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

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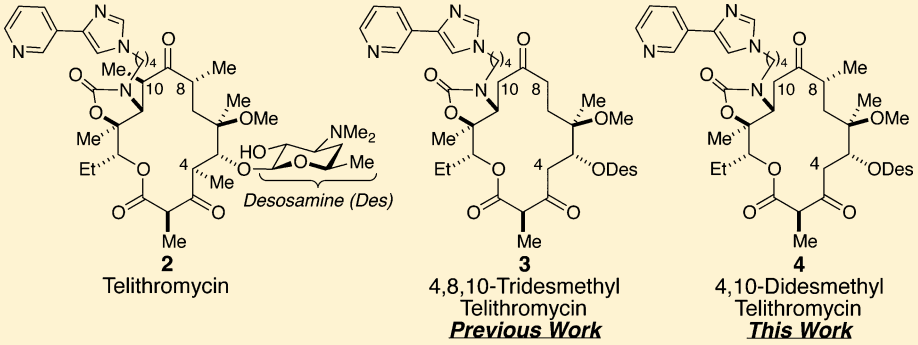
Supporting Information



ABSTRACT: Novel sources of antibiotics are required to keep pace with the inevitable onset of bacterial resistance. Continuing with our macrolide desmethylation strategy as a source of new antibiotics, we report the total synthesis, molecular modeling, and biological evaluation of 4,10-didesmethyl telithromycin (**4**), a novel desmethyl analogue of the third-generation drug telithromycin (**2**). Telithromycin is an FDA-approved ketolide antibiotic derived from erythromycin (**1**). We found 4,10-didesmethyl telithromycin (**4**) to be four times more active than previously prepared 4,8,10-tridesmethyl congener (**3**) in MIC assays. While less potent than telithromycin (**2**), the inclusion of the C-8 methyl group has improved biological activity, suggesting that it plays an important role in antibiotic function.

KEYWORDS: *total synthesis, ketolide antibiotics, antibiotic resistance, telithromycin, molecular modeling, desmethyl analogues*

The rapid development of bacterial resistance to antibiotic drugs, which is a natural consequence of their use and abuse, has resulted in a global health crisis. To exacerbate the problem, many pharmaceutical companies have terminated their antimicrobial research programs due to economic pressures. Thus, new sources of antibiotics are critical.[1] To address this need, we recently launched a structure-based drug design program wherein desmethyl analogues (i.e., $CH_3 \rightarrow H$) of the third-generation macrolide antibiotic telithromycin (TELI) (**2**) are made via synthesis (Figure 1).[2,3] We have reported the total synthesis, molecular modeling, and biological evaluation of 4,8,10-tridesmethyl TELI (**3**) against both wild-type and macrolide-resistant bacteria, which was found to be active against various bacterial strains (vide infra). Herein, we report the total synthesis, molecular modeling, and biological evaluation of 4,10-didesmethyl TELI (**4**), our second desmethyl analogue of **2**. Importantly, we have installed the (*R*)-C-8 methyl group in a chemo- and stereoselective manner by means of substrate-controlled asymmetric synthesis,[4] enabling us to test the consequences of a single methyl group on bioactivity. All macrolide antibiotics target the 50S subunit of the bacterial ribosome by reversibly binding in the peptidyl transferase center, thus blocking protein synthesis.[5] TELI (**2**) is a third generation semisynthetic drug used clinically since 2004 and is derived from the flagship macrolide antibiotic erythromycin (**1**).[6]

All macrolide antibiotics based on the erythromycin scaffold are semisynthetic (i.e., prepared from erythromycin). Our approach distinguishes itself from others in that total synthesis is employed to access each analogue, which allows significant flexibility and provides ample opportunity to explore the chemical space of this important class of safe and effective broad-spectrum antibiotics.[7,8]

Antibiotic resistance mechanisms fall into three major categories: (1) drug modification, (2) drug efflux, and (3) target (i.e., ribosomal) modification arising from either ribonucleotide *N*-methylation of residues critical for binding (e.g., A2058) or single point mutations (e.g., A2058G).[9]

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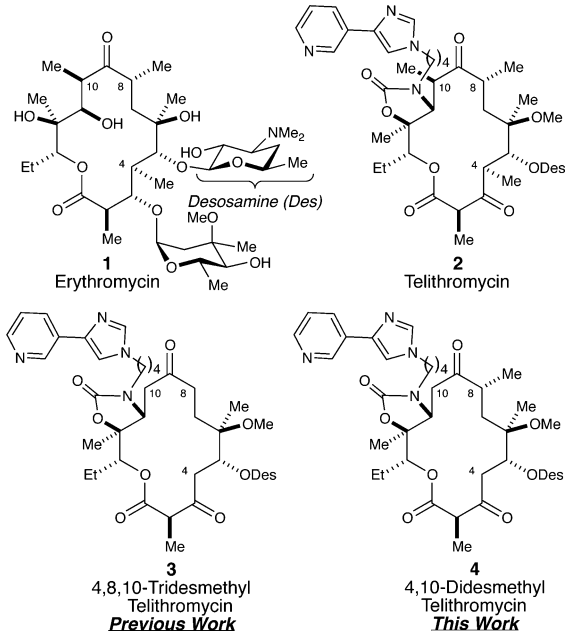


Figure 1. Structures of erythromycin (**1**), TELI (**2**), and novel analogues 4,8,10-tridesmethyl TELI (**3**) and 4,10-didesmethyl TELI (**4**).

Inspiration for our desmethylation strategy is derived from Steitz's elegant structural studies of macrolide drugs (e.g., **1** and **2**) cocrystallized with 50S ribosomal subunits of the archaeon *Haloarcula marismortui* (Hm).[10]

Unlike eubacteria that possess 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 TELI (**2**) bound to the HmA2058 mutant at 2.6 Å resolution to be obtained (Figure 2A). Thus,

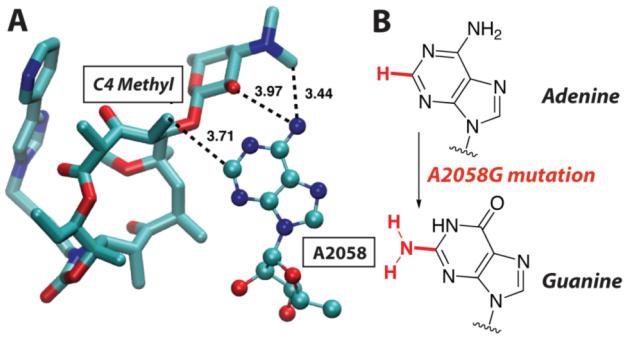


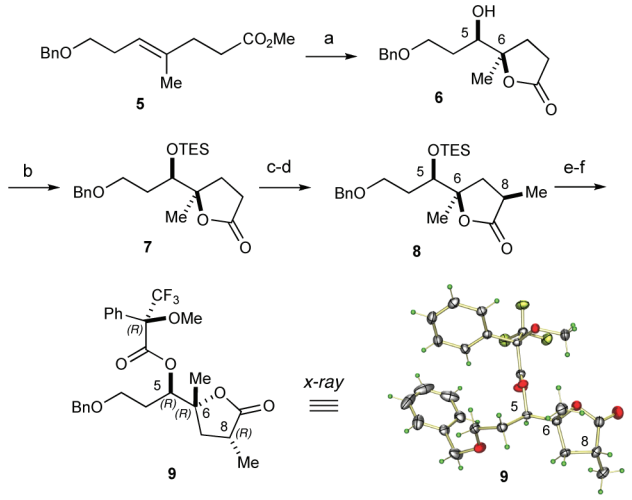
Figure 2. (A) TELI and A2058 interactions in *H. marismortui* with select distances in Angstroms (Steitz et al. PDB = 1YIJ). (B) Steric consequences of A2058G mutation.

Steitz showed that A2058G mutations in bacteria confer resistance by (1) loss of hydrogen bonding to the C-2′ hydroxyl of desosamine and (2) a steric clash of the exocyclic C-2 amino group of guanine 2058 with C-4 methyl of the macrolide drug (Figure 2B). We in turn hypothesized that replacing the C-4 methyl group with hydrogen (i.e., desmethylation) should relieve the steric clash component as other residues within the ribosome (e.g., 2059) can also form hydrogen bonds with desosamine's hydroxyl.[11] The rationale behind removing methyls at C-8 and C-10 was to (1) simplify chemical synthesis and (2) understand their roles in antibiotic function.

To test the consequences of adding another methyl vis-à-vis our simplest analogue **3** while probing the effects of removing the C-4 and C-10 methyl groups of TELI (**2**), we launched a total synthesis of **4** to realize biological evaluation (i.e., minimum inhibitory concentrations, or MICs).[12]

The installation of the requisite (*R*)-C-8 methyl group commenced with enoate **5**, which was utilized in the synthesis of tridesmethyl analogue **3** (Scheme 1).[2,3] Sharpless dihydrox-

Scheme 1. Installation of (*R*)-C-8 Methyl Group and Stereochemical Confirmation[a]



[a]Reagents and conditions: (a) AD mix-*β*, 85%, er >20:1. (b) TESCl, imidazole. (c) LDA, MeI. (d) LDA, Me_3CCO_2H (dr = 6:1), 54% over three steps or LDA, Ph_3CCO_2H (dr = 14:1, 82% over three steps). (e) TBAF, THF. (f) DCC, (*R*)-MTPA, DMAP, 60% over two steps.

ylation (AD mix-*β*) of **5** furnished *γ*-lactone **6** by in situ lactonization of the newly formed C-6 hydroxyl in 85% yield (er > 20:1).[13,14] Protection of the C-5 alcohol as its triethylsilyl (TES) ether was accomplished with TESCl and imidazole. Treatment of **7** with lithium diisopropylamide (LDA) at −78 °C and alkylation with MeI afforded a (*S*)-C-8 methyl intermediate. Inversion at C-8 to obtain the (*R*) configuration was accomplished by (1) re-enolization with LDA and (2) quenching with trimethylacetic acid (dr = 6:1, 54% overall yield from **6**).

Alternatively, quenching the enolate with a bulklier acid source (e.g., triphenylacetic acid) greatly improved the selectivity of epimerization (dr = 14:1, 82% overall from **6**). Confirmation of the stereochemical course of the experiment was obtained by preparing Mosher ester **9** and subsequent single crystal X-ray analysis, which was realized by removing the TES ether on the C-5 hydroxyl with tetrabutylammonium fluoride (TBAF) and esterification with (*R*)-*α*-methoxy-*α*-(trifluoro-methyl)phenylacetic acid (MTPA).[13,14]

Subsequent steps in the synthesis of 4,10-didesmethyl TELI (**4**) were guided by the previously reported synthesis of tridesmethyl congener **3** (Scheme 2).[2,3] Thus, lactone **8** was reduced with $LiAlH_4$ and the primary alcohol chemoselectively protected as its *tert*-butyldimethylsilyl (TBS) ether to afford **10** (78% yield over two steps). Methylation of the tertiary C-6 alcohol was accomplished with MeOTf and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) to furnish **11** in 75% yield. The use

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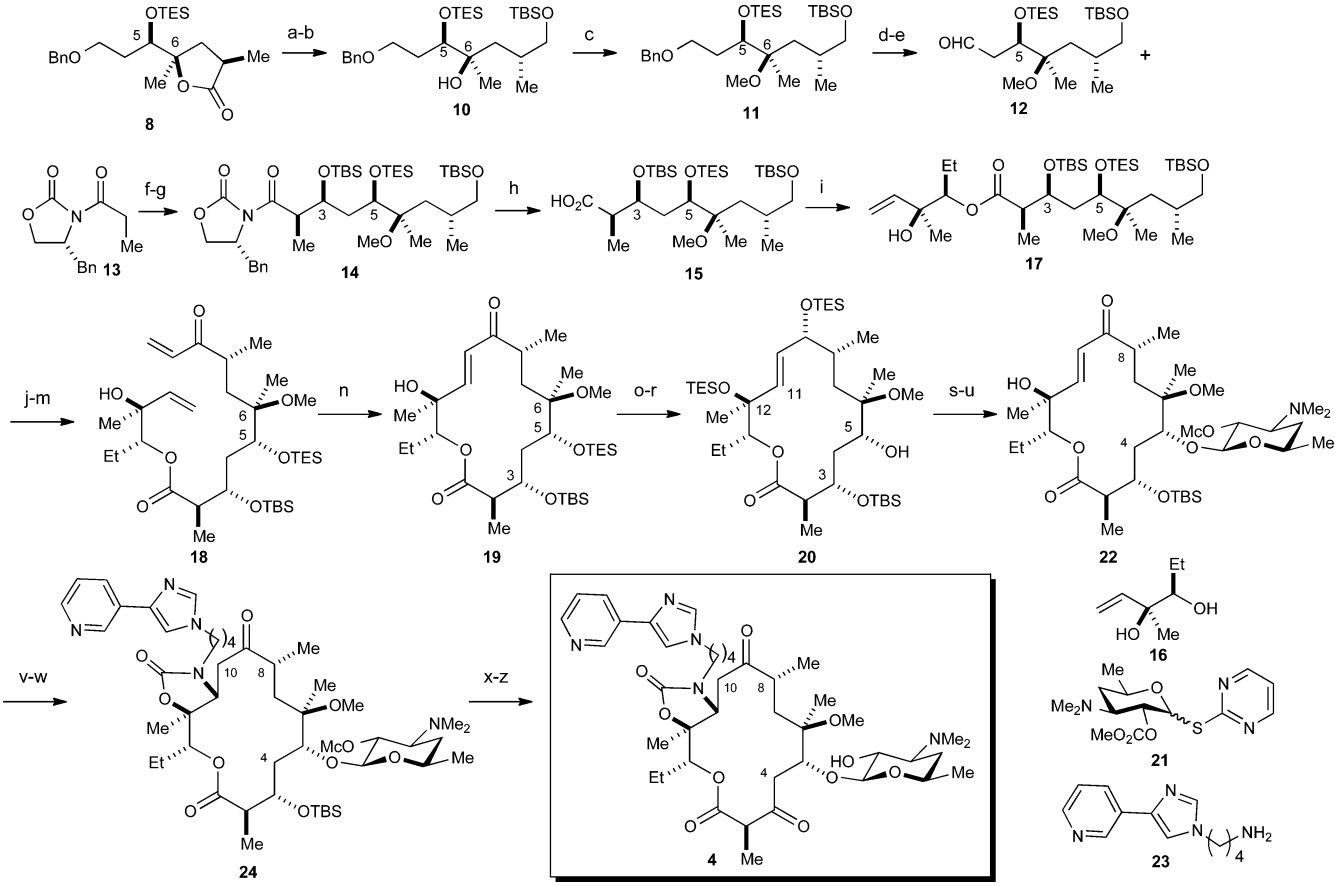
Scheme 2. Synthesis of 4,10-Didesmethyl TELI (4)[a]



[a]Reagents and conditions: (a) $LiAlH_4$, THF. (b) TBSCl, imidazole, 78% over two steps. (c) MeOTf, DTBMP, 75%. (d) H_2, Pd/C, 80%. (e) $(COCl)_2$, DMSO, Et_3N. (f) Bu_2BOTf, Et_3N, dr > 20:1, 76% over two steps. (g) TBSOTf, 2,6-lutidine. (h) LiOOH, THF, H_2O, 88% over two steps. (i) $Cl_3PhCOCl$, Et_3N, DMAP, **16**, 78%. (j) TBAF, AcOH, 20%. (k) DMP, $NaHCO_3$. (l) Vinyl MgBr, THF. (m) DMP, 50% over three steps. (n) 20 mol % Grubbs' second generation catalyst, 60%. (o) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, dr = 5:2. (p) TESOTf, 2,6-lutidine. (q) *p*-TsOH, 30% over three steps. (r) TESCl, imidazole. (s) Compound **21**, AgOTf, DTBMP, 50% over two steps. (t) $HF{\cdot}Et_3N$, Et_3N, CH_3CN. (u) DMP, CH_2Cl_2, 60% over two steps. (v) NaH, CDI, THF/DMF. (w) Compound **23**, 45% over two steps. (x) TAS-F, DMF/H_2O, 75%. (y) NCS, Me_2S, Et_3N, 70%. (z) MeOH, 80%.

of Proton Sponge, previously employed in the synthesis of **3**, gave an inferior 35% yield. Hydrogenolysis of benzyl ether **11** (80% yield) afforded an intermediary alcohol that was oxidized to aldehyde **12** with the Swern method.

At this stage, the Evans aldol reaction with propionimide **13** was employed to set the configurations at both C-2 and C-3 positions.[15] In the event, the aldol adduct was isolated in 78% yield (dr > 20:1). Protection of the C-3 hydroxyl with TBSOTf to access **14** and removal of the auxiliary furnished acid **15** in 88% yield (two steps). Chemoselective Yamaguchi esterification of **15** with known diol **16** delivered ester **17** in 78% yield.[2,3]

To prepare the 14-membered macrolactone ring, we recruited the ring-closing metathesis (RCM) strategy previously employed in the simpler congener **3**.[2,3] To this end, we removed the primary TBS ether in **17** with TBAF buffered with AcOH (20% yield, unoptimized).[16] The resulting alcohol was oxidized to the aldehyde with the Dess–Martin periodinane (DMP);[17] moreover, the addition of vinyl MgBr and subsequent DMP oxidation afforded vinyl ketone **18** in 50% overall yield. Treatment of dienone **18** with 20 mol % Grubbs' second-generation catalyst effected the desired RCM, affording macroketolactone **19** in 60% yield.[18]

Stereo- and regioselective installation of desosamine at the C-5 hydroxyl paralleled the approach taken for **3**. Specifically, the C-9 ketone in **19** was reduced under Luche conditions to avoid ketalization with the C-5 hydroxyl with a dr of 5:2, and the C-12 hydroxyl was protected as its TES ether to prevent glycosylation with desosamine donor **21**.[2,3] Silylation of both C-9 and C-12 hydroxyls with TESOTf and subsequent treatment with *p*-toluenesulfonic acid (*p*-TsOH) selectively deprotected the C-5 and the C-9 silyl ethers while leaving the tertiary C-12 hydroxyl TES-protected (30% yield over three steps).

Site-selective silylation of the secondary, allylic C-9 alcohol in the presence of the secondary C-5 alcohol with TESCl and imidazole furnished **20**, which was subjected to glycosylation with known desosamine donor **21** (50% yield over two steps) under the agency of AgOTf and DTBMP.[19,20] When removing both C-9 and C-12 silyl ethers, we found that $HF{\cdot}3Et_3N$ gave better selectivity as compared to TBAF. DMP oxidation afforded glycosylated macroketolactone **22** in 60% yield over two steps.

Installation of the C-11/C-12 carbamate was accomplished by employing methods originally developed by Baker at Abbott and later adapted by Hoechst Marion Roussel to prepare **2**.[21,6] Thus, treatment of **22** with NaH and carbonyldiimidazole

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(CDI) and subsequent addition of butylamine **23** effected a tandem carbamoylation/intramolecular aza-Michael sequence to stereoselectively afford oxazolidinone **24** in 45% overall yield.[22] Removal of the C-3 TBS ether with tris-(dimethylamino)sulfonium difluorotrimethylsilicate (TAS-F) proceeded in 75% yield.[23] Corey-Kim oxidation afforded the C-3 ketone (70% yield), and methanolysis of the methyl carbonate on the C-2′ position of desosamine delivered target molecule 4,10-didesmethyl TELI (**4**) in 80% yield.[24]

With **4** in hand, we initiated biological evaluation by testing its activity against several bacterial strains of *E. coli* and *Staphylococcus aureus*.[12] TELI (**2**) and 4,8,10-tridesmethyl TELI (**3**) were used as comparators (Table 1).[2,3]

Table 1. MIC Values in μg/mL for 4,8,10-Tridesmethyl Analogue (3), 4,10-Didesmethyl Analogue (4), and TELI (2)

				Previous work		*This work*	
Entry	Strain	Bacteria	wt/mutant	MIC trides (3)[a]	MIC TELI (2)[a]	MIC dides (4)[b]	MIC TELI (2)[b]
1	SQ171/2058G	*E. coli*	A2058G	>512	>512	>512	>512
2	DK/pKK3535	*E. coli*	wt	32	0.5	8	0.5
3	DK/2058G	*E. coli*	A2058G	64	1	16	1
4	UCN14	*S. aureus*	A2058T	32	>256	>256	>128
5	ATCC33591	*S. aureus*	*ermA*	>128	>128	>128	>128

[a]Previous experiment carried out in EtOH. [b]Current experiment carried out in DMSO.

Although both resistant strains (entries 1 and 5) were not susceptible to any of the macrolides, both *E. coli* wild-type and A2058G mutant (entries 2 and 3) were inhibited by tridesmethyl analogue **3**, didesmethyl analogue **4**, and TELI (**2**). In these strains, the didesmethyl congener was 4-fold more potent than tridesmethyl variant. As compared to TELI (**2**), the novel analogue was 16-fold less potent. While the current evaluation was carried out in DMSO and not EtOH, the MIC values for TELI (**2**) were found to be identical in both, thus suggesting the potency of didesmethyl analogue **4** vis-à-vis **3** is not derived from solvent change. Thus, these data illustrate the consequences of deleting methyl groups from the erythronolide scaffold. Interestingly, **4** did not inhibit the growth of the UCN14 strain that carries an A2058T mutation as compared to **3**.

To facilitate interpretation of the MICs, we employed the conformationally sampled pharmacophore (CSP) approach using Hamiltonian Replica Exchange Dynamics (HREX MD), from which conformational properties of **2**, **3**, and **4** were obtained.[25] Probability distributions of distances between select points on TELI (**2**, black), 4,8,10-tridesmethyl (**3**, blue), and 4,10-didesmethyl (**4**, red) TELI are shown in Figure 3A–D. The distributions for both desmethyl analogues overlap well with that of TELI, consistent with both analogues binding to the ribosome. The additional conformational flexibility of **3** and **4** as seen in panels A–C of Figure 3 are suggested to contribute to the decrease in their MICs vs **2**. Consistent with the additional methyl in didesmethyl **4** vs tridesmethyl **3**, **4** has decreased conformational flexibility, which is supported by the improved MIC values. Altogether, these results indicate a model where removal of the methyl groups lead to increased conformational flexibility, which has a negative impact on the biological activity, a consideration that must be taken into account when removing the C-4 methyl group to avoid resistance associated with the A2058G mutation.

In conclusion, 4,10-didesmethyl TELI (**4**) was prepared via total synthesis using a RCM approach in 44 steps overall (32 steps in the longest linear sequence) from commercially available starting materials. The novel analogue was evaluated for biological activity and found to inhibit the growth of two bacterial strains, including an A2058G mutant. Significantly, the addition of an extra methyl group at C-8 resulted in a 4-fold increase in activity as compared to tridesmethyl congener, suggesting that it plays an important role in antibiotic function. We are currently working toward the synthesis of other desmethyl TELI analogues. Those results will be reported in due course.

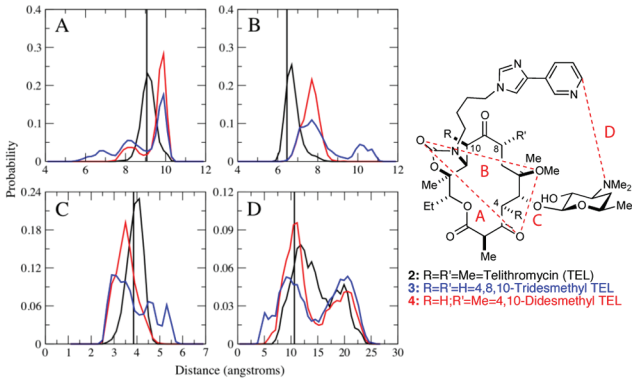

Figure 3. CSP probability distributions for TELI (**2**, black); 4,8,10-tridesmethyl TELI (**3**, blue); and 4,10-didesmethyl TELI (**4**, red). The vertical line corresponds to the crystallographic distances from PDB 1YIJ. Atom pairs represented in A–D are shown in the inset figure.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General experimental protocols, computational methods, C-8 epimerization studies, crystallographic details of **9**, full structural assignment of **22**, 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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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

TBS, *tert*-butyldimethylsilyl; TES, triethylsilyl; LDA, lithium diisopropylamide; DTBMP, 2,6-di-*tert*-butyl-4-methylpyridine; RCM, ring-closing metathesis; MIC, minimum inhibitory concentration; CSP, conformationally sampled pharmacophore; DMP, Dess–Martin periodinane; Tf, trifluoromethanesulfonyl; TBAF, tetrabutylammonium fluoride; HREX MD, Hamiltonian Replica Exchange Dynamics; TELI, telithromycin; CDI, carbonyldiimidazole; TAS-F, tris(dimethylamino)-sulfonium difluorotrimethylsilicate

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