Journal of Computer-Aided Molecular Design **18:** 1–11, 2004.

Theoretical study of selective methylation in the synthesis of azithromycin

Dilek Duran[a], Viktorya Aviyente[a] & Canan Baysal[b,*]
[a]*Chemistry Department, Faculty of Art and Sciences, Bogaziçi University, 34342, Bebek, Istanbul, Turkey;* [b]*Laboratory of Computational Biology, Faculty of Engineering and Natural Sciences, Sabanci University, Orhanli, 34956, Tuzla, Istanbul, Turkey*

Received 31 July 2003; accepted in revised form 8 December 2003

Key words: azalide antibiotics, conformational analysis, methylation, molecular dynamics, solvent effect

Summary

Azithromycin is a 15-membered macrolide antibiotic which is active *in vitro* against clinically important gram-negative bacteria. In this study, the selectivity of the methylation mechanism was analyzed computationally on the 2′-*O*Cbz-3′-*N*MeCbz derivative of azithromycin in vacuum and in DMF. We have shown that the methylation of the hydroxy group on C-6 is energetically unfavorable compared to the other hydroxy groups in vacuum; the softness values further showed that the C-6 anion is not reactive towards CH_3I in the methylation mechanism. To understand the effect of the solvent on the methylation process, detailed molecular dynamics simulations were performed in DMF using the anions at the C-4″, C-6, C-11 and C-12 positions. We find the conformations of the anions not to be affected by the presence of the solvent. The radial distribution functions of the solvent molecules around the O^- of the anions demonstrate that DMF molecules cluster around the C-6 anion. The relative strength of the anion–solvent interactions reveal that the solvent molecules provide the largest stabilization to the C-6 anion and prevent the methylation at this position. The latter descriptor was found to be an important factor in explaining the experimentally observed selectivity towards the methylation of the C-4″, C-6, C-11 and C-12 anions.

Abbreviations: Cbz – benzyloxycarbonyl; TMS-Cl – chlorotrimethylsilane; CVFF – Consistent Valence Force Field; DMF – dimethylformamide; GEM – global energy minimum; CH_3I – methyl iodide; MD – molecular dynamics; RDF – radial distribution function.

Introduction

Azithromycin is the first member of a new class of antibiotics called azalides [1]. It is an effective therapeutic agent for oral treatment of sexually transmitted diseases, upper and lower respiratory tract infections, and skin structure infections [2, 3]. It exerts its effect by reversibly binding to the 50S subunit of the bacterial ribosome. This action interferes with microbial protein synthesis by preventing transpeptidation and translocation reactions.

Azithromycin (**1**) differs structurally from erythromycin A by the insertion of a methyl-substituted nitrogen at position 9 in the lactone ring to create a 15-membered macrolide [4]. This modification results in a significant improvement in potency against gram-negative bacteria. To improve the pharmacokinetic and antibacterial activity of azithromycin, the *O*-methyl derivatives of the azalide antibiotics have been investigated, as a result of the great commercial success of clarithromycin (the 6-*O*Me derivative of erythromycin A) [5]. The synthesis of the *O*-methyl derivatives of azithromycin proceeds by the initial protection of the reactive sites on the desosamine, typically as 2′-*O*Cbz-3′-*N*MeCbz (**2**). This protected compound is then *O*-methylated with sodium hydride and methyl iodide (CH_3I) in dimethylformamide (DMF); methylation of the 6-OH was not observed, whereas with erythromycin A, the 6-OH was the first hydroxy

*To whom correspondence should be addressed. Phone: +90 (216) 4839523; Fax: +90 (216) 4839550; E-mail: canan@sabanciuniv.edu

group to be methylated. Finally, the methyl derivatives of azithromycin are recovered by removing the Cbz groups.

We have recently studied erythromycin A derivatives using computational methodologies [6, 7]. The structures proposed as well as the methodology used reproduced the experimental findings and paved the way to study the reaction mechanisms of azithromycin derivatives. In the present study, starting with the 2′-*O*Cbz-3′-*N*MeCbz derivative of azithromycin, the selectivity in the methylation mechanism is investigated in vacuum by semi-empirical quantum mechanical calculations; energy differences, barrier heights, and local softness values are used as descriptors of selectivity. Results from MD simulations in DMF are reported to gain insight into how the presence of the solvent affects the methylation process. The solvent effect is investigated by a detailed conformational analysis and by an analysis of the distribution of solvent molecules around the methylation sites.

Methodology

Conformational analysis of azithromycin using high temperature MD

To obtain a reliable initial three-dimensional structure for azithromycin, we have used a simple procedure that has proven very efficient in the conformational search of cyclic molecules [8]. In this procedure, first an MD simulation is carried out at high temperature, and various structures are recorded during the run. The high temperature MD ensures that the generated structures efficiently sample the potential energy surface. Next, the recorded structures are energy minimized with a stringent minimization criterion (10^{-4} kcal/mol/Å of the derivative is used here). Finally, the energy-minimized structures are arranged in the order of the size of the energy, and only the structures that are significantly different from each other are retained. A comparison of the various structures obtained in this manner gives an idea about the character of the energy surface, especially at the low energy end. For example, if there are two structures that are ca. 1 kcal/mol apart from each other and the rest of the structures are above the 2 kcal/mol region, then using the Boltzmann factors it may be computed that at 300 K, the lowest energy one contributes ca. 80% to the populations sampled by the molecule, the second lowest ca. 20%, and the contributions of the rest of the structures are insignificant.

Using the approach outlined above, we first carried out vacuum MD simulations at 1000 K using the X-ray structure of azithromycin [4] as the initial structure and treating all atoms explicitly. We used the Consistent Valence Force Field (CVFF) implemented within the Molecular Simulations Inc. InsightII 98.0 package for both the MD and minimization stages [9, 10]. We used a time step of 1 fs, and kept the temperature fixed at 1000 K by using the temperature control method of Andersen [11]. Initial velocities were generated from a Boltzmann distribution with an average temperature of 1000 K. Integration was carried out by the velocity Verlet algorithm [12]. Atom-based cutoffs were used with a 9.5 Å cutoff distance. A switching function, a polynomial function of distance that multiplies the non-bonded terms of the potential energy function, is used to eliminate discontinuities in the energy and force. The spline width over which the potential is smoothly switched to zero at the cutoff distance, and the buffer width determining how far any atom must move before the neighbor list is updated, are set to 1.0 and 0.5 Å, respectively. The high temperature MD simulation was carried out for 1 ns and the structures were recorded every 1 ps. The resulting 1000 structures were energy-minimized with the truncated Newton method implemented within the InsightII package. They were then listed according to the size of the energy and classified as significantly different if any one of the dihedrals from a pair of structures differs by more than 60° from each other.

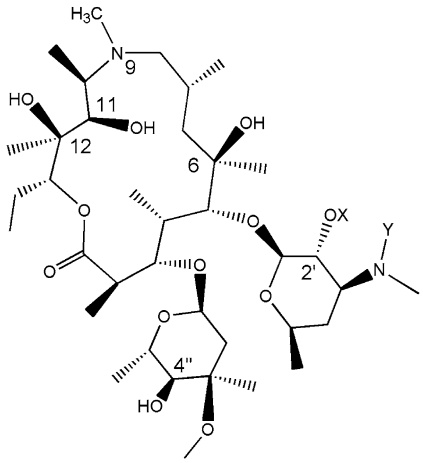
For azithromycin, all the 15 rotatable dihedrals on the backbone as well as five selected dihedrals on the side chains, which may influence the conformation of the backbone, have been considered in the analysis. Our conformational search yields 82 significantly different structures, the one with lowest energy being found at 14.9 kcal/mol. Note that we can go so far as to *assume* that the lowest energy structure obtained from the conformational search performed is the global energy minimum (GEM). In general, for cyclic molecules of the current size, it is relatively easy to locate the GEM using standard conformational search methods [13], one of which is the high temperature MD approach we used earlier [6]. Compared to the reported X-ray structure [4], all the dihedrals of the lowest energy structure of azithromycin are within ±6° except for the χ_1 angle which has a 160° instead of a 90° rotational angle. This dihedral is far from the reactive site. Thus, this structure was taken as the initial structure in the next stage of our study.

Quantum mechanical calculations on azithromycin derivatives

Structure generation

In our previous work [6], the selectivity of *O*-methylation on the synthesis of clarithromycin was studied. Therein, we initially performed a set of calculations using various quantum mechanical methods. We found the AM1 method to be optimal in both treating the systems of the current size and the precision with which the experimental findings were reproduced. Since the size and the chemical composition of the current system are similar, the same semiempirical method was used here. We have minimized the structure of azithromycin obtained from the MD calculations with GAUSSIAN 98 using the AM1 method [14] and verified that there are no major conformational changes during the process. This optimized structure was used as an initial structure for the rest of the study. Note that, apart from computational cost requirements, the use of AM1 for studying various stages during the synthesis of macrolide antibiotics is justified because (i) we compare the energy differences of very similar reactions (i.e. methylation of the anions of the same molecule), rather than absolute values of the energies, to deduce the methylation trends in these reactions; (ii) our previous work on the synthesis of dirithromycin and epidirithromycin gave the energy difference between the two compounds in favor of the former as 2.7 kcal/mol with both AM1 and B3LYP/6-31G*//AM1 [15]; and (iii) our model of the synthesis of clarithromycin with AM1 confirmed the experimental fact that the methylation at C-6 is favored over methylation at C-11 and C-12 [6].

For modeling the methylation mechanism, first 2′-*O*Cbz-3′-*N*MeCbz azithromycin was generated by adding Cbz groups at C-2′ and C-3′ on the side chain (Scheme 1). At this stage, the conformational states of the Cbz groups at C-2′ and C-3′ were investigated by freezing the macrolide and searching for all the possible orientations of the Cbz groups with respect to the ring with the MMFF94 force field in the SPARTAN 5.1.3 program [16]. The conformers located were fully optimized by the AM1 method and the lowest energy structure of the 2′-*O*Cbz-3′-*N*MeCbz azithromycin was selected for subsequent stages. The C-4″, C-6, C-11 and C-12 anions of 2′-*O*Cbz-3′-*N*MeCbz azithromycin were created by the deletion of a hydrogen atom at the corresponding hydroxy position of this structure. The resulting structures were reoptimized with AM1.



1. X= H, Y= CH_3
2. X, Y= Cbz (benzyloxycarbonyl)

Scheme 1. 1. X = H, Y = CH_3. 2. X, Y = (benzyloxycarbonyl).

Methylation mechanism

S_N2 reactions of nucleophiles with methyl halides are enhanced when polar aprotic solvents such as DMF are used. These solvents will also depress the reaction rate of a nucleophilic substitution operating under S_N1 conditions. We have therefore modeled the reaction of each anion with CH_3I as an S_N2 reaction (see Ref. 17 and references cited therein), as in our previous study [6]. Both theoretical and experimental studies indicate that the preferred gas-phase reaction pathway involves a backside attack of the ion ($-O^-$) at the carbon atom followed by the 'Walden Inversion' of the CH_3 group. Using constrained geometry optimizations at different stages of the reaction all the stationary points located along the potential energy surface (reactants, transition structures, complexes and products) were generated and the structures were optimized with the AM1 method. The vibrational frequencies of all these compounds were calculated and the results were used to identify the nature of the structure.

Hardness and softness as a measure of selectivity

To explain the selectivity, an analysis has been carried out using the reactivity descriptors such as the global hardness (η) and softness (S) [18], calculated from

$$\eta = 1/2(IE - EA) \quad (1)$$

$$S = 1/\eta \quad (2)$$

where IE and EA are the first vertical ionization energy and electron affinity of the molecule, respectively [18]. There is a conceptual relationship between the

properties called nucleophilicity and basicity, the most useful qualitative approach for making predictions of this type being the hard acid and soft acids and bases (HSAB) concept [18]. Hard nucleophiles prefer hard electrophiles, while soft nucleophiles prefer soft electrophiles. In S_N2 reactions, the results involving the HSAB principle have been interpreted by correlating the energy differences ΔE_m between the two ion-molecule complexes ($X^-\cdots CH_3Y$ and $Y^-\cdots CH_3X$), with the group hardness difference between X and Y [19]. Their correlation has been interpreted such that increasing the hardness of Y also hardens the neighboring C atom of the CH_3 group, favoring the attack of a harder nucleophile [20]. In this study, the global softness and hardness values were calculated for the anions (X^-) and I (Y^-) by using the AM1 method.

Molecular dynamics simulations of the anions in DMF

To understand the solvent effects on the selective *O*-methylation, we performed MD simulations on the C-4″, C-6, C-11 and C-12 anions of 2′-*O*Cbz-3′-*N*MeCbz azithromycin in DMF. The starting conformations of the anions are those described in the *Structure generation* subsection, and used in modeling the methylation processes.

Each anion was first solvated in a periodic cubic box with DMF molecules using the Amorphous Cell Module version 10.0 [9], treating all the atoms explicitly. The number of solvent molecules was set using DMF density under experimental conditions (d_{DMF} = 0.94 g/cm^3 at 0 °C). This results in 778 DMF molecules placed around each structure in a cubic box of length 46.7 Å on each side. Electro neutrality was achieved by distributing the positive charge uniformly in the box. The systems were minimized through 200 steps of the steepest descent method using CVFF. CVFF is a classical, first generation force field, derived by fitting experimental data sets. It handles a wide range of organic systems; due to its extensive use, it has been widely tested. It is primarily intended for studies of structures and binding energies, although it predicts vibrational frequencies and conformational energies reasonably well. In recent years, similar force fields (e.g. AMBER) were used to rationalize polar, aprotic solvent–organic solute interactions with success [21, 22].

At this initial optimization stage, the nonbonded interactions were treated with a simple 8.5 Å atom-based cutoff distance; a switching function was used with the buffer and spline widths set to 0.5 and 1.0 Å, respectively. Thus, a starting structure for the MD simulation for each of the four anions was prepared. MD simulations were carried out at constant volume and at 273 K using the temperature control method of Andersen [11]. Initial velocities were generated from a Boltzmann distribution at an average temperature of 273 K. Newton's equations of motion were integrated with the velocity Verlet algorithm [12]. Nonbonded interactions were treated with a simple 15 Å atom-based cutoff distance; a switching function was used with the buffer and spline widths set to 0.5 and 1.0 Å, respectively. After energy minimization, 600 ps of MD simulation was run at 273 K; the first 100 ps correspond to the equilibration stage followed by 500 ps of data collection. A 500 ps simulation takes 37 days of CPU time on a Silicon Graphics O2 workstation with a 360-MHz R12000 CPU and 2048 MB RAM. The solute and solvent coordinates were saved every 1 ps, thus resulting in 500 snapshots for each system.

Table 1. Heats of reaction (kcal/mol) for the anion formation through the deprotonation of 2′-*O*Cbz-3′-*N*MeCbz azithromycin.

	$\Delta E = E_{products} - E_{reactants}$
C-4″	−47.9
C-6	−39.1
C-11	−48.2
C-12	−51.8

Results and discussion

Modeling the O-methylation in vacuum

In the experimental studies, the methylation process was carried out by using methyl iodide in DMF in basic conditions starting with the 2′-*O*Cbz-3′-*N*MeCbz azithromycin [5]. We study this mechanism by a two-step procedure: first, the deprotonation followed by formation of the anions has been modeled; then, the attack of CH_3I to the anions has been investigated. This procedure was applied to each of the hydroxy groups on C-4″, C-6, C-11 and C-12.

The formation of the anions by deprotonation in basic medium is a very rapid reaction and does not contribute to the rate determining step of the overall reaction. As mentioned within the context of short-strong hydrogen bonds, these types of reactions have

Table 2. Activation barriers (kcal/mol) during the methylation of the anions.

	$\Delta E^{\neq} = E_{ts} - E_{reactants}$
C-4″	23.1
C-6	33.1
C-11	26.9
C-12	29.5

Table 3. Selected bond lengths (Å) for the structures formed along the methylation reaction of the 2′-*O*Cbz-3′-*N*MeCbz azithromycin derivatives.

	C-4″	C-6	C-11	C-12
		Pre-complex		
O- - - - - -CH_3	2.606	3.000	2.649	2.454
CH_3- -I	2.070	2.063	2.067	2.070
		TS		
O- - -CH_3	1.784	1.799	1.761	1.771
CH_3- - -I	2.383	2.384	2.410	2.405
		Post-complex		
O- -CH_3	1.423	1.420	1.421	1.421
CH_3- - - - -I	3.532	3.952	3.636	3.535

low activation barriers [23]. Thus, we have examined the heats of reaction for the formation of the anions corresponding to deprotonation from C-4″, C-6, C-11 and C-12 (Table 1). Our findings have revealed the fact that the anion formation at the C-6 position was not the most exothermic process. We made single point calculations to identify the potential energy surface of the deprotonation reaction of each anion. This was modeled by deprotonating the hydroxy group step by step through the base. The results showed that the C-6 anion passes through a barrier height of 5 kcal/mol whereas C-11 has a 1 kcal/mol barrier and other anions do not pass through a barrier. Thus, the formation of the anion proceeds slower at C-6.

We have then modeled the reaction of each anion with CH_3I as an S_N2 reaction as outlined in the Methodology section. The reaction path (Figure 1) exhibits two local minima, the pre- and the post-reaction ion-molecule complexes [O⋯⋯CH_3I] and [OCH_3⋯⋯I] and proceeds via the transition structure [O⋯⋯CH_3⋯⋯I]. The energy barriers and the bond lengths of the structures between the pre-reaction ion-molecule complex and the transition structure are listed in Tables 2 and 3, respectively. The results have shown that the *O*-methylation of the C-6 anion is not preferred over the *O*-methylation of the C-4″, C-11, and C-12 anions.

Table 4. Calculated global softness, *S* (Hartree), and hardness, η(Hartree^{-1}), for the anions compared with those of I^-.

Descriptor	C-4″	C-6	C-11	C-12	I^-
S	46.490	54.054	41.493	34.734	45.246
η	0.0215	0.0185	0.0241	0.0287	0.0221

To explain the selectivity of the anions, the HSAB principle is applied. The results in Table 4 are in line with the HSAB principle as proved by Pearson et al. [18] in the case of S_N2 reactions, stating that when the nucleophile and the leaving group have similar hardness, the reaction rates are relatively high. Increasing the hardness of Y also hardens the neighboring C atom of the CH_3 group, favoring the attack of a harder nucleophile. The hardness values of the C-4″, C-11 and C-12 anions are closer to I^- than the one of C-6. This finding corroborates that the C-4″, C-11 and C-12 anions are more reactive than the C-6 anion towards CH_3I in the methylation reaction.

Conformational preferences of the anions in DMF

For each of the anions (C-4″, C-6, C-11 and C-12) of 2′-*O*Cbz-3′-*N*MeCbz azithromycin, MD simulations were carried out in DMF. Since the starting structures were taken as the optimized ones from AM1 calculations, we first check if the 100 ps equilibration period is adequate for the molecules to settle into their equilibrium conformations dictated by the solvent environment. Trajectories of selected dihedral angles of the anions were monitored for this purpose. For each anion, all 15 dihedral angles on the backbone (ϕ_n) as well as five dihedral angles on the side chains (χ_n), which may influence the conformation of the backbone, were considered. These angles are shown schematically in Figure 2. We find that all the 20 dihedral angles that were monitored fluctuate within ca. $\pm 10°$ of their final average value; i.e. after 100 ps, the molecules attain their new equilibrium state.

We next compare the average structures with the AM1 optimized ones from our study to discern the effect of solvent on molecular conformations. The comparison is shown in Table 5 for the C-4″, C-6, C-11, and C-12 anions. We find that once equilibrium is

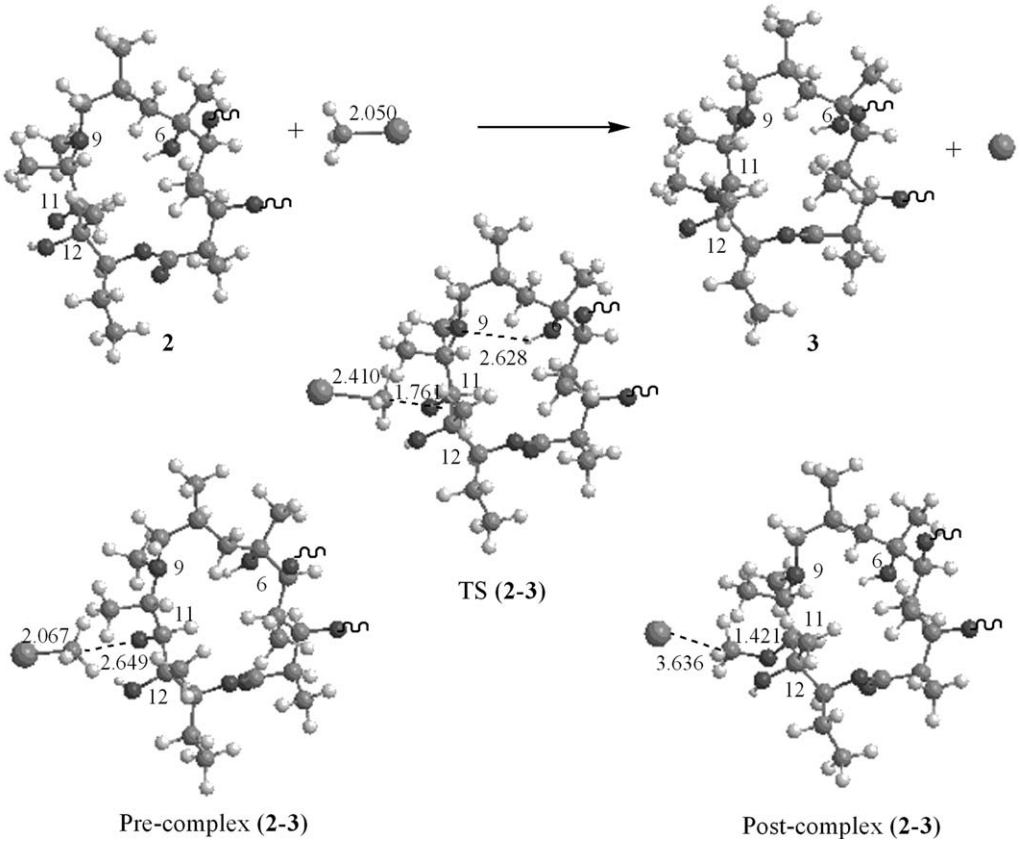


Figure 1. Three-dimensional view of the methylation mechanism of C-11 anion of 2′-*O*Cbz-3′-*N*MeCbz azithromycin (**4**).

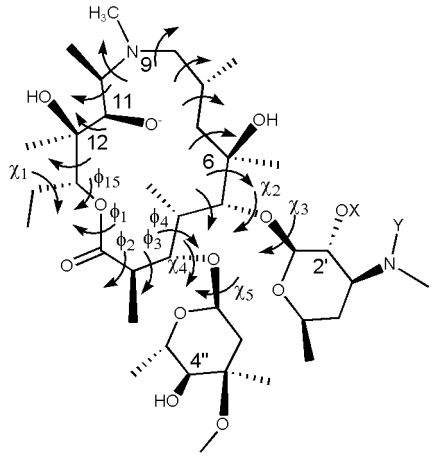


Figure 2. Essential dihedral angles defining the conformation of the anion. 15 dihedrals from the backbone (ϕ_1-ϕ_{15}) and 5 dihedrals from the side-chains (χ_1-χ_5) are selected. The C-11 anion is shown here; the dihedrals in the C-4″, C-6, C-12 anions follow the same numbering scheme.

established, all the conformationally important angles in the C-4″, C-6, C-11 and C-12 anions fluctuate ±10° around the optimized value. In fact, to a good approximation, the optimal structure is within the bounds of the fluctuations of the dynamics for these anions. This analysis demonstrates that the stable conformations of the C-4″, C-6, C-11, and the C-12 anions are not affected by the presence of the solvent. We finally analyze the structure of the solvent around the methylation site to get a deeper understanding of the processes involved.

To understand the molecular basis of the solvent effect, the radial distribution functions (RDFs) were computed for the distribution of the solvent molecules around the O^- of the anions. The RDF, $g_{ij}(r)$, is given by $g_{ij}(r) = \rho_{ij}(r)/\langle\rho_j\rangle$, where $\rho_{ij}(r)$ is the number density of atom j at a distance r from an atom i, and $\langle\rho_j\rangle$ is the average number density of atom j. The $g_{ij}(r)$ are averaged over the 500 recorded snapshots of each trajectory. Since the number of conformations we average over is relatively small, we apply a third degree, five-point smoothing to the data [24]. The RDF

Table 5. Optimized dihedral angles of the anions compared with the average angles obtained from MD simulations in DMF.

	C-4″		C-6		C-11		C-12	
	Optimized	MD	Optimized	MD	Optimized	MD	Optimized	MD
ϕ_1	−167.8	−172.7	−167.9	−168.0	−171.1	−169.8	−169.5	−172.3
ϕ_2	−95.4	−92.9	−73.8	−88.9	−80.8	−94.5	−74.9	−90.2
ϕ_3	431	60.7	48.1	54.5	60.6	59.7	58.5	60.5
ϕ_4	−167.9	−168.7	−169.8	−168.8	−177.7	−169.9	−171.2	−171.9
ϕ_5	107.2	102.5	95.7	97.9	102.7	107.8	102.3	98.6
ϕ_6	77.8	82.4	71.9	70.5	78.4	77.9	76.6	80.9
ϕ_7	−172.1	−175.8	−172.8	−177.8	−177.1	−174.9	−177.5	−174.7
ϕ_8	123.8	111.4	142.3	139.4	127.9	112.5	132.7	112.5
ϕ_9	−65.7	−62.7	−72.3	−68.2	−69.0	−62.8	−67.8	−63.3
ϕ_{10}	133.5	130.4	116.6	118.6	121.4	133.1	120.2	133.9
ϕ_{11}	−148.2	−144.3	−133.3	−135.8	−158.7	−141.5	−148.2	−138.4
ϕ_{12}	158.3	159.4	163.1	164.8	159.1	157.8	153.1	154.2
ϕ_{13}	−171.1	−172.7	−174.8	−172.3	−156.7	−172.8	−169.7	−177.7
ϕ_{14}	67.5	69.9	67.2	69.5	82.9	67.2	85.4	65.9
ϕ_{15}	−104.9	−117.7	−123.3	−118.3	−137.3	−177.9	−139.9	−112.1
χ_1	160.9	167.5	160.7	168.1	157.3	169.5	156.1	169.2
χ_2	72.9	73.3	72.3	75.8	75.5	75.3	76.8	78.4
χ_3	81.0	78.7	82.5	83.8	86.5	84.4	79.2	86.1
χ_4	98.6	101.5	96.5	97.5	90.5	105.1	92.3	94.6
χ_5	77.8	76.4	71.9	77.9	76.5	80.6	75.7	82.1

of the anion oxygen distances in any direction from all the seven hydrogen atoms of the solvent molecules are displayed in Figures 3a and 3b for determining the structure of the solvent around the four anions.

We find that the solvent density approaches the bulk value [$g(r) \rightarrow 1$] at distances of ca. 10 Å in all four cases in DMF. On the other hand, at distances shorter than ca. 5 Å, the solvent molecules cluster predominantly around the O^- of the C-6 and C-11 anions [$g(r) = 1$; Figure 3a]; whereas the solvent shows less clustering around the O^- of the C-4″ and C-12 anion at these distances [$g(r) < 0.6$; Figure 3b]. In other words, there are no prevailing favorable interactions between the solvent and the anion in the latter two, so that methylation may proceed smoothly at these sites. Conversely, the solvent molecules tend to cluster around the C-6 and C-11 anions with a peak at 2.6 Å, preventing the approach of the methylating agent. However, the number density of solvent molecules around the C-6 anion is greater than that of the C-11 anion; moreover, there is a second sharp peak at ca. 4 Å in the former case pointing to the existence of a tight second coordination shell formed by the solvent molecules around the C-6 anion. Thus, at distances shorter than 5 Å, there is a substantial organization of DMF molecules around the C-6 anion, preventing methylation at this side.

Mechanisms of solvent obstruction

To gain deeper insight into the results of the RDFs of the anions with the solvent molecules, specific interactions between the solvent molecules and the anions were analyzed in the trajectories of the four anions. In each trajectory, the distances up to the 5 Å region of the anion were considered in detail. In this analysis, we define favorable O⋯⋯H interactions if the distance between the anion O^- and the solvent H atom is less than 3 Å. Of these 'favorable interactions', the stronger hydrogen bonds are defined for distances shorter than 2.7 Å and (O⋯⋯H-C) angles greater than 140°.

As the DMF molecule has a planar geometry, it can approach the anions either from the H atom of the carbonyl group or from the hydrogens of the N-methyl groups on the DMF. In fact, our analysis of the trajectories reveals that the solvent molecules prefer to block the C-4″, C-6 and C-12 anions by forming

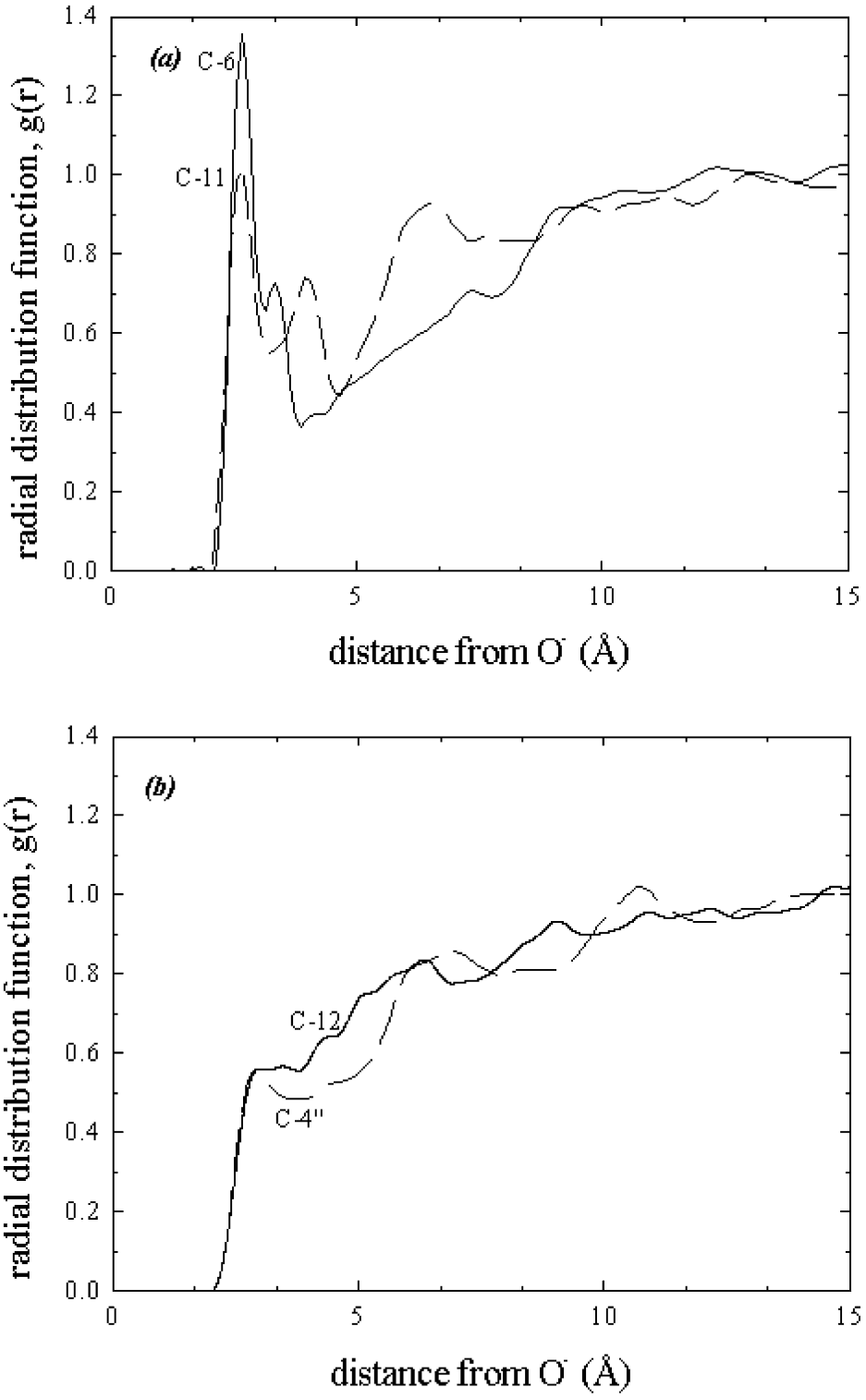
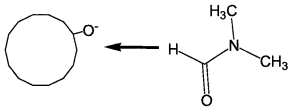
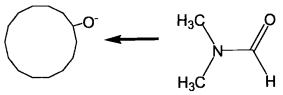


Figure 3. Radial distribution functions (RDFs), $g(r)$, for all the hydrogens of DMF molecules around the O^- of the anions (a) at the C-6 and C-11 positions, (b) at the C-4″ and C-12 positions. The solvent clustering is the most pronounced for the C-6 anion.

Table 6. Energetics (kcal/mol) for the formation of the stable complex between the DMF molecule and the anions of the 2′-OCbz-3′-NMeCbz azithromycin.[a]

		C-4″	C-6	C-11	C-12
ΔE_1[b]		−13.31	−11.01	−6.77	−4.21
ΔE_2[b]		−10.64	−11.48	−10.97	−10.40
p_1/p_2		0.14/0.86	0.14/0.86	0.35/0.65	0.14/0.86
ΔE_{avg}[c]		−11.0	−11.4	−9.5	−9.5

[a] 1 and 2 refer to the approach of DMF to the anion from the carbonyl and methyl sides, respectively; p_1 and p_2 are calculated from the relative weights of the approach directions deduced from the MD trajectories.
[b] $\Delta E = \Delta E_{products} - \Delta E_{reactants}$.
[c] $\Delta E_{avg} = (p_1 \Delta E_1 + p_2 \Delta E_2)$.

a six-membered ring with the hydrogens of the N-methyl groups. Conversely, the DMF molecules tend to approach the C-11 anion from the H of the carbonyl more frequently. To quantify the directional preference of DMF towards the anion, we compute the RDFs between the anion-carbonyl hydrogen (1) and anion-methyl hydrogen (2) separately. Since these provide the distribution of the solvent molecules, we integrate the curves at distances shorter than 5 Å, and express their relative weights in the two separate cases as probabilities. These probabilities are given in Table 6. Note that for the C-4″, C-11, and C-12 anions, the relative weights of the two instances are 1:6 (0.14:0.86), reflecting the fact that for these anions there is no directional preference of the solvent towards the anion, and the orientation of the DMF molecule is random (there is one carbonyl hydrogen for every six methyl hydrogens). On the other hand, there is a strong preference for the DMF molecules to approach to the C-11 anion from the hydrogen of the carbonyl, as reflected by the relative weights of the approach directions (0.35:0.65).

To quantify the stabilization introduced by the presence of DMF molecules around the anions, we modeled the complex formation reactions of the anions with DMF molecules by using the AM1 method in GAUSSIAN 98 in vacuum. The reactions were studied based on the interactions observed in the snapshots for each anion. Thus, two types of mechanisms were modeled: one of them is the approach of the DMF molecule to the anion from the hydrogen of the carbonyl (1) and the other is from the N-methyl hydrogens (2). The reactants and the products were fully optimized by the AM1 method. A frequency analysis has been made and the nature of the minima has been confirmed by the presence of real vibrational frequencies, whereas the structures corresponding to the transition structures have one imaginary frequency. The heats of complexation (ΔE_1 and ΔE_2) between the reactants and the products of the two mechanisms and their weighted average were evaluated (Table 6).

As the DMF molecule approaches the anion from the hydrogen of the carbonyl and the hydrogens of the N-methyl groups, they form a stable complex for each case. The results show that the solvent molecule stabilizes the C-6 and C-4″ anions in both complexation reactions by ca. 1.5–1.9 kcal/mol more than the C-11 and C-12 anions (the exact energy differences are listed in Table 6). As a result, it requires less energy for the methylating agent to destabilize the solvent-anion complex for the latter two anions, leading to the methylation to proceed smoothly at these sites. It is these results that closely mimic the experimentally observed methylation yields which are in the order of C-11, C-12, C-4″.

Conclusions

In modeling complicated mechanisms, it is often necessary to consider phenomena that take place at different time and length scales. Our recent work on clarithromycin [6, 7] and the current study on azithromycin target finding well grounded procedures to model the

synthesis of macrolide antibiotics in particular and medium sized organic molecules in general. We have shown that molecular modeling has predictive power on how the synthesis of a certain organic molecule will proceed under prescribed environmental conditions, using the well established modeling tools of quantum mechanics and molecular dynamics hand-in-hand. The former will model a chemical reaction; the latter will give the solvent effect on the intermediates.

Our prescription for treating these problems involves several steps: (i) A detailed conformational search is performed at the classical limit. (ii) Alternative chemical reactions are studied *in vacuo* using quantum mechanical tools, the level of theory depending on the number and the size of the systems of interest. (iii) A general trend of the alternative pathways will emerge at this point, and it will be relatively easy to discriminate some of the alternatives if the barrier heights are too high (kinetic control) or the energy differences are too different (thermodynamical control). (iv) If energy differences are on the order of only several kcal/mol, then the environment plays a discriminating role in the different alternatives. We study the local environment of the most important reaction intermediates in the solvent of interest using MD simulations. If the solvent has a strong effect on the solute, it will cause significant conformational changes necessitating further quantum mechanical calculations to be conducted on the new conformation of the solute. If the solvent has a weak effect, it will cluster around preferred regions without initiating significant conformational change. This situation is diagnosed by the use of radial distribution functions. (v) MD trajectories are analyzed in detail to identify the interaction geometries of solvent–solute atoms. These are compared with the geometries obtained from small molecule models. The rule of thumb used at this stage is that solvent molecules that are held tighter by the solute (lower ΔE) will be harder to displace by the reacting agent.

Following this hybrid procedure, the selective methylation of the 2′-*O*Cbz-3′-*N*MeCbz azithromycin derivatives has been modeled in vacuum and MD simulations of the C-4″, C-6, C-11 and C-12 anions of 2′-*O*Cbz-3′-*N*MeCbz azithromycin have been carried out in pure DMF solution. Solvent–solute interactions and optimal geometries were also studied by small molecule models. The combined studies allow a better understanding of the selectivity of the methylation reaction.

Experiments show that, unlike clarithromycin, methylation of the C-6 position in azithromycin is not observed. With all the descriptors we have used in this study, we found this position to be the least prone to methylation due to its high energy of formation and the high energy barrier that needs to be surmounted during its reaction with CH_3I (Tables 1–3); the softness values corroborate this finding (Table 4). Moreover, MD simulations in DMF solution showed that the solvent further stabilized the C-6 anion (Figure 3a). The energetics of local solvent–solute interactions also support this result. However, one cannot comment on how the transition states would be affected by the presence of the solvent, as these interactions cannot be modeled with the current approach. On the other hand, selectivity towards the methylation of all the anions, which was qualitatively found to be in the order $11 > 12 > 4'' \gg 6$ from experiments [5], cannot be readily discriminated by these methods, as different descriptors point to different ordering. We find that this ordering is captured most closely by considering the relative strength of anion–solvent interactions (Table 6), an uncommon descriptor of reaction yields.

Acknowledgements

The authors would like to thank Bogazici Arastirma Fonu project 01M101 and TUBITAK (The Scientific and Technical Research Council of Turkey) Munir Birsel Foundation for financial support.

References

1. Dokic, S., Kobrehel, G., Lazarevski, G., Lopotar, N., Tamburesev, Z., Kamenar, B., Nagl, A. and Vickovic, I., J. Chem. Soc. Perkin Trans. I, (1986) 1881.
2. Kirst, H.A. and Sides, G.D., Antimicrob. Agents Chemother., 33 (1989) 1419.
3. Kremer, C.J., Antibiot. Rev., 9 (2002) 174.
4. Dokic, S., Kobrehel G., Lopotar, N., Kamenar, B., Nagl, A. and Mrvos, D., J. Chem. Res. (S) (1988) 152.
5. Waddell, S.T., Santoreli, G.M., Blizzard, T.A., Graham, A. and Occi, J., Bioorg. Med. Chem. Lett., 8 (1998) 549.
6. Duran, D., Aviyente, V. and Baysal, C., J. Chem. Soc., Perkin Trans. 2 (2002) 670.
7. Duran, D., Aviyente, V. and Baysal, C., J. Comput-Aided Mol. Des., revised.
8. Baysal, C. and Meirovitch, H., J. Comput. Chem., 20 (1999) 1659.
9. Molecular Simulations Inc., San Diego, CA.
10. Dauber-Osguthorpe, P., Roberts, V.A., Osguthorpe, D.J., Wolff, J., Genest, M. and Hagler, A.T., Proteins, 4 (1988) 31.
11. Andersen, H.C., J. Chem. Phys., 72 (1980) 2384.

12. Swope, W.C., Andersen, H.C., Berens, P.H. and Wilson, K.R., J. Phys. Chem., 76 (1982) 637.
13. Saunders, M., Houk, K.N., Wu, Y.D., Still, W.C., Lipton, M., Chang, G. and Guida, W.C., J. Am. Chem. Soc., 112 (1990) 1419.
14. GAUSSIAN 98 Revision A.1, Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Zakrzewski, V.G., Montgomery, J.A. Jr., Stratmann, R.E., Burant, J.C., Dapprich, S., Millam, J.M., Daniels, A.D., Kudin, K.N., Strain, M.C., Karkas, O., Tomasi, J., Barone, V., Cossi, M., Cammi, R., Mennucci, B., Pomelli, C., Adamo, C., Clifford, S., Ochterski, G., Petersson, G.A., Ayala, P.Y., Cui, Q., Morokuma, K., Malick, D.K., Rabuck, A.D., Raghavachari, K., Foresman, J.B., Cioslowski, J., Ortiz, J.V., Stefanov, B. B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Gomperts, R., Martin, R.L., Fox, D.J., Keith, T.A., Al-Laham, M.A., Peng, C.Y., Nanayakkara, A., Gonzalez, V., Challacombe, M., Gill, P.M.W., Johnson, B.G., Chen, W., Wong, M.W., Andres, J.L., Head-Gordon, M., Replogle E.S. and Pople, J.A., Gaussian Inc., Pittsburgh, PA, 1998.
15. Duran, D., Aviyente, V. and Baysal, C., J. Mol. Model., in press. DOI 10.1007/s00894-003-0176-3.
16. SPARTAN Version 5.1.3. Wavefunction, Inc., Irvine, CA.
17. Partiban, S., Oliveira, G. and Martin, J.M.L., J. Phys. Chem. A, 105 (2001) 895.
18. Pearson, R.G., J. Am. Chem. Soc., 85 (1963) 3533.
19. Pearson, R.G. (Ed.), Hard and Soft Acids and Bases. Dowden, Hutchinson, and Ross, Inc., Stroudsburg, PA, 1973.
20. Safi, B., Choho, K. and Geerlings, P., J. Phys. Chem. A, 105 (2001) 591.
21. Colombo, G., Toba, S. and Merz, Jr., K.M., J. Am. Chem. Soc., 121 (1999) 3486.
22. Digilio, G., Bracco, C., Barbero, L., Chicco, D., Del Curto, M.D., Esposito, P., Traversa, S. and Aime, S., J. Am. Chem. Soc., 124 (2002) 3431.
23. Chen, J., McAllister, M.A., Lee, J.K. and Houk, K.N., J. Org. Chem., 63 (1998) 4611.
24. Allen, M.P. and Tildesley, D.J. (Eds.), Computer Simulation of Liquids, Clarendon Press, Oxford, UK, 1987, p. 204.