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Synthesis and in vitro antibiotic activity of 16-membered 9-*O*-arylalkyloxime macrolides

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Abstract—A series of novel 9-*O*-arylalkyloxime analogs based on three different 16-membered macrolide scaffolds—5-*O*-mycaminosyltylonolide (OMT), tilmicosin, and 20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT—was synthesized. In vitro antibiotic activities were assayed against Gram-positive *Streptococcus pneumoniae* and *Staphylococcus aureus* and Gram-negative *Haemophilus influenzae* bacterial strains. Analogs derived from OMT (**3**–**15**) showed similar or better antibacterial activities against macrolide-susceptible strains and enhanced activities against macrolide-resistant strains compared with erythromycin A, tylosin, or OMT. Similar results were observed for tilmicosin 9-*O*-arylalkyloxime analogs (**18**–**24**). In contrast, most of the 20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT analogs (**25**–**33**) showed reduced antibacterial activities compared with OMT. Ribosome-binding studies were performed on compounds **12** (OMT derivative), **20** (tilmicosin derivative), and **29** [20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT derivative]. It was found that these compounds interacted with both domain V and domain II of the *Escherichia coli* 23S rRNA.

We describe the preparation and evaluation of a series of novel functionalized 16-membered macrolides based on the tylonolide scaffold. These compounds show a promising spectrum of activity against a range of important pathogenic bacteria. Both 14- and 16-membered macrolide antibiotics have been used extensively in human and veterinary medicine. These compounds bind to bacterial ribosomes and inhibit protein synthesis.[1] The steady and continuous emergence of strains resistant to macrolides has prompted a large effort in the design of semisynthetic analogs, mainly around erythromycin A, the prototype 14-membered macrolide, resulting in the creation of the recently approved ketolide telithromycin.[2]

The two most important mechanisms by which bacteria become resistant to macrolides are through N6-mono or N6,6-dimethylation of the adenosine residue at nucleotide 2058 of the 23S rRNA component of the 50S subunit of the bacterial ribosome[3] and through the acquisition of efflux pumps that export macrolides from the cells before they reach their target.[4] It is believed that the ketolides overcome macrolide resistance in *Streptococcus pneumoniae* and *Streptococcus pyogenes* through enhanced binding to the bacterial ribosome via their aromatic side chains.[5] These compounds also evade the efflux pumps. In our search for novel macrolides to combat resistance, we have designed and synthesized a series of 16-membered macrolides functionalized with 9-*O*-arylalkyloximes, wherein the aryl group of the oxime side chain may mimic the function of the aryl groups of the ketolides.

The 9-*O*-ether oxime derivatives of tylosin[6] and erythromycin[7] were prepared previously and showed antibacterial activity comparable to that of their parent, tylosin (Tyl) or Ery A. The 9-*O*-arylalkyloxime compounds in this study were prepared from 5-*O*-mycaminosyltylonolide (OMT), tilmicosin, and 20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT scaffolds. The preparation of OMT and tilmicosin from tylosin and desmycosin, respectively, has been described previously.[8,9] 20-Deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT was prepared from OMT using a similar protocol as that for preparing tilmicosin from desmycosin.[9] Synthesis of 9-*O*-arylalkyloxime OMTs (compounds **3**–**15**) starting from OMT is depicted in Scheme 1.

Keywords: 16-Membered macrolide antibiotics; Oximes; Ribosome binding.
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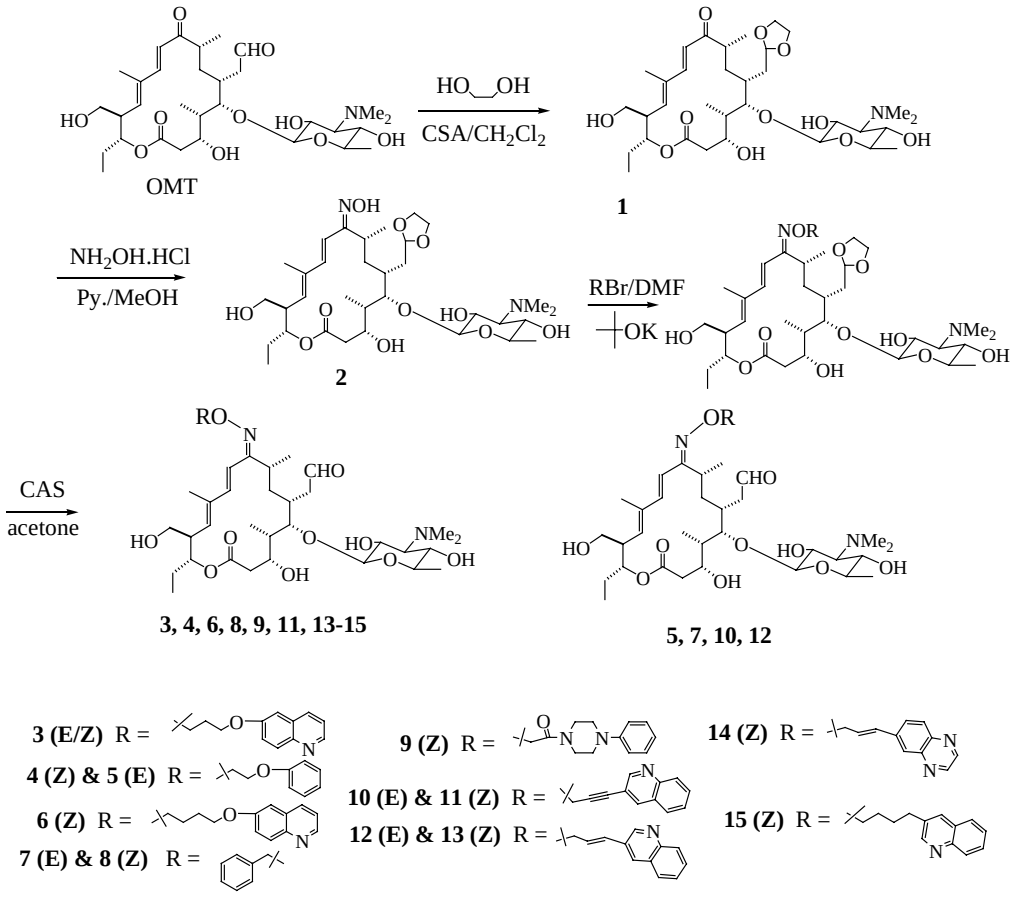


Scheme 1. Synthesis of 9-*O*-arylalkyloxime OMTs.

The C20 aldehyde functional group of OMT was protected as the 1,3-dioxolane by treatment with ethylene glycol in the presence of camphorsulfonic acid (CSA) in dichloromethane. Conversion of the C-9 ketone to the oxime was carried out using hydroxylamine hydrochloride in the presence of pyridine.[10] The oxime could then be alkylated selectively on oxygen using arylalkylbromides and potassium *t*-butoxide in DMF to generate the arylalkyloxime analogs. In many cases, the *E* and *Z* oximes generated could be separated by reversed-phase HPLC. The oxime configuration was determined by comparison of the ^{1}H and ^{13}C NMR spectra of both *E* and *Z* isomers according to the literature.[11,12] In general, the chemical shift for H8 in *E* isomers is more down-field (ca. 1 ppm) than that in the *Z* isomer. In contrast, the chemical shift for C8 in the E isomer is more up-field (7–10 ppm) than that in the *Z* isomer. Finally, de-protection of the 20-aldehyde was achieved by stirring the alkylated oxime in acetone and CSA.[13] The in vitro activities of compounds **3**–**15** are summarized in Table 1.

Many of the arylalkyloxime OMT analogs have comparable or better activity than that of Ery A or OMT against the macrolide-susceptible strains *S. pneumoniae* ATCC6301, ATCC700671, and ATCC49618, *Staphylococcus aureus* ATCC 6538p and ATCC 29213, and *Staphylococcus epidermidis* ATCC12228. In addition, the analogs, in particular **3** and **15**, show substantially improved potency, equal to that of telithromycin, against a number of macrolide-resistant strains of *S. pneumoniae* (ATCC700676, ATCC700677, and ATCC700905). The basis of resistance to macrolides in these strains has not been determined. It is worth noting that the optimal chain length between the aromatic side chain and the oxime oxygen is four atoms, with compounds **3** and **15** (4-atom linker) showing potencies superior to those of compound **6** (5-atom linker), and compounds **12** and **13** (3-atom linker). The *Z* oximes consistently show better potencies than those of their *E* counterparts (i.e., **4** > **5**, **8** > **7**, and **11** > **10**).

The improved potencies of **3** and **6** are also maintained against the macrolide-susceptible *S. aureus* strains ATCC 6538p and ATCC29213, and *S. epidermidis* ATCC12228. In general, *staphylococci* are inherently less susceptible to macrolides than *streptococci*. The basis for erythromycin/azithromycin/clarithromycin resistance in *S. aureus* ATCC141541 is ribosome methylation induced by the presence of the 14-membered macrolide. The 16-membered macrolides (tylosin and OMT) analogs are poor inducers of macrolide resistance in this strain, hence the modest activities (MIC = 1.56 μg/ml). The OMT analogs, in particular **3** and **15**, are very potent against this strain, likely due to their enhanced activities as antibiotics and, possibly, to their weakened ability to act as inducers of resistance. The basis of resistance in the remaining *S. aureus* strains in Table 1, ATCC33591, ATCCBAA-39,

Table 1. Antibacterial activities of compounds **3**–**9** and **10**–**15**

	MIC (μg/ml)															
Compound:	EryA	Tyl	OMT	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
S. pneumoniae																
ATCC6301	0.025	0.098	0.025	0.025	0.025	0.098	0.025	0.20	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
ATCC700671	0.049	0.20	0.049	0.025	0.025	0.39	0.025	0.39	0.20	0.025	0.049	0.025	0.049	0.025	0.049	0.025
ATCC700676[a]	6.25	0.20	0.78	0.025	0.025	0.20	0.025	0.20	0.025	0.39	0.049	0.025	0.049	0.049	0.049	0.025
ATCC700677[a]	6.25	>12.5	6.25	0.20	0.39	1.56	0.39	3.12	1.56	6.25	>12.5	>12.5	>12.5	>12.5	>12.5	0.025
ATCC700905[a]	3.12	0.20	0.78	0.025	0.025	0.78	0.025	0.39	0.20	0.20	0.049	0.049	0.049	0.049	0.049	0.025
ATCC700906[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC49619	0.049	0.098	0.098	0.01	0.01	0.20	0.01	0.20	0.20	0.01	0.049	0.025	0.025	0.025	0.025	0.049
S. aureus																
ATCC6538p	0.098	0.20	0.39	0.025	0.098	1.56	0.20	0.78	0.39	0.78	0.098	0.098	0.098	0.20	0.20	0.025
ATCC33591[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC141541[a]	>12.5	1.56	1.56	0.20	0.39	3.12	0.78	1.56	1.56	1.56	0.78	0.39	0.39	0.78	0.39	0.20
ATCCBAA-39[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCCBAA-44[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC29213	0.20	1.56	0.78	0.098	0.20	3.12	0.78	1.56	1.56	1.56	0.39	0.39	0.39	0.78	0.39	0.20
S. epidermidis																
ATCC12228	0.20	0.39	0.39	0.098	0.20	1.56	0.20	1.56	0.78	0.20	0.20	0.098	0.049	0.39	0.20	0.20
E. faecalis																
ATCC51575	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5

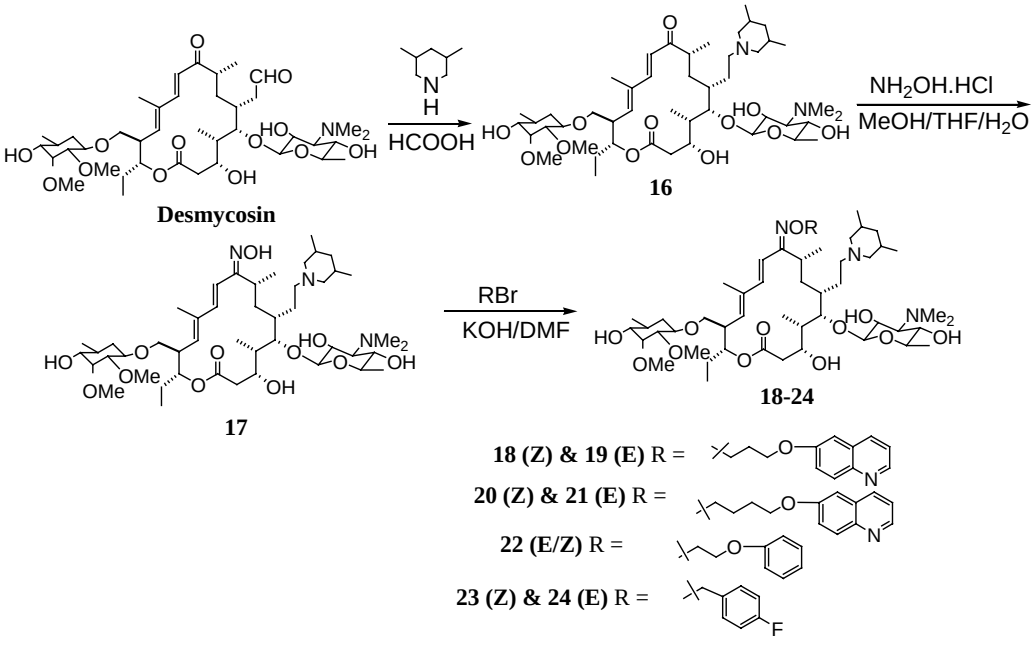
Abbreviations: Ery A, erythromycin A; Tyl, tylosin, *S. pneumoniae*, *Streptococcus pneumoniae*; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; *E. faecalis*, *Enterococcus faecalis*.
[a] Macrolide-resistant strains.

and ATCCBAA-44, is constitutive ribosomal methylation. These strains are resistant to 14- and 16-membered macrolides, the ketolides, and the arylalkyloxime OMT analogs, indicating that no macrolide or analog can bind to dimethylated *S. aureus* ribosomes. The *Enterococcus faecalis* ATCC51575 strain in Table 1, resistant to all macrolides and OMT analogs, likely also contains methylated ribosomes.

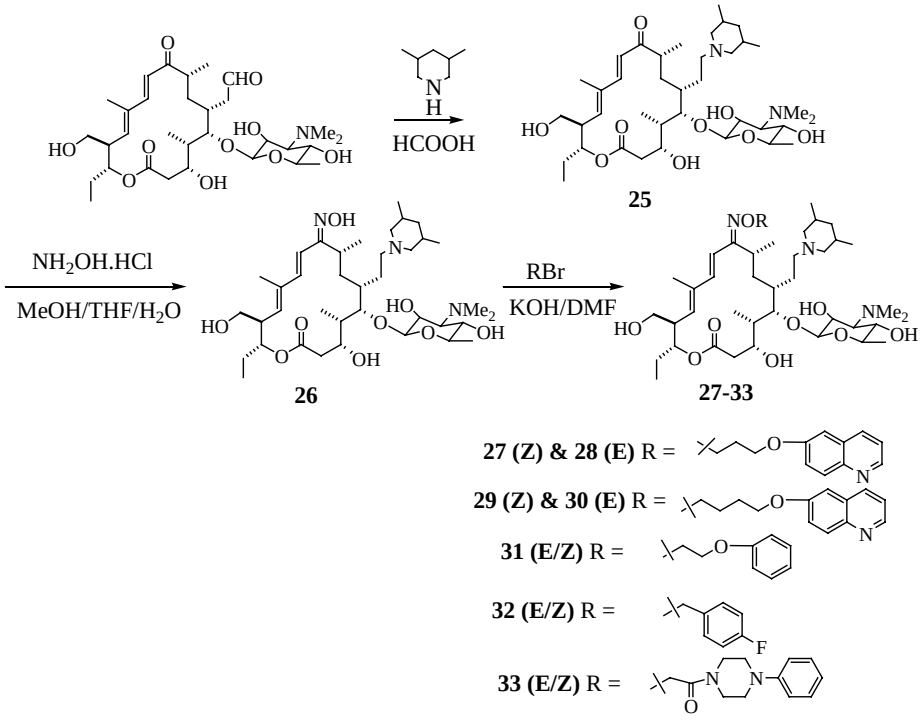
The synthesis of 9-*O*-arylalkyloxime tilmicosin analogs follows a straightforward path (Scheme 2). Tilmicosin was prepared from desmycosin via reductive amination, using 3,5-dimethylpiperidine (a mixture of *cis* and *trans* isomers) in the presence of formic acid. Formation of the oxime of tilmicosin was achieved by treatment of tilmicosin with hydroxylamine hydrochloride in MeOH/THF/H_2O.[14] Alkylation of tilmicosin 9-oxime with arylalkylbromides gave rise to the corresponding 9-*O*-arylalkyloxime tilmicosins **18**–**24**. Similarly, 9-*O*-arylalkyloxime-20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT analogs were prepared from OMT via reductive amination of the 19-aldehyde followed by oximation and alkylation leading to compounds **27**–**33** (Scheme 3). The in vitro antibacterial activities of compounds **18–24** and compounds **27–33** are summarized in Table 2 and Table 3, respectively.

In general, the 9-arylalkyloxime tilmicosin analogs show significantly increased antibacterial potencies over the parent tilmicosin against both macrolide-susceptible and macrolide-resistant *S. pneumoniae* strains (Table 2), but none of the compounds exhibited the overall potencies of **3** and **15**. Interestingly, 20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT (**25**) and 20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT 9-oxime (**26**) exhibited reduced antibiotic activity compared to OMT, and addition of aromatic side chains to 20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT 9-oxime (**31**–**33**) restored antibacterial activity only slightly (Table 3). Steitz et al. have suggested that the C-6 ethylaldehyde of 16-membered macrolides forms a covalent bond with the N6 atom of the A2103 residue (corresponding to A2062 in *Escherichia coli*) in the 23S rRNA component of the ribosome of *Haloarcula marismortui*.[1] They also suggested that the mycinosyl moiety of tylosin interacts with A841 (A748, *E. coli* numbering) in domain II of the 50S ribosome. It is possible that the binding of 16-membered macrolides to ribosomes requires the mycinose residue if the 20-aldehyde is missing. Addition of extensions at C-9 would therefore not restore binding substantially, resulting in the poor activity of **25**. On the other hand, when the scaffold contains either the 20-aldehyde (OMT) or the mycinose residue (tilmicosin), addition of arylalkyl side chains at C-9 via the oxime appeared to enhance binding to the ribosome. It is also possible that the poor activities of the 20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT 9-oxime derivatives are due to reduced penetration into the bacterial cell.

One compound from each scaffold was chosen for ribosome binding studies. Results are shown in Figure 1 and Table 4. The studies were performed as described previously using reconstituted *E. coli* 70S ribosomes.[15] Briefly, the ribosomes are first methylated with dimethylsulfate in the presence or absence of saturating amounts of a given macrolide. The RNA is then extracted from the ribosomes and subjected to transcription employing a ^{32}P-labeled primer, deoxyribonucleotides, and reverse transcriptase. Transcription stops when it encounters a methylated nucleotide; thus a band is observed on the autoradiograms of a polyacrylamide sequencing gel.[16] Quantitation of the bands is achieved by scanning phosphorimages with a gel reader. Sites on the rRNA that are methylated can be uncovered by use of primers corresponding to domain II, domain V, etc. Binding of macrolides protects



Scheme 2. Synthesis of 9-*O*-arylalkyloxime tilmicosins.



Scheme 3. Synthesis of 9-*O*-arylalkyloxime-20-deoxo(3,5-dimethyl-1-piperidine)OMTs.

Table 2. Antibacterial activities of compounds **18**–**24**

	MIC (μg/ml)									
Compound:	EryA	Tyl	Til	**18**	**19**	**20**	**21**	**22**	**23**	**24**
S. pneumoniae										
ATCC6301	0.025	0.098	0.39	0.01	0.01	0.01	0.025	0.39	0.39	0.20
ATCC700671	0.049	0.20	0.78	0.025	0.01	0.01	0.049	0.39	0.78	0.39
ATCC700676[a]	6.25	0.20	0.78	0.049	0.049	1.56	1.56	1.56	3.12	0.78
ATCC700677[a]	6.25	>12.5	6.25	0.049	0.025	6.25	6.25	6.25	6.25	6.25
ATCC700905[a]	3.12	0.20	1.56	0.20	0.098	1.56	0.78	0.78	3.12	0.78
ATCC700906[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	12.5	12.5	12.5
ATCC49619	0.049	0.098	1.56	0.098	0.025	0.01	0.098	0.78	0.78	0.39
S. aureus										
ATCC6538p	0.098	0.20	0.098	0.20	0.20	0.78	0.78	0.20	0.39	0.20
ATCC33591[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	12.5
ATCC141541[a]	>12.5	1.56	0.39	0.39	0.20	1.56	1.56	0.78	6.25	0.78
ATCCBAA-39[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCCBAA-44[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC29213	0.20	1.56	0.20	0.39	0.20	1.56	3.12	0.39	0.78	0.78
S. epidermidis										
ATCC12228	0.20	0.39	0.098	0.39	0.20	1.56	3.12	0.39	0.78	0.78
E. faecalis										
ATCC51575	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5

Strains and abbreviations as in Table 1; Til, tilmicosin.
[a] Macrolide-resistant strains.

sites from methylation, resulting in loss of intensity of bands in the gel. Both 14- and 16-membered macrolides interact with nucleotides A2058 and A2059 in domain V of 23S RNA via their desosamine or mycaminose moieties, respectively.[1]

As expected, erythromycin A protects both A2058 and A2059 from methylation[16] and also causes slight enhancement of methylation at A752 in domain II. The ketolide cethromycin (ABT-773) shows protection not only of A2058 and 2059 but also of A752 in domain II. Our data indicate that tylosin, tilmicosin (**16**), and tilmicosin 9-oxime (**17**) also interact with A752, probably via the mycinose moiety. The OMT analog **12**, which lacks the mycinose sugar at the 23-position, has a very similar protection pattern to ABT-773. The interaction

Table 3. Antibacterial activity of compounds **25**–**33**

	MIC (μg/ml)										
Compound:	EryA	Tyl	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
S. pneumoniae											
ATCC6301	0.025	0.098	0.39	0.78	6.25	6.25	1.56	6.25	0.20	0.39	0.049
ATCC700671	0.049	0.20	0.78	1.56	6.25	6.25	3.12	12.5	0.39	0.39	0.098
ATCC700676[a]	6.25	0.20	1.56	6.25	>12.5	12.5	6.25	>12.5	0.78	0.39	1.56
ATCC700677[a]	6.25	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC700905[a]	3.12	0.20	1.56	6.25	>12.5	12.5	6.25	12.5	0.39	0.39	0.78
ATCC700906[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC49619	0.049	0.098	0.78	1.56	6.25	6.25	6.25	12.5	0.39	0.39	0.049
S. aureus											
ATCC6538p	0.098	0.20	0.39	3.12	12.5	>12.5	6.25	>12.5	0.78	0.78	0.39
ATCC33591[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC141541[a]	>12.5	1.56	1.56	>12.5	>12.5	>12.5	>12.5	>12.5	6.25	3.12	3.12
ATCCBAA-39[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCCBAA-44[a]	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC29213	0.20	1.56	1.56	12.5	>12.5	>12.5	12.5	>12.5	6.25	3.12	1.56
S. epidermidis											
ATCC12228	0.20	0.39	0.78	>12.5	>12.5	>12.5	>12.5	>12.5	6.25	3.12	0.78
E. faecalis											
ATCC51575	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5

Strains and abbreviations as in Table 1.
[a] Macrolide-resistant strains.

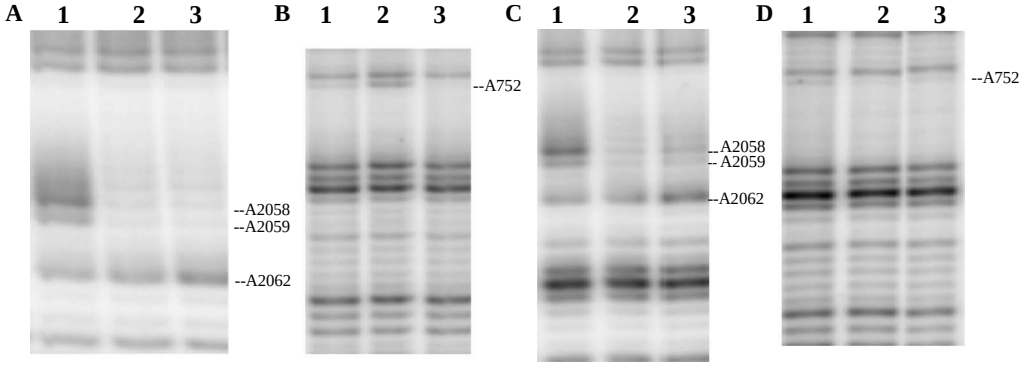


Figure 1. Polyacrylamide gels showing antibiotic-induced alterations in the degree of methylation of nucleotides in domain V (A and C) and domain II (B and D) of *Escherichia coli* 23S rRNA. Antibiotics were used at concentrations of 50 μM. (A and B) lane 1, no antibiotic; lane 2, erythromycin A; lane 3, compound **12**. (C and D) lane 1, no antibiotic, lane 2, compound **20**; lane 3, compound **29**.

Table 4. Ribosome (*Escherichia coli* MRE600 23S rRNA) accessibility study[a] of selected compounds

	A752	A2058	A2059	A2062
No drug	1	1	1	1
EryA	1.28 ± 0.02	0.08 ± 0.03	0.22 ± 0.07	1.46 ± 0.32
ABT773	0.105 ± 0.002	0.033 ± 0.020	0.11 ± 0.03	1.83 ± 0.30
Tyl	0.069 ± 0.027	0.082 ± 0.020	0.16 ± 0.03	0.15 ± 0.07
12	0.10 ± 0.03	0.031 ± 0.011	0.054 ± 0.037	2.17 ± 0.19
Til	0.44 ± 0.16	0.070 ± 0.032	0.14 ± 0.02	1.30 ± 0.20
17	0.35 ± 0.11	0.035 ± 0.025	0.073 ± 0.046	1.90 ± 0.10
20	0.23 ± 0.12	0.12 ± 0.020	0.30 ± 0.05	1.10 ± 0.06
29	0.44 ± 0.12	0.093 ± 0.022	0.28 ± 0.17	1.8 ± 0.7

The intensities of A782 and A2082 were not affected by drug binding[16] and were therefore used to normalize the intensities of A752 or A2058, A2059, and A2062 within each lane.
[a] Quantification was obtained by scanning the phosphorimages of the sequencing gels.

of compound **12** with A752 is probably via the aromatic side chain extending from the 9-oxime. Likewise, tilmicosin analog **20** and 20-deoxy-20-(3,5-dimethyl-1-piperidin-1-yl)-OMT analog **29** protect A752 to a lesser degree than does **12**.

The two most potent analogs (**3** and **15**) were tested against two *Haemophilus influenzae* strains (ATCC9006 and ATCC49766) (Table 5). Each showed ca. 4-fold decreased activity against *H. influenzae* compared to OMT. It has been documented that replacement of the

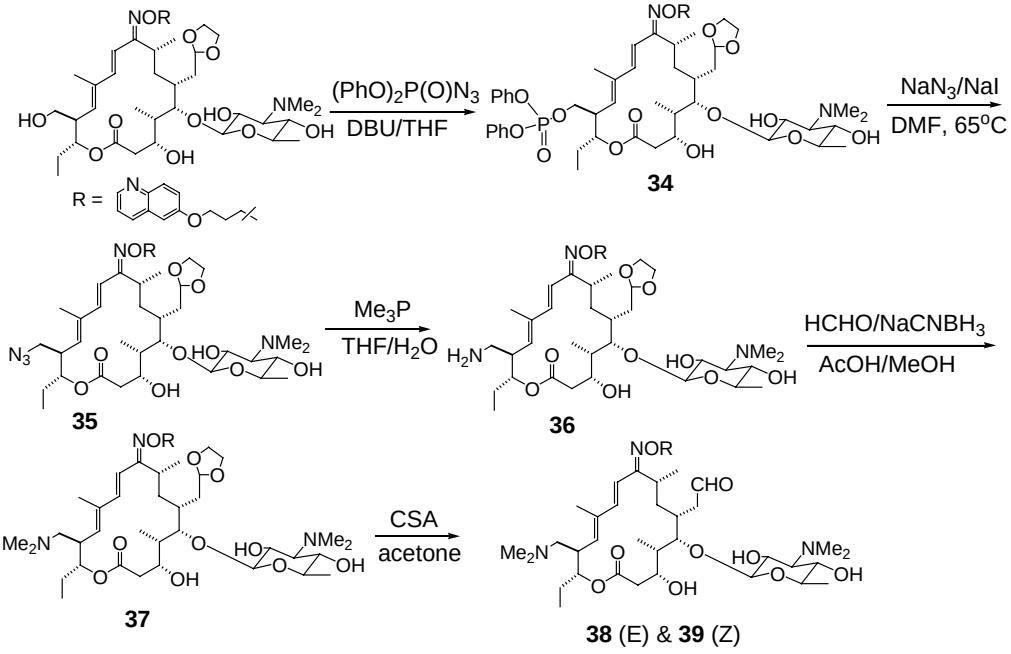
Table 5. In vitro activities of **3**, **15**, **38**, and **39**

	MIC (μg/ml)						
Compound:	EryA	Teli	OMT	**3**	**15**	**38**	**39**
S. pneumoniae							
ATCC6301	0.025	0.025	0.025	0.025	0.025	0.025	0.025
ATCC700671	0.049	0.025	0.049	0.025	0.025	0.098	0.049
ATCC700676[a]	6.25	0.098	0.78	0.025	0.025	0.025	0.025
ATCC700677[a]	6.25	0.025	6.25	0.20	0.025	>12.5	>12.5
ATCC700905[a]	3.12	0.025	0.78	0.025	0.025	0.025	0.025
ATCC700906[a]	>12.5	0.20	>12.5	>12.5	>12.5	>12.5	>12.5
ATCC49619	0.049	0.049	0.098	0.01	0.049	0.025	0.025
H. influenzae							
ATCC9006	1.56	1.56	1.56	6.25	6.25	1.56	1.56
ATCC49766	6.25	3.12	1.56	6.25	6.25	3.12	3.12
EH001	3.12	1.56	3.12	N/D	N/D	3.12	3.12
EH002	6.25	6.25	6.25	N/D	N/D	6.25	3.12
EH003	3.12	1.56	1.56	N/D	N/D	3.12	1.56

Abbreviations as in Tables 1 and 2. *H. influenzae*, *Haemophilus influenzae*.
[a] Macrolide-resistant strains.

23-OH of OMT by a basic group such as dialkylamine enhances potency against Gram-negative bacteria.[17,18] Therefore, **3** was converted to its 23-deoxy-23-dimethylamino analogs (**38** and **39**). The activity of **38** and **39** against a panel of five *H. influenzae* strains was found to have regained potencies similar to those of OMT and telithromycin; their potencies against *S. pneumoniae* essentially remained unchanged compared to **3** (except against ATCC700677). The preparation of **38** and **39** is illustrated in Scheme 4. The 19-protected **3** was converted to **34** by treating it with diphenylphosphoryl azide and subsequently converted to the 23-azido compound **35** by heating **34** in the presence of sodium azide and a catalytic amount of sodium iodide in DMF. Reduction of **35** with trimethylphosphine resulted in **36**, followed by reductive amination with $NaCNBH_3$/HOAc/HCHO to give **37**. Deprotection of **37** using CSA/acetone yielded **38** and **39**, which were separated by HPLC.

The work presented here represents an initial attempt to produce potent macrolide analogs that can be used against macrolide-resistant bacterial pathogens. Three scaffolds were used to produce a limited series of C9-arylalkyloximes. The most potent compounds employed the simplest scaffold, OMT, and contained a 4-atom linker between the aryl group and the oxygen atom, and exhibited properties similar to those of the recently developed ketolides: clinically relevant activity against macrolide-resistant *S. pneumoniae* and inducible-resistant *S. aureus* strains, although their in vivo potencies, pharmacology, and toxicities have yet to be examined. Footprinting data



Scheme 4. Synthesis of compounds **38** and **39**.

suggest that the restoration of potency against the macrolide-resistant strains are through binding to the ribosome in domain II to offset the disruption of macrolide–ribosome interaction in domain V that takes place through methylation at nucleotide 2058. As in the case of the ketolides, the OMT analogs were not active against the constitutive-resistant *staphylococci* and potencies against *H. influenzae*, an important respiratory disease causing agent, were somewhat lower than that of the ketolides, but could be improved through modification of the scaffold. Additional modifications, including optimization of the aryl group, should also enhance the properties of this exciting class of molecules.

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