32	2	8
Peptostreptococcus intermedius 1264	0.5	0.5	0.5	≦0.06	0.5	1	8	0.5	8
Propionibacterium acnes 79	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	0.5	≦0.06	2
Bacteroides fragilis 111	32	1	4	1	16	4	>32	>32	>32
B. thetaiotaomicron 1438	32	0.5	2	0.25	16	2	16	16	>32
B. melaninogenicus 2736	32	2	8	4	16	8	>32	>32	>32
B. vulgatus 1211	16	2	4	2	8	4	>32	16	>32
B. corrodens 1874	32	1	4	0.5	16	4	>32	32	>32
Fusobacterium symbiosum 1470	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.5	≦0.06	0.5
F. necrophorum 6054A	8	0.5	1	2	2	≦0.06	≦0.06	8	16

	23	28	29	30	31	34	35	36	37
Clostridium difficile 2994	2	1	1	2	1	0.5	0.5	1	1
C. perfringens 81	0.5	0.25	0.25	0.5	0.25	<0.25	0.5	0.5	0.5
C. septicum 1128	2	1	1	2	1	1	0.5	1	0.25
Eubacterium aerofaciens 1235	0.12	0.12	≦0.06	0.5	0.12	0.5	0.5	0.5	0.5
Peptococcus prevotii 1281	2	1	1	1	1	4	1	1	0.5
P. asaccharolyticus 1302	>32	>32	16	>32	>32	128	32	1	0.5
Peptostreptococcus intermedius 1264	1	0.25	0.25	0.5	0.25	0.5	0.5	0.25	0.25
Propionibacterium acnes 79	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	0.5	<0.25	≦0.06	≦0.06
Bacteroides fragilis 111	4	0.25	0.5	2	2	4	1	0.5	1
B. thetaiotaomicron 1438	0.5	≦0.06	0.12	0.5	0.25	0.5	1	0.5	0.5
B. melaninogenicus 2736	4	2	4	4	2	2	0.5	2	1
B. vulgatus 1211	2	1	2	2	1	2	0.5	0.5	1
B. corrodens 1874	4	≦0.06	0.5	2	2	4	1	0.5	0.5
Fusobacterium symbiosum 1470	1	≦0.06	1	2	≦0.06	<0.25	<0.25	0.5	0.5
F. necrophorum 6054A	1	≦0.06	1	1	≦0.06	<0.25	<0.25	0.25	0.5

Abbreviations: See footnote in Table 5.

Table 6. (Continued)

	38	39	40	42	43	45	46	48	50
Clostridium difficile 2994	1	1	0.5	1	1	8	4	0.5	2
C. perfringens 81	0.25	0.5	0.25	0.5	0.5	2	1	0.12	2
C. septicum 1128	1	1	0.5	1	1	8	4	0.5	2
Eubacterium aerofaciens 1235	≦0.06	≦0.06	≦0.06	0.12	0.25	4	0.5	≦0.06	2
Peptococcus prevotii 1281	0.5	1	1	2	1	16	4	0.25	1
P. asaccharolyticus 1302	8	16	4	128	32	>32	>32	8	8
Peptostreptococcus intermedius 1264	0.25	0.25	0.12	0.5	0.5	8	2	0.12	<0.5
Propionibacterium acnes 79	≦0.06	≦0.06	≦0.06	0.12	≦0.06	2	≦0.06	≦0.06	<0.5
Bacteroides fragilis 111	1	1	1	0.25	0.5	>32	16	1	2
B. thetaiotaomicron 1438	0.12	≦0.06	≦0.06	0.12	0.25	16	8	0.25	1
B. melaninogenicus 2736	4	2	2	4	4	>32	16	4	8
B. vulgatus 1211	2	0.12	0.12	4	1	32	16	2	1
B. corrodens 1874	1	0.5	0.5	0.5	0.25	32	16	1	2
Fusobacterium symbiosum 1470	1	1	1	≦0.06	1	0.5	8	2	<0.5
F. necrophorum 6054A	1	≦0.06	≦0.06	≦0.06	1	8	8	2	2

	53	54	58	60	61	62	63	Ros	EM
Clostridium difficile 2994	0.5	0.5	0.5	<0.12	<0.12	<0.12	<0.12	0.5	0.5
C. perfringens 81	≦0.06	0.25	0.25	<0.12	<0.12	0.25	<0.12	0.12	2
C. septicum 1128	0.25	0.5	0.5	<0.12	<0.12	0.25	<0.12	0.12	1
Eubacterium aerofaciens 1235	≦0.06	0.25	0.12	<0.12	<0.12	<0.12	<0.12	0.12	0.5
Peptococcus prevotii 1281	0.12	0.5	0.5	0.5	0.5	1	8	1	16
P. asaccharolyticus 1302	32	4	8	0.5	1	1	4	0.5	1
Peptostreptococcus intermedius 1264	0.12	0.25	0.25	<0.12	0.25	0.25	0.25	0.25	0.25
Propionibacterium acnes 79	≦0.06	≦0.06	≦0.06	1	<0.12	<0.12	<0.12	0.5	0.12
Bacteroides fragilis 111	1	8	2	4	0.5	1	2	0.5	2
B. thetaiotaomicron 1438	0.25	1	0.5	2	0.25	0.5	0.25	0.5	1
B. melaninogenicus 2736	2	8	4	4	0.5	2	4	0.5	2
B. vulgatus 1211	0.5	4	2	2	0.5	1	4	0.5	2
B. corrodens 1874	1	4	2	2	0.5	0.5	0.5	0.5	4
Fusobacterium symbiosum 1470	0.5	4	2	2	0.5	2	1	0.25	2
F. necrophorum 6054A	1	≦0.06	≦0.06	1	0.5	2	1	0.5	2

Abbreviations: See footnote in Table 5.

Table 7. *In vitro* activity of 23-modified derivatives of OMT against *Chlamydia trachomatis*.

Compound number	MIC (μg/ml)	Compound number	MIC (μg/ml)
OMT	1	**38**	<0.12
DOMT	<0.12	**39**	<0.12
7	<0.12	**40**	<0.12
10	<0.12	**42**	<0.12
13	0.25	**43**	<0.12
16	<0.12	**45**	4
17	2	**46**	0.25
18	1	**48**	<0.12
19	>4	**50**	<0.12
23	<0.12	**53**	<0.12
28	<0.12	**54**	0.25
29	<0.12	**58**	<0.12
30	<0.12	**60**	<0.5
31	<0.12	**61**	<0.5
34	<0.12	**62**	<0.5
35	<0.12	**63**	<0.5
36	<0.12	Ros	<0.12
37	<0.12	EM	<0.12

Abbreviations: See footnote in Table 5.

though most compounds containing an ionizable basic amino group at C-23 showed unexpectedly good *in vitro* activity, the more basic, but more lipophilic, tertiary amino derivatives were more active than the primary amino derivative (**17**) or a bis-amino derivative (**41**). Furthermore, several examples illustrated a decrease of *in vitro* activity upon introduction of a basic amino group (**19** vs. **18** or **20**; **21** vs. **22**; **57** vs. **48** or **56**), whereas introduction of a weakly- or non-basic nitrogen atom gave variable results (**11**~**13** vs. **14**; **32** vs. **33**; **54**, **58**, **59** vs. **4**). These results indicate that all of the factors which are responsible for maximum activity are not fully understood, but suggest that a balance between lipophilicity and polarity plays an important role.

The moderately good *in vitro* activity of the structurally rearranged macrolide derivatives **45**~**47** was unexpected and suggests some new directions for modifications of the lactone ring.

In Vivo Evaluation

In spite of their excellent improvement of *in vitro* activity, few of the C-23 modified derivatives of OMT showed any substantial improvement over OMT in treating experimental infections in rodents (Table 8). When administered parenterally, many of the derivatives treated at levels comparable to that achieved with OMT, while the rest of the derivatives treated poorly, if at all, at the levels tested. The C-23 amino derivatives appeared to be the most active series *in vivo*. In contrast, the derivatives of OMT modified at C-23 by very lipophilic substituents appeared to be the least active *in vivo*. The most efficacious derivatives appeared to contain some type of polar or ionizable group at C-23 (perhaps to achieve higher solubility in body fluids) moderated by some degree of surrounding lipophilic character (perhaps to increase membrane permeability and distribution into the tissues and organs of the animal). This optimization of activity from a tertiary amino substituent, both *in vitro* and *in vivo*, has been observed in another series of macrolide derivatives.[20]

None of the derivatives treated the experimental infections when given orally. Furthermore, none of the derivatives tested produced any reasonable concentration or duration in plasma of mice after oral administration, even at a relatively high dose (Table 9). A similar result was obtained after

Table 8. *In vivo* antimicrobial activity of 23-modified derivatives of OMT against experimental infections in mice induced by *Streptococcus pyogenes* C203.

Compound number	ED_{50} value (mg/kg×2)		Compound number	ED_{50} value (mg/kg×2)	
	Subcutaneous	Oral		Subcutaneous	Oral
OMT	2.6	97	**35**	>10	>100
DOMT	20	62	**36**	2.2	87
3	15	146	**37**	1.3	87
7	4.6	>100	**38**	2.3	77
8	>20	>150	**39**	7.7	79
10	25.7	>100	**40**	5.9	78
12	12.6	>100	**41**	5.5	>100
13	13.7	>100	**42**	9.1	>100
15	21.4	>100	**43**	2.5	100
16	6.3	>100	**44**	7.4	63
17	2.7	>100	**45**	>30	>100
18	5.9	>100	**46**	>25	>100
19	6.3	>100	**48**	>10	>100
23	1.4	>100	**50**	18.4	100
25	6.8	>100	**53**	>10	>100
26	>10	>100	**54**	>10	>100
27	2.2	>100	**57**	>10	>100
28	8.5	>100	**58**	>10	87
29	7.5	59	**60**	3.3	109
30	1.1	71	**61**	6.0	>100
31	1.9	60	Ros	6.3	83
32	10.0	>100	EM	0.9	10
34	1.3	60			

Abbreviations: See footnote in Table 5.

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

Compound number	5 minutes	15 minutes	30 minutes	1 hour	2 hours
OMT	0.63	1.68	NZ	NZ	NZ
17	NZ	NZ	NZ	NZ	NZ
23	NZ	NZ	NZ	NZ	NZ
28	NZ	NZ	NZ	NZ	NZ
34	1.62	0.2	0.2	0.07	NZ
35	0.83	NZ	NZ	NZ	NZ
42	NZ	NZ	NZ	NZ	NZ
48	NZ	NZ	NZ	NZ	NZ
50	0.45	NZ	NZ	NZ	NZ
60	0.51	1.31	0.42	NZ	NZ
61	0.39	NZ	NZ	NZ	NZ
62	NZ	NZ	NZ	NZ	NZ
63	NZ	NZ	NZ	NZ	NZ
Ros	2.1	NZ	NZ	NZ	NZ

Abbreviations: See footnote in Table 5.
NZ: No measurable zone of inhibition in microbiological assay.

oral administration of selected derivatives to beagle dogs (Table 10). Consequently, although this series of derivatives of OMT has an excellent spectrum of *in vitro* antimicrobial activity, none of the compounds appear to have a sufficiently useful level of oral efficacy or bioavailability. The best explanation for this appears to be rapid first-pass metabolism in the liver. Since these compounds

Table 10. Plasma levels (μg/ml) of derivatives of OMT in beagle dogs administered orally at 15 mg/kg in capsules.

Compound number	30 minutes	1 hour	2 hours	3 hours
OMT	<0.1	0.1	0.6	<0.1
23	0.2	0.7	0.3	<0.1
34	0.6	0.5	0.3	0.1
35	0.4	0.5	0.3	0.1
38	0.7	0.6	0.4	0.2
42	0.1	0.2	0.2	0.1
48	1.3	1.7	1.5	<0.2
60	0.3	0.1	<0.1	<0.1
61	<0.1	<0.1	0.1	<0.1
62	<0.1	0.1	<0.1	<0.1
63	<0.1	<0.1	<0.1	<0.1
Ros	0.5	0.4	0.2	<0.1
EM	2.0	0.9	0.5	0.2

Abbreviations: See footnote in Table 5.

are no larger than erythromycin and are more stable in acidic environments, the lack of oral activity comparable to that of erythromycin suggests that they may be metabolized more rapidly.

Experimental

Physico-chemical Determinations and Chromatography

^{1}H NMR spectra were measured in $CDCl_3$ solution on a Bruker WH-360 or Jeol FX90A NMR spectrometer. Field desorption mass spectra (FD-MS) were obtained on a Varian-MAT 731 spectrometer using carbon dendrite emitters. UV spectra were measured in 95% ethanol solution on a Cary 219 spectrometer. IR 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. 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[21] (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 5 and 6 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 hours post-infection, 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*. Concentrations in Table 9 represent average values from 5 mice per time period. Concentrations in Table 10 are an average of 2 dogs (1 per sex).

23-Pyridinium-23-deoxy-2′-*O*-acetyl-OMT Trifluoromethanesulfonate (6)

A solution of 2′-*O*-acetyl-OMT[8] (640 mg, 1.0 mmol) and pyridine (0.40 ml, 5.0 mmol) in dichloromethane (10 ml) at 0°C under argon was treated dropwise with trifluoromethanesulfonic anhydride (0.18 ml, 1.1 mmol). After 10 minutes, the reaction appeared incomplete by TLC, and additional trifluoromethanesulfonic anhydride (0.20 ml, 1.2 mmol) was added dropwise. Ten minutes after addition had been completed, the reaction was quenched with saturated sodium bicarbonate solution. The organic layer was separated, dried over sodium sulfate, and filtered. Partial evaporation of the filtrate gave a precipitate which was separated by filtration and air-dried to yield 343 mg (40%) of 2′-*O*-acetyl-23-pyridinium-23-deoxy-OMT trifluoromethanesulfonate (**6**): MP 139~145°C; $[\alpha]_D^{22}$ +106°

(*c* 1.0, MeOH); elemental *Anal*, found C 54.89, H 6.85, N 3.35, calcd for $C_{39}H_{57}N_2O_{13}F_3S$: C 55.04, H 6.75, N 3.29.

23-Fluoro-23-deoxy-OMT (7)

A solution of OMT (2.39 g, 4.0 mmol) and *sym*-collidine (1.6 ml, 12.0 mmol) in dichloromethane (50 ml) was cooled to −78°C under argon and treated dropwise with trifluoromethanesulfonic anhydride (1.0 ml, 6.0 mmol). Ten minutes after addition had been completed, solid tetraethylammonium fluoride dihydrate (1.48 g, 8.0 mmol) was added to the reaction. The cooling bath was removed, and the reaction was allowed to come to room temp over a 2-hour period. The reaction mixture was extracted with saturated sodium bicarbonate solution, dried over sodium sulfate, filtered and evaporated. The residue was evaporated again from cyclohexane to give a glass which was purified by silica gel chromatography (Waters Prep 500). Elution with a linear gradient of dichloromethane (4 liters) and methanol - dichloromethane (15 : 85, 4 liters) yielded 520 mg (22%) of 23-fluoro-23-deoxy-OMT (**7**): MP (softened) ~145°C; $[\alpha]_D^{22}$ −25.9° (*c* 1.0, MeOH); elemental *Anal*, found C 61.84, H 8.34, N 2.14, F 3.27, calcd for $C_{31}H_{50}NO_9F$: C 62.08, H 8.40, N 2.34, F 3.17.

23-(5-Methyl-1,3,4-oxadiazolyl-2-thio)-23-deoxy-OMT (**12**)

A solution of OMT (2.39 g, 4.0 mmol) and *sym*-collidine (2.0 ml, 15.1 mmol) in dichloromethane (40 ml) was cooled to −78°C under argon and treated dropwise with trifluoromethanesulfonic anhydride (1.05 ml, 6.2 mmol). One hour later, 2-mercapto-5-methyl-oxadiazole (511 mg, 4.4 mmol) was added to the reaction. The cooling bath was removed, and the reaction was allowed to come to room temp over a 2-hour period. The reaction mixture was extracted with saturated sodium bicarbonate solution (50 ml), dried over sodium sulfate, filtered and evaporated. The residue was evaporated again from cyclohexane to give a glass.

The resulting crude product was purified by silica gel chromatography (Waters Prep 500). Elution with a linear gradient of dichloromethane (4 liters) and methanol - dichloromethane (15 : 85, 4 liters) yielded 1.70 g (61%) of 23-(5-methyl-1,3,4-oxadiazolyl-2-thio)-23-deoxy-OMT (**12**): MP (softened) ~105°C; $[\alpha]_D^{22}$ +115.5° (*c* 1.0, MeOH); elemental *Anal*, found C 58.78, H 7.84, N 5.95, S 4.72, calcd for $C_{34}H_{53}N_3O_{10}S$: C 58.68, H 7.68, N 6.04, S 4.61.

23-Acetylthio-23-deoxy-OMT (**15**)

A solution of OMT (2.39 g, 4.0 mmol) and *sym*-collidine (2.0 ml, 15.1 mmol) in dichloromethane (50 ml) was cooled to −78°C under argon and treated dropwise with trifluoromethanesulfonic anhydride (1.05 ml, 6.2 mmol). Ten minutes after addition had been completed, solid potassium thioacetate (460 mg, 4.0 mmol) was added to the reaction, and the cooling bath was removed. Three hours later, additional potassium thioacetate (90 mg, 0.8 mmol) was added to the reaction, and stirring was continued for 1 hour. The reaction mixture was extracted with saturated sodium bicarbonate solution, dried over sodium sulfate, filtered and evaporated. The residue was evaporated again from cyclohexane to give a glass. The glass was purified by silica gel flash chromatography, eluting with a linear gradient of dichloromethane (1 liter) and methanol - dichloromethane (15 : 85, 1 liter) to give 465 mg (18%) of 23-acetylthio-23-deoxy-OMT (**15**) and 245 mg (9%) of the elimination-rearrangement product (**45**). For compound **15**: MP (softened) ~125°C; $[\alpha]_D^{22}$ +59.5° (*c* 1.0, MeOH); elemental *Anal*, found C 60.73, H 8.44, N 2.23, S 4.64, calcd for $C_{33}H_{53}NO_{10}S$: C 60.43, H 8.15, N 2.14, S 4.89.

23-Azido-23-deoxy-OMT (**16**)

Following the procedure above, OMT (20.0 g, 33.5 mmol) and *sym*-collidine (9.0 ml, 67 mmol) were reacted with trifluoromethanesulfonic anhydride (8.4 ml, 50.0 mmol) and then with lithium azide (3.3 g, 67 mmol). The cooling bath was removed, and after 30 minutes, the reaction mixture was diluted with acetonitrile to bring the lithium azide into solution. After 2 hours, the solution was evaporated to dryness, dissolved in dichloromethane and worked up as usual. The crude product was purified by silica gel chromatography (Waters Prep 500). Elution with a linear gradient of dichloromethane (4 liters) and methanol - dichloromethane (5 : 95, 4 liters), adding 100 ml of methanol to the latter solution after elution volumes of 3 and 5 liters, yielded 12.0 g (58%) of 23-azido-23-deoxy-OMT (**16**): MP 135~138°C; $[\alpha]_D^{22}$ +7.4° (*c* 1.0, MeOH); elemental *Anal*, found C 60.00, H 8.27, N 7.79,

calcd for $C_{31}H_{50}N_4O_9$: C 59.79, H 8.09, N 9.00.

23-Amino-23-deoxy-OMT (17)

A solution of 23-azido-23-deoxy-OMT (**16**) (12.0 g, 19.3 mmol), triphenylphosphine (5.3 g, 20.3 mmol) and water (0.37 ml, 20.3 mmol) in distilled tetrahydrofuran (200 ml) was stirred at room temp for 4 days. The solution was evaporated to give a glass which was partitioned between ethyl acetate and 0.1 M acetic acid solution. The aqueous layer was separated, washed with ethyl acetate and carefully poured into saturated sodium bicarbonate solution. The resulting mixture was extracted with dichloromethane. The extract was dried over sodium sulfate, filtered and evaporated to give 10.5 g (91%) of 23-amino-23-deoxy-OMT (**17**): MP (softened) ~185°C; $[\alpha]_D^{22}$ +14.5° (*c* 1.0, MeOH); elemental *Anal*, found C 63.58, H 8.50, N 4.58, calcd for $C_{31}H_{52}N_2O_9$: C 62.39, H 8.78, N 4.70.

Alternate Synthesis of 23-Amino-23-deoxy-OMT (17)

A solution of 23-azido-23-deoxy-OMT (**16**) (622 mg, 1.0 mmol) in degassed 20% aqueous methanol (25 ml) was treated with solid chromous chloride (290 mg, 2.4 mmol) under argon. After stirring for 30 minutes, additional chromous chloride (135 mg, 1.1 mmol) was added. A final addition of chromous chloride (90 mg, 0.7 mmol) was made after another 10 minutes. Thirty minutes after the last addition, the reaction mixture was evaporated to an aqueous solution which was diluted with saturated sodium bicarbonate solution and filtered through a Celite pad. The filtrate was extracted with ethyl acetate. The combined ethyl acetate extracts were dried over sodium sulfate, filtered and evaporated to give 350 mg (59%) of 23-amino-23-deoxy-OMT (**17**).

23-(D-Phenylglycyl)amino-23-deoxy-OMT (19)

A solution of 23-amino-23-deoxy-OMT (**17**) (3.0 g, 5.0 mmol) in 10% aqueous acetone (50 ml) was treated with *N*-(*tert*-butoxycarbonyl(BOC)-D-phenylglycyloxy)succinimide (1.75 g, 5.0 mmol) and stirred at room temp for 2 hours. After the addition of a few drops of methanol, the reaction mixture was evaporated to an aqueous solution and then was extracted with dichloromethane. The dichloromethane layer was extracted with saturated sodium bicarbonate solution, dried over sodium sulfate, and filtered. The filtrate was evaporated to give a glass. The glass was purified by silica gel flash chromatography, eluting with a linear gradient of dichloromethane (1 liter) and methanol - dichloromethane (1 : 4, 1 liter) to give 2.5 g (60%) of 23-(*tert*-BOC-D-phenylglycyl)amino-23-deoxy-OMT (**20**). A portion of **20** (1.0 g, 1.2 mmol) was dissolved in trifluoroacetic acid (10 ml) at 0°C and stirred for 30 minutes. The reaction mixture was diluted with diethyl ether. The resulting precipitate was collected on a filter, washed with *n*-hexane and air-dried to give a quantitative yield of **19** as its bis-trifluoroacetate salt: MP (softened) ~90°C; $[\alpha]_D^{22}$ +38.7° (*c* 1.0, MeOH).

23-Iodo-23-deoxy-OMT (9)

OMT (**1**) (40 g, 67 mmol) was dissolved in dimethylformamide (60 ml) and triphenylphosphine (35.2 g, 134 mmol, powder) was subsequently dissolved with gentle heating. The resultant solution was stirred rapidly at ice-bath temperature under a nitrogen atmosphere as iodine (34 g, 134 mmol) in DMF (40 ml) was added dropwise over a 30-minute period. The thick yellow slurry which formed initially eventually redissolved. The ice bath was removed after addition had been completed and the mixture was stirred at room temp for 2.5 hours. It was then carefully poured into cold saturated sodium bicarbonate solution (2 liters) and the product was extracted into dichloromethane (2 portions, 1 liter total). The dichloromethane layer was extracted with 0.1 M sodium thiosulfate solution (2 liters) to remove excess iodine, then dried (sodium sulfate), filtered and evaporated to give a red-brown oil.

Some of the triphenylphosphine oxide (by-product) was removed at this point by crystallization from toluene. The remaining material was purified by silica gel chromatography (Waters Prep 500) to give 28.4 g (60%) of 23-iodo-23-deoxy-OMT as a tan foam: MP ~115°C; $[\alpha]_D^{22}$ +40.0° (*c* 1.0, MeOH); elemental *Anal*, found C 52.88, H 7.11, N 1.72, I 17.82, calcd for $C_{31}H_{50}NO_9I$: C 52.62, H 7.12, N 1.98, I 17.93.

23-(3-Azabicyclo[3.2.2]nonan-3-yl)-23-deoxy-OMT (42)

23-Iodo-23-deoxy-OMT (**9**) (50.0 g, 70.7 mmol) was dissolved in ethanol (500 ml); 4A molecular

sieves and *p*-toluenesulfonic acid monohydrate (20.2 g, 106 mmol) were then added. The reaction mixture was stirred at room temp for 1.25 hours before triethylamine (20 ml) was added. After 15 minutes, the reaction mixture was filtered and the filtrate was evaporated. The residue was dissolved in dichloromethane (600 ml) and extracted with saturated sodium bicarbonate solution (1 liter). The dichloromethane layer was separated, dried (sodium sulfate), filtered and evaporated to give the diethylketal of 23-iodo-23-deoxy-OMT (53 g, 96%). A sample of this material (6.2 g, 8 mmol) was dissolved in acetonitrile (60 ml) and 3-azabicyclo[3.2.2]nonane (3.0 g, 24 mmol) was added. The reaction mixture was stirred at reflux temperature for 2.5 hours and then was cooled and filtered. The filtrate was evaporated and the brown residue was dissolved in dichloromethane and extracted with saturated sodium bicarbonate solution. The organic layer was separated, dried (sodium sulfate), filtered and evaporated to give a brown foam which was purified by silica gel flash chromatography [gradient from methanol - dichloromethane (1 : 49) to methanol - dichloromethane - conc ammonium hydroxide (12 : 87.5 : 0.5)] to give 5.2 g (84%) of 23-(3-azabicyclo[3.2.2]nonan-3-yl)-23-deoxy-OMT-20-diethylketal. A portion of this product (4.5 g, 5.8 mmol) was dissolved in acetonitrile (40 ml), 0.1 N HCl (75 ml) was added, and the reaction mixture was stirred at room temp. 1 N HCl (10 ml) was added during the initial stages of the reaction after TLC showed it was progressing very slowly. The reaction was then complete within 2 hours and the reaction mixture was evaporated under vacuum to aqueous. The acidic solution was neutralized with saturated sodium bicarbonate solution and the product was extracted into dichloromethane. The organic layer was separated, dried (sodium sulfate), filtered, and evaporated to give 23-(3-azabicyclo[3.2.2]nonan-3-yl)-23-deoxy-OMT (3.9 g, 96%): MP (softened) ~130°C; $[\alpha]_D^{22}$ +38.6° (*c* 0.9, MeOH); elemental *Anal*, found C 66.71, H 9.19, N 3.85, calcd for $C_{39}H_{64}N_2O_9$: C 66.45, H 9.15, N 3.97.

Alternative Methods of Purification of 23-Dialkylamino Derivatives

The crude products obtained from aqueous-organic partition of the reaction mixtures could be further purified by extraction from ethyl acetate solution into a 0.5 M NaH_2PO_4 buffer solution (pH 6.5). The aqueous layer was separated and made basic with 5 N NaOH until precipitation was complete. The precipitate was then filtered and redissolved in dichloromethane; the organic solution was dried and evaporated to dryness *in vacuo*. Alternatively, crystallization from ethyl acetate, ethyl acetate - hexane, dichloromethane - petroleum ether or acetonitrile was used to accomplish purification.

Elimination-Rearrangement Product (**45**)

A solution of OMT (2.39 g, 4.0 mmol) and *sym*-collidine (1.5 ml, 11.3 mmol) in dichloromethane (40 ml) was cooled to −78°C under argon and treated dropwise with trifluoromethanesulfonic anhydride (1.05 ml, 6.2 mmol). Ten minutes after the addition had been completed, the reaction mixture was treated with diazabicycloundecene (66.9 mg, 4.4 mmol). The cooling bath was removed, and the reaction was allowed to come to room temp over a 2-hour period. The reaction mixture was worked up as usual to give a glass which was purified by silica gel flash chromatography. Elution with a linear gradient of dichloromethane (1 liter) and methanol - dichloromethane (15 : 85, 1 liter) gave 475 mg (21%) of the elimination-rearrangement product **45**: MP 135~142°C; $[\alpha]_D^{22}$ −58.7° (*c* 1.0, MeOH); elemental *Anal*, found C 64.43, H 8.32, N 2.67, calcd for $C_{31}H_{49}NO_9$: C 64.22, H 8.52, N 2.42.

Methanol Adduct of Rearrangement Product (**46**)

A solution of OMT (6.0 g, 10.05 mmol) and *sym*-collidine (4.0 ml, 30.0 mmol) in dichloromethane (150 ml) was cooled to −78°C under argon and treated dropwise with trifluoromethanesulfonic anhydride (2.4 ml, 14.1 mmol). Five minutes after the addition had been completed, the reaction mixture was allowed to warm to 0°C. A portion (40 ml, 2.5 mmol theoretical) of this reaction solution was transferred to another flask and treated with methanol (1.0 ml, excess) at room temp under argon. After being stirred for 5 hours, the reaction mixture was worked up as usual; elution of the silica gel chromatography column with a linear gradient of dichloromethane (600 ml) and methanol - dichloromethane (1 : 9, 600 ml) gave 400 mg (29%) of **46**: MP 116~120°C; $[\alpha]_D^{22}$ −29.8° (*c* 1.0, MeOH);

elemental *Anal*, found C 62.62, H 8.50, N 2.50, calcd for $C_{32}H_{53}NO_{10}$: C 62.82, H 8.73, N 2.29.

2-Phenylethanol Adduct of Rearrangement Product (**47**)

Using the above procedure, a portion of the solution of 23-*O*-trifluoromethanesulfonyl-OMT (40 ml, 2.5 mmol theory) was reacted with 2-phenylethanol (2 ml, excess) at room temp under argon. The reaction mixture was stirred for 5 hours and worked up as usual. Elution of the silica gel column with a linear gradient of dichloromethane (700 ml) and methanol - dichloromethane (1 : 9, 700 ml) gave 500 mg (29%) of **47**: MP 90~93°C; $[\alpha]_D^{22}$ −24.8° (*c* 1.0, MeOH); elemental *Anal*, found C 66.82, H 8.48, N 1.90, calcd for $C_{39}H_{59}NO_{10}$: C 66.73, H 8.47, N 2.00.

OMT-20-diethylketal

OMT (20.0 g, 33.5 mmol) was dissolved in absolute ethanol (150 ml), and 4A molecular sieves (1 g) and *p*-toluenesulfonic acid (9.5 g, 50.5 mmol) were added. After the reaction mixture had been stirred for 1.5 hours at room temp, triethylamine (10 ml) was added, and stirring was continued for 10 minutes. The mixture was filtered, and the filtrate was evaporated. The residue was dissolved in dichloromethane (150 ml), and the solution was extracted with saturated sodium bicarbonate (150 ml). The organic layer was separated, dried over sodium sulfate, filtered and evaporated to give 18.9 g (84%) of OMT-20-diethylketal: MP 90~100°C; $[\alpha]_D^{22}$ −7.0° (*c* 1.0, MeOH); elemental *Anal*, found C 62.43, H 8.91, N 1.97, calcd for $C_{35}H_{61}NO_{11}$: C 62.57, H 9.15, N 2.08.

23-*O*-Phenyl-OMT (**48**)

To a solution of OMT-20-diethylketal (12.6 g, 18.8 mmol), triphenylphosphine (9.8 g, 37.6 mmol), and phenol (3.6 g, 37.6 mmol) in tetrahydrofuran (300 ml) under argon was added diethyl azodicarboxylate (6.0 ml, 37.6 mmol). The mixture was stirred at room temp for 1 hour. Methanol (1 ml) was added, and stirring was continued for 5 minutes. Solvent was evaporated, and the resulting glass was dissolved in dichloromethane (150 ml). This solution was extracted with saturated sodium bicarbonate solution (200 ml) and the organic layer was separated, dried over sodium sulfate, filtered and evaporated to yield 22.1 g of crude 23-*O*-phenyl-OMT-20-diethylketal. This material was dissolved with stirring in 0.1 N HCl (200 ml) with addition of acetonitrile (125 ml). After stirring for 90 minutes at room temp, solvent was evaporated. This residue was dissolved in dichloromethane (200 ml) and extracted with saturated sodium bicarbonate solution (250 ml). The organic layer was separated, dried over sodium sulfate, filtered and evaporated to give 25 g of crude product. This was purified by preparative HPLC, eluting with a gradient of dichloromethane to methanol - dichloromethane (1 : 9) to yield 2.3 g (18%) of 23-*O*-phenyl-OMT: MP 121~124°C; $[\alpha]_D^{22}$ −4.4° (*c* 0.9, (MeOH); elemental *Anal*, found C 65.77, H 8.47, N 2.24, calcd for $C_{37}H_{55}NO_{10}$: C 65.95, H 8.23, N 2.08.

23-*O*-(4-Formylphenyl)-OMT (**56**)

Using the procedure described previously, OMT-20-diethylketal (6.5 g, 9.7 mmol), was reacted with *p*-hydroxybenzaldehyde (2.4 g, 19.4 mmol) triphenylphosphine (5.1 g, 19.4 mmol), and diethyl azodicarboxylate (3.1 ml, 19.4 mmol) for 30 minutes to give 17.9 g of crude product as an oil. Preparative HPLC of this product [using a gradient of dichloromethane to dichloromethane - methanol (98 : 2)] gave 1.9 g of 23-*O*-(4-formylphenyl)-OMT-20-diethylketal as a foam (25%). Hydrolysis as described above for 3 hours yielded 1.2 g of 23-*O*-(4-formylphenyl)-OMT as a foam (89%): MP 115~122°C; $[\alpha]_D^{22}$ −3.7° (*c* 1.0, MeOH); elemental *Anal*, found C 65.32, H 8.19, N 2.13, calcd for $C_{38}H_{55}NO_{11}$: C 65.03, H 7.90, N 2.00.

23-*O*-[4-[(Hexahydro-1*H*-azepin-1-yl)methyl]phenyl]-OMT (**57**)

23-*O*-(4-Formylphenyl)-OMT-20-diethylketal (1 g, 1.3 mmol) was dissolved with stirring in methanol (30 ml). Molecular sieves 4A (1 g) were added and stirring was continued under argon for 30 minutes. Hexamethyleneimine (0.23 ml, 2 mmol) was added, and sodium cyanoborohydride (0.13 g, 2 mmol) was added slowly over 1 minute. The mixture was stirred for 20 hours at which time additional sodium cyanoborohydride (0.1 g, 1.6 mmol) was added slowly. After a total reaction time of 22 hours, the solvent was evaporated; the resulting foam was taken up in ethyl acetate (25 ml) and

extracted with water (25 ml) and then with pH 6.5 phosphate buffer solution (25 ml). The latter aqueous extract was made basic by dropwise addition of 1 N NaOH until no further precipitation was evident and the resulting mixture was extracted with dichloromethane (30 ml). The organic layer was dried over sodium sulfate, filtered and evaporated to give 200 mg of foam (15%). After it had been dissolved with stirring in 0.1 N hydrochloric acid (15 ml), the reaction mixture was stirred at room temp for 2 hours and worked up as usual to yield 173 mg (100%) of 23-*O*-[4-[(hexahydro-1*H*-azepin-1-yl)methyl]phenyl]-OMT as an amorphous solid foam.

Alternate Synthesis of 23-Azido-23-deoxy-OMT (**16**)

OMT-20-diethylketal (10.0 g, 15.0 mmol) and triphenylphosphine (7.9 g, 30.0 mmol) were dissolved in tetrahydrofuran (200 ml) under argon. The mixture was cooled in an ice water bath for 30 minutes. Diethyl azodicarboxylate (4.7 ml, 30 mmol) was added dropwise, and the reaction mixture was stirred for 5 minutes. Diphenylphosphorylazide (6.5 ml, 30 mmol) was added dropwise, and stirring was continued for 10 minutes. The mixture was removed from the cooling bath and stirred for another 1.5 hours. Methanol (1 ml) was added, and the solution was evaporated. The resulting glass was dissolved in dichloromethane (200 ml) and this solution was extracted with saturated sodium bicarbonate solution (200 ml). The organic layer was dried over sodium sulfate, filtered and evaporated to give 23-azido-23-deoxy-OMT-20-diethylketal as an oil. It was then dissolved in 0.1 N HCl (200 ml) and hydrolysis was effected by addition of 1 N HCl (10 ml) and stirring for 1.5 hours. The crude product was purified by preparative HPLC to give 3.0 g of 23-azido-23-deoxy-OMT as a foam (32%).

23-*S*-Phenyl-23-deoxy-OMT (**10**)

OMT-20-diethylketal (6.5 g, 9.7 mmol), triphenylphosphine (5.1 g, 19.4 mmol) and diethyl azodicarboxylate (3.1 ml, 19.4 mmol) were dissolved with stirring in tetrahydrofuran (150 ml). Benzenethiol (1.9 ml, 19.4 mmol) was added, and stirring was continued for 30 minutes. Solvent was evaporated and the resulting glass was taken up in dichloromethane (15 ml) (aided by sonication). The mixture was cooled at 0°C for 30 minutes, and the resulting precipitate (diethyl hydrazinedicarboxylate) was removed by filtration. The filtrate was diluted with additional dichloromethane (85 ml) and extracted with saturated sodium bicarbonate solution (100 ml). The reaction was worked up as described above to yield the crude product as an oil. This material was purified by flash chromatography, eluting with a linear gradient of dichloromethane (1 liter) to methanol - dichloromethane (15 : 85, 1 liter) to yield 982 mg of a glass (13%). After it had been dissolved with stirring in 0.1 N HCl (35 ml), stirring was continued at room temp for 2 hours. The reaction mixture was worked up as described in previous examples to give 800 mg of 23-*S*-phenyl-23-deoxy-OMT as a foam (90%): MP (softened) ~100°; $[\alpha]_D^{22}$ +127.8° (*c* 1.0, MeOH); elemental *Anal*, found C 64.31, H 7.81, N 1.84, S 4.65, calcd for $C_{37}H_{55}NO_9S$: C 64.41, H 8.04, N 2.03, S 4.65.

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