# Modeling the selective methylation in the synthesis of clarithromycin

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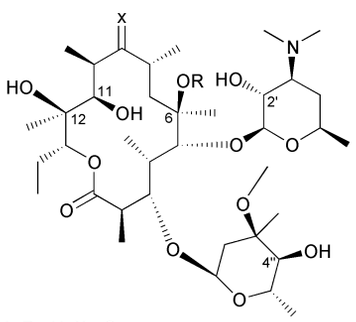
The synthesis of clarithromycin from erythromycin A oxime derivatives has been investigated in order to improve the antibacterial and pharmacokinetic properties of erythromycin A. In this study, the protection and the methylation reactions of each of the hydroxy groups in erythromycin A have been analyzed computationally by the AM1 method by using erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] in the gas phase. Our results have shown the protection of the C-2′ and C-4″ hydroxy groups to be faster than for the other hydroxy groups, in agreement with the experimental studies. Furthermore, methylation of the hydroxy group on C-6 was found to be preferred over that of the other hydroxy groups.

## Introduction

Erythromycin A (**1**) is a 14-membered ring macrolide of an important family of oral antibiotics. It is used in the treatment of infectious diseases caused by aerobic Gram-positive bacteria and some anaerobic Gram-negative bacteria, mycoplasma and chlamydia.[1] It is unstable under acidic conditions and undergoes dehydration into 6,9:9,12-spiroketal, anhydroerythromycin A, when administered orally.[1] Clarithromycin (**2**), a new semi-synthetic derivative of erythromycin A, has an improved range of pharmacokinetic properties. This new antibiotic differs from erythromycin A only in the substitution of the C-6 OH by C-6 OMe.[2] The presence of the methoxy group makes clarithromycin more hydrophobic than erythromycin A and it becomes more active towards Gram-negative bacteria such as *Haemophilus ducreyi*. Clarithromycin, the 6-*O*-methyl derivative of erythromycin, has been prepared in attempts to overcome one source of its instability to acids.[1] Blocking the C-6 hydroxy prohibits its interaction with the C-9 carbonyl and improves the stability to acids of erythromycin as well as its antibacterial and pharmacokinetic properties.[3] The purpose of the synthetic work was to achieve *O*-methylation with high regioselectivity at C-6 OH, while protecting only the hydroxy groups in the sugar units. Sugar-protected erythromycin gave the undesired 11-*O*-methyl ether as the major product, but 9-allyloxyiminoerythromycin (X = $NOCH_2CH{=}CH_2$ in Fig. 1) gave predominantly the desired 6-*O*-methyl ether.[1,2,4]

The selectivity of the methylation of the C-6 hydroxy group was studied by using a variety of erythromycin A derivatives.[4] Before methylation of C-6, the 2′ and 4″ hydroxy groups were protected starting with erythromycin 9-oxime (**3**) and protecting the OH groups on the cladinose and desosamine rings with chlorotrimethylsilane [$(CH_3)_3SiCl$]. This protected compound was allowed to react with methyl iodide ($CH_3I$) and potassium hydroxide (KOH) in a mixture of dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) (1 : 1).[5] In the presence of a bulky derivative of an oxime group (9-[*O*-(dimethylthexylsilyl)])† the C-11 and C-12 hydroxy groups were automatically protected against the attack of the methylating reagent ($CH_3I$)[6] (Scheme 1).

† The IUPAC name for thexyl is 1,1,2-trimethylpropyl.




**1** R= H, X= O
**2** R= $CH_3$, X= O
**3** R= H, X= $NOSi(CH_3)_2C(CH_3)_2CH(CH_3)_2$

**Fig. 1** Structures of erythromycin A (**1**), clarithromycin (**2**) and erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] (**3**).

In this study, starting with the 9-[*O*-(dimethylthexylsilyl)-oxime] derivative of erythromycin A (**3**), the protection of each hydroxy group in the macrolide with $(CH_3)_3SiCl$ was modeled. Furthermore, the selectivity in the methylation mechanism was also investigated. Quantum mechanical tools were used to rationalize the aforementioned mechanisms.

## Methodology

The following procedure was followed in order to obtain a reliable initial three-dimensional structure for erythromycin A.

### Conformational search using molecular dynamics simulations and energy minimization

To understand the extent of the stability of the X-ray structure[7] as described by the potential energy functions used, we carried out a conformational search of clarithromycin using the CFF91 forcefield (Consistent Forcefield 91) implemented within the Molecular Simulations Inc. InsightII 98.0 software package.[8,9] Note that the conformational search was performed on clarithromycin rather than erythromycin, as a detailed X-ray structure has been reported in literature only for the former. We used a simple procedure that has proven very efficient in conformational searches of cyclic molecules.[10] According to

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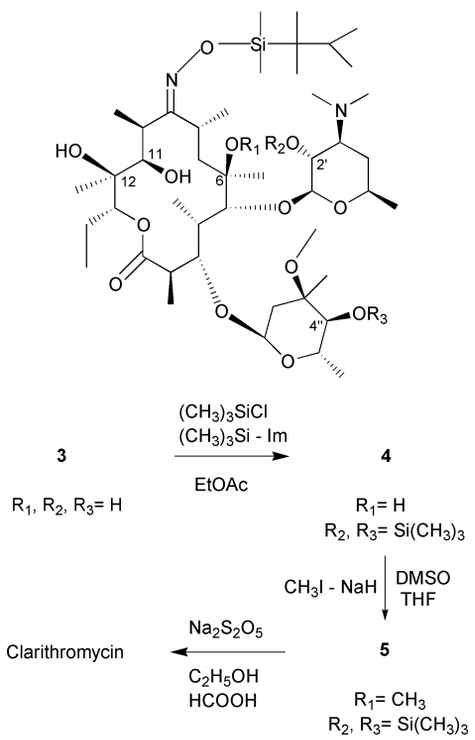


**Scheme 1**

this procedure, first a molecular dynamics (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 samples a wide range on the potential energy surface. Thus, high energy barriers are easily surmounted, preventing the search from being stuck in a given energy well. Next, the recorded structures are energy-minimized with a stringent minimization criterion (such as $10^{-4}$ kcal mol$^{-1}$ Å$^{-1}$ of the derivative). Finally, the energy-minimized structures are arranged in order of 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. In previous work, structures within the region of 2 kcal mol$^{-1}$ of the global energy minimum have been found to contribute significantly to the partition function.[11,12] For more details on these computations, see ref. 13 and references cited therein.

Thus, first, we randomly generated a clarithromycin structure such that the bond lengths and bond angles were consistent with the equilibrium values, whereas the dihedrals assume random values, also ensuring that the ring structure of the backbone is formed. We next carried out MD simulations at 1000 K, treating all of the atoms explicitly. A time step of 1 fs was used, and the temperature was fixed at 1000 K by using the temperature control method of Andersen.[14] Initial velocities were generated from a Boltzmann distribution with an average temperature of 1000 K. Integration was carried out by the velocity Verlet algorithm. Group-based cutoffs were used with a 9.5 Å cut-off distance; a switching function was used with the spline and buffer widths set to 1.0 and 0.5 Å, respectively. The neighbor list is updated whenever any atom moves more than one-half of the buffer width. The system was equilibrated for 100 ps, and data were collected for the next 500 ps. Atomic positions were saved every 100 steps, yielding 5000 coordinate sets. We then energy-minimized these 5000 coordinates to within 0.0001 kcal mol$^{-1}$ Å$^{-1}$ of the derivative using the truncated Newton method.[15] We next ordered the structures according to energy, and retained the structures that were significantly different from each other. The criterion for this classification is that if any of the corresponding dihedrals from a pair of structures differ by more than 60° from each other, the two structures are located at separate potential energy wells.[16]

Clarithromycin has 20 rotatable bonds. Of these, 14 are located along the backbone of the ring structure, and six are on various side chains. When we apply our criterion to all of these dihedrals, our conformational search yields 564 significantly different structures, the one with lowest energy being found at −16.3 kcal mol$^{-1}$. In this structure, all of the dihedral angles are within ±4° of the reported X-ray structure,[1] except for the $\chi_2$ angle of the cladinose ring which has a minimum of *ca.* 180° instead of *ca.* 270°. If we apply the criterion to the backbone of the molecule only (14 dihedrals), we find 97 structures that differ significantly from each other, *i.e.* reside in different wide energy wells. Analysis of this subset suggests that the nearest structure that belongs to a different backbone motif from that of the X-ray structure has a potential energy minimum at an energy of −10.2 kcal mol$^{-1}$. The energy difference between the two lowest energy motifs is therefore 6.1 kcal mol$^{-1}$, and it is highly unlikely that the entropic contribution, which is not computed in this part of the study, will compensate for this difference. Thus, we conclude that the X-ray structure of clarithromycin is stable, and contributions to the populations from structures in the closest energy wells will be negligible. Moreover, since erythromycin and clarithromycin differ by only a methyl group, we used the geometry of the lowest energy structure obtained from the analysis described above as the starting structure for the rest of the analysis. Having carried out our calculations *in vacuo*, we also concluded that the solvent effect is minimal since the X-ray structure was easily located at the lowest potential energy well. We therefore carried out the rest of our analysis *in vacuo*.

### Quantum mechanical calculations

As we intended to carry out further work using a semiempirical quantum mechanical method, we had to decide on the nature of the semiempirical method to be adopted. We have minimized the structure of clarithromycin obtained from the MD calculations with GAUSSIAN 98[17] using the AM1 and the PM3 methods. The output was further compared with the available X-ray structure. The bond lengths and angles generated with AM1 were seen to mimic better the experimental results. Thus, AM1 was used in the rest of the project. For the stationary points corresponding to local minima located along the potential energy surface (reactants, complexes and products), the conformational search was performed using Molecular Mechanics calculations with the MMFF94 force field in the SPARTAN 5.1.3 program.[18] The conformers located with MMFF94 were fully optimized by the AM1 method and the global minimum was chosen for each structure. Structures corresponding to saddle points between reactants and products of interest have been located for a small model (methanol) by using the linear synchronous dihedral angle transit (LST) method in Spartan. Then, the critical parameters (distance, angle and dihedral angle) were applied to the macrolide and the corresponding transition structures were located. The vibrational frequencies of all the compounds of interest were calculated and the results were used to identify the nature of the structures.

## Results and discussion

### Protection of the hydroxy groups

The structure of clarithromycin generated by MD calculations and optimized with AM1 was chosen as the initial structure for the QM calculations. The methyl group at C-6 was replaced by a hydrogen to generate erythromycin and a conformational search was performed in order to determine the orientation of the substituent at position 9. Each of the five hydroxy groups of

**Table 1** Selected bond lengths (Å) for the transition structures formed along the protection reaction of the erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] derivatives

| | C-2′ | C-4″ | C-11 | C-12 | C-6 |
|---|---|---|---|---|---|
| O–H | 1.255 | 1.255 | 1.259 | 1.283 | 1.275 |
| H–Cl | 1.577 | 1.577 | 1.571 | 1.563 | 1.557 |
| Si–Cl | 2.713 | 2.713 | 2.738 | 2.700 | 2.716 |
| O–Si | 1.866 | 1.866 | 1.861 | 1.876 | 1.879 |

**Table 2** Energetics (kcal mol$^{-1}$) for the formation of the erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] derivatives with $(CH_3)_3SiCl$

| | $\Delta E^a$ | $\Delta E^{\ddagger b}$ |
|---|---|---|
| C-2′ | 12.107 | 32.439 |
| C-4″ | 17.452 | 34.482 |
| C-11 | 15.711 | 35.473 |
| C-12 | 19.045 | 38.991 |
| C-6 | 17.005 | 39.570 |

[a] $\Delta E = E_{products} - E_{reactants}$. [b] $\Delta E^{\ddagger} = E_{TS} - E_{reactants}$.

the erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] (**3**) was protected one by one with chlorotrimethylsilane $(CH_3)_3SiCl$. In order to assess the protection mechanism of alcohols by $(CH_3)_3SiCl$, several pathways were considered for which methanol was adopted as a model alcohol. An $S_N1$ mechanism through which $(CH_3)_3Si^+$ attacks methanol did not yield a stationary transition structure. An $S_N2$ mechanism in which $(CH_3)_3SiCl$ attacks the O atom of methanol as Cl leaves, yielded a transition structure which was 51 kcal mol$^{-1}$ higher in energy than the reactants. Finally, a four-membered transition structure was located in which the Si–O and H–Cl bonds form while the Si–Cl and O–H bonds are broken. This last stationary structure was found to have a barrier of 31.5 kcal mol$^{-1}$. Similar four-membered transition structures have already been proposed in the literature for the formation of formamide and water from ammonia and formic acid.[19] Thus, in the course of the reaction, the attack of the $(CH_3)_3SiCl$ on the hydroxy group was simulated by a four-membered transition state (Fig. 2). In each case (C-6, C-11, C-12, C-2′, C-4″), the geometries of the four-membered ring have been analyzed. The results show that the O–Si bond in the transition state is longer for the C-6 case rather than for the other hydroxy groups (Table 1). The energy barrier ($\Delta E^{\ddagger}$) between the reactants and the transition states for each hydroxy group was also evaluated (Table 2). The energy barriers for the protection of C-2′ and C-4″ are somewhat lower than those for the protection of C-6, C-11 and C-12. Even though the difference in energy between the barriers for the protection of C-4″ and C-11 is only 1 kcal mol$^{-1}$, the general trend is such that C-2′ and C-4″ tend to be protected faster than the others. This result is in agreement with the experimental findings[5] of Watanabe *et al.* where the protection of these two hydroxy groups precedes that of the others. The difference in energy between the barriers for C-2′ and C-6 protection is 5 kcal mol$^{-1}$ and this corresponds to a rate enhancement of 4000. In further reactions, 2′,4″-bis(*O*-trimethylsilyl)erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] (**4**) was used.

### Modeling the methylation reaction

In the experimental studies, the methylation process was carried out by methyl iodide and potassium hydroxide in a mixture of DMSO–THF (1 : 1) in basic conditions using 2′,4″-bis(*O*-trimethylsilyl)erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] (**4**).[6] In this study, this process was reproduced in two steps: First, the hydrogen abstraction followed by formation of the anions at C-6, C-11 and C-12 (Fig. 2) was modeled. Then, the attack of $CH_3I$ on the anions was investigated. This process was examined for each of the hydroxy groups on C-6, C-11 and C-12.

**Table 3** Energetics (kcal mol$^{-1}$) for the methylation of the 2′,4″-bis(*O*-trimethylsilyl)erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] derivatives

| | $\Delta E^a$ | $\Delta E^{\ddagger b}$ | $E_T$ [c] |
|---|---|---|---|
| C-11 | −45.645 | 25.980 | −757.889 |
| C-12 | −43.647 | 21.663 | −755.708 |
| C-6 | −49.747 | 21.370 | −760.086 |

[a] $\Delta E = E_{products} - E_{reactants}$. [b] $\Delta E^{\ddagger} = E_{TS} - E_{reactants}$. [c] $E_T$ = Total energy of the methylated compounds.

**Table 4** Selected bond lengths (Å) for the structures formed during the methylation reaction of the 2′,4″-bis(*O*-trimethylsilyl)erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] derivatives

| | C-11 | C-12 | C-6 |
|---|---|---|---|
| Pre-complex | | | |
| O ··· $CH_3$ | 2.749 | 2.591 | 3.067 |
| $CH_3$ ··· I | 2.066 | 2.071 | 2.063 |
| TS | | | |
| O ··· $CH_3$ | 1.835 | 1.775 | 1.824 |
| $CH_3$ ··· I | 2.364 | 2.392 | 2.373 |
| Post-complex | | | |
| O ··· $CH_3$ | 1.425 | 1.419 | 1.423 |
| $CH_3$ ··· I | 3.439 | 3.713 | 3.621 |
| Product | | | |
| O ··· $CH_3$ | 1.419 | 1.418 | 1.416 |

The formation of the anions by abstraction of hydrogen in a 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,[20] these types of reactions have low activation barriers. Thus, we examined the heats of reaction for the formation of the anions corresponding to hydrogen abstraction from C-6, C-11 and C-12 (Table 3, column 2). Our findings reveal that anion formation at the C-6 position is the most exothermic process.

We then modeled the reaction of each anion with $CH_3I$. This process was considered as an $S_N2$ reaction.[21] 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 familiar 'Walden inversion' of the $CH_3$ group.[21] The reaction path (Fig. 3) exhibited two local minima, the pre- and the post-reaction ion–molecule complexes [O ··· $CH_3$I] and [$OCH_3$ ··· I], and proceeds *via* the transition structure [O ··· $CH_3$ ··· I]. The bond lengths of the structures and the energy barriers between the pre-reaction ion–molecule complex and transition structure are presented in Table 4 and the third column of Table 3, respectively. Methylation of the C-6 anion was preferred over that of the other anions.

Allevi *et al.*[6] have claimed that the bulkiness of the 9-[*O*-(dimethylthexylsilyl)oxime] group protects the C-11 and C-12 hydroxy groups from attack by $CH_3I$ and induces selective methylation at C-6. Comparison of the energetics for the methylation at C-6, C-11 and C-12 has shown that the $OCH_3$ group at C-6 is more stable than the other methylated carbon atoms (Table 3, column 4).

### The effect of internal hydrogen bonds on selective methylation

The reactivity of alcohols in the *O*-methylation in a basic medium is influenced by the stability of the intermediate alkoxide anion as well as by the ease of deprotonation. A secondary

hydroxy group, such as that on C-11, is more reactive than a tertiary hydroxy group, such as those on C-6 and C-12. The ease of deprotonation depends upon the intramolecular hydrogen bonding. Prompted by the work of Gotō *et al.*[22] we examined three types of internal hydrogen bonds in erythromycin A (**1**), 9-[*O*-(dimethylthexylsilyl)oxime] (**3**) and 2′,4″-bis(*O*-trimethylsilyl)erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] (**4**). The hydrogen atom of the hydroxy group on C-12 and the oxygen atom of the hydroxy group on C-11 form a five-membered chelate ring. This distance is 2.145 in **1**, 2.176 in **3** and 2.223 Å in **4**. The hydrogen of the hydroxy group on C-11 is held by C-9. This distance is 2.161 in **1**, 2.080 in **3** and 2.178 Å in **4**. The hydrogen of the hydroxy group on C-11 and the carbonyl oxygen on C-1 interact through a long-distance hydrogen bond. This distance is 3.947 in **1**, 3.237 in **3** and 2.543 Å in **4**.

As claimed by Gotō *et al.*,[22] a network of hydrogen bond interactions within and along the macrocyclic lactone ring is present in compounds **1**, **3** and **4**. These distances confirm the

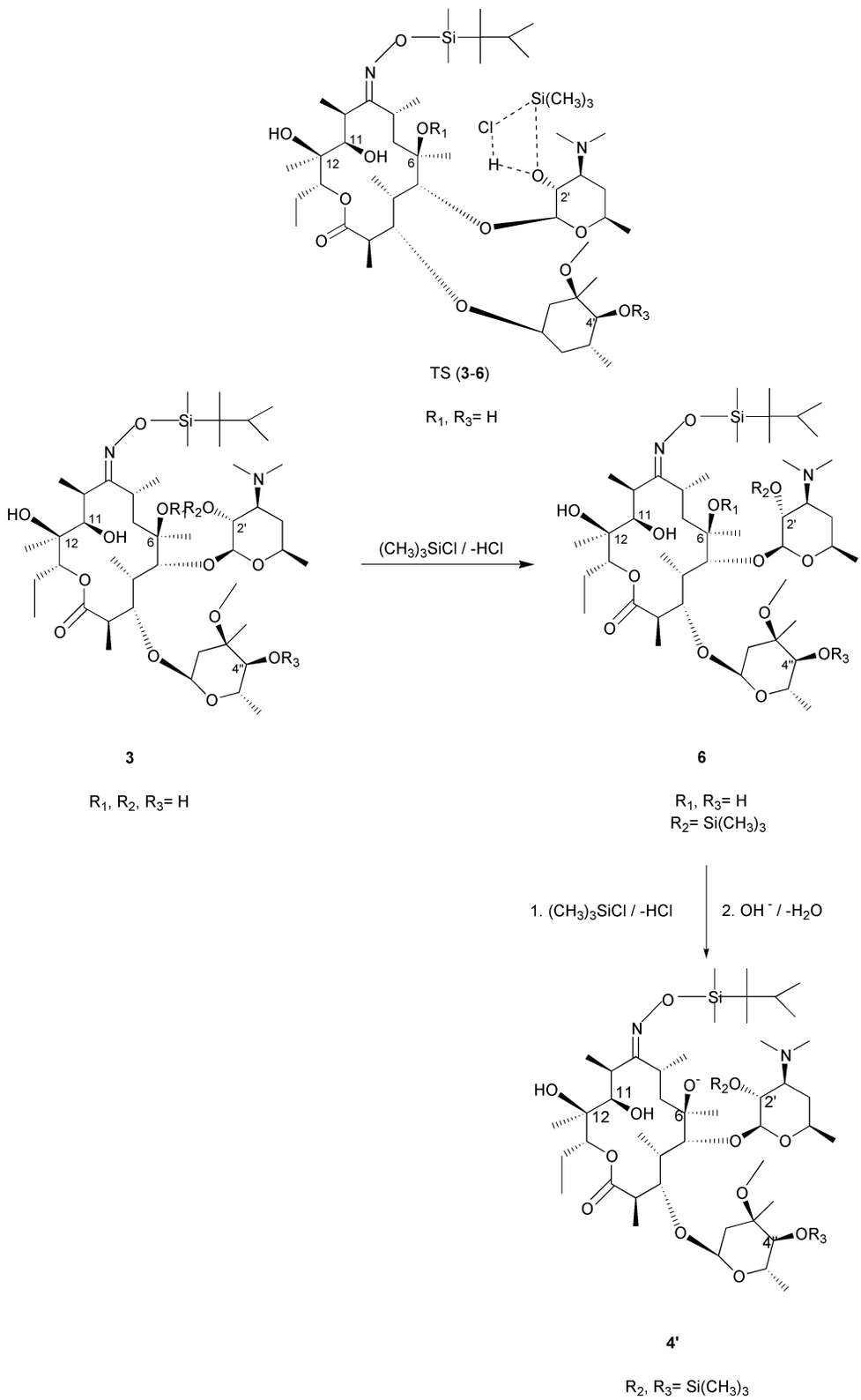


**Fig. 2** Protection and anion formation at C-6 for erythromycin A 9-[*O*-(dimethylthexylsilyl)oxime] (**3**).

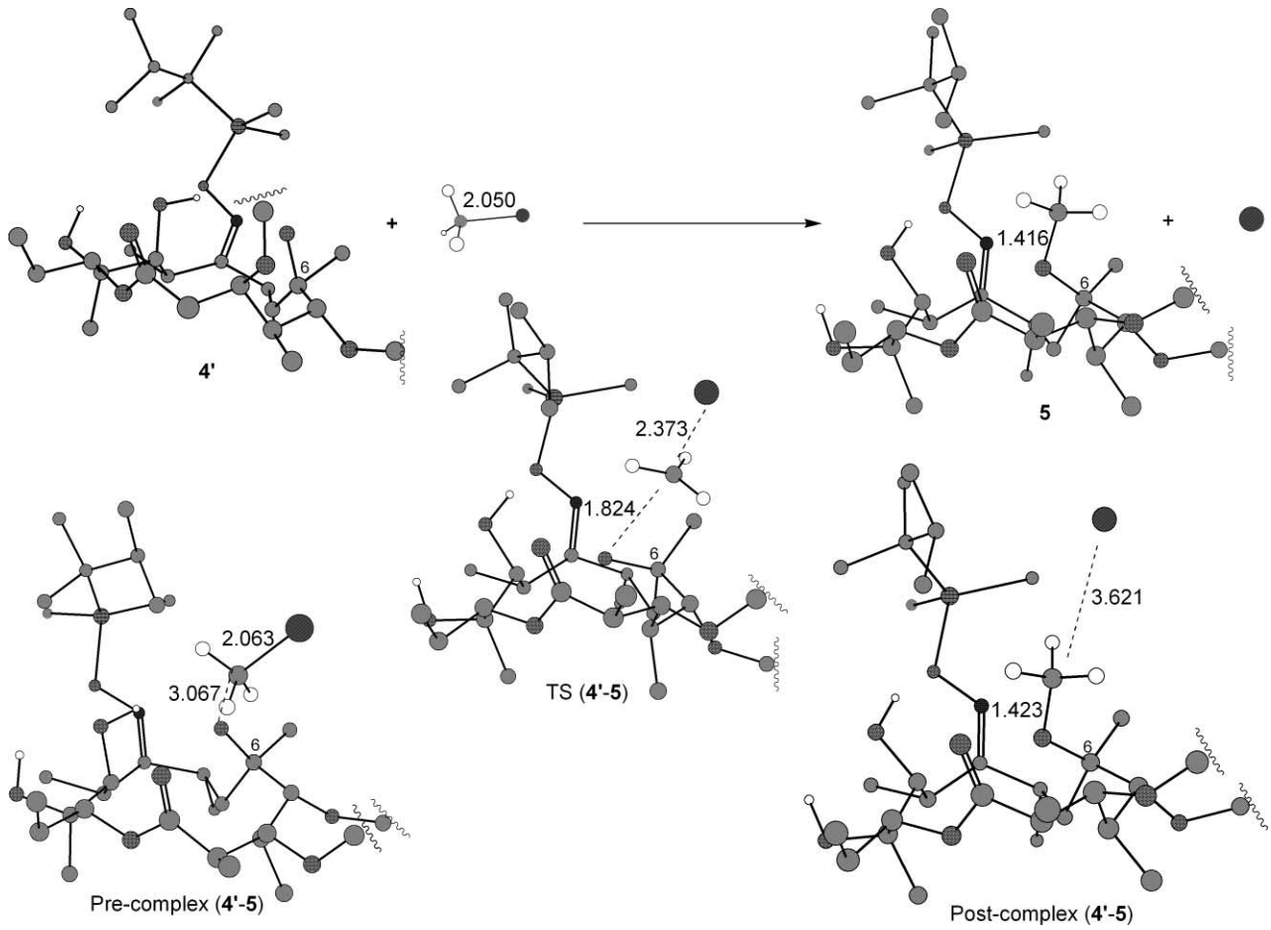


**Fig. 3** Three dimensional view of the methylation mechanism of C-6-O$^-$ of 2′,4″-bis(*O*-trimethylsilyl)erythromycin A 9-[*O*-(dimethylthexylsilyl)-oxime] (**4′**).

protection of 11-OH and 12-OH through long-range interactions. Furthermore, the introduction of the oxime at C-9 and the protection of 2′ and 4″ enhances the bonding between the 11-OH and the carbonyl oxygen on C1. On the other hand, the fact that the structures of **1**, **3** and **4** located with AM1 reproduce the experimental expectations lends further support to the use of AM1 for more certain geometry optimization of stationary points along the reaction path of macrolides.

## Conclusions

We have modeled the synthesis of clarithromycin by suggesting a four-membered transition structure for the protection of erythromycin and an $S_N2$ mechanism for the methylation reaction. The structures proposed as well as the methodology used reproduce the experimental findings. Thus, the methodology used in this study can be extended to modeling the synthesis of other potential semi-synthetic 14-membered ring macrolide antibiotics such as roxithromycin and azithromycin.

The approach used in this work ignores the effect of solvent. In particular, solvent shielding, which may have a considerable influence on the interactions of side chains and therefore lead to different conformers, cannot be reproduced. However, *ab initio* methods that incorporate solvent effects do so through implicit solvation models which have so far been developed for a limited number of solvents such as water. We are therefore performing molecular dynamics simulations of the C-6, C-11, and C-12 anions in explicit DMSO–THF (1 : 1) solution (a solvent cage of DMSO–THF) to gain insight into how the dynamic behavior of the molecules would affect the side chain conformers leading to the methylation process.

It is also desirable to use the methodology described in this study and test the selectivity of the methylation reaction in the presence of several oxime groups that have performed favorably in experimental studies. In all the approaches described above, the ultimate goal would be to guide experimentalists through the synthesis of macrolides.

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## References

1 S. Morimoto, Y. Takahashi, Y. Watanabe and S. Omura, *J. Antibiot.*, 1984, **37**, 187; S. Morimoto, Y. Misawa, T. Adachi, T. Nagate, Y. Watanabe and S. Omura, *J. Antibiot.*, 1990, **43**, 286; S. Morimoto, T. Nagate, K. Sugita, T. Ono, K. Numata, J. Miyachi, Y. Misawa and K. Yamada, *J. Antibiot.*, 1990, **43**, 295.
2 Y. Watanabe, S. Morimoto, T. Adachi, M. Kashimura and T. Asaka, *J. Antibiot.*, 1993, **46**, 647.
3 P. Kurath, P. H. Jones, R. S. Egan and T. J. Perun, *Experientia*, 1971, **27**, 362; P. J. Atkins, T. O. Herbert and N. B. Jones, *Int. J. Pharm.*, 1986, **30**, 199.
4 Y. Watanabe, T. Adachi, T. Asaka, M. Kashimura and S. Morimoto, *Heterocycles*, 1990, **31**, 2121; Y. Watanabe, M. Kashimura, T. Asaka, T. Adachi and S. Morimoto, *Heterocycles*, 1993, **36**, 243; S. Omura, S. Morimoto, T. Nagate, T. Adachi and Y. Kohno, *Yakugaku Zasshi*, 1992, **112**, 593.
5 Y. Watanabe, T. Adachi, T. Asaka, M. Kashimura, T. Matsunaga and S. Morimoto, *J. Antibiot.*, 1993, **46**, 1163.
6 P. Allevi, A. Longo and M. Anastasia, *Bioorg. Med. Chem.*, 1999, **7**, 2749.
7 H. Iwasaki, Y. Sugawara, T. Adachi, S. Morimoto and Y. Watanabe, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1993, **49**, 1227.

8 J. R. Maple, M. J. Hwang, T. P. Stockfisch, U. Dinur, M. Waldman, C. S. Ewig and A. T. Hagler, *J. Comput. Chem.*, 1994, **15**, 162.
9 M. J. Hwang, T. P. Stockfisch and A. T. Hagler, *J. Am. Chem. Soc.*, 1994, **116**, 2515.
10 C. Baysal and H. Meirovitch, *J. Comput. Chem.*, 1999, **20**, 1659.
11 H. Meirovitch, E. Meirovitch and J. Lee, *J. Phys. Chem.*, 1995, **9**, 4847.
12 E. Meirovitch and H. Meirovitch, *Biopolymers*, 1996, **38**, 69.
13 C. Baysal and H. Meirovitch, *Biopolymers*, 2000, **53**, 423.
14 H. C. Andersen, *J. Chem. Phys.*, 1980, **72**, 2384.
15 S. G. Nash, User's Guide for TN/TNBC: FORTRAN Routines for Nonlinear Optimization, Report 397, Mathematical Sciences Department, Johns Hopkins University, Baltimore, MD, 1984.
16 C. Baysal and H. Meirovitch, *Biopolymers*, 1999, **50**, 329.
17 GAUSSIAN 98 Revision A.1, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Karkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, G. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. A. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, V. Gonzalez, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle and J. A. Pople, Gaussian Inc., Pittsburgh, PA, 1998.
18 SPARTAN Version 5.1.3, Wavefunction, Inc., 18401 Von Karman Ave., #370 Irvine, CA 92715.
19 T. Oie, G. H. Loew, S. K. Burt, J. Stephen Binkley and R. D. MacElroy, *J. Am. Chem. Soc.*, 1982, **104**, 6169.
20 J. Chen, M. A. Mcallister, J. K. Lee and K. N. Houk, *J. Org. Chem.*, 1998, **63**, 4611.
21 S. Partiban, G. Oliveira and J. M. L. Martin, *J. Phys. Chem. A*, 2001, **105**, 895.
22 H. Gotō, Y. Kawashima, M. Kashimura, S. Morimoto and E. Ōsawa, *J. Chem. Soc., Perkin Trans. 2*, 1993, 1647.