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Conformation and Absolute Configuration of Nocathiacin I Determined by NMR Spectroscopy and Chiral Capillary Electrophoresis

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Nocathiacin I is a cyclic thiazolyl peptide antibiotic[1] isolated from *Nocardia sp.* (ATCC-202099) that displays potent activity *in vitro* and *in vivo* against Gram-positive bacteria, including a number of antibiotic-resistant strains. This compound disrupts bacterial protein biosynthesis by interacting directly with the L11 protein and 23S rRNA region of the ribosome.[2] Other cyclic thiazolyl peptide antibiotics are known to interact directly with the ribosome,[3] or with elongation transfer factor. [4]

Nocathiacin I possesses a cyclic thiazolyl peptide framework that is similar to that of other members of this antibiotic family;[5] it is distinguished by an indole ring within the cyclic scaffold. The two internal cross-links produce three chiral centers within the cyclic scaffold. In addition, there is a chiral center where the amino sugar attaches to the cyclic scaffold, four chiral centers within the amino sugar moiety, and two chiral centers in the threonine residue. In total, nocathiacin I contains 10 chiral centers (Figure 1).

Knowledge of the structure of nocathiacin I, in terms of both its conformation and stereochemistry, is essential for further chemical modification and development of this compound. Efforts to determine the structure by X-ray crystallography have proven unsuccessful; therefore, solution NMR studies were undertaken. To facilitate the assignment of NMR resonances, a uniformly $^{13}C/^{15}N$-labeled sample of nocathiacin I was prepared with $^{13}C/^{15}N$ Celtone^{R} growth media, as described.[6] NMR assignments (Supporting Information) were obtained by analysis of 2D $^{1}H-^{1}H$ COSY, $^{1}H-^{1}H$ NOESY, $^{1}H-^{13}C$ HMBC, and $^{1}H-^{15}N$ HSQC spectra. The ^{1}H NMR spectrum is well dispersed, and numerous cross-peaks are observed in the NOESY spectra. Qualitatively, these results indicate that nocathiacin I adopts a relatively rigid, compact conformation.

To obtain accurate distance restraints, a series of 2D $^{1}H-^{1}H$ NOESY spectra were recorded by using an unlabeled nocathiacin I sample. Mixing times of 30, 60, 90, 120, 160, 200, 250, and 300 ms were employed. Diagonal peak normalized NOE volumes[7] were then fit by using an expression that correctly treats equivalent or degenerate protons.[8] This procedure minimizes the effects of one-step spin diffusion pathways. Distance restraints were calibrated by using NOEs between protons of the indole ring (Figure 1). The resulting distance estimates were adjusted by +10% and −20% to establish upper and lower bounds, respectively. Lower bounds were not allowed to exceed 3.5 Å. A total of 135 distance restraints were obtained (Supporting Information). Thirty-eight of these contain one or more assignment ambiguities due to resonance overlap; these were incorporated by using the ambiguous restraint capability of X-PLOR.[9]

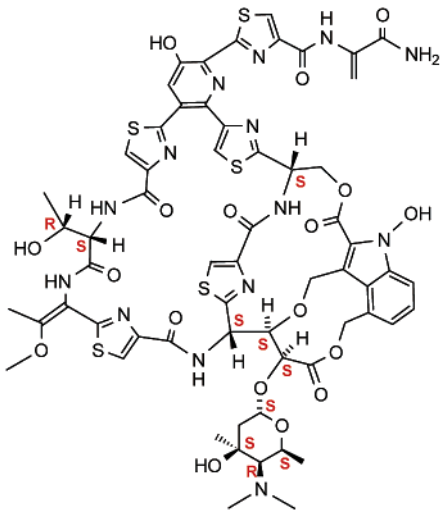

Figure 1. Chemical structure of nocathiacin I. The 10 chiral centers are labeled (red letters) to show the absolute configurations determined in this study.

Nocathiacin I structures consistent with the NOE restraints were calculated by using a modified simulated annealing protocol[10] with the X-PLOR program.[9] No assumptions regarding the chiral center configurations were made in the annealing procedure. A preliminary structure containing arbitrary chiral center configurations was generated with QUANTA (Accelrys). This preliminary structure was diversified by using unrestrained simulated annealing with X-PLOR. Improper torsion terms for the chiral centers were excluded from all stages of the calculation. Bond angle terms were scaled to very small values during the high-temperature stage and subsequently increased during the cooling stages and final energy minimization. This annealing procedure was found to allow efficient conversion between the *R* and *S* configurations of the chiral centers.

With use of the 135 NOE distance estimates (Supporting Information), 80 structures were refined by using restrained simulated annealing, following the same protocol as described above for the unrestrained calculations. Out of 80 structures, 24 were selected for further analysis based on the following criteria: total energy <270.0 kcal/mole, NOE restraint energy <12.0 kcal/mole, and no NOE violations >0.20 Å. Statistics for the accepted structures are reported in Table 1.

The 24 accepted structures can be grouped into two mutually exclusive clusters, as shown in Figure 2. Within each cluster, all 10 chiral centers are uniquely defined. An examination of the energy distribution over all 80 computed structures revealed that those containing alternate configurations have significantly higher total and restraint energies than the accepted structures.

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Table 1. Statistics for the 24 Accepted Nocathiacin I Structures

statistic	value
av heavy-atom RMSD (L threonine cluster)[a]	0.64 Å
av heavy-atom RMSD (D threonine cluster)[b]	0.73 Å
av RMS distance restraint violation[c]	0.038 Å
av no. of violations >0.20 Å	0
av total energy	264.2 kcal/mole
av restraint energy	9.6 kcal/mole
av bond angle deviation	1.90°
av bond length deviation	0.45°
av improper torsion deviation[d]	0.45°

[a] Root-mean-square difference (RMSD) averaged over all heavy atoms of the cluster of 7 structures containing L-threonine. The RMSD of each individual structure was computed to the average structure of this cluster. [b] Same as footnote *a*, but computed for the cluster of 17 structures containing D-threonine. [c] All of the remaining statistics were averaged over all 24 accepted structures. [d] Improper torsions for the chiral and prochiral centers were excluded.

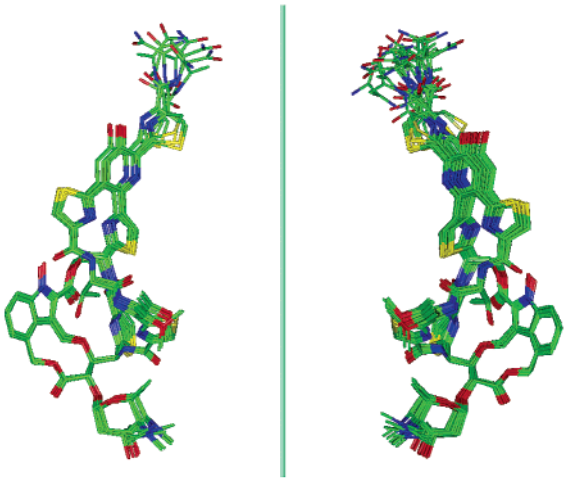

Figure 2. "Mirror image" clusters of nocathiacin I NMR structures. A cluster of 7 structures containing L-threonine is shown on the left, and a cluster of 17 structures containing D-threonine is shown on the right. Atoms are colored according to type.

The two clusters of accepted structures are in essence mirror images of each other: all of the chiral centers that have the *R*(*S*) configuration in one cluster have the *S*(*R*)configuration in the other. One cluster contains L-threonine, and the other contains D-threonine. Nocathiacin I displays a compact conformation, with the threonine methyl group buried.

After the structure calculations, $^{1}H-^{1}H$ ^{3}J coupling constants were measured from 1D spectra and 2D PE-COSY spectra. All of the relevant coupling constants were found to be reasonably consistent with the dihedral angles observed in the accepted structures (Table 2), as all large (>8.0 Hz) couplings are associated with dihedrals within 40° of the *trans* conformation, and all small (<5.0 Hz) couplings are associated with dihedrals within 20° of a *gauche* conformation.

Given the results described above, it was clear that an independent determination of the absolute configuration of any chiral center would allow the absolute configurations of all of the chiral centers to be defined. Metal chelate chiral capillary electrophoresis (Supporting Information) was used to determine the chirality of the threonine residue of nocathiacin I. These data demonstrate conclusively that nocathiacin I contains L-threonine.

Taking into account both the NMR structures (Figure 2) and the results of the chiral capillary electrophoresis experiments, the absolute configurations of all 10 chiral centers were defined (Figure 1). The information provided by these studies proved useful in the design of chemical modifications of nocathiacin I, and for modeling the binding of nocathiacin I to the ribosome. Regarding the latter, a cleft between the N-terminal domain of the ribosomal L11 protein and the 23S RNA has also been implicated in the binding of thiostrepton and micrococcin.[11] By using the available crystal structure of the L11-RNA complex[11] and our nocathiacin I NMR structures, the interactions between the ribosome and the thiazolyl peptide have been computationally modeled.[12] Full details on the chemical and biological investigations of the nocathiacin antibiotics will be described elsewhere.

Table 2. $^{1}H-^{1}H$ ^{3}J Coupling Constants and Associated Dihedrals

$^{1}H-^{1}H$ pair[a]	^{3}J[b]	dihedral (L)[c]	dihedral (D)[d]
H12, H15	8.6	−145.5	145.1
H14, H16	8.5	156.2	−156.1
H9, H12	2.1	−63.5	63.7
H9, H13	9.7	−174.9	175.2
H14, HM1	3.9	−76.3	76.2
H14, HM2	1.2	43.5	−43.5
H18, HI1	4.7	−47.6	48.0
H18, HI2	1.4	68.0	−67.7

[a] Three-bond coupled proton pair. See the Supporting Information for the atom naming convention used. [b] Coupling constant value in Hz. [c] Dihedral angle (degrees) observed in the average structure derived from the cluster of 7 structures containing L-threonine. [d] Dihedral angle (degrees) observed in the average structure derived from the cluster of 17 structures containing D-threonine.

Supporting Information Available: ^{1}H, ^{13}C, and ^{15}N NMR assignments, experimental NMR conditions, distance restraints, coordinates for the seven accepted structures containing L-threonine, and a figure and experimental details for the chiral capillary electrophoresis studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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