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On the Macrocyclization of the Erythromycin Core: Preorganization is *Not* Required**

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Graphical Abstract

Keywords

erythromycin; natural products; C—H oxidation; lactonization; preorganization; cyclization

The erythromycins, discovered and isolated in the early 1950's, are the best-known members of the clinically important macrolide class of antibiotics.^[1] The 14-membered macrolactone core imbedded in these natural products has inspired new synthetic methodology for the construction of large ring lactones, beginning with the landmark synthesis of erythronolide B by the Corey group in 1978.^[2] During these studies, a single acetonide protecting group was utilized at the C-3/C-5 position. Similarly, this protecting group was used by the Masamune group years later for the synthesis of 6-deoxyerythronolide B (6-dEB).^[3] While no rationale was given for the use of this acetonide at the time, its function was revealed during the Woodward group's historic synthesis of erythromycin A in 1981. In three consecutive communications, the Woodward group extensively explored the conformational requirements for efficient acylation-based macrolactonization of erythromycin A seco acid derivatives. In particular, cyclic protecting groups were placed at varying positions in order to serve as biasing elements, *i.e.* artificial structural features intended to aid macrocyclic ring closure through substrate preorganization.^[4] The results from this study led the Woodward group to conclude that "certain structural features such as... cyclic protecting groups at C-3/C-5 and C-9/C-11 are *required for efficient lactonization*" and that "these structural

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requirements probably arise from conformation requirements for lactonization.” This conclusion – that preorganization is required for efficient cyclization – has become a well-accepted doctrine that has influenced the planning of all ensuing erythromycin syntheses (*vide infra*). We now report for the first time that conformational restraining elements are in fact *not required* for attaining efficient lactonization of the erythromycin core structure, 6-deoxyerythronolide B (6-dEB). Moreover, we demonstrate that the removal of biasing elements allows for more stereochemical flexibility in the cyclization of 6-dEB, enabling access of stereochemical analogues not readily accessible with them in place. Overall these findings require the revision of the thirty-year-old dogma that preorganization is mandatory for achieving macrocyclization of the erythromycins.

Inspired by the Woodward report, synthetic endeavors by Stork, Nakata, Yonemitsu, Danishefsky, Kochetkov, Hoffmann, Evans, Woerpel, Nelson, and our labs have reduced conformational space available to the seco acid backbones of the erythronolide series (*i.e.* 6-dEB, erythronolide B, and erythronolide A) through the use of six-membered ring protecting groups on C-3/C-5 and C-9/C-11 (Figure 1).^[5,6] In addition to cyclic protecting group scaffolds, other types of biasing elements (e.g. heterocycles, olefins, etc.) have been employed in similar positions to rigidify the hydroxy acid backbone.^[7,8] In a particularly notable example, Paterson validated this approach using two olefinic rigidifying elements in place of cyclic protecting groups.^[9] Furthermore, the Martin group demonstrated that steric bulk at C-5 (a desosamine sugar appendage) coupled to a C-9/C-11 cyclic acetal could enable cyclization.^[10] The steadfast application of one^[3] or more structural biasing elements in erythromycin’s synthetic history demonstrates the resonating impact of Woodward’s cyclization studies.

We recently reported a late-stage C–H oxidation strategy for the total synthesis of 6-deoxyerythronolide B (6-dEB), using a palladium(II)/bis-sulfoxide(**1**)-catalyzed C–H oxidative macrolactonization reaction developed in our labs.^[6, 11] As a part of our synthetic planning, we also chose to employ traditional cyclic protecting groups at C-9/C-11 and C-3/C-5 (**2**) in order to facilitate macrocyclic ring closure (Figure 3, *vide infra*). In the presence of these biasing elements, the 14-membered macrolide product was formed in 34% yield (45% recovered starting material, rSM; 56% yield recycled 2x) and with *>40:1 d.r. in favor of the natural C-13 diastereomer 3* (Figure 3). Based on the Hammond postulate, we attributed the inability to form the unnatural C13 diastereomer under the chelate-controlled C–H oxidative macrolactonization conditions to the large difference in ground-state product energies between the C13 diastereomers (the natural C-13 diastereomer was calculated to be 3 kcal/mol more stable than the C13 epimer). Similarly, while acylation-based Yamaguchi cyclization of **5** provided the natural macrolide **3** in high yield, the unnatural C-13 diastereomer (**4**) *could not be formed*.

Upon revisiting the Woodward studies, in which the positioning of cyclic protecting groups had been optimized for the natural erythromycin structure, we questioned whether the biasing elements were in fact hampering the cyclization of stereochemical analogues. In this vein, we recognized the absence of a *key control experiment*: attempted cyclization of a substrate completely devoid of biasing elements. Surprisingly, this experiment has remained unreported in the literature despite over 30 years of erythromycin syntheses. We therefore

set out to test the well-accepted idea that preorganization is necessary for cyclization of the erythromycin structure.

6-Deoxyerythronolide B, the aglycone precursor to the erythromycins, serves as the archetypical core of the polyketide macrolide antibiotics. In Nature, a seco acid bearing unadorned hydroxyl groups at C3, C5, and C11 and ketone functionality at C9 is cyclized to form 6-dEB, which is then hydroxylated at the C6 and C12 positions through enzymatic C–H functionalization to form erythronolides B and A, respectively. In addition to practical considerations of eliminating the formation of unwanted ring sizes^[12], the introduction of protecting groups was deemed necessary to prevent preorganization *via* 1,3-hydrogen bonding.^[13] Polypropionate molecules typically adopt conformations that minimize *syn*-pentane interactions, and thus will have inherent preorganization that may aid cyclization.^[14] However, in attempts to minimize artificial bias (bias not present in the native polypropionate structure), we selected methyl ether protecting groups for this study because of their inability to induce electrostatic preorganization while maintaining similar steric properties to the natural substrate's free hydroxyls. We reasoned that the use of any other protecting group, albeit potentially more synthetically useful, might inadvertently enable cyclization through either steric or electronic preorganization of the substrate.^[15] Accordingly, we synthesized a tetra-methyl ether protected hydroxy acid **8** and alkenoic acid **9** as the unbiased cyclization precursors (Figure 2).

The syntheses of both unbiased cyclization precursors **8** and **9** proceeded conveniently *via* a common intermediate, terminal olefin **7**. Global deprotection of a previously synthesized bis-acetal intermediate (**6**)^[6], followed by permethylation with Me₃OPF₆ furnished tetramethylated terminal olefin **7** (Figure 2). Straightforward chiral auxiliary removal with LiOOH provided the C–H oxidative cyclization substrate **9** in 99% yield. Intermolecular palladium(II)/bis-sulfoxide(**1**)-catalyzed C–H oxidation provided the C-13 oxidized products as diastereomeric allylic *p*-nitrobenzoates in 59% yield (1.2:1 d.r.). Chiral auxiliary hydrolysis with LiOOH and methanolysis of the *p*-nitrobenzoates furnished the unbiased seco acid **8** in 89% yield (over 2-steps, 1.2:1 d.r.). Notably, C–H oxidation greatly aided these studies by circumventing *de novo* syntheses of both epimeric Yamaguchi precursors **8**.^[6,16,17]

In order to evaluate if preorganization is needed for efficient macrolactonization of erythromycin precursors, we attempted a traditional acylation-based macrolactonization with unbiased hydroxy acids **8** (1.2:1 d.r., Figure 3).^[18,19] Strikingly, both the natural and unnatural C-13 diastereomeric hydroxy acids cyclized efficiently under standard Yamaguchi macrolactonization conditions, to afford the 14-membered macrolide products **10** and **11** in 70% yield (2:1 d.r.)! The ease with which these hydroxy acids cyclized in the absence of biasing elements is remarkable; matching the best yield obtained from Woodward's original preorganization studies.^[4] Despite decades of erythromycin syntheses, this is the first reported case where precursors to any member of the erythromycins have been cyclized successfully without the use of biasing elements to aid in 14-membered macrolide formation.

The C–H oxidative macrolactonization of unbiased alkenoic acid (**9** → **10/11**) also proceeded in the absence of biasing elements, providing *comparable yields* (36% yield, 44% recovered SM) to the analogue containing biasing elements (**2** → **3**, Figure 3). More importantly, in contrast to previous results with cyclic protecting groups at C-9/C-11 and C-3/C-5, the unnatural C13-diastereomer **11** could now be accessed from this unbiased precursor (1:3.3 d.r. from **9** vs. 1:>40 d.r. from **2**). Based on these results, we may conclude that Pd/bis-sulfoxide-catalyzed C–H oxidative macrolactonization of erythromycin precursors also does not require biasing elements, although such elements can significantly improve the diastereomeric outcome of the cyclization.

These results definitively demonstrate that artificial preorganization is not a requirement for the efficient cyclization of erythromycin's polypropionate core (6-dEB). In other words, the inherent conformation of the linear polypropionate structure is sufficient for facile macrolactonization.^[20] Significantly, we show that designed preorganization dramatically impacts the cyclization outcome of stereochemical analogues of the erythromycins. Removal of artificial biasing elements allows for increased stereochemical flexibility in the macrocyclization process. We anticipate that empowered with the knowledge that preorganization is not a requirement for cyclization, a broader evaluation of protecting groups^[15] will lead to the syntheses of stereochemically modified and/or functional group deficient analogues of erythromycin that may have been difficult and/or impossible to generate under the former perceived constraints.

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19. Allylic alcohol **5** (Figure 3) gave a comparable macrolactonization yield to that of an analogous saturated alcohol under identical Yamaguchi conditions (i.e. 87% and 86%, respectively, ref. 5g).
20. It is clear that the polypropionate core of the erythromycins (6-dEB) does not require artificial preorganization for macrocyclization. While we cannot exclude the possibility that erythronolide B or A would require preorganization due to the presence of a C6 and/or C12 hydroxyl(s), the fact that linear precursors to erythronolide B, A, and 6-dEB have all been cyclized under comparable Yamaguchi conditions using the same cyclic biasing elements in the same positions, strongly suggests that they will continue to behave in a very similar manner to 6-dEB (see ref. 5f, 5g, and 5i).

Figure 1.
Cyclic Biasing Elements in Erythromycin's Synthetic History

Conditions: (a) 1M HCl_(aq), 70% yield (b) Me₃OBF₄, proton spongeTM, 62% yield (c) **1** (10 mol%), BQ (2.0 equiv), *p*-NO₂BzOH (1.5 equiv), 45°C, 72 h, 1.2:1 d.r., 59% yield (d) LiOOH_(aq) (e) K₂CO₃, MeOH, 89% (2-steps) (f) LiOOH_(aq), 99% yield.

Figure 2.
Synthesis of hydroxy acid **8** and alkenoic acid **9**

Figure 3.
Macrocyclization Studies on Biased and Unbiased 6-dEB precursors