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Desmethyl Macrolides: Synthesis and Evaluation of 4,8,10-Tridesmethyl Telithromycin

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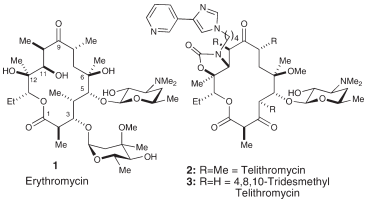
ABSTRACT There is an urgent need to discover new drugs to address the pressing problem of antibiotic resistance. Macrolide antibiotics such as erythromycin (**1**) are safe, broad-spectrum antibiotics used in the clinic since 1954. Herein, we report the synthesis and evaluation of 4,8,10-tridesmethyl telithromycin (**3**), a novel desmethyl analogue of the third-generation drug telithromycin (**2**), which is a semisynthetic derivative of **1**. Analogue **3** was found to possess antibiotic activity and was superior to telithromycin (**2**) when tested against resistant strains of *Staphylococcus aureus* possessing an A → T mutation at position 2058 (*Escherichia coli* numbering).

KEYWORDS Total synthesis, ketolide antibiotics, antibiotic resistance, telithromycin, desmethyl, analogues



The rapid emergence of antibiotic-resistant bacteria represents a serious public health threat.[1] The problem is compounded by the sharp decline in drug companies with active antimicrobial research programs; therefore, new sources of antibiotics are critical.[2,3] To address this need, we have initiated a structure-based drug design program wherein desmethyl analogues (i.e., $CH_3 \rightarrow H$) of the third-generation macrolide antibiotic telithromycin (**2**) are prepared via chemical synthesis (Figure 1). Herein, we report the synthesis and biological evaluation of 4,8,10-tridesmethyl telithromycin (**3**) against both wild-type and macrolide-resistant bacteria.

All macrolide antibiotics target the bacterial ribosome and reversibly inhibit the 50S subunit by binding in the peptidyl transferase center, thus blocking protein synthesis.[4] Telithromycin (**2**) is a third-generation semisynthetic drug derived from the classic macrolide antibiotic erythromycin (**1**) and has been used in the clinic since 2004.[5]

Macrolide antibiotic resistance mechanisms fall into three major categories: (1) enzymatic modification of the drug, (2) drug efflux from the cell encoded by *mef* genes, and (3) ribosomal modification arising from either ribonucleotide N-methylation of residues critical for binding (e.g., A2058) or single point mutations (e.g., A2058G). Third-generation ketolides have largely addressed the first two issues yet remain susceptible to ribosomal modification.[4,5]

The rationale behind our desmethylation strategy is grounded in structural data obtained by Steitz and co-workers who successfully cocrystallized various macrolide drugs with mutant large ribosomal subunits of the archaeon *Haloarcula marismortui* (Hm).[6] Unlike eubacteria that possess an adenine at 2058, all archea possess a guanine (*Escherichia coli* numbering) and do not efficiently bind macrolides **1** and **2**. However, a point mutation of guanine to adenine at position 2058 of 23S rRNA (i.e., G2058A) rendered mutants susceptible to the antibiotics, allowing the structure of telithromycin (**2**) bound to the HmA2058 mutant at 2.6 Å resolution to be obtained (Figure 2A). From these data, Steitz argued that in bacteria, A2058G mutations confer resistance due to a steric clash of the amino group of guanine 2058 with C4 methyl of the drug (Figure 2B, both in red). In turn, we hypothesize that replacement of the C4 methyl group in **2** with hydrogen should relieve this steric clash and thus offer a means of addressing antibiotic resistance arising from mutation.

To rapidly access analogues for biological testing by facilitating synthesis, we first targeted 4,8,10-tridesmethyl telithromycin (**3**). Prior to embarking on our synthetic campaign, we examined the conformational consequences of replacing the methyl groups in telithromycin (**2**) with hydrogens (Figure 3).

A priori, the removal of any methyl group from the 14-membered macrolactone scaffold of telithromycin (**2**) has two major consequences that directly impact binding: (1) conformational changes caused by removing *syn*-pentane interactions that serve to bias the classical Perun−Celmer modified diamond lattice structure and (2) loss of potential van der Waals contributions (ΔG_{vdW}) to the overall binding.[7] To address the former, we employed the conformationally sampled pharmacophore (CSP) protocol that uses molecular dynamics to look at distribution of distances (Figure 3A−D).[8]

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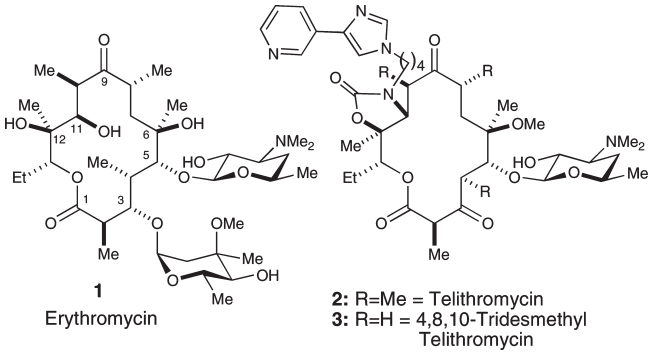

Figure 1. Structures of erythromycin (**1**), telithromycin (**2**), and novel analogue 4,8,10-tridesmethyl telithromycin (**3**).

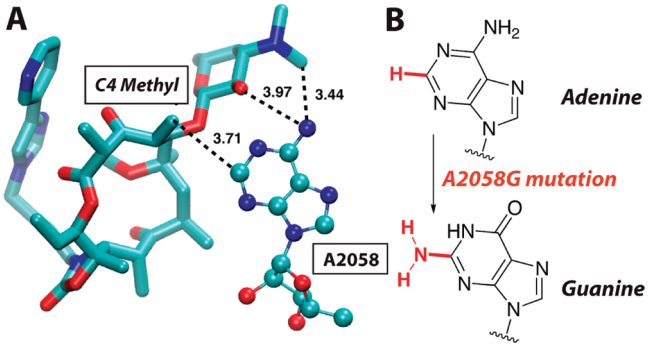

Figure 2. (A) Telithromycin and A2058 interactions in Hm with select distances in Angstroms (Steitz et al., PDB = 1YIJ). (B) Steric consequences of A2058G mutation.

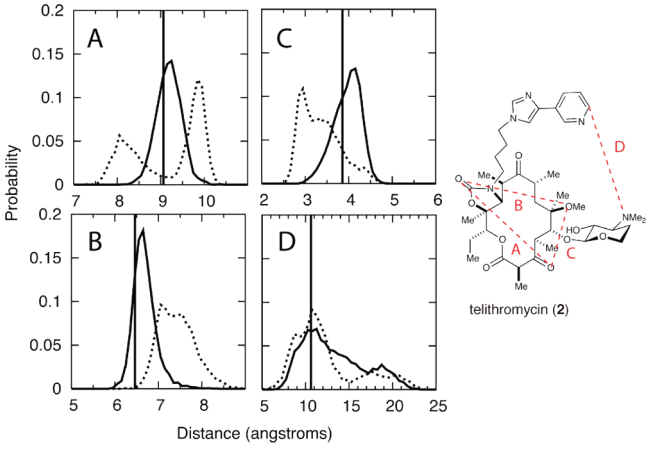

Figure 3. Conformationally sampled pharmacophore (CSP) analysis of telithromycin (**2**, solid line) and analogue **3** (dashed line).

While the distributions for desmethyl analogue (**3**, dashed lines) differ from those of telithromycin (**2**, solid lines), analogue **3** does sample conformations that overlap with **2**, suggesting that **3** can bind the ribosome.

To test our hypothesis and probe the consequences of a desmethylation strategy, we employed chemical synthesis to access material for biological evaluation (i.e., minimum inhibitory concentrations or MICs).[9] The total synthesis of 4,8,10-tridesmethyl telithromycin (**3**) is shown in Scheme 1.

We began with the preparation of known diol **10** via known aldehyde **8** (Scheme 1).[10−12] Swern oxidation of commercially available 3-benzyloxy-propanol (**11**), addition of 2-propenyl MgBr, and subsequent Johnson−Claisen orthoester rearrangement afforded enoate **12** in 48% overall yield. Reduction of the ester and protection of the newly formed alcohol as its *tert*-butyldimethylsilyl (TBS) ether provided **13** (78% yield over two steps).[13] Sharpless dihydroxylation (AD mix-β) established the requisite stereochemistry of hydroxyls at C5 and C6 in 91% yield (er > 20:1).[14,15] Selective protection of the secondary C5 alcohol with triethylsilylchloride (TESCl) followed by methylation of the tertiary C6 alcohol with Me_3OBF_4 and Proton Sponge afforded **14** in 80% over two steps.[16] Hydrogenolysis of the benzyl ether (85% yield) followed by Swern oxidation furnished aldehyde **15**, which was subjected to an Evans aldol reaction with **16** to set the stereochemistry at C2 and C3.[17] In the event, desired aldol product **17** was obtained in 78% yield (dr > 20:1) over two steps. Protection of the aldol with TBSOTf and removal of the auxiliary secured acid **18** in 85% yield (two steps). Chemoselective Yamaguchi esterification of **18** and diol **10** afforded ester **19** in 78% yield.

To prepare the 14-membered macroketolactone, we employed a ring-closing metathesis (RCM) strategy first established by Kang.[18] Toward this end, we first removed the primary TBS ether chemoselectively with tetrabutylammonium fluoride (TBAF) and AcOH to furnish an alcohol (60% yield, 86% borsm) that was oxidized to the aldehyde with the Dess−Martin periodinane (DMP).[19] The addition of vinyl MgBr and subsequent DMP oxidation afforded vinyl ketone **20** in 60% yield overall. Treatment of dienone **20** with 20 mol % Grubbs' second-generation catalyst effected the desired RCM, affording macroketolactone **21** in 90% yield (borsm).[20]

To chemoselectively install desosamine at the C5 position, it was critical to (1) reduce the C9 ketone to avoid ketalization with the hydroxyl at C5 and (2) protect the tertiary C12 hydroxyl to avoid undesired glycosylation.[21] Toward these goals, the C9 ketone was first subjected to a Luche reduction. Silylation of C9 and C12 hydroxyls with TESOTf and subsequent treatment with *p*-toluenesulfonic acid (PTSA) selectively left only the tertiary C12 hydroxyl protected (52% yield over three steps). Chemoselective silylation of the allylic C9 alcohol with TESCl furnished **22**, which was subjected to glycosylation with known thiopyrimidine desosamine donor **23** (50% yield over two steps) under the agency of AgOTf and 2,6-di-*t*-Bu-4-Me-pyridine (DTBMP).[22,23] Fluoride-mediated cleavage of silyl ethers at C9, C12 and DMP oxidation afforded glycosylated macroketolactone **24** (84% yield over two steps).

The end game for desmethyl analogue **3** began with a sequence employed by Aventis[24] to prepare C11−C12 oxazolidinones originally developed by Baker and co-workers at Abbott.[25] Activation of the C12 alcohol with NaH and carbonyldiimidazole (CDI) and treatment with primary amine **25**[26] effected a tandem carbamoylation/intramolecular aza-Michael sequence to stereoselectively afford oxazolidinone **26** in 35% overall yield. Removal of the C3 TBS ether with tris(dimethylamino)sulfonium difluorotrimethylsilicate (TAS-F) proceeded in 70% yield.[27] Corey−Kim

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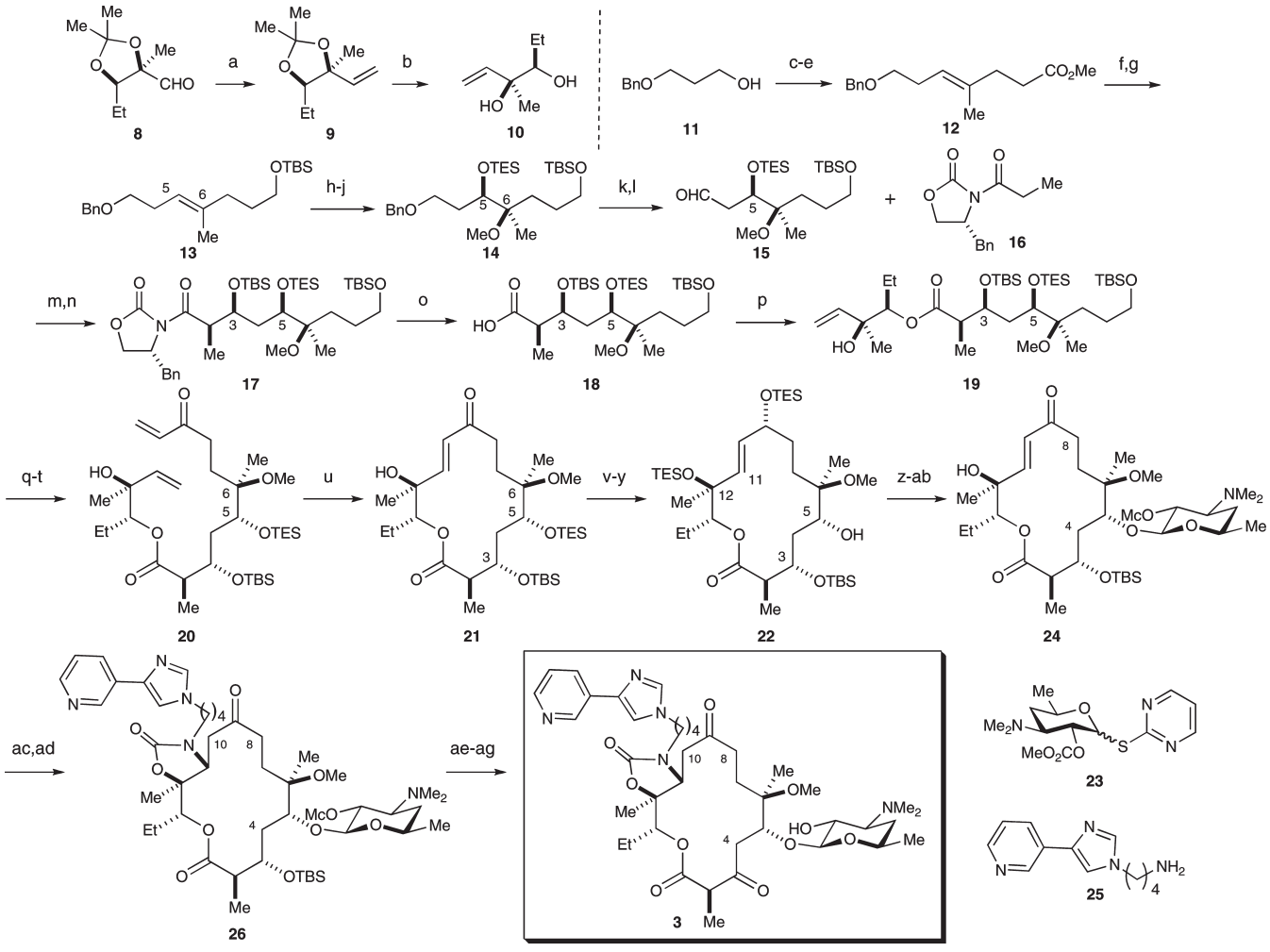
Scheme 1. Synthesis of 4,8,10-Tridesmethyl Telithromycin (**3**)[a]



[a] Reagents and conditions: (a) $Ph_3P=CH_2$, THF. (b) 1 N HCl(aq), MeOH, 53% from **7** (see Supporting Information). (c) $(COCl)_2$, DMSO, Et_3N. (d) 2-Propenyl MgBr, THF. (e) $(MeO)_3CCH_3$, $EtCO_2H$, 48% from **11**. (f) LAH, Et_2O. (g) TBSCl, imidazole, 78% from **12**. (h) AD mix-β, 91%, er > 20:1. (i) TESCl, imidazole. (j) Me_3OBF_4, Proton Sponge, 80% (two steps). (k) H_2, Pd/C, 85%. (l) $(COCl)_2$, DMSO, Et_3N. (m) Bu_2BOTf, Et_3N, 78%, dr > 20:1 (two steps). (n) TBSOTf, 2,6-lutidine. (o) LiOOH, THF, H_2O, 85% (two steps). (p) $Cl_3PhCOCl$, Et_3N, DMAP, **10**, 78%. (q) TBAF, AcOH, 86% (borsm). (r) DMP, $NaHCO_3$. (s) vinyl MgBr, THF. (t) DMP, 60% (three steps). (u) 20 mol % Grubbs II cat., 90% (borsm). (v) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, dr = 7:1. (w) TESOTf, 2,6-lutidine. (x) PTSA, 52% (three steps). (y) TESCl, imidazole. (z) Compound **23**, AgOTf, DTBMP, 50% (two steps). (aa) 1 M TBAF, THF, 95%. (ab) DMP, CH_2Cl_2, 88%. (ac) NaH, CDI, THF/DMF. (ad) Compound **25**, 35% over two steps. (ae) TASF, DMF/H_2O, 70%. (af) NCS, DMS, Et_3N, 75%. (ag) MeOH, 60%.

oxidation furnished the C3 ketone; methanolysis of the methyl carbonate on the C2′-position of desosamine delivered 4,8,10-tridesmethyl telithromycin (**3**) in 45% yield over two steps.[28] With desmethyl analogue **3** in hand, we tested it against several bacterial strains of *E. coli* and *Staphylococcus aureus* using telithromycin (**2**) as comparator (Table 1).

While both resistant strains (entries 1 and 5) were not susceptible to either macrolide, both *E. coli* wild-type and A2058G mutant (entries 2 and 3) were inhibited by both analogue **3** and telithromycin (**2**). Moreover, desmethyl analogue **3** was less potent than telithromycin (**2**) by a factor of 64 (entries 2 and 3). As described, this may be due to the conformational flexibility of **3** vis-à-vis **2**, in addition to loss of van der Waals contacts at C4, C8, and C10. Curiously,

Table 1. Minimum Inhibitory Concentration (MIC) Values in μg/mL for 4,8,10-Tridesmethyl Analogue (**3**) and Telithromycin (**2**)

entry	strain	bacteria	wt/mutant	MIC (**3**)	MIC (**2**)
1	SQ171/2058G	*E. coli*	A2058G	> 512	> 512
2	DK/pKK3535	*E. coli*	wt	32	0.5
3	DK/2058G	*E. coli*	A2058G	64	1
4	UCN14	*S. aureus*	A2058T	32	> 256
5	ATCC33591	*S. aureus*	*ermA*	> 128	> 128

desmethyl analogue **3** was found to be more potent than telithromycin (**2**) against *S. aureus* clinical strain UCN14 with an A2058T mutation (entry 4).[29] These results demonstrate that structural simplification (i.e., function-oriented synthesis)[30] and/or molecular editing[31] of established antibiotics can result in

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analogues with improved activity against A2058T ribosomal mutants.

In conclusion, we have prepared 4,8,10-tridesmethyl telithromycin (**3**), a desmethyl analogue of ketolide antibiotic telithromycin (**2**), by chemical synthesis. We were able to prepare a total of 12.1 mg of analogue **3** in 23 operations (42 steps overall, 31 steps in the longest linear sequence), which was found to inhibit bacterial growth. In addition, our analogue was more potent than telithromycin against an A2058T mutant. While the bioactivity data do not directly support our original hypothesis, the synthesis and evaluation of telithromycin analogues bearing methyl at C8 and C10 are needed to address this. We are currently working toward that end; results will be reported in due course.

SUPPORTING INFORMATION AVAILABLE

General experimental protocols, including the preparation of diol **10**, and characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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