# *Letters*

## Synthesis and Antibacterial Activity of 6-*O*-Arylbutynyl Ketolides with Improved Activity against Some Key Erythromycin-Resistant Pathogens

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**Abstract:** A series of novel 6-O-substituted homopropargyl ketolides was synthesized and evaluated against various erythromycin-resistant pathogens. Promising in vitro antibacterial activity was demonstrated for compounds bearing this structural motif.

**Introduction.** Macrolide antibiotics have been used effectively and safely for the treatment of respiratory tract infections for more than 40 years.[1] The first macrolide, erythromycin A (Figure 1), was introduced in the 1950s and has enjoyed widespread clinical use especially in patients with allergic reactions to penicillin. However, a major problem with erythromycin is its instability in the acidic environment of the stomach. The acid degradation products generated by intramolecular hemiketal formation between the hydroxyls at C-6 and C-12 with the C-9 carbonyl are responsible for its poor bioavailibility and gastrointestinal (GI) side effects.[2] To minimize the acid instability and hemiketal formation, the second generation macrolide antibiotics, clarithromycin and azithromycin, were developed in the 1980s. These antibiotics successfully addressed the problem of acid-catalyzed hemiketal formation resulting in an improved pharmacokinetic profile and improved GI tolerability.[3]

During the past decade, however, the emergence of macrolide resistance has prompted further research directed toward the discovery of third generation macrolides that can effectively address resistance and other issues associated with current macrolide regimens.[4] In 1995 a novel series of macrolides was introduced. These compounds, known as ketolides, possess 3-keto and 11,-12-carbamate functionalities and show excellent activity against the major macrolide-resistant organisms.[5] In addition, the two most prominent ketolides, ABT-773[6] and telithromycin[7] (Figure 1), also have aromatic groups tethered to the macrolide core.

Macrolide resistance arises from two main mechanisms, the first being ribosome methylation by *erm* methyltransferases and the second being efflux by macrolide pumps. It is believed that the enhanced activity of ketolides toward macrolide-resistant organisms is due to the interaction of the tethered aryl group or anchor group with a secondary ribosomal binding site. This inhibits the *erm* mechanism by creating tighter ribosomal binding. The tighter binding also inhibits the *mef* mechanism by creating an influx rate of the ketolide that exceeds its efflux rate.[8] In this communication we wish to report the synthesis of a series of novel 6-*O*-homopropargyl ketolides and their activity against some key erythromycin-resistant pathogens (Figure 1).

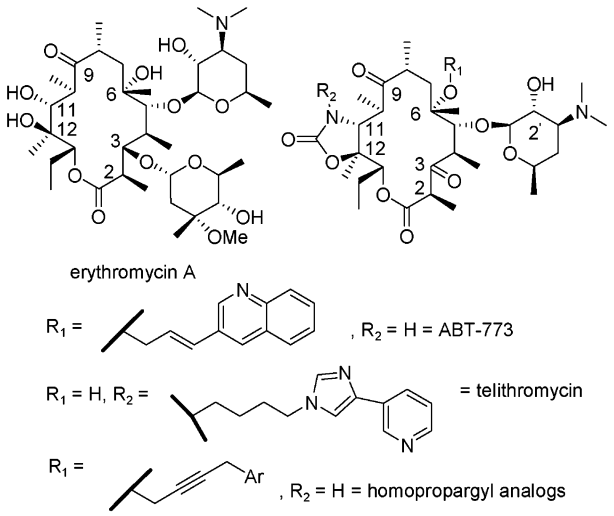


**Figure 1.** Structure of erythromycin A, ABT-773, telithromycin, and homopropargyl analogues.

**Chemistry.** Methodology was recently developed in our laboratories for the regioselective alkylation of the 6-hydroxyl group in erythromycin A with an allyl or propargyl group.[9] The propargyl compound provided a convenient source of starting material for further chemical modification leading to the homopropargyl series. The ketolide **1** was readily prepared in five steps from 6-*O*-propargyl erythromycin A (Scheme 1).[9] Since anion-generated stannylation was not feasible, the propargyl stannane **2** was chemoselectivly synthesized from **1** by treatment with tributyltin ethoxide at 110 °C in the absence of solvent.[10] The desired stannane **2** was obtained in 60% yield after column chromatography. The structure of **2** was confirmed via DQCOSY, HSQC, and gHMBC NMR experiments. Stille coupling[11] of **2** with various aryl methyl halides followed by deprotection of the 2′-benzoate in refluxing methanol provided the target compound **3** in yields ranging from 24% to 78% over two steps from **2**. Compound **2** was further elaborated to a series of biaryl compounds by a regioselective Stille coupling with 4-bromobenzyl bromide followed by deprotection of the 2′-benzoate to give **5**. Another palladium-catalyzed coupling of compound **5** with various arylstannanes then provided compounds of the general structure **6**. The 2-fluoro series represented by structure **4** was synthesized in the same

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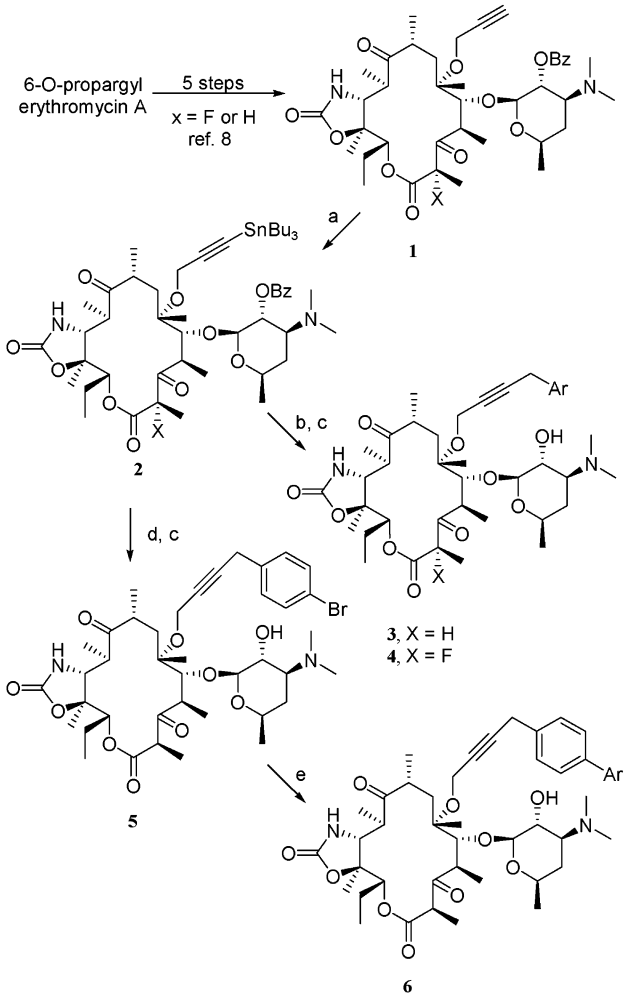
**Scheme 1**[a]



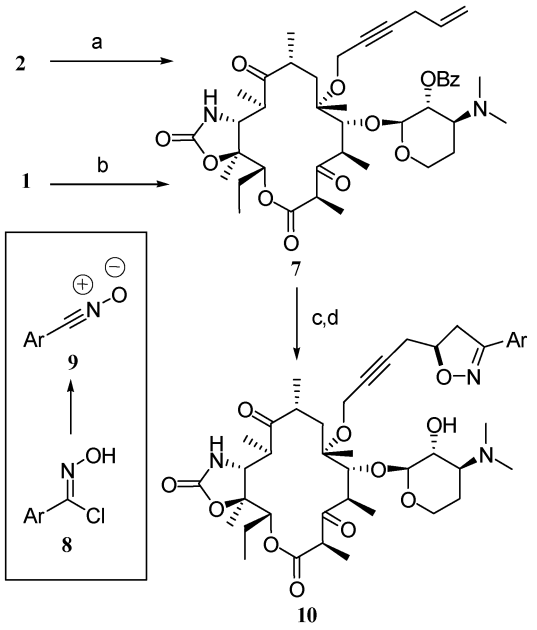

[a] Conditions: (a) $Bu_3SnOEt$, 110 °C, 60%. (b) $ArCH_2X$, $Pd(PPh_3)_4$, toluene, reflux. (c) MeOH, reflux. (d) bromobenzyl bromide, $Pd(PPh_3)_4$, toluene, reflux, 75%. (e) ArX, $Pd(PPh_3)_4$, toluene, reflux.

manner as described above by starting with the 2-fluoro-6-*O*-propargyl ketolide.

A second series of homopropargyl analogues was prepared by synthesizing the enyne intermediate **7** (Scheme 2). Compound **7** was generated by two protocols. The first method involved palladium-mediated coupling of stannane **2** with allyl bromide to give the enyne in a 60% yield. Alternatively, the enyne was prepared in a novel one-step reaction by treating **1** with allyl acetate, bis(1,5-cyclooctadiene)nickel (0) ($Ni(COD)_2$), and $P(O\text{-}iPr)_3$ in THF at 75 °C to give a 52% yield of **7** according to the procedure of Newman-Evans et al.[12] This method offers the advantage of shortening the synthesis and avoiding the use of tin. 1,3-Dipolar cycloaddition of **7** with the nitrile oxide **9** (generated in situ from arylchloroaldoxime **8**) followed by deprotection of the 2′-benzoate in refluxing methanol afforded isoxazole **10** as a 1:1 ratio of diastereomers in good yield (50%-70%).

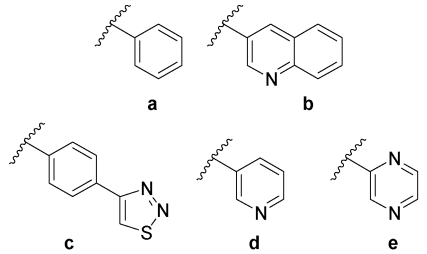
**Microbiology.** The antibacterial activity of the homopropargyl analogues was tested against a panel of representative pathogens selected from the Abbott clinical culture collection. To evaluate each analogue's potential to overcome macrolide-resistance, various macrolide-resistant strains were included. *Staphylococcus aureus* ATCC 6538P, *Streptococcus pyogenes* EES61, and *Streptococcus pneumoniae* ATCC 6303 are erythromycin-susceptible strains (Ery-S). *Staphylcoccus aureus* A5177 is an inducibly macrolide-lincosamide-streptogramin B ($MLS_B$) resistant strain encoded by an *erm*A gene. *Streptococcus pyogenes* 930 and *Streptococcus pneumoniae* 5979 are $MLS_B$ resistant strains encoded by *erm*B genes. *Streptococcus pyogenes* PIU 2548 and *Streptococcus pneumoniae* 5649 are efflux-resistant strains encoded by *mef*A and *mef*E genes, respectively. *Staphylococcus aureus* 1775 is a methicillin-resistant (MRSA) strain. *Haemophilus influenzae* DILL is an ampicillin-resistant strain with a $\beta$-lactamase positive determinant. The in vitro antibacterial activities are reported as minimum inhibitory concentrations (MICs) that were determined by the agar dilution method as recommended by the National Committee for Clinical Laboratory Standards. Table 1 shows the in vitro antibacterial activities of the homopropargyl analogues and reference compounds, erythromycin and telithromycin.

**Scheme 2**[a]




[a] Conditions: (a) allyl bromide, $Pd(PPh_3)_4$, toluene, reflux, 60%. (b) allyl acetate, $Ni(COD)_2$, $P(O\text{-}iPr)_3$, THF, 75 °C, 52%. (c) **8**, $NEt_3$, PhH, rt (two diastereomers in a 1:1 ratio). (d) MeOH, reflux, 60% over two steps.

**Chart 1.** Structures of Aryl (Ar) Groups




**Results and Discussion.** Since the majority of macrolide resistance results from methylation (*erm*) of the ribosome and efflux (*mef*), the reference compounds and all the homopropargyl ketolides (Chart 1) were tested against the pathogens listed in the microbiology section. The results tabulated in Table 1 show that

**Table 1.** In Vitro Antibacterial Activity of Homopropargyl Ketolides against Selected Pathogens

| | | MIC ($\mu$g/mL) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| organism | | **3a** | **3b** | **3c** | **4c** | **6d** | **6e** | **10a** | **10b** | Ery[a] | Teli[b] |
| *S. aureus* ATCC 6538P | Ery-S | 0.2 | 0.1 | 0.05 | 0.05 | 0.02 | 0.02 | 0.2 | 0.1 | 0.5 | 0.1 |
| *S. aureus* A5177 | *erm*A-i | 0.2 | 0.1 | 0.1 | 0.05 | 0.02 | 0.02 | 0.2 | 0.1 | >128 | 0.1 |
| *S. aureus* 1775 | *erm*A-c | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >128 | >100 |
| *S. pyogenes* EES61 | Ery-S | 0.06 | 0.015 | 0.03 | 0.03 | <0.004 | <0.004 | 0.02 | 0.01 | 0.015 | 0.004 |
| *S. pyogenes* 930 | *erm*B | 128 | 16 | 4 | 4 | 8 | 4 | >100 | >100 | >128 | 8 |
| *S. pyogenes* PIU 2548 | *mef*A | 0.25 | 0.25 | 0.125 | 0.25 | 0.125 | 0.015 | 0.2 | 0.2 | 8 | 2 |
| *S. pneumo* ATCC 6303 | Ery-S | 0.03 | <0.004 | <0.004 | 0.015 | <0.004 | <0.004 | <0.004 | <0.004 | 0.015 | 0.004 |
| *S. pneumo* 5979 | *erm*B | 16 | 2 | 8 | 4 | 0.125 | 0.03 | 128 | 128 | >128 | 8 |
| *S. pneumo* 5649 | *mef*E | 0.25 | 0.25 | 0.25 | 0.5 | 0.125 | 0.03 | 0.25 | 0.25 | 4 | 0.5 |
| *H. flu* DILL | Amp-R | 2 | 2 | 2 | 2 | 4 | 4 | 8 | 2 | 4 | 2 |

[a] Ery = erythromycin A. [b]Teli = telithromycin.

although erythromycin A is very potent against erythromycin-susceptible strains it is only weakly active against *mef*-resistance and inactive against *erm*-resistance. On the other hand, telithromycin showed activity against all the resistant pathogens tested except methicillin-resistant *S. aureus*.

Homopropargyl compound **3a** where the aryl group is an unsubstituted phenyl shows weak activity against erythromycin-resistant pathogens encoded by the *erm* gene. Clearly, its activity was also less affected by the efflux pump as its activity against *mef*-resistance pathogens was greater than that of telithromycin. Activities against *erm* as well as *mef* improved when the phenyl group was replaced by the 3-quinolyl group, **3b**. When the aryl group was converted to a biaryl system, typified by compounds **3c**, **6d**, and **6e,** activity against *erm*- and *mef*-resistance could be further increased with the greatest activity being found in **6d** and **6e**. Most importantly, compounds **6d** and **6e** demonstrated about 100-fold better activity than telithromycin against *S. pneunoniae* 5979, an *erm*-containing strain. We believe that the improved activity achieved by these homopropargyl analogues against *erm* strains was a result of an improved secondary interaction between the anchor group and the ribosome.

When fluorine was introduced into the 2-position, excellent activity against the resistant pathogens was retained. However, compound **4c** shows comparable activity to its desfluoro counterpart **3c**, indicating that introduction of a fluoro group at the C-2 position has no significant effect on its antibacterial activity.

The presence of an isoxazole in the compounds generated via [3 + 2] cycloadditions (**10a** and **10b**) resulted in marked decreases in activity against the *erm*-containing strains of *S. pyogenes* and *S. pneumoniae*. However, the activity remained the same for inducibly resistant *S. aureus*. Moreover, the compounds were active against efflux-resistant strains containing *mef*A or *mef*E genes.

The most active compounds were obtained when a heteroatom-containing biaryl was appended to the propargyl stannane. The activities of the compounds tested (compounds **3c**, **4c**, **6d**, and **6e**) were greater for *mef*-resistant *S. pyogenes* and *mef*-resistant *S. pneumoniae* as compared to telithromycin with compound **6e** showing a greater than 15-fold improvement against *mef*-resistant *S. pneumoniae*. Activity against *erm*-resistance in the biaryl systems was also improved as compared to that of telithromycin. Compound **6e** represents the most potent compound in the series exhibiting significant improvement in activity against both *erm*- and *mef*-resistant organisms as compared to telithromycin.

Typical of macrolides and ketolides, all the compounds tested showed no activity against methicillin-resistant *S. aureus*. They were, however, about one dilution better than erythromycin against the Gram-negative strain, *H. influenzae*.

**Conclusion.** A new series of homopropargyl ketolides was synthesized and evaluated for antibacterial activity against macrolide-susceptible and macrolide-resistant pathogens. Compounds containing either quinolyl or heteroatom-substituted biaryls were the most active against *erm*- and *mef*-containing strains with activities exceeding those of telithromycin. Compounds containing the isoxazole ring showed weak activity against *erm*-resistant *S. pyogenes* and *S. pneumoniae*. However, they did show excellent activity against inducibly *erm*-resistant *S. aureus* and both *mef*-resistant *S. pneumoniae* and *S. pyogenes*. The homopropargyl series of compounds offers enhanced activity as compared to telithromycin against resistant pathogens. This series presents a considerable opportunity for the development of new macrolide antibiotics to effectively combat the growing problem of macrolide resistance.

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**Supporting Information Available:** Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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