# CONFORMATIONAL FLEXIBILITY OF THE METHYLTETRAZOLETHIOMETHYL SIDE CHAIN OF β-LACTAM ANTIBIOTICS

## A COMPUTER GRAPHICS STUDY

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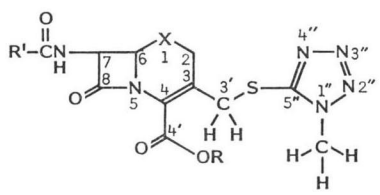
Analysis of X-ray crystallographic data for cephalosporins and 1-oxacephalosporins shows that, although the 1-methyl-1*H*-tetrazol-5-ylthiomethyl side chain at position 3 of a bicyclic β-lactam nucleus has certain conformational preferences, it also has considerable flexibility. Both the $C_4=C_3-C_{3'}-S$ and $C_{3'}-S-C_{5''}$ torsional angles are frequently observed in the vicinity of $\pm 90°$. The distance between the tetrazole ring carbon $C_{5''}$ and the 4-carboxyl carbon ranges from 4.07 to 5.65 Å. Mean bond lengths and bond angles for the 3 and 4 side chains are tabulated.

One of the most important side chains in cephalosporins (X=S) and 1-oxacephalosporins (X=O) is 3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]. Not only does it impart to the antibiotic broad spectrum activity, it also contributes remarkable potency. On the Transition State Energy (TSE) scale[1] this side chain confers reactivity to the β-lactam ring which is nearly optimal for Gram-negative activity. The side chain occurs in numerous antibacterial agents, such as cefamandole, latamoxef, cefoperazone,[2~4] as well as cefazaflur, cefmetazole, cefotetan (YM-09330), cefpiramide (SM-1652), and cefmenoxime.[2] Derivatized at the 1-methyl position, analogs of the side chain occur in cefotiam (SCE-963), ceforanide (BL-S786), and cefonicid (SKF 75073).[2] Despite its widespread occurrence in semisynthetic and synthetic antibiotics, only recently have enough X-ray crystallographic data accumulated to study the conformational preferences of this side chain.



The purpose of this work is to analyze the solid state conformations of the methyltetrazolethiomethyl side chain (abbreviated $R_3$). Systematic analysis of X-ray crystallographic data provides information about structural trends and mean geometrical data.[5] The present analysis demonstrates a wide latitude in conformational possibilities for $R_3$, but also shows that the various torsional angles are more prevalent in certain regions of conformational space. An appreciation of the spatial extent of the side chain is obtained by comparing it to glutaric acid. Although capable of spanning approximately the same distance between the terminal groups, no energetically reasonable conformational analogy between the two structures is found.

### Analysis of Crystallographic Data

Eight independent $R_3$-containing structures that have been solved by X-ray diffraction are listed in Table 1. Structures **1** and **2** are the two independent molecules A and B, respectively, in each unit

Table 1. Compounds.

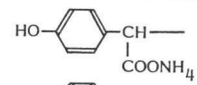
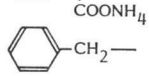
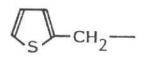
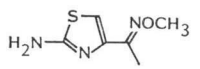
| Structure | R′ | X | R |
|---|---|---|---|
| **1, 2** |  | O ($7\alpha$-$OCH_3$) | $NH_4$ |
| **3** |  | O ($7\alpha$-$OCH_3$) | $CHPh_2$ |
| **4, 5** |  | S | H |
| **6, 7, 8** |  | S | H |

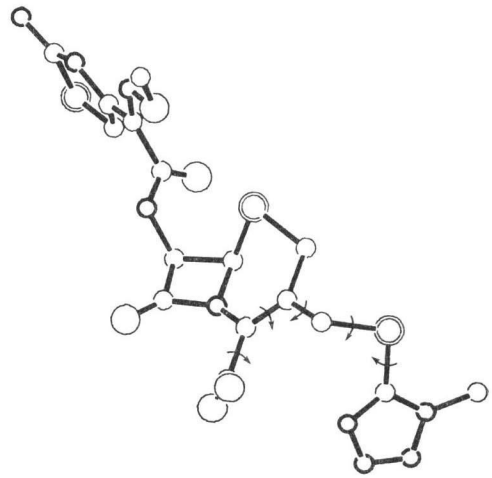
Table 2. Bond lengths (in Å) and bond angles (in degrees) in structures **1**~**8**: Mean and standard deviation

| | | | |
|---|---|---|---|
| $O{=}C_4{'}$[a] | 1.23±0.03 | $O{=}C_4{'}{-}O$ | 125±2 |
| $C_4{'}{-}O$[a] | 1.28±0.04 | $O{=}C_4{'}{-}C_4$ | 119±4 |
| $C_4{-}C_4{'}$ | 1.50±0.05 | $O{-}C_4{'}{-}C_4$ | 116±4 |
| $C_4{-}N_5$ | 1.41±0.03 | $C_4{'}{-}C_4{-}N_5$ | 115±2 |
| $C_4{=}C_3$ | 1.35±0.04 | $N_5{-}C_4{=}C_3$ | 118±3 |
| $C_3{-}C_3{'}$ | 1.52±0.03 | $C_4{'}{-}C_4{=}C_3$ | 126±3 |
| $C_3{'}{-}S$ | 1.82±0.04 | $C_4{=}C_3{-}C_3{'}$ | 121±4 |
| $S{-}C_5{''}$ | 1.73±0.02 | $C_3{-}C_3{'}{-}S$ | 112±2 |
| $C_5{''}{=}N_4{''}$ | 1.31±0.02 | $C_3{'}{-}S{-}C_5{''}$ | 101±1 |
| $N_4{''}{-}N_3{''}$ | 1.36±0.02 | $S{-}C_5{''}{=}N_4{''}$ | 127±4 |
| $N_3{''}{=}N_2{''}$ | 1.30±0.03 | $S{-}C_5{''}{-}N_1{''}$ | 122±2 |
| $N_2{''}{-}N_1{''}$ | 1.36±0.02 | $N_4{''}{=}C_5{''}{-}N_1{''}$ | 110±2 |
| $N_1{''}{-}C_5{''}$ | 1.33±0.02 | $C_5{''}{=}N_4{''}{-}N_3{''}$ | 104±3 |
| $N_1{''}{-}C$ | 1.46±0.03 | $N_4{''}{-}N_3{''}{=}N_2{''}$ | 113±5 |
| | | $N_3{''}{=}N_2{''}{-}N_1{''}$ | 105±5 |
| | | $N_2{''}{-}N_1{''}{-}C$ | 121±3 |
| | | $C_5{''}{-}N_1{''}{-}C$ | 131±2 |
| | | $C_5{''}{-}N_1{''}{-}C_2{''}$ | 108±2 |

[a] $C{=}O_4{'}$ and $C_4{-}O$ are the shorter and longer bond lengths, respectively, in the carboxyl group.

Fig. 1. Torsional angles pertaining to the 3 and 4 side chains of a cephalosporin or 1-oxacephalosporin.

The symbols used are: small circles—carbon, small bold circles—nitrogen, large circles—oxygen, large double circles—sulfur. Hydrogens are not shown in this figure.



cell of the diammonium salt of latamoxef (6059-S or moxalactam).[6,7] Structure **3** is another 1-oxacephalosporin analog.[8] Structures **4** and **5** are the two independent molecules 1 and 2, respectively, of a 7-(thien-2-ylacetyl)cephalosporin.[9] Finally, structures **6**, **7**, and **8** are the three independent molecules A, B, and B′, respectively, of cefmenoxime.[10]

Our primary interest is in the three-dimensional structure of the "lower right-hand" portion of the molecules, namely, $R_4{-}C_4{=}C_3{-}R_3$, where $R_4$ abbreviates the carboxyl group at the 4 position of the cephem ring. The torsional angles shown in Fig. 1 are the ones of interest. In our notation, a positive dihedral angle A–B–C–D is measured by a clockwise rotation from the A–B–C plane to the B–C–D plane as one looks from B toward C. A dihedral angle of 0° corresponds to atoms A and D being *syn*.

The fractional atomic coordinates of **1**~**8** from the references[6,8~10] were transformed to Cartesian coordinates using the respective unit cell dimensions. Internal geometrical parameters (distances, angles) were then computed. A FORTRAN program called GEOM was used for these calculations and for interfacing to graphics software.

First the published crystallographic coordinates and internal geometries were checked for internal consistency. Agreement of ±0.004 Å and ±0.3° was found between the published bond lengths and angles and what are computed using the atomic coordinates appearing in the same papers. This is typical for crystallographic papers.

Averaged bond lengths and bond angles in Table 2 pertaining to the $R_4{-}C_4{=}C_3{-}R_3$ substructure

Table 3. Torsional angles in crystalline state conformations of **1**~**8**.

| Structure | $O=C_4'-C_4=C_3$[a] | $C_4'-C_4=C_3-C_3'$ | $C_4=C_3-C_3'-S$ | $C_3-C_3'-S-C_5''$ | $C_3'-S-C_5''=N_4''$ |
|---|---|---|---|---|---|
| **1** | 199° | 3° | 279° | 258° | 354° |
| **2** | 198° | 6° | 280° | 240° | 3° |
| **3** | 8° | 7° | 278° | 273° | 12° |
| **4** | 32° | 359° | 65° | 75° | 13° |
| **5** | 224° | 13° | 227° | 78° | 359° |
| **6** | 220° | 0° | 119° | 273° | 350° |
| **7** | 35° | 0° | 128° | 111° | 341° |
| **8** | 41° | 8° | 264° | 240° | 137° |

[a] The bond denoted $O=C_4'$ is whichever is shorter in the carboxyl; the torsional angle to the longer $C_4'-O$ bond is essentially 180° away from the value tabulated for $O=C_4'$.

are reasonable compared to standard geometrical values.[11] It is interesting to note that among the data in Table 2, the $C_3'-S$ bond length (1.82 Å) is considerably longer than the $S-C_5''$ bond (1.73 Å). The two bond lengths are typical for a C–S single bond and a C–S conjugated acyclic bond, respectively.[11] The presence of double bond character in the latter bond is brought about by delocalization of the $\pi$ electron density between the tetrazole ring and the sulfur $3p\pi$ orbital. Consistent with the conventional valence bond structure of the tetrazole, the nominal double bonds, $C_5''=N_4''$ and $N_3''=N_2''$, have slightly shorter bond lengths than the other ring bonds. The carboxyl group is essentially planar as expected. Also, the methyltetrazolethiol substructure is nearly coplanar (except for the methyl hydrogens). In other words, the three bond angles at $C_5''$ and $N_1''$ each sum to 360° within experimental uncertainty.

It is common for conformational degrees of freedom to be more subject to crystal packing forces than are bond lengths and bond angles. This is because less energy is required to twist a single bond than to stretch or bend it. Consequently, in the following discussion it should be kept in mind that the observed solid state conformations are influenced to some extent by intermolecular contacts that occur in the crystal. Frequently observed conformations are more likely to stem from inherent properties of an isolated molecule.

The values of the torsional angles defined in Fig. 1 are given in Table 3. The interrelationships of the angles can be even better appreciated by the plots in Fig. 2. The dihedral angles are observed over a wide range. Even the ethylenic moiety of the six-membered ring is not exactly planar and is twisted up to 13°. This degree of deformation is not uncommon for cyclic olefins.[11]

Despite the conformational freedom of the side chains, the torsional angles tend to cluster in specific regions. For instance, as seen in Fig. 2 the plane of the carboxyl group is turned, on the average, 30° with respect to the enamine plane. The shorter carboxyl carbon-oxygen bond can be either *syn* or *anti* to $C_3=C_4$, but the carboxyl oxygen on the $\beta$ face of the molecule is proximal to $C_3$, rather than $N_5$, in all eight cases.

The $C_4=C_3-C_3'-S$ dihedral angle is often such that the sulfur is either directed down from the $\alpha$ face of the molecule (90°) or up from the $\beta$ face (270°$=-90°$). In effect, these two conformations put the methyltetrazolethiol leaving group in an orientation where departure can be concerted stereo-electronically with opening of the $\beta$-lactam ring.[12]

The $C_3-C_3'-S-C_5''$ torsional angle is observed (Fig. 2) to cluster even closer to $\pm 90°$. This means that the $R_3$ side chain is not in a fully extended conformation, but rather the tetrazole is turned so as to be nearer $C_2$ or $C_4$ of the cephem ring. The conformational energy minima for rotation about

Fig. 2. Torsional angles observed in the solid state.
The circumferential numbers correspond to the structures in Table 1.

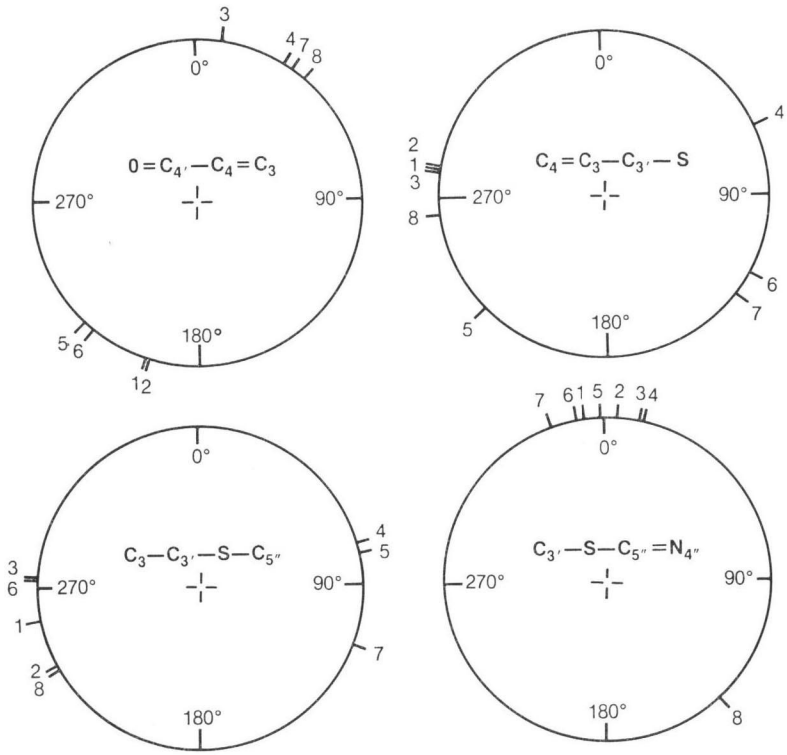
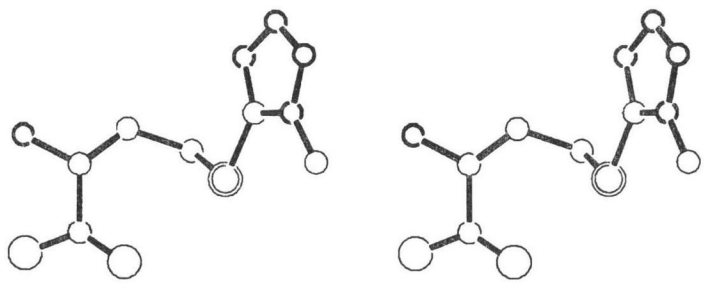



Fig. 3. Stereoview of the $R_4$–$C_4$=$C_3$–$R_3$ substructure of **3**.
The hydrogens are not shown for sake of clarity. This view shows the $\beta$ face of the molecule.

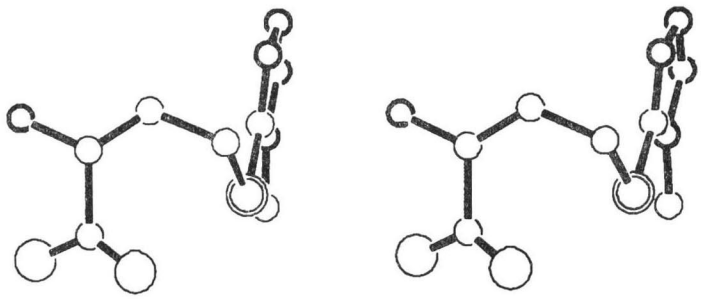


the $C_{3'}$–S bond may not be very deep because in a simpler molecule, methyl ethyl sulfide, the gauche $C_{sp^3}$–S conformer is only about 0.2 kcal/mol more stable than the *trans* in the gas phase.[13]

The $C_{3'}$ methylene carbon is in all cases, except one (**8**), *syn* to $N_{4''}$. That is, the $C_{3'}$–S–$C_{5''}$=$N_{4''}$ dihedral angle is *ca* 0°. In this coplanar arrangement, the 3p-type lone pair on sulfur[14] can overlap with the tetrazole $\pi$ electron cloud. (The other sulfur lone pair, which has $sp^2$ character, is in the $C_{3'}$–S–$C_{5''}$ plane.) The apparent conformational preference at the S–$C_{sp^2}$ bond is consistent with theoretical calculations on internal rotation in methyl vinyl sulfide.[15,16]

Typical $R_4$–$C_4$=$C_3$–$R_3$ substructures corresponding to **3** and **4** are shown in Figs. 3 and 4, respectively. The dihedral angles of both fall in the more populated regions of the circles in Fig. 2.

Fig. 4. Stereoview of the $R_4$–$C_4$=$C_3$–$R_3$ substructure of **4**.
This view shows the $\beta$ face of the molecule.



## Spatial Extent

The dimensions of the receptor sites[17~19] of the bacterial enzymes that are inhibited by cephalosporins and 1-oxacephalosporins must be able to accommodate both the 4-carboxyl group, which is known to be important for antibacterial activity, and the $R_3$ side chain. It is of interest, therefore, to examine the distance between these groups. In Table 4 are given the distances between $C_{4'}$ and $C_{5''}$ observed for structures **1**~**8**. The tetrazole ring is as close as 4.07 Å and as far as 5.65 Å from the carboxyl group.

In order to put these dimensions in perspective, one can compare it to the glutaric acid. When fully extended, the carboxyl carbons of HOOC–$CH_2$–$CH_2$–$CH_2$–COOH are no more than 5.12 Å apart. Thus, a glutaric acid or related molecule could, in principle, span about the same distance at the $R_4$–$C_4$=$C_3$–$R_3$ substructure. *A priori* it is not chemically reasonable that glutaric acid should match the spatial relationship of the terminal groups in $R_4$–$C_4$=$C_3$–$R_3$, but it is of interest to make such a hypothetical comparison in the following computer experiment. The experiment will entail using the molecular mechanics method MM2[20~22] to fit glutaric acid to the same spatial relationship as observed for the $R_3$ and $R_4$ side chains of the antibiotics. In order to assure the accuracy of the results, the MM2 steric energies are computed with even the small VAN DER WAALS interactions included as provided by the computer program.

Table 4. Distance between the 4-carboxyl carbon and the ring carbon of the tetrazole and relative MM2 steric energy of glutaric acid with the carboxyl groups in a spatial relationship analogous to **1**~**8**.

| Structure | C···C (Å) | Energy (kcal/mol) |
|---|---|---|
| **1** | 5.25 | 22.3 |
| **2** | 5.27 | 27.1 |
| **3** | 5.14 | 24.2 |
| **4** | 4.55 | 10.2 |
| **5** | 4.33 | 32.4 |
| **6** | 4.07 | 16.9 |
| **7** | 5.65 | 31.2 |
| **8** | 5.30 | 22.1 |

Fig. 5. Stereoview of glutaric acid in a fully extended conformation.
This is the most stable configuration as predicted by MM2.

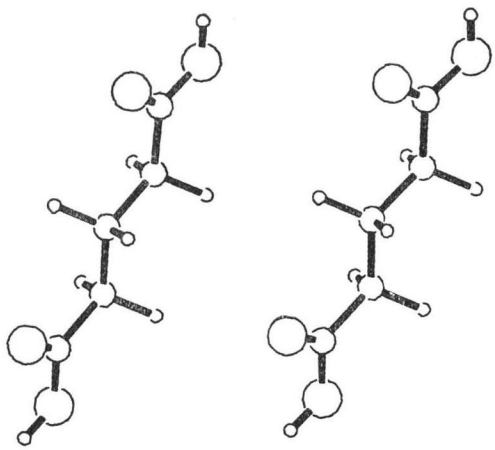
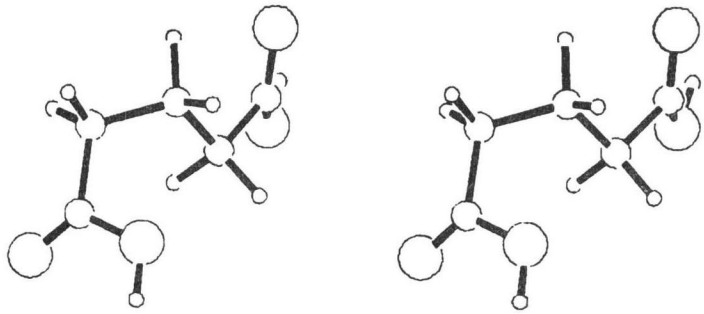
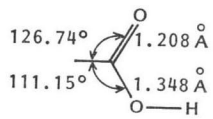


Fig. 6. Stereoview of glutaric acid with the carboxyl groups fixed at MM2 optimized internal geometries in a spatial relationship that coincides with **4**.

The atomic coordinates of the COO groups are held fixed while the rest of the coordinates, including those for the two lone pairs on –O–, are allowed to vary until the steric energy is minimized. The smallest circles represent hydrogen.



First, the most stable conformation of glutaric acid was determined to be the fully extended one (Fig. 5). Putting one gauche C–C–C–C twist in the molecule costs only 0.3 kcal/mol. The fully optimized internal geometry averaged for the two carboxyl groups is shown below. MM2 also predicts that the carboxyl proton prefers by 6.2 kcal/mol to be *syn* rather than *anti* to the doubly bonded oxygen. The *syn* coplanar conformation of each COOH allows formation of an intramolecular hydrogen bond. The 6.2 kcal/mol is reasonable compared to the intramolecular hydrogen bond stabilization of about 8.1 kcal/mol found by *ab initio* molecular orbital calculations on formic acid.[23,24]




Next two carboxyl groups with the above MM2 optimized bond lengths and angles were positioned such that the distance between the carbons is identical to each of the C ··· C distances in Table 4. The spatial orientation of the carboxyls was such that one was in the same plane as the 4-COO group, and the other was in the same plane as the tetrazole ring of structures **1**~**8**. Within these two planes, the carboxyls were oriented with the open coordination site at each carbon directed along the $C_4$–$C_{4'}$ or S–$C_{5''}$ axis. Then a chain of three methylenes was built between the carboxyls. In effect, one has glutaric acid with the ends overlapping the $R_3$ and $R_4$ groups of the $R_4$–$C_4$=$C_3$–$R_3$ substructure. The MM2 program allows the equilibration of the bridging methylene geometry and the carboxyl hydrogen positions, while the two COO groups are held fixed. The resulting steric energies, which are the sum of the stretching, bending, torsional, *etc.*, energy terms,[20~22] can be converted to values relative to the most stable conformer of glutaric acid, the fully extended one. The final relative energies are shown in Table 4. These values show how much energy is required to fit the termini of glutaric acid exactly to the spatial positions of $R_3$ and $R_4$.

One sees in Table 4 that none of the energies is very small. The fit least costly in terms of energy is to structure **4**. The corresponding glutaric acid structure is shown in Fig. 6, where pyramidal deformations contributing to the strain energy are clearly evident at the carboxyl carbons. It must be concluded that none of the spatial analogies appears to be energetically reasonable with the sort of interactions commonly associated with drug-receptor complexes. Fitting glutamic acid rather than glutaric acid gives a similar result. Thus, these calculations indicate that a receptor site appropriate for binding a glutaric or glutamic acid residue would not be appropriate for binding any of the observed

conformations of $R_3$-containing cephalosporins or 1-oxacephalosporins.

The structural data for the 3 and 4 side chains analyzed in this paper will hopefully prove useful in rationalizing the fit of $R_3$-containing antibiotics in the binding sites of target enzymes.

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