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ORIGINAL PAPER

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A computational approach to the synthesis of dirithromycin

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Abstract Dirithromycin is a macrolide antibiotic derived from erythromycin A. Dirithromycin is synthesized by the condensation of 9(*S*)-erythromycylamine with 2-(2-methoxyethoxy)-acetaldehyde. To gain insight into the synthesis, the condensation mechanism has been analyzed computationally by the AM1 method in the gas phase. First, the formation of the Schiff bases of dirithromycin and epidirithromycin from 9(*S*)-erythromycylamine and 2-(2-methoxyethoxy)-acetaldehyde were modeled. Then, the tautomerization of the Schiff bases to dirithromycin and epidirithromycin were considered. Finally, the epimerization of the Schiff base of epidirithromycin to the Schiff base of dirithromycin was investigated. Our results show that, even though carbinolamine forms faster for epidirithromycin than the corresponding structure for dirithromycin, dirithromycin is the major product of the synthesis.

Keywords Dirithromycin · Epidirithromycin · Schiff base · Ring–chain tautomerization · Epimerization

Introduction

Macrolide antibiotics are a class of antimicrobial compounds widely used against infectious diseases caused by a number of different microorganisms [1]. Erythromycin is the most commonly-used compound derived from natural sources by McGuire et al. in 1952 [2]. It binds to the larger 50S ribosomal subunit and prevents the translocation step in protein synthesis [3]. The major problem associated with erythromycin is its acid instability, leading to the formation of a 6,9-hemiacetal and its consequential degradation into products that are known for their poor pharmacokinetic profiles and gastrointestinal side effects [4]. The first attempt to improve acid stability and antibacterial activity of erythromycin was made by Sigal et al. in 1956 [5]. The 9-keto of erythronolide was replaced by an amino group, giving rise to 9(*S*)-erythromycylamine (**1**) which is poorly absorbed after oral administration to humans [6]. The limitations of this macrolide have been partly overcome by the preparation of a prodrug, dirithromycin, an oxazine derivative of erythromycylamine that affords high levels of the antibiotic in the tissue after oral administration [7].

Dirithromycin (**3**) is a semisynthetic derivative of erythromycin that is prepared by first converting erythromycin to 9(*S*)-erythromycylamine (**1**) and then condensing this intermediate with 2-(2-methoxyethoxy)-acetaldehyde (**2**), thereby creating a 9-*N*,11-*O*-oxazine chair-like ring system, which has the same macrocyclic conformation as 9(*S*)-erythromycylamine [8]. The stereochemistry of the methoxyethoxymethyl substituent attached to the newly formed oxazine ring has been established as the *R* configuration by X-ray crystallography [9].

The isomer of dirithromycin in which the methoxyethoxymethyl substituent on the oxazine boat-like ring possesses the opposite (*S*) stereochemistry is epidirithromycin (**4**). The macrocyclic conformation of epidirithromycin resembles the conformation of erythromycin A and it was initially detected as a minor constituent by chromatographic and NMR spectroscopic studies of the isomerization of dirithromycin in solution [10].

Dirithromycin is synthesized by treating 9(*S*)-erythromycylamine (**1**) with 2-(2-methoxyethoxy)-acetaldehyde (**2**) for several hours in acetonitrile (Scheme 1). HPLC analysis of the reaction mixture revealed that epidirithromycin (**4**) is formed very rapidly as the initial product and that it subsequently epimerizes until an 85:15 equilibrium ratio in favor of dirithromycin (**3**) is reached

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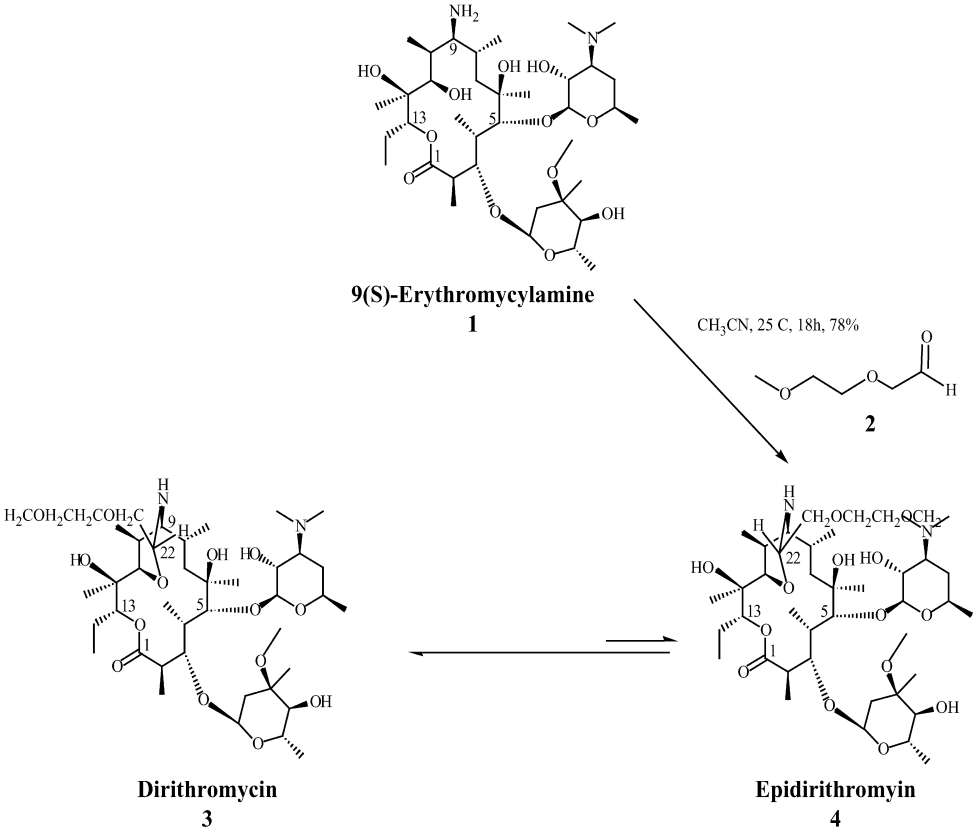
Scheme 1 Synthesis of dirithromycin



in solution [10]. The formation and the epimerization of epidirithromycin (**4**) is known to occur via a Schiff-base intermediate. The cyclization of the C-11 hydroxyl to the *Re* face of the Schiff-base intermediate gave dirithromycin (**3**), whereas the *Se* face of the intermediate gave epidirithromycin (**4**). The formation of the Schiff base controls the facial selectivity of the cyclization yielding oxazine. There is experimental evidence for the formation of the Schiff base of epidirithromycin, which immediately cyclizes to give epidirithromycin (**4**). The latter equilibrates to the thermodynamically more stable dirithromycin (**3**) at the end of the reaction [10].

In this study, the synthesis of epidirithromycin (**4**) and dirithromycin (**3**) via the condensation of the 9(*S*)-erythromycylamine (**1**) with 2-(2-methoxyethoxy)-acetaldehyde (**2**) has been rationalized with quantum mechanical calculations. The pathways for the formation of the Schiff bases for dirithromycin (**3im**) and epidirithromycin (**4im**) have been displayed and modeled. Interconversion of epidirithromycin (**4**) to dirithromycin (**3**) via epimerization of the Schiff bases has also been considered.

Methodology

For the calculations, the X-ray structure of dirithromycin was adopted from the literature [9]. On the other hand, due to the lack of X-ray structure of epidirithromycin, the backbone conformation of erythromycin was used. This choice is justified based on the similarity of the backbone structures for erythromycin and epidirithromycin [9, 11]. In order to generate the three dimensional structure of epidirithromycin, a boat-like oxazine ring with an alkyl group on the axial position was introduced between C-9 and C-11 on the backbone. At this stage, the conformational behavior of the alkyl group at C-22 was investigated by freezing the macrolide and searching for all the possible orientations of the alkyl group with respect to the ring with the MMFF94 force field in the SPARTAN 5.1.3 program [12]. The conformers located were fully optimized by the AM1 method in Gaussian 98 [13] and the global minimum for epidirithromycin was chosen among the structures thus generated.

Regarding the formation of the Schiff bases, first 9(*S*)-erythromycylamine (**1**), which has the same macrocylic conformation with dirithromycin (**3**), was generated by removing C-22 and the substituents on it and then optimized with the AM1 methodology. In order to find the stationary points corresponding to the local minima of the 2-(2-methoxyethoxy)-acetaldehyde (**2**), a conformational search was performed with the AM1 method. The conformers were fully optimized, the structures corresponding to local minima have been located and the global minimum was chosen among them.

The tautomerization of the Schiff bases was modeled for a small model in vacuum using density functional theory with the B3LYP/6-31G* [14, 15] method and solvent energies were estimated for acetonitrile (ε=35.9)

Scheme 2 Synthesis of a Schiff base

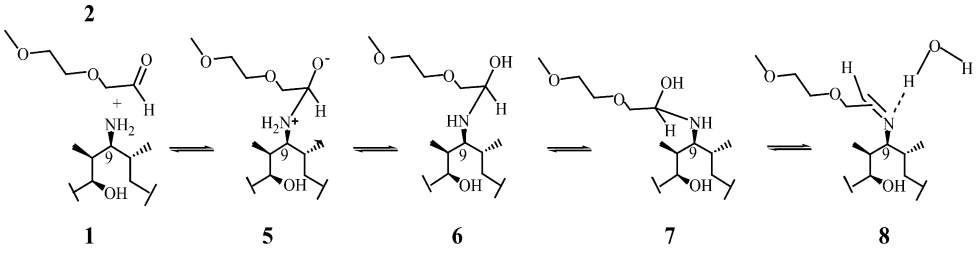
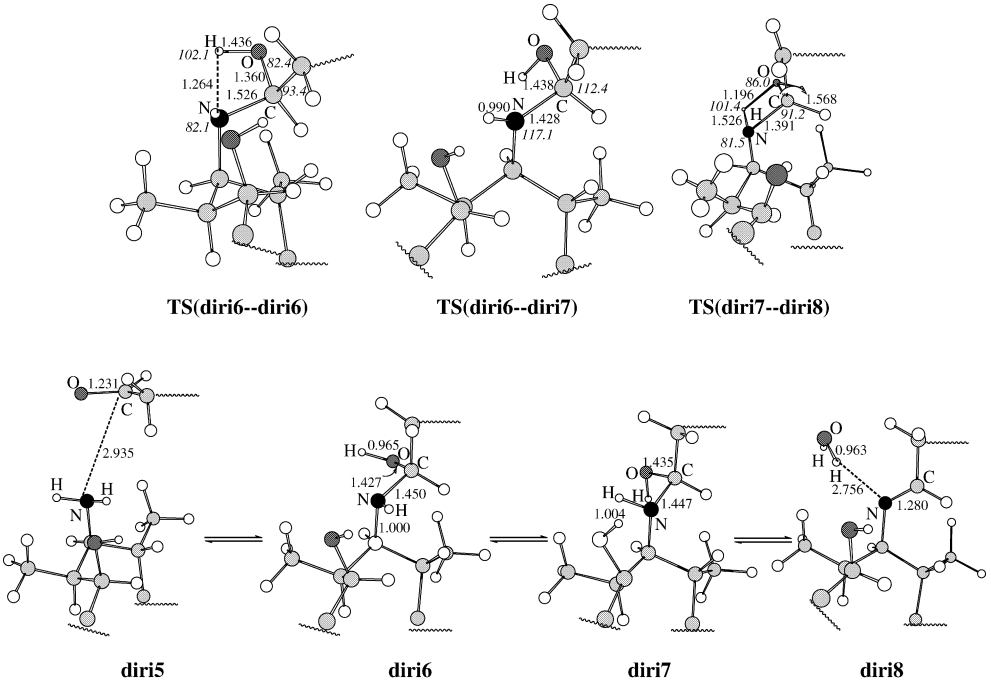


Fig. 1 Reaction pathway for the formation of **diri8**



using the solvation model CPCM [16] as implemented in Gaussian 98.

The three-dimensional structures of the Schiff bases of dirithromycin (**3im**) and epidirithromycin (**4im**) were considered as initial structures for the epimers of the Schiff bases of dirithromycin (**3im'**) and epidirithromycin (**4im'**). The conformational preference of the imine group around the C9–N bond was searched with the MMFF94 force field. The conformers located were fully optimized by the AM1 method, the structures corresponding to local minima were identified and the global minimum chosen among them.

All of the stationary points were characterized by a frequency analysis, which is also used to provide the Gibbs free energies. The intrinsic reaction coordinate (IRC) approach was used for the transition structures in all the mechanisms [17].

The condensation of the 9(*S*)-erythromycylamine (**1**) with 2-(2-methoxyethoxy)-acetaldehyde (**2**) yielding a Schiff base is shown in Scheme 2. The intermediates along these steps are denoted as (**5**), (**6**), (**7**) and (**8**). The prefix "**diri**" is used for the species modeled for the Schiff base of dirithromycin (**diri5**–**diri8**) (Fig. 1) and "**epi**" (**epi5**–**epi8**) for the corresponding species along the formation of the Schiff base of epidirithromycin. Furthermore, the perhydro 1,3-oxazines **9** and **11** were used as models for the tautomerization of the Schiff bases (**3im** and **4im**), as shown in Scheme 3 and Fig. 2. In this model system, the two conformers of the perhydro 1,3-oxazines (**9** and **11**) tautomerize yielding **10** and **12**. The tautomerization of the Schiff bases (**3im**) and (**4im**) followed by the formation of dirithromycin (**3**) and epidirithromycin (**4**) was also investigated. Finally, the epimerization of the Schiff base of epidirithromycin (**4im**) yielding the Schiff base of dirithromycin (**3im**) was modeled in two different pathways, as shown in Fig. 3.

Scheme 3 Chain–ring tautomerization of perhydro 1,3-oxazine

The energetics related to the formation of dirithromycin, **3** and epidirithromycin, **4** are shown in Table 1 and Table 2 by considering Gibbs free energies (AM1) and

Fig. 2 Tautomerization of the perhydro 1,3-oxazines (**9** and **11**)

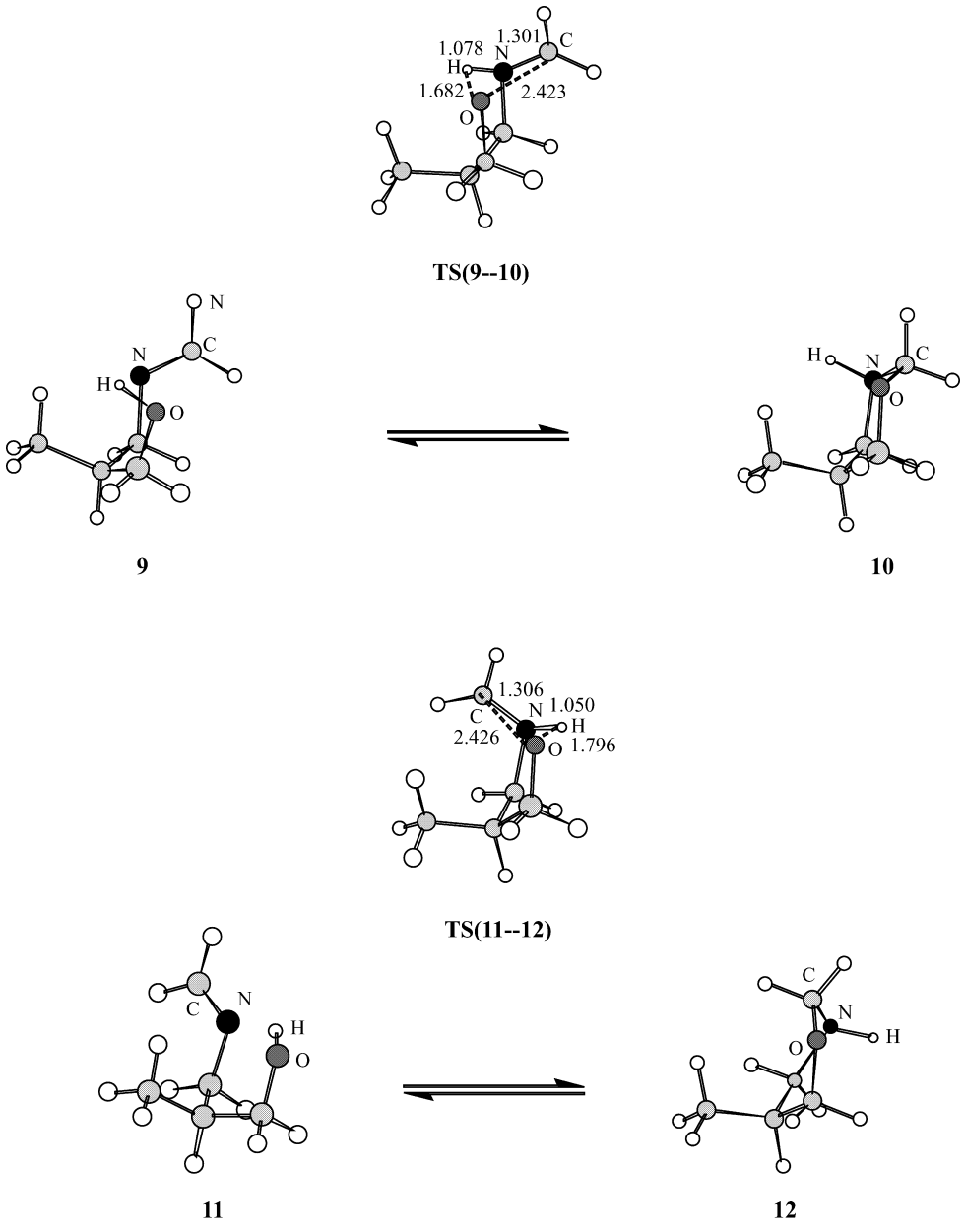


Table 1 Relative Gibbs free energies, G (AM1) and electronic energies, E_{el} (B3LYP/6-31G*//AM1) for the formation of **diri8** (in kcal mol^{-1})

	AM1 G	B3LYP/6-31G*//AM1 E_{el}
diri5	0.00[a]	0.00[b]
TS(diri5–diri6)	56.8	42.2
diri6	7.4	4.1
TS(diri6–diri7)	8.0	6.9
diri7	5.2	1.6
TS(diri7–diri8)	72.4	64.9
diri8	3.2	6.9

[a] The Gibbs free energy for **diri5** is −34.0 kcal mol^{-1}
[b] E_{el} for **diri5** is −203.9 kcal mol^{-1}

Table 2 Relative Gibbs free energies, G (AM1) and electronic energies, E_{el} (B3LYP/6-31G*//AM1) for the formation of **epi8** (in kcal mol^{-1})

	AM1 G	B3LYP/6-31G*//AM1 E_{el}
epi5	0.00[a]	0.00[b]
TS(epi5–epi6)	45.7	27.5
epi6	−0.7	−11.6
TS(epi6–epi7)	0.3	−9.0
epi7	−2.3	−13.8
TS(epi7–epi8)	67.9	62.4
epi8	3.0	3.0

[a] The Gibbs free energy for **epi5** is −29.3 kcal mol^{-1}
[b] E_{el} for **epi5** is −197.7 kcal mol^{-1}

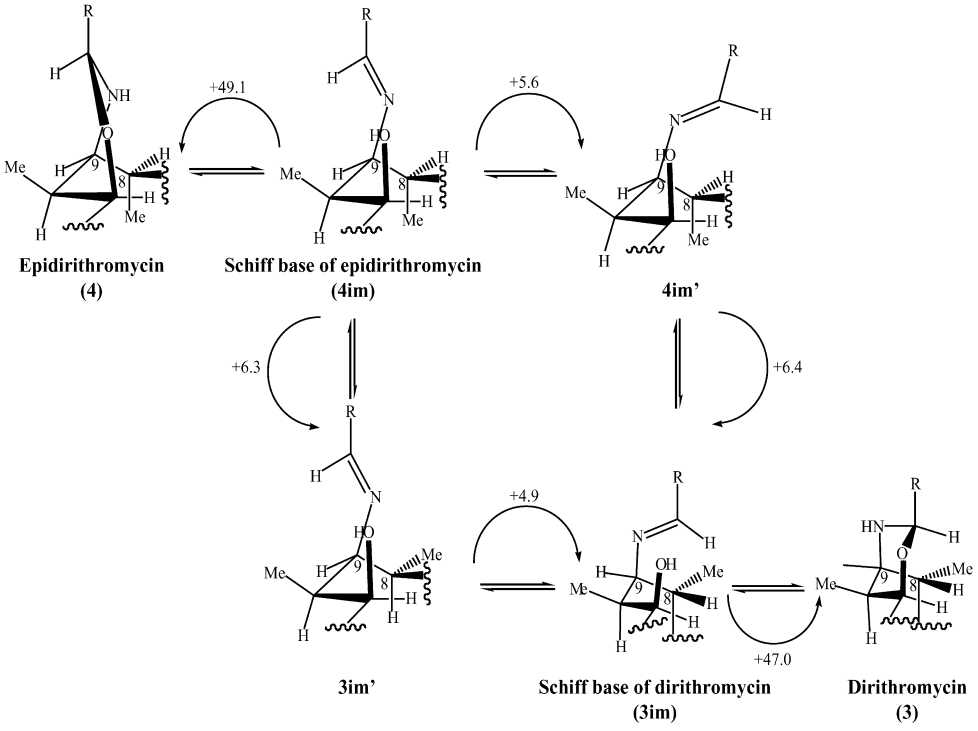


Fig. 3 Tautomerization and epimerization of the Schiff base of epidirithromycin

electronic energies (B3LYP/6-31G*//AM1). The relative stability of dirithromycin **3** versus epidirthromycin **4** is discussed based on the energetics of the fully optimized structures with AM1 and B3LYP/6-31G*

Results and discussion

Formation of the Schiff bases

The formation of the Schiff bases from 9(*S*)-erythromycylamine (**1**) and 2-(2-methoxyethoxy)-acetaldehyde (**2**) was found to proceed through three steps in vacuum. First, a zwitterionic species (**5**) forms and undergoes an internal proton transfer from the nitrogen of the 9(*S*)-erythromycylamine (**1**) to the oxygen of the 2-(2-methoxyethoxy)-acetaldehyde (**2**) followed by the formation of the carbinolamine (**6**). Then, the reaction proceeds by the inversion of nitrogen yielding the new carbinolamine (**7**) followed by the formation of the Schiff base by the loss of a water molecule (**8**) (Scheme 2). This process was examined for the Schiff base formation of both epidirithromycin (**4im**) and dirithromycin (**3im**) (Fig. 1).

The condensation of **1** and **2** yields a complex (**5**). The C–N bond distance is 2.935 Å for **diri5** and 2.854 Å for **epi5**. The formation of the carbinolamines (**diri6** and **epi6**) proceeds through a concerted proton transfer reaction via four-membered transition states well-established in the literature (Fig. 1) in vacuum [18]. Since the experimental medium for the synthesis is aprotic (CH_3CN), the same mechanism has been used as in vacuum. The transition states **TS**(**diri5**–**diri6**) and **TS**(**epi5**–**epi6**) leading to the formation of the carbinolamines, **diri6** and **epi6**, lie 56.8 kcal mol^{-1} above **diri5** and 45.7 kcal mol^{-1} above **epi5** (Fig. 4 and Table 1). In the transition structures, the H–N and H–O bond distances are 1.264 Å, 1.436 Å for **TS**(**diri**–**diri6**) and 1.278 Å, 1.427 Å for **TS**(**epi5**–**epi6**), respectively.

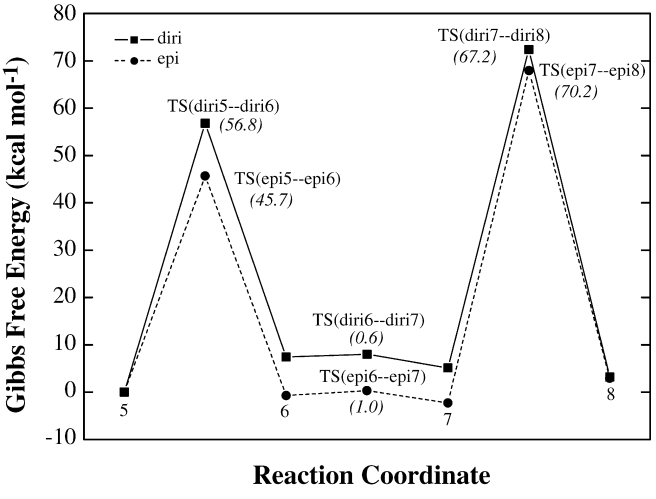


Fig. 4 Free energy pathway for the formation of **diri8** and **epi8**

The nitrogen of the carbinolamine **diri6** and **epi6** must first undergo inversion through **TS**(**diri6–diri7**) and **TS**(**epi6-epi7**) so as to have the amine hydrogen and the hydroxyl group on the same side of the molecule, yielding carbinolamines **diri7** and **epi7**, respectively. The transition structures, **TS**(**diri6–diri7**) and **TS**(**epi6–epi7**), located for nitrogen inversion have trigonal planar geometries where the HNC angles are 117.1° and

118.9°, respectively (Fig. 1). The calculated barriers for the nitrogen inversion are 0.6 kcal mol^{-1} for **diri6** and 1.0 kcal mol^{-1} for **epi6** (Fig. 4, Table 1, Table 2).

Finally, the Schiff bases (**diri7** and **epi7**) eliminate water via four-membered transition states **TS(diri7–diri8)** and **TS(epi7–epi8)**. In this process, the hydrogen linked to the nitrogen of the carbinolamine (**diri7** and **epi7**) is transferred to the oxygen of the carbinolamine, resulting in the elimination of a water molecule (Fig. 1). The four-membered transition states **TS(diri7–diri8)** and **TS(epi7–epi8)** are 67.2 kcal mol^{-1} and 70.2 kcal mol^{-1} above **diri7** and **epi7** respectively (Fig. 4 and Table 1). In **TS(diri7–diri8)** and **TS(epi7e–epi8)**, the internal bond angles are 81.5° and 86.0° for CNH and HOC, for both cases. The complexes **diri8** and **epi8** eliminate water molecules, yielding the Schiff bases of dirithromycin (**3im**) and epidirithromycin (**4im**), respectively.

Gibbs free energies have been used to consider the energetics along the formation of Schiff bases of dirithromycin and epidirithromycin as well as their interconversion. Provided that compounds **1** and **2** are introduced into the reaction vessel, they interact immediately (barrierless) to form **diri5** (0.0 kcal mol^{-1}) and **epi5** (4.7 kcal mol^{-1}). The interconversion of conformers **diri5** and **epi5** can be rationalized based on the modeling of the conformational changes of **3im** to **4im**. As seen from Fig. 3, the rotation around the C8–C9 bond requires about 6 kcal mol^{-1} and this much energy can be gained easily under the reaction conditions. Thus, even though **diri5** is found to be more stable than **epi5** by 4.7 kcal mol^{-1}, it will be partially converted to **epi5** before it undergoes proton transfer from the nitrogen of the ammonium group to the oxygen anion. Compound **epi5** undergoes proton transfer 11 kcal mol^{-1} more easily than **diri5** (Table 1). In the proton transfer of **epi5**, while the nitrogen looses one of its hydrogens, the other hydrogen makes a hydrogen bond with the C6 hydroxyl group and this causes the stabilization of **TS(epi5–epi6)** as compared to **TS(diri5–diri6)**, **epi6** is expected to form faster than **diri6**. The next high barrier to be overcome is that for water elimination, yielding **diri8** and **epi8**. This step is even slower than in the case of the proton transfer, but it is almost isoenergetic for both isomers **diri7** and **epi7**. Elimination of water is expected to be more facile by 3 kcal mol^{-1} for the complexes **diri8** as compared to **epi8** in order to yield the Schiff bases **4im** and **3im**. Finally, observation of the Gibbs free energies for the species formed along the synthesis of dirithromycin reveals the fact that dirithromycin (**3**) is the thermodynamically stable product along this synthesis. On the other hand, the Schiff base of epidirthromycin (**4im**) is expected to form faster than the Schiff base of dirithromycin (**3im**) due to the fact that proton transfer in **diri5** is more hindered by 11 kcal mol^{-1} as compared to **epi5**. These computational findings are in agreement with the experimental results, where dirithromycin is the final product of the synthesis and epidirithromycin was found to be the kinetically formed product.

The aforementioned mechanism has been rationalized by refining the energetics with B3LYP/6-31G*//AM1 (Table 1 and Table 2). This methodology confirmed the relative stability of **diri5** versus **epi5** (6.3 kcal mol^{-1}). The barrier for proton transfer is in favor or **epi5** where **TS(epi5–epi6)** is 14.8 kcal mol^{-1} lower in energy than **TS(diri5–diri6)**. Thus, **epi6** is expected to be the kinetically favored intermediate, as predicted by AM1. Water elimination is energetically more demanding than proton transfer and the second barriers **TS(diri7–diri8)** and **TS(epi7–epi8)** are higher than the former ones. These barriers are almost isoenergetic, confirming the fact that the transition from **diri5** to **epi5** and from **diri6** to **epi6** constitutes the bottleneck of this reaction. Full geometry optimization of dirithromycin, **3**, and epidirithromycin, **4** confirmed the stability of dirithromycin by 17.6 kcal mol^{-1}. Thus, refinement of the energetics confirmed the qualitative trend produced with AM1, while pointing out a lowering of the barriers in general, justifying the experimentally attainable conditions.

Tautomerization of the Schiff bases

The Schiff bases of dirithromycin (**3im**) and epidirithromycin (**4im**) are derivatives of perhydro 1,3-oxazines, which are in the chain form. In this study, the chain-ring tautomerization was modeled at the B3LYP/6-31G* level by choosing a small model where the perhydro 1,3-oxazine is used (Scheme 3). It is known that the perhydro 1,3-oxazines exist in the mobile tautomeric equilibrium of the ring form, with the open-chain Schiff base in the gas phase and in the solution [19]. Two conformers of the perhydro 1,3-oxazines were chosen as model compounds, the Schiff base of dirithromycin (**3im**) was represented by compound **9** and the Schiff base of epidirithromycin (**4im**) by compound **11** in order to analyze their tautomerization (Fig. 2). Compound **9** tautomerizes through a chair-like transition structure **TS(9–10)** to form the ring conformer (**10**), which has the chair conformation, whereas conformer **11** cyclizes via the boat-like transition structure **TS(11–12)** to form ring **12,** which has the twisted boat conformation. The mechanism of the chain–ring tautomerism is a concerted reaction involving both proton transfer from the hydroxyl proton to the Schiff base and C–O bond formation in a single step in vacuum. This type of transition states involving proton transfer and bond formation have been studied theoretically in the primary enamine-mediated intermolecular aldol reaction of acetaldeyde with *N*-methylvinylamine [20]. The forming N–H bond has a distance of 1.078 Å and 1.050 Å in **TS(9–10)** and **TS(11–12)**, respectively. The strong hydrogen bond between the N-hydrogen and the developing alkoxide (N..H...O) is 1.682 Å in **TS(9–10)** and 1.796 Å in **TS(11–12)**. The forming C–O bond is 2.423 Å in **TS(9–10)** and 2.426 Å in **TS(11–12)**. The energy barrier for **TS(9–10)** is 50.4 kcal mol^{-1}, whereas that for **TS(11–12)** is 54.5 kcal mol^{-1}. The perhydro 1,3-oxazine cyclizes preferentially through a chair transition

structure rather than a boat transition structure. To understand the effect of the medium, the cyclization of the conformers **9** and **11** was modeled in acetononitrile (ε=35.9) with the CPCM methodology. The geometries of the transition structures were analyzed and compared with the ones in the gas phase. The results show that the bond distances of the transition structures are slightly longer (the forming bond 2.56–2.65 Å) and the hydrogen bond is much longer (2.07–2.11 Å) than in the gas phase. The energy barrier in solution is lowered by 5 kcal mol^{-1} as compared to that in the gas phase for the tautomerization of the two conformers of the perhydro 1,3-oxazine.

Compounds **diri8** and **epi8** are the Schiff bases complexed with water for dirithromycin (**3**) and epidirithromycin (**4**), respectively. These complexes lose water to yield the Schiff bases **3im** and **4im** in the chain form (Fig. 3). Concerted reactions similar to the ones for the model reaction were modeled for the chain–ring tautomerization in epidirithromycin and dirithromycin. In the transition states, **TS(3im–3)** and **TS(4im–4)**, the N–H bond distance is around 1.030 Å and there is also a hydrogen bond between the N-hydrogen. The developing alkoxide (N..H...O) is 2.026 Å in **TS(3im–3)** and 2.104 Å in **TS(4im–4)**, respectively. The forming C–O bond is 2.720 Å in **TS(3im–3)** and 2.623 Å in **TS(4im–4)**. The activation barrier for the tautomerization of dirithromycin is 46.7 kcal mol^{-1} whereas for epidirithromycin, the energy barrier is 49.1 kcal mol^{-1} (Table 3). Thus, the tautomerization of the Schiff base of dirithromycin is slightly more rapid than the Schiff base of epidirithromycin.

Table 3 Relative Gibbs free energies, *G* (AM1) for the tautomerization of the Schiff base of dirithromycin (**3im**) and epidirithromycin (**4im**) (in kcal mol^{-1}); also see Fig. 3

	G		*G*
3im	0.0[a]	**4im**	0.0[b]
TS(3im–3)	47.0	**TS(4im–4)**	49.1
3	−2.6	**4**	−3.4

[a] The Gibbs free energy for **3im** is 21.7 kcal mol^{-1}
[b] The Gibbs free energy for **4im** is 25.2 kcal mol^{-1}

Table 4 Relative Gibbs free energies, *G* (AM1) for the epimerization of the Schiff base of dirithromycin (**3im**) and epidirithromycin (**4im**) (in kcal mol^{-1}); also see Fig. 3

	Path1 *G*		Path2 *G*
4im	0.0[a]	**4im**	0.0*
TS(4im–4im')	5.6	**TS(4im–3im')**	6.3
4im'	−7.8	**3im'**	−2.7
TS(4im'–3im)	−1.4	**TS(3im'–3im)**	2.2
3im	−3.6	**3im**	−3.6

[a] The Gibbs free energy for **4im** is 25.2 kcal mol^{-1}

Epimerization of the Schiff bases

In the synthesis of dirithromycin, after the condensation of the 9(*S*)-erythromycylamine (**1**) with 2-(2-methoxyethoxy)-acetaldehyde (**2**), the Schiff base of epidirthromycin (**4im**) is formed. Two different mechanisms have been suggested for the epimerization of the Schiff base of epidirithromycin (**4im**) into the Schiff base of dirithromycin (**3im**) (Fig. 3). In the first mechanism, the rotation of the imine group around the C9–N bond is followed by the rotation of the methyl group around the C8–C9 bond (**4im–4im'–3im**). In the second mechanism, rotation of the methyl group around the C8–C9 bond followed by the C9 imine bond rotation, (**4im–3im'–3im**) was observed. The Schiff base of dirithromycin (**3im**) is obtained by either mechanism since the two paths are quasi-isoenergetic.

These two mechanisms were modeled starting from the Schiff base of epidirithromycin **4im** (Fig. 3). In the first mechanism, the rotation of the imine was very rapid with a 5.6 kcal mol^{-1} barrier followed by rotation around the C8–C9 bond of the methyl group on the backbone with a barrier of 6.4 kcal mol^{-1} (Table 4). The rotation of the methyl group was slower due to the presence of the imine group. In the second mechanism, the rotation of the methyl group was slower with a 6.3 kcal mol^{-1} barrier followed by the rotation of the imine with a barrier height of 4.9 kcal mol^{-1} (Table 4). The rotation of the methyl group requires a higher barrier as compared to the rotation of the imine. The results showed that the rotation of the imine is easier than the rotation of the methyl group. This can be explained by the location of the methyl group, which is on the 14-membered ring. The rotation of the methyl group causes a change in the conformation of the 14-membered ring. Thus, the epimerization reaction can take place from either the first or the second path.

Conclusions

We have modeled the synthesis of dirithromycin by following first the formation of the Schiff bases from 9(*S*)-erythromycylamine (**1**) and 2-(2-methoxyethoxy)-acetaldehyde (**2**), and then the tautomerization of the Schiff bases to dirithromycin (**3**) and epidirithromycin (**4**). On the experimental side, the reaction path leading to the synthesis of dirithromycin was not completely identified; rather epidirithromycin was detected as a minor product, and only parts of the complete reaction pathway were hypothesized. Our calculations allow a better understanding of the synthesis of dirithromycin and epidirithromycin and show the importance of the Schiff bases in their synthesis.

We find that the condensation of 9(S)-erythromycylamine (**1**) with 2-(2-methoxyethoxy)-acetaldehyde (**2**) yielding the complexes (**diri5** and **epi5**) leading to the formation of the Schiff base of dirithromycin (**3im**) and epidirithromycin (**4im**) proceeds through two paths. Due to the kinetic environment during the progress of the reaction, the Schiff base of epidirithromycin (**4im**) is expected to form faster than the Schiff base of

dirithromycin (**3im**). Notice that during the Schiff-base formation (pathway 5–8; Scheme 2), the formation of the carbinolamine **epi6** is preferred over **diri6** since **TS**(**diri5**–**diri6**) is ca. 11 kcal higher than **TS**(**epi5**–**epi6**). Furthermore, **diri5** is also expected to convert to **epi5**. For the epimerization of the Schiff base of epidirithromycin (**4im**), both paths will be preferred. The Gibbs free energies for the species formed along the synthesis of dirithromycin show that dirithromycin (**3**) is thermodynamically 2.7 kcal mol^{-1} more stable than epidirithromycin (**4**) along the synthesis and a 99:1 equilibrium ratio in favor of dirithromycin is found both with AM1 and B3LYP/6-31G*//AM1. The experiments, on the other hand, yield an 85:15 equilibrium ratio for the two products. The reaction medium is expected to play a role in the relative stabilization of these two structures.

This study, together with our earlier work on clarithromycin [11], lays the foundations of a general approach to the modeling of the synthesis of macrolide antibiotics. These molecules are found to have rather stable conformations, which can be well described at various levels of theory. This property gives us the flexibility to study many aspects of these very important class of macrolides, such as their reaction pathways at the quantum mechanical level, solvent effect on their dynamics at the classical limit by detailed molecular dynamics simulations [21], and their conformational properties by both approaches.

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