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# A new insight into solid-state conformation of macrolide antibiotics

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**Abstract**—Quantitative analysis of the molecular conformations of the 14-membered macrolide antibiotics erythromycin A and B, clarithromycin, and roxithromycin in the solid state was performed. While the erythronolide macrocycle adopts a very similar folded-out conformation in all the macrolides studied, the proximity of the monosaccharide moieties, L-cladinose and D-desosamine, to each other is demonstrated to be the distinctive feature of their molecular conformations, based on atom–atom interaction energy analysis. More surprisingly, the common features in the relative orientation of the monosaccharide moieties (in terms of non-bonded atom–atom interactions) were revealed between the 14- and 15-membered (azithromycin) macrolide antibiotics. Herein we report on the details of the spatial arrangement of the monosaccharide moieties in these structurally related drug molecules and their influence on the biopharmaceutical properties of erythromycin derivatives.


## 1. Introduction

Modern methods of structural analysis and the advances in molecular biology allow us to determine the structure of a target enzyme or receptor for a particular disease, which can be used to establish a suitable model for a new drug. Once the molecular target is identified, medicinal chemists use a variety of computational methods[1,2] to modify the chemical structure of the hit and lead compounds to maximize their in vitro activity. However, good in vitro activity cannot be extrapolated satisfactorily to in vivo activity unless a drug possesses desirable absorption, distribution, metabolism, and elimination (ADME) characteristics.[3,4] Overall, around half of all drugs in development fail to make it to the market because of ADME deficiencies.[3,5]

In contrast to the majority of other drugs, the target receptors of antibiotics are not located in human tissues, but in the cells of microbes which possess high genetic variability. Due to this, therapeutic use of antibacterial antibiotics is often compromised by the emergence of drug resistance in various pathogenic bacteria. The structural basis for antibacterial action of some macrolide antibiotics that could be utilized in the drug development to overcome resistance problem has recently been reported.[6,7] Nevertheless, the ability to predict ADME profile from a molecular structure remains an essential goal.[8]

The macrolides, especially erythromycin derivatives, are among the most extensively used antibacterial antibiotics that have proved to be very well tolerated and safe.[9] They inhibit bacterial polypeptide translation by binding selectively to the bacterial 50S ribosomal subunit.[6] In addition, erythromycin inhibits the assembly of 50S subunits of the bacteria through the specific interaction with the 23S rRNA of the bacterial ribosomes.[6] Meanwhile, macrolide antibiotics do not bind to the mammalian ribosomes. Erythromycin, the first natural macrolide antibiotic, has been used clinically since 1952. In 1980–1990s, a resurgence of interest in macrolides driven by the recognition of pathogens such as *Legionella*, *Mycoplasma*, *Chlamydia*, and *Campylobacter* resulted in the launching of a number of new semisynthetic erythromycin derivatives (e.g., roxithromycin, clarithromycin, azithromycin). More recently, macrolides have been also considered for other uses in the

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areas of neurology,[10] gastroenterology,[11] rheumatology,[12] cardiology,[13] and cancer therapy,[14,15] facilitating the development of new synthetic methods[16,17] as well as the design of new therapeutic agents possessing non-antibacterial activities.

The distinguishing feature of erythromycin derivatives is the presence of a 14-membered lactone ring (erythronolide) and two monosaccharide moieties, L-cladinose (CL, 2,3,6-trideoxy-3-methoxy-3-*C*-methyl-L-*ribo*-hexose) and D-desosamine (DA, 3,4,6-trideoxy-3-dimethylamino-D-*xylo*-hexose), attached to the macrocycle (Fig. 1a). The intricate structures of the erythromycin derivatives suggest the variety of possible conformations and the ways they can be spatially packed. Considering organic crystals as solid-state supermolecules self-assembled by intermolecular interactions,[18] one can assume that supramolecular motifs in crystalline substances as well as molecular conformations may have a substantial effect on pharmacological activity and bioavailability of drug molecules.[19,20] In particular, erythromycin derivatives are an example of the structurally related drugs, which have the same mode of action, but significantly differ in their pharmacokinetic properties.

In this study, quantitative analysis of the molecular conformations of 14-membered macrolide antibiotics erythromycin A and B, clarithromycin, and roxithromycin in the solid state was performed to advance our understanding of the structural features, which can affect the pharmacological activity and in vivo performance of these drugs. In addition, spatial arrangement of the monosaccharide moieties (in terms of non-bonded atom–atom interactions) in a 15-membered macrolide antibiotic, azithromycin (Fig. 1c), was further analyzed to ascertain whether or not conformational similarities are conserved between the 14- and 15-membered erythromycin derivatives.

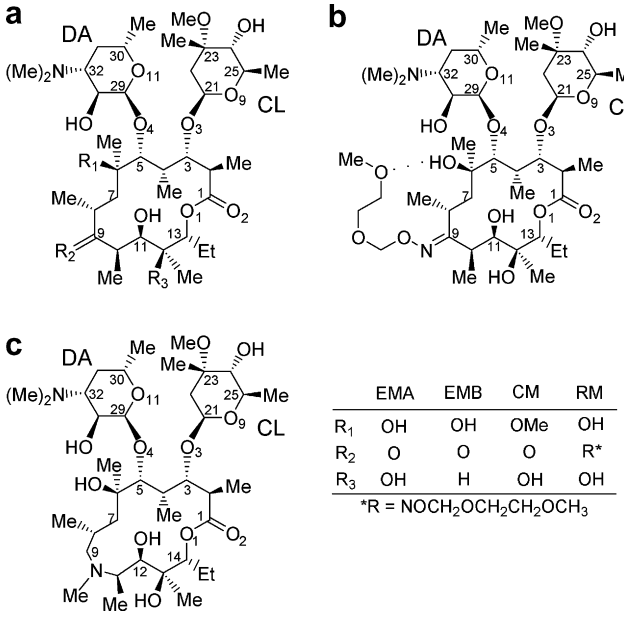


**Figure 1.** General structural formula of the 14-membered macrolide antibiotics with the numbering system of the atoms used in this study (a), structural formula of roxithromycin showing an intramolecular hydrogen bond (b), and chemical structure of a 15-membered macrolide antibiotic, azithromycin (c).

## 2. Results and discussion

### 2.1. Conformation of 14-membered lactone rings

The search of Cambridge Structural Database (CSD) has retrieved four clinically relevant macrolide antibiotics—erythromycin A dihydrate (EMA, refcode: NAVTAF[21]), erythromycin B dihydrate (EMB, refcode: NAVTEJ[21]), clarithromycin (CM, refcode: NAVSUY[21]), and roxithromycin monohydrate (RM, refcode: FUXYOM[22])—which satisfy the general molecular structure depicted in Figure 1a. Since their distinguishing groups $R_1$, $R_2$, and $R_3$ are fairly small, these molecules have rather similar contours with the sole exception of the RM molecule, the contour of which differs due to the bulky substituent $R_2$ (=N–O–$CH_2$–O–$CH_2$–$CH_2$–O$CH_3$) of ~11 Å in length. However, as deduced from the crystallographic data, this *N*-2,5-dioxahexyloxime residue is tightly bound over the macrocycle due to an intramolecular hydrogen bond between the O atom of the terminal methoxy group and the hydroxy group $R_1$ (see Fig. 1b). For this reason, the contour of the RM molecule is approaching that of EMA, EMB, and CM.

Furthermore, it would seem that a 14-membered macrolide ring must display considerable conformational mobility. However, as seen in Table 1 and Figure 2, the configuration of the macrocycle is essentially the same in all the macrolide antibiotics considered (the distinctions for deviations from the mean plane of the macrocycle as a rule do not exceed 0.5 Å). Interestingly, the RM molecule with its bulky substituent $R_2$ did not constitute an exception. It should be added that the crystal structures of these macrolides markedly differ in cell parameters and in the features of molecular packing. Nevertheless, the erythronolide macrocycle appears to be sufficiently rigid to retain its conformation in this series of the macrolides. The analysis of torsion angles in

**Table 1.** Deviations (Å) of atoms of the 14-membered erythronolide macrocycle from the mean square plane

| Atoms of the macrocycle | EMA | EMB | CM | RM |
|---|---|---|---|---|
| C(1) | 0.64 | 0.63 | 0.60 | 0.62 |
| C(2) | 0.15 | 0.13 | 0.02 | 0.04 |
| C(3) | 0.42 | 0.43 | 0.42 | 0.25 |
| C(4) | −0.68 | −0.68 | −0.68 | −0.64 |
| C(5) | −0.28 | −0.26 | −0.15 | −0.17 |
| C(6) | 0.40 | 0.41 | 0.52 | 0.52 |
| C(7) | −0.60 | −0.59 | −0.56 | −0.53 |
| C(8) | 0.09 | 0.08 | −0.07 | −0.01 |
| C(9) | 0.71 | 0.69 | 0.63 | 0.61 |
| C(10) | −0.16 | −0.18 | −0.20 | −0.26 |
| C(11) | 0.31 | 0.31 | 0.33 | 0.34 |
| C(12) | −0.60 | −0.58 | −0.51 | −0.56 |
| C(13) | −0.04 | −0.03 | −0.02 | 0.09 |
| O(1) | −0.36 | −0.36 | −0.35 | −0.31 |

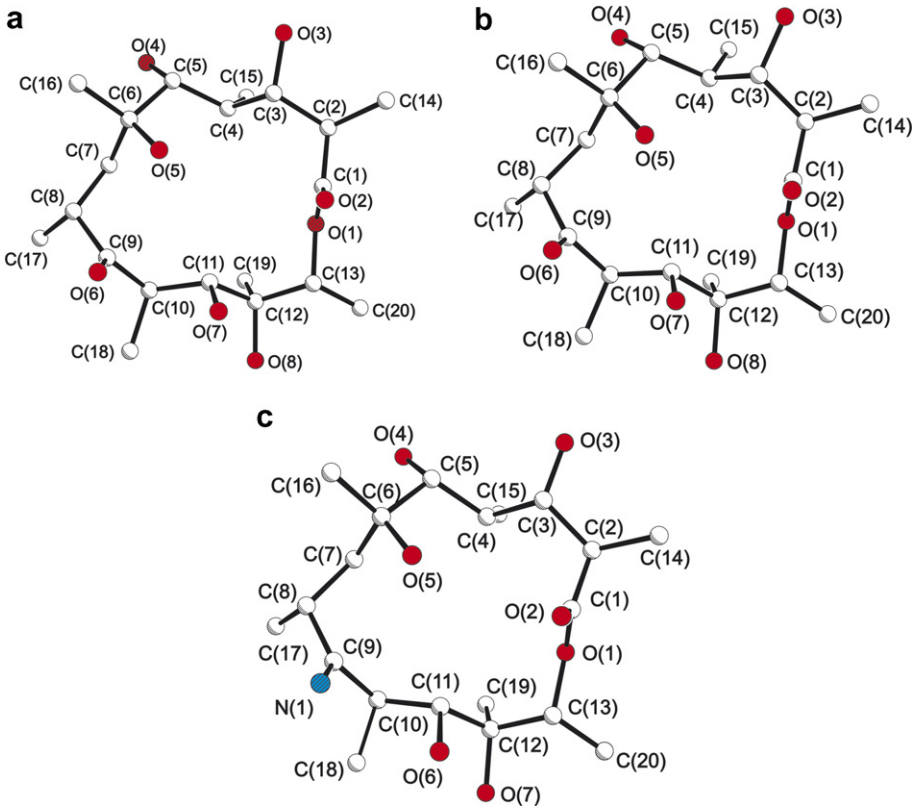


**Figure 2.** Projections of the macrocycles and of the adjusted atoms of erythromycin A (a), clarithromycin (b), and roxithromycin (c) onto the mean square plane of the macrocycle. Color scheme: C, white; O, red; N, blue; H, not shown.

the macrocycle as well as the closest superposition of the compared atomic systems leads us to a similar conclusion; the distances between atoms with the same labels do not exceed 0.3–0.4 Å. In particular, the comparison of macrocycles in molecules of CM and RM gives a maximal incompatibility value of 0.43 Å. Finally, the conclusion about the rigidity of the macrocycle is well supported by the previous reports, showing these macrolide molecules to adopt predominantly the same conformation in both the solid state and aqueous solution.[23,24] This rigid conformation of the macrocycle is called a 'folded-out' conformation[25] and is characterized by a large torsion angle H(2)C(2)C(3)H(3) of ~160° and a close proximity of H(4) and H(11) atoms.

## 2.2. Conformation of monosaccharides and their attachment to macrocycles

The essential structural components of erythromycin derivatives are the cyclic monosaccharides, D-desosamine (DA), and L-cladinose (CL). The comparison of their spatial structures showed that the conformation of both monosaccharide moieties remains practically unchanged in all the macrolides considered and can be defined as a chair conformation. Next, spatial configurations of the 'hinges' (α-glycosidic bonds) that connect the 14-membered erythronolide macrocycle to the monosaccharide moieties were compared. The most descriptive structural characteristics of these fragments are torsion angles (Table 2), which considerably influence the general shape of the molecule. The CM molecule most significantly differs from the other macrolide molecules studied by the torsion angles between the erythronolide macrocycle and L-cladinose monosaccharide. For example, the torsion angles C(2)C(3)O(3)C(21) and C(4)C(3)O(3)C(21) differ in the case of CM by ~15° from the corresponding angles in the macrolide molecules EMA, EMB, and RM.

**Table 2.** Torsion angles (degrees) between the erythronolide macrocycle and monosaccharide moieties of macrolide antibiotics

| Torsion angles | EMA | EMB | CM | RM |
|---|---|---|---|---|
| L-*Cladinose* | | | | |
| C(2)C(3)O(3)C(21) | −94.5 | −94.5 | −108.4 | 90.9 |
| C(4)C(3)O(3)C(21) | 143.2 | 143.1 | 131.2 | −147.6 |
| C(3)O(3)C(21)O(9) | −79.9 | −80.5 | −80.8 | 71.7 |
| C(3)O(3)C(21)C(22) | 155.0 | 153.0 | 154.3 | −163.0 |
| O(3)C(21)C(22)C(23) | 79.0 | 80.1 | 72.2 | −78.3 |
| O(3)C(21)O(9)C(25) | −76.8 | −77.5 | −68.5 | 73.7 |
| D-*Desosamine* | | | | |
| C(4)C(5)O(4)C(29) | −100.5 | −99.9 | −108.8 | 106.9 |
| C(6)C(5)O(4)C(29) | 133.0 | 133.1 | 127.8 | −129.8 |
| C(5)O(4)C(29)O(11) | −81.9 | −81.2 | −75.6 | 74.8 |
| C(5)O(4)C(29)C(33) | 160.5 | 161.5 | 165.6 | −165.5 |
| O(4)C(29)O(11)C(30) | 178.4 | 179.4 | −178.9 | 179.7 |
| O(4)C(29)C(33)C(32) | 177.0 | 178.1 | 176.6 | −173.1 |

Furthermore, when the backbone atoms in the three rings are fitted to the mean planes, the L-cladinose ring in the CM molecule forms an angle of ~14° with the mean plane of the macrocycle, whereas in the RM molecule this angle is close to 38°, and in the EMA

and EMB molecules it is close to 20°. Meanwhile, the D-desosamine ring is nearly perpendicular to the macrocycle, which is a common conformational feature for all the erythronolide molecules considered (105° for EMA and EMB, 82° for CM, 83° for RM).

### 2.3. Relative orientation of monosaccharide moieties and in vivo behavior of erythromycin derivatives

The most striking differences in the molecular conformation among erythromycin derivatives were revealed in the relative orientation of the monosaccharide moieties, L-cladinose (CL) and D-desosamine (DA) (Fig. 3). In all the macrolide molecules considered, close atom–atom contacts between two monosaccharides occur (Table 3). We took the van der Waals radii of atoms C, H, and O to be 1.72, 1.16, and 1.29 Å, respectively,[26] and regarded the distance $r(X\ldots Y)$ as a contact if $r \leqslant R_X + R_Y + 0.2$ Å (the distances shown by thick broken and dot-and-dash lines in Fig. 3). The distances $r(X\ldots Y)$ that are approximate contacts, that is, for which $R_X + R_Y + 0.2$ Å $< r \leqslant R_X + R_Y + 0.3$ Å (shown by thin broken and dot-and-dash lines in Fig. 4), were also taken into account. In the case of RM, several contacts O...H (in Fig. 4 they are shown by dotted lines) between CL and DA were revealed, with two of them, O(13)...H(39) and O(10)...H(38), corresponding to intramolecular hydrogen bonds.

The systems of contacts between monosaccharide moieties in the EMA and EMB molecules are very similar—the five of six contacts present in each compound are almost identical; hereafter, they are called 'recurring' contacts (in Table 3 they are asterisked). These contacts are mostly realized in the interring region (CL–DA); also, there are short non-bonded contacts between methyl groups ($M_1$–$M_2$). In the RM molecule, the number of the contacts and approximate contacts is much larger. In addition to all the recurring contacts, there are the contacts between the methoxy group and DA, which provide compactness to the RM molecule. The structure of the CM molecule differs more significantly from the other macrolides studied. Specifically, the CM molecule reproduces only two contacts typical (i.e., 'recurring') for the triad EMA–EMB–RM, a contact H...C between rings CL and DA and a contact C...H between methyl groups $M_1$ and $M_2$. Furthermore, there is a region of close interaction $M_1$–DA, which is unique for the CM molecule. Finally, the comparison of monosaccharide moieties' arrangements in these antibiotics shows that, unlike the other macrolides, CL monosaccharide in the CM molecule is rotated relative to the amino sugar DA (the mean planes of CL and DA are almost perpendicular).

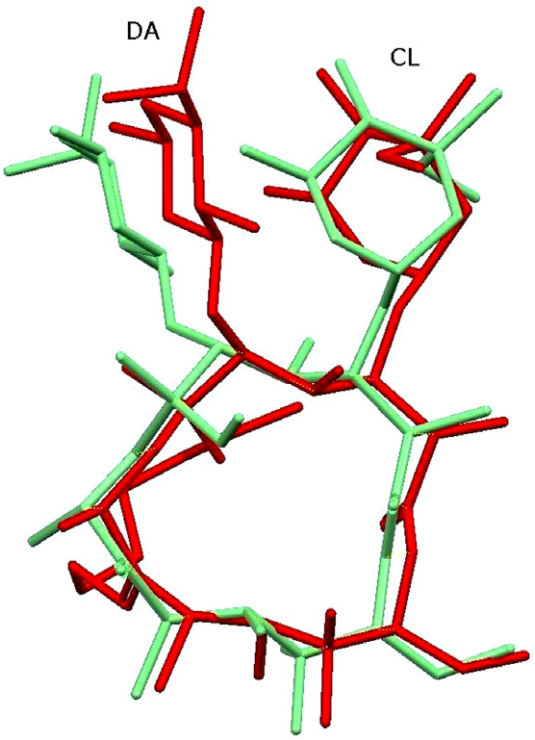


**Figure 3.** Superimposition of clarithromycin (green) and roxithromycin (red) showing differences in relative orientation of the monosaccharide moieties, L-cladinose (CL) and D-desosamine (DA). Atoms of the erythronolide macrocycle were used for superimposition; the H atoms are not shown for clarity.

To get a more complete picture of the relative orientation of the monosaccharide moieties, non-bonded atom–atom interactions were further evaluated in terms of interaction energy. For the EMA, EMB, and CM molecules, interaction energy corresponding to the atom–atom contacts between CL and DA is rather small (predominantly less than 0.1 kJ $mol^{-1}$) and mostly negative (Table 3) indicating that the arrangements of the monosaccharide moieties are not rigid. In the case of RM, the situation is fairly different. There are a number of atom–atom contacts, mostly those observed in the EMA and EMB macrolide molecules, but the distances are shortened. As a result, the values of atom–atom interaction energy, which correspond to these shortened contacts, range up to 1.7 kJ $mol^{-1}$. Hence, the relative arrangement of CL and DA in the RM molecule is very rigid. However, it should be noted here that Novak's previous conformational NMR analysis of the related macrolide oleandomycin with an exocyclic epoxide moiety has revealed that oleandomycin showed a certain amount of conformational flexibility both in the erythronolide and monosaccharide parts. Folded-in conformation was found to be the predominant conformation in DMSO, and mixtures of folded-in and folded-out conformers were observed in buffered $D_2O$, acetone-$d_6$, and $CDCl_3$.[27]

Our study demonstrates that the configurations of three structural components of erythromycin derivatives (the 14-membered erythronolide-type lactone ring, L-cladinose, and D-desosamine) are rather similar. Moreover, the ways the monosaccharides are linked to the macrocycles are conformationally similar (with exception of CM). This is a possible reason for the same mode of action and a similar spectrum of antibacterial activity of the macrolides investigated. Nevertheless, it is known that the second-generation macrolides, CM and especially RM, are pharmacokinetically superior to their precursor EMA[28] (Table 4). Following oral administra-

**Table 3.** Non-bonded atom–atom contacts between monosaccharide moieties[a]

| Interacting fragments | Atom–atom contacts | Distances (Å) and energy values (kJ mol$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | EMA | EMB | CM | RM[b] | AM[c] |
| L-Cladinose–D-desosamine (CL–DA) | C(25)...H(39) | 3.183 (−0.096) | | | 2.859 (0.059)† | 3.123 (−0.089) |
| | H(25)...C(29) | 3.119 (−0.088)* | 3.106 (−0.084)* | 3.197 (−0.077)* | 2.798 (0.134)*† | 2.874 (0.041)*† |
| | H(25)...C(30) | 2.960 (−0.029)*† | 2.957 (−0.025)*† | | 2.896 (0.021)*† | 2.896 (0.021)*† |
| | H(25)...H(38) | | | 2.407 (0.498)† | 2.141 (1.691)† | 2.227 (1.151) † |
| | H(25)...H(39) | 2.401 (0.511)*† | 2.444 (0.414)*† | | 2.169 (1.491)*† | 2.345 (0.667)*† |
| Methyl–methyl (M1–M2) | C(27)...H(46) | 3.063 (−0.075)*† | 3.036 (−0.063)*† | 3.090 (−0.080)*† | 2.927 (−0.004)*† | 3.188 (−0.098)* |
| | H(29)...C(34) | | | | 2.887 (0.029)† | |
| | H(29)...H(46) | 2.427 (0.448)*† | 2.276 (0.921)*† | | 2.147 (1.650)*† | 2.439 (0.424)*† |
| | C(27)...C(30) | | | 3.626 (−0.123) | | |
| Methyl–D-desosamine (M1–DA) | C(27)...H(39) | | | 3.011 (−0.054)† | 3.180 (−0.096) | |
| | H(29)...C(30) | | | 3.176 (−0.096) | | |
| | H(29)...H(39) | | | 2.421 (0.461)† | | |

[a] Contacts that satisfy the condition $r \leqslant R_X + R_Y + 0.3$ Å are presented in Table: (†) the contacts which are less than $R_X + R_Y + 0.2$ Å; (*) 'recurring' contacts.

[b] In the RM molecule, there are additionally the following short contacts: CL–DA: C(25)...C(30) 3.673 (−0.163), C(24)...H(39) 3.148 (−0.092), C(25)...H(38) 3.134 (−0.092), H(25)...O(11) 2.719 (−0.243)†, O(13)...H(39) 2.470 (0.029)†, C(27)...C(34) 3.718 (−0.176); methoxy group-D-desosamine (MO–DA): C(35)...H(38) 3.079 (−0.080)†, O(10)...H(38) 2.482 (0.004)†.

[c] In the AM molecule, there are additionally the following short contacts: CL–DA: H(25)...O(11) 2.716 (−0.242)†, O(13)...H(39) 2.575 (−0.152)†; methoxy group-D-desosamine (MO–DA): C(35)...H(38) 3.137 (−0.091), O(10)...H(38) 2.747 (−0.249)†, O(10)...H(39) 2.996 (−0.225).

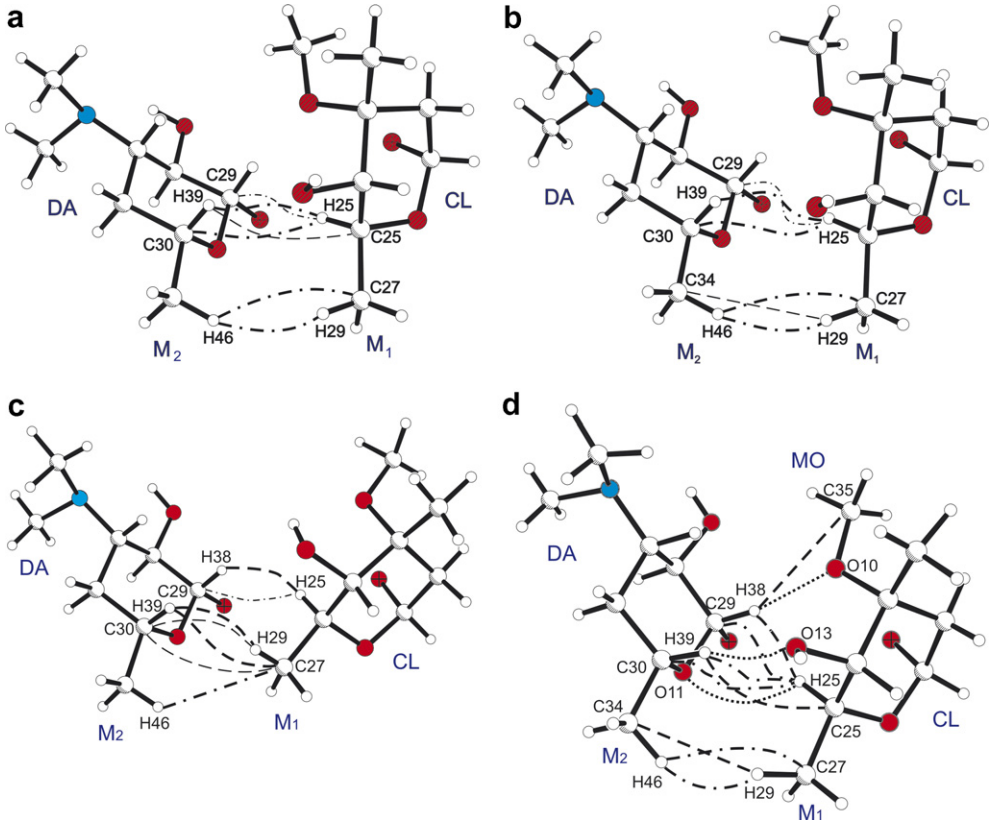


**Figure 4.** Arrangement of monosaccharide moieties, L-cladinose (CL) and D-desosamine (DA), in erythromycin A (a), erythromycin B (b), clarithromycin (c), and roxithromycin (d). The shortest atom–atom distances between monosaccharide moieties are shown (see text). For roxithromycin, only the contacts that satisfy the condition $r \leqslant R_X + R_Y + 0.2$ Å are represented for clarity. Crosses indicate the atoms of the macrocycle to which the monosaccharide moieties are attached. Color scheme: C, white; O, red; N, blue; H, white.

tion, CM and RM reach peak plasma concentrations within the same time period (at about 2 h), while EMA is slower with its peak levels being reached at 1–5 h. The concentration maxima indicating the significant difference in absorption properties increase in the following sequence: EMA–CM–RM (after an oral dose of 500, 500, and 150 mg, respectively). Accordingly, RM has an oral bioavailability of 81%, which is the

**Table 4.** Mean pharmacokinetic parameters of erythromycin A, clarithromycin, and roxithromycin following single-dose oral administration[a]

| Parameter | EMA[30,31] | CM[32,33] | RM[30] |
|---|---|---|---|
| Dose (mg) | 500 | 500 | 150 |
| Bioavailability (%) | 18–45 | 55 | 72–85 |
| Peak plasma concentration ($C_{max}$, mg/l) | 1.9–3.8 | 2.41 | 7.9 |
| Time to reach peak plasma concentration ($T_{max}$, h) | 1–5 | 2.0 | 2.2–2.4 |
| Area under the pharmacokinetic curve (AUC, mg/h L) | 5.8–11.2 | 18.9 | 81 |
| Elimination half-life ($T_{1/2}$, h) | 1.5–3.0 | 4.9 | 10.5 |

[a] Erythromycin B is an active impurity and a biosynthetic precursor of erythromycin A;[29] however, pharmacokinetic data for its administration in humans have not been published.

highest of these three macrolide antibiotics. Overall, RM and CM have more predictable absorptions (AUC 81 and 18.9 mg/h L, respectively) compared to EMA (AUC 5.8–11.2 mg/h L). Finally, EMA has a significantly shorter elimination half-life than either CM or RM (1.5–3 h vs 4.9 and 10.5 h).

It is well known that a suitable pharmacokinetic profile of a compound is a complex function of its properties such as dissolution, intestinal absorption, and cellular permeability,[4] just to name a few. These properties are, in turn, influenced by the physicochemical properties of the compound, that is, molecular weight, lipophilicity, and hydrogen-bonding capacity.[8,34] In addition, the impacts of molecular surface properties[35] and of molecular flexibility[36] on intestinal absorption have been recognized recently. In the case of erythromycin A and B, their low bioavailability is also a result of decomposition under the acidic conditions present in the abdomen.[37,38] Since the degradation pathway involving 6-OH and the 9-keto group is blocked in the second-generation macrolides, it is considered to be the major reason for their superiority.[24] The pharmacokinetic differences between RM and CM may be related to their distinct physicochemical properties arising from the chemical modification. For example, RM is assumed to keep the high serum levels owing to its high affinity to plasma protein (protein binding is approximately 95% and 50% for RM[39] and CM,[40] respectively), whereas CM is likely to distribute extensively from the blood to other tissues due to its low affinity to plasma protein and a higher lipophilicity ($\log D$ of *n*-octanol–water partition coefficient at pH 8.0 amounts to 2.5 and 3.9 for RM[41] and CM,[42] respectively), resulting in the low serum levels.[43]

Meanwhile, the results of this study indicate that the relative orientation of monosaccharide moieties in these macrolide antibiotics affects the molecular properties, which are essential for membrane permeation. More specifically, a number of short atom–atom contacts, including two intramolecular hydrogen bonds between CL and DA in the RM molecule, lead simultaneously to the reduced polar surface area and restricted molecular flexibility of this molecule as compared with those of EMA, EMB, and CM. Both these molecular properties have been shown to be prerequisites for good oral bioavailability; reduced polar surface area correlates with increased permeation rate, while increased molecular flexibility has a negative effect on the permeation rate.[36] The improved pharmacokinetic profile of RM, therefore, can be partly attributed to the specific conformation of this molecule.

To further verify or refute this statement, an additional model drug, azithromycin dihydrate (AM; CSD refcode: GEGJAD[44]), was selected, and the non-bonded atom–atom contacts between the monosaccharide moieties in this molecule were analyzed. AM provides a good model for this study for several reasons: (i) it belongs to a different subclass of macrolides—azalides—with the 15-membered lactone ring (Fig. 1c); (ii) possesses enhanced, rather unique pharmacokinetic profile with excellent tissue distribution and extended elimination half-life;[45] and (iii) adopts the similar (folded-out) conformation in the solid state and aqueous solutions.[23] Surprisingly, the system of contacts between CL and DA appeared to be almost identical (especially in terms of distances) in the molecules of RM and AM (see Table 3). Specifically, 11 out of 15 contacts and approximate contacts between the monosaccharide moieties in AM reproduce the contacts that are characteristic of RM, with all the 'recurring' contacts of the 14-membered macrolides being present. Meanwhile, there is an increased amount (4 vs 3) of the O...H contacts in AM as compared with that of RM, leading to the more reduced polar surface area of the AM molecule. Furthermore, among these macrolides AM appeared to be distinguished by the highest proximity and thus the most restricted conformational mobility of the monosaccharide moieties due to the three contacts, C(35)...H(42), C(35)...H(42), and O(3)...H(38), distinctive of AM (Fig. 5). This study therefore demonstrates that the relative orientation of the monosaccharide moieties is similar in the different

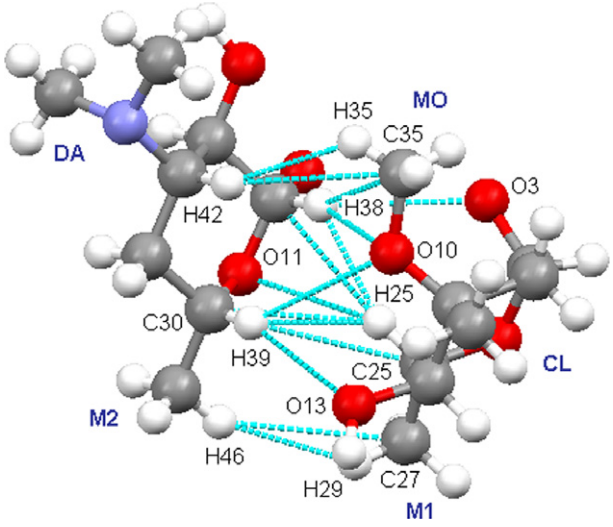


**Figure 5.** Arrangement of monosaccharide moieties, L-cladinose (CL) and D-desosamine (DA), in a 15-membered macrolide, azithromycin. The atom–atom contacts ($r \leqslant R_X + R_Y + 0.3$ Å) between the monosaccharide moieties are shown by dotted lines. Color scheme: C, grey; O, red; N, blue; H, white.

subclasses of the macrolide antibiotics and is likely to be an important structural feature with respect to biopharmaceutical properties. Hence, spatial arrangement of the monosaccharide moieties should be taken into account when establishing the relationships between the molecular structure and pharmacokinetic properties of the macrolide antibiotics.

## 3. Conclusions

A systematic study of the solid-state conformation of the 14-membered macrolide antibiotics has been undertaken, and three key conclusions have been made. The erythronolide macrocycle has a very similar folded-out conformation in all the macrolide molecules studied thus demonstrating its rigidity. The molecular conformations of clarithromycin and especially roxithromycin are shown to differ from those of erythromycin A and B in the relative orientation of their monosaccharide moieties, L-cladinose and D-desosamine. Furthermore, the analysis of non-bonded atom–atom contacts in terms of interaction energy reveals that the arrangement of the monosaccharide moieties in roxithromycin has an effect on the properties of biopharmaceutical importance such as molecular flexibility and polar surface area. This finding was further verified by analyzing spatial arrangement of the monosaccharide moieties in azithromycin, a 15-membered macrolide antibiotic. Overall, our study underlines that, for any drug molecule adopting similar conformations in the solid state and solution, all aspects of its solid-state conformation should be given full consideration in the drug design and development.

## 4. Experimental

### 4.1. Conformation of molecules

X-ray structural data for the 14-membered macrolide antibiotics, which satisfy the general formula shown in Figure 1a, were retrieved from the Cambridge Structural Database (CSD) (November 2004, v.5.26) using the ConQuest program.

All calculations were run on a computer using a set of programs available for molecular modeling. The computations included: (i) comparing conformations of 14-membered erythronolide macrocycles, (ii) comparing conformations of monosaccharide moieties, (iii) comparing the ways in which the monosaccharides are connected to the macrocycle, and (iv) comparing the relative arrangements of monosaccharide moieties (in terms of non-bonded atom–atom contacts). For this purpose, the projections of molecules and their fragments onto root-mean-square planes of the 14-membered cycle and the rings of monosaccharide moieties were used and the deviations of atoms from these planes were subsequently calculated using the interactive graphics program XP. Information on the attachment of the monosaccharides to the macrocycle was obtained from the corresponding values of torsion angles.

Another effective means of comparing molecules and their parts is the closest superposition of the compared atomic systems. This superposition was performed using the SUSY program,[46] which minimizes the $S$ criterion:

$$S = \frac{1}{N}\left(\sum_{i=1}^{N}\sigma_i^2\right)^{1/2}, \quad (1)$$

where $\sigma_i$ is the distance between atoms of the compared molecules with the same label. The values of $\sigma_i$ generating the minimum value $S$ are called 'incompatibilities'; correspondingly, $S_{\min}$ is the minimal root-mean-square incompatibility.

Calculations for the distances between the atoms of D-desosamine and L-cladinose were carried out using the Mercury 1.3 program.

### 4.2. Energy calculation procedure

To quantitatively evaluate the contacts between the monosaccharide moieties of the macrolides, the energies of non-bonded atom–atom interactions ($U_{AA}$) have been calculated. Following the classic work by Williams,[47] atom–atom approximation is most often used for such calculations:

$$U_{AA} = \varphi_{ij} + \psi_{ij}, \quad (2)$$

where $i$ and $j$ are the indeces of interacting atoms, $\varphi_{ij}$ is the energy of van der Waals interaction taken in the form of '6-exp' potential, and $\psi_{ij}$ is the energy of electrostatic interaction of residual atomic charges $q_i$ and $q_j$. Hence,

$$\phi_{ij} + \psi_{ij} = -Ar_{ij}^{-6} + B\mathrm{e}^{-\alpha rij} + q_iq_j/r_{ij}, \quad (3)$$

where $A$, $B$, $\alpha$ are empirical parameters dependent on the nature of interacting atoms. However, since the obtaining of very precise absolute values of the energy is certainly not required in such investigations (as a rule, relative values are of concern), the simplified procedure proposed by Filippini and Gavezzotti[48] can be considered as sufficient for this purpose. The special feature of the approach recommended by these authors is the ignoring of effective atomic charges and, consequently, of the term which takes into account electrostatic interaction ($\psi_{ij}$). Filippini and Gavezzotti demonstrated that the parameters of the 6-exp potentials selected in an appropriate way ensure correct calculation of interaction energy, even though atomic charges are ignored. This means that the atom–atom potential (3) is replaced by the potential, which contains only the first two of the three terms. In should be noted that while the parameters $A$, $B$, and $\alpha$ differ in these two calculation procedures, the profiles of both curves $\phi_{ij} + \psi_{ij}$ (the first approach) and $\phi_{ij}$ (the second approach) are similar. In the present study, the approach proposed by Filippini and Gavezzotti (i.e., '6-exp' potential) has been applied.

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