

NMR Studies of the Anticancer Drug Pepleomycin and its Complexes with DNA

Pepleomycin (PEP) is a metalloglycopeptide that has stronger anticancer activity and less pulmonary toxicity than bleomycin (BLM). PEP, like BLM, exerts its action by binding to and degrading DNA in the presence of oxygen and certain metals. Two cobalt-PEP species, $\text{HO}_2\text{-Co(III)-PEP}$ and its deglycosylated $\text{HO}_2\text{-Co(III)-dPEP}$, have been studied by 2D-NMR and NOE refined structures were obtained. Two chiral conformers (form A or B) can exist for these molecules with either the β -aminoalanine primary amine (A_1NH_2) or the mannose carbamoyl nitrogen (M_1NH_2) as the axial ligand. Analysis of our NOESY data shows convincingly that form A is the most probable conformer with the mannose carbamoyl M_1NH_2 and the β -aminoalanine primary amine A_1NH_2 as the axial ligands in CoPEP and CodPEP, respectively. The NOE crosspeaks resulting from the interactions between the N-terminus (*i.e.*, the metal binding domain) and the C-terminus of CoPEP and CodPEP have similar patterns, suggesting that they both adopt compact structures with the bithiazole group folded back over the N-terminus(1). We have treated the self-complementary oligonucleotide $\text{d}(\text{CGTACG})_2$ with one equivalent of CoPEP or CodPEP and carefully examined these complexes — denoted CGTACG-CoPEP and CGTACG-CodPEP, respectively — by one- and two- dimensional ^1H NMR. Refined solution structures of CGTACG-CoPEP and CGTACG-CodPEP have been obtained by NOE restrained refinement procedures. Addition of CoPEP or CodPEP to $\text{d}(\text{CGTACG})_2$ disrupts the two-fold symmetry of the DNA duplex, consistent with binding of the drugs to the DNA duplex. The binding mode of both CoPEP and CodPEP with respect to the DNA duplex is defined by approximately 60 inter-molecular NOEs. The NOE data is consistent with a partial intercalative binding mode for both DNA-drug complexes with the drugs' metal-binding domain and peptide linker bound in the minor groove of the DNA close to residues G8 and T9, and the drug's bithiazole tail intercalated between the base pairs A4-T9 and T3-A10. A structural comparison between CGTACG-CoPEP and CGTACG-

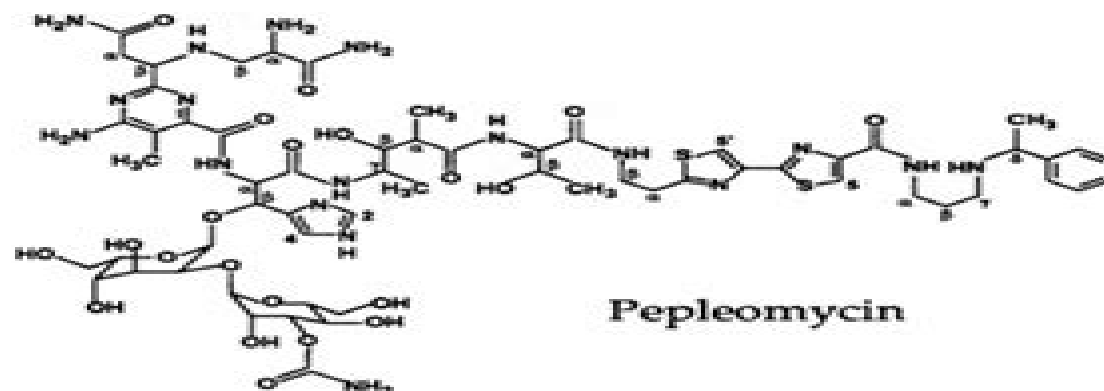
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Table of Contents Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987

1. Understanding the eBook Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - The Rise of Digital Reading Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Advantages of eBooks Over Traditional Books
2. Identifying Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - User-Friendly Interface
4. Exploring eBook Recommendations from Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Personalized Recommendations
 - Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 User Reviews and Ratings
 - Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 and Bestseller Lists
5. Accessing Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 Free and Paid eBooks

- Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 Public Domain eBooks
- Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 eBook Subscription Services
- Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 Budget-Friendly Options
- 6. Navigating Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 eBook Formats
 - ePub, PDF, MOBI, and More
 - Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 Compatibility with Devices
 - Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987 Enhanced eBook Features
- 7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Highlighting and Note-Taking Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Interactive Elements Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
- 8. Staying Engaged with Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
- 9. Balancing eBooks and Physical Books Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Setting Reading Goals Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Fact-Checking eBook Content of Of Abstracts Fifth Conversation In Biomolecular Stereodynamics June 26 1987
 - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning

- Utilizing eBooks for Skill Development
- Exploring Educational eBooks

14. Embracing eBook Trends

- Integration of Multimedia Elements
- Interactive and Gamified eBooks

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