Methods in Stereochemical Analysis 6

Applications of NMR Spectroscopy to Problems in Stereochemistry and Conformational Analysis

Edited by
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To the memory of
Alvin R. Marchand

Preface

Few instrumental techniques and developments have had the dramatic impact upon progress in stereochemical and structural analysis that has been evidenced by nuclear magnetic resonance (NMR) spectroscopy. Recent advances in hardware (high sensitivity, high magnetic field instrumentation) and in software (data acquisition, development of new pulse sequences in Fourier transform nuclear magnetic resonance (FT-NMR) spectroscopy, and the like) have revolutionized the field and its applications to structural problems of interest to organic and bio-organic chemists. NMR elucidation of conformation and stereochemistry in high molecular weight polymers and biopolymers, both in solution and in the solid state, is now routinely pursued; often, nuclei that possess low sensitivity and/or low natural abundance are employed for such studies. The advent of two dimensional (2-D) NMR spectroscopy has had a profound effect upon NMR applications; molecules whose NMR spectra had been regarded only a few years ago as being intractably complex are now routinely analyzed by the use of 2-D NMR techniques.

As a result of these and related developments, there has been an explosion of interest in applications of NMR spectroscopy to structural and stereochemical problems. It is certainly appropriate to state that maintaining current awareness of developments in this burgeoning interdisciplinary field is vitally important to chemists and biochemists whose research programs are concerned with the determination of gross molecular structure as well as with details of molecular stereochemistry and conformational analysis. It is in this spirit that the preparation of the present volume was undertaken.

Given the scope of recent developments in NMR spectroscopy, it did not seem reasonable to attempt an exhaustive exposition of the subject in a single volume. In addition the interdisciplinary nature of NMR applications virtually mandated that we approach this subject through a multiauthored treatise format. To this end, we sought contributions from diverse segments of the chemical community.

In the first chapter Professor I. O. Sutherland (University of Liverpool) presents new NMR applications to the study of conformational analysis of guest-host molecular systems, many of which are of intense current interest to investigators in the life sciences. In Chapter 2 Professor P. Diehl (University of Basel) leads us through the intricacies of NMR investigations in liquid crystal media and demonstrates how the study of partially oriented molecules can provide information pertaining to their molecular geometry. In Chapter 3 Professor R. Kitamaru (Kyoto University) illustrates the use of ^{13}C relaxation phenomena as a source of information pertaining to chain dynamics and conformation in macromolecules. In the fourth chapter Professor T. Terao

(Kyoto University) examines the use of NMR spectroscopy to study stereochemical problems in the solid state. Two-dimensional NMR spectroscopy is treated in the last two chapters: in Chapter 5 Professor K. Nagayama (University of Tokyo) introduces the basic concepts of 2-D NMR spectroscopy and presents a number of applications of 2-D NMR techniques; in the final chapter Professor H. Kessler (Johann Wolfgang Goethe University, Frankfurt) examines problems in conformational analysis of peptides using 2-D NMR techniques.

The editors acknowledge with pleasure the contributions and suggestions made by numerous colleagues and by the individual authors whose names appear above. We are especially grateful for the support and encouragement which we received from members of our respective families. We thank Mrs. Rosalind B. Marchand for assistance in preparing the Author Index. Finally, we thank Ms. Mary Stradner, Marie Stilkind, and other persons associated with the Production Division of VCH Publishers, Inc.

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CONTENTS

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GUEST–HOST CHEMISTRY AND CONFORMATIONAL ANALYSIS

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Introduction

Conformational analysis in organic chemistry has generally been based upon the conformational preference of molecules in their ground states and the stereochemical requirements of transition states. Ground state conformations are usually considered for isolated molecules and the conformational analysis of transition states is based upon the formation of more or less well-defined partial bonds between reacting centers in either a single molecule or a pair of molecules. Such considerations[1] have played an important part in the appreciation of structure and reactions in organic chemistry over the last three decades, since the publication of the seminal paper by Barton[2] in 1950. The development of guest–host chemistry[3–7] over the past 10 years has focused attention on systems involving two or more molecules, bound together in a noncovalent fashion. This requires the introduction of methods for studying the conformations of multimolecular systems or intermolecular conformational analysis. Information about the solid phase is, of course, readily available through x-ray crystallography, but information for multimolecular systems in solution must be obtained by more indirect methods; this chapter reviews the application of NMR spectroscopy to the study of intermolecular conformational analysis. This chapter is restricted to cases in which the lifetime of the complex is

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significant (τ ca. 1 s) on the NMR time scale at a temperature within the range ($-110°C$ to $+100°C$) over which complexes may be studied in solution by NMR spectroscopy. These limits are somewhat arbitrary and they exclude, for example, collision complexes and charge-transfer complexes with very short lifetimes, as well as strongly bound metal–ligand complexes with very long lifetimes.

The range of lifetimes selected above is of the same order as that found in biological processes involving complex formation, and one of the incentives for the study of guest–host chemistry is that of providing analogs of biological systems. Examples are the modification of a substrate, $S \rightarrow S'$, by an enzyme E (eq. 1-1, where the formation of a complex is indicated by $E \cdot S$ and $E + S$ indicates a mixture of the free components); the interaction of a substrate S, such as a drug, with a biological receptor R to initiate a biological response (eq. 1-2); or even the transport of a substrate S through a cell membrane or across a phase boundary by the formation of a complex with a carrier molecule C (eq. 1-3, where the vertical lines represent phase boundaries).

$$E + S \rightleftharpoons E \cdot S \rightleftharpoons E \cdot S' \rightleftharpoons E + S' \quad (1\text{-}1)$$

$$R + S \rightarrow R \cdot S \rightarrow \text{biological response} \quad (1\text{-}2)$$

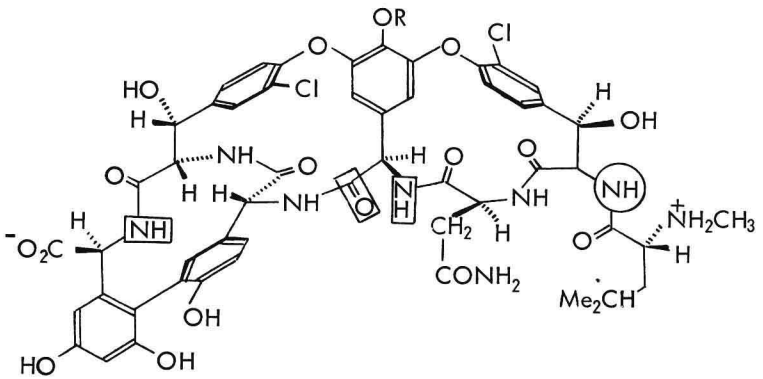
$$S + |C \rightleftharpoons C \cdot S \rightleftharpoons C| + S \quad (1\text{-}3)$$

These processes summarized here are related to other important biological systems, including immune response and the biological activity of antibiotics, toxins, hormones, and neurotransmitters. The discussion in this chapter, however, is centered upon the systems involving synthetic host molecules.

The binding energy required for the formation of a complex of the type to be discussed is derived primarily from (a) coulombic attraction, (b) hydrogen bonding, and (c) hydrophobic interactions. The first two of these binding forces are possible in all types of solvent but are particularly favored in organic solvents of low dielectric constant; the third binding force is restricted to aqueous systems. The best studied complexes in organic solvents are those formed between cations and crown ethers, whereas systems that have been studied in aqueous solvents include complexes of cyclodextrins[8-10] and of water-soluble cyclophanes.[11-13] Before specific examples of these systems are discussed, it is worth noting that a number of natural products besides proteins owe their biological activity to their ability to function as host molecules. The first group of these, the ionophoric antibiotics,[14] function as hosts for the simplest guests of all, spherical metal cations. Information on the conformational aspects of complexation generally has been derived from crystal structures of free and complexed host molecules, rather than from NMR studies. The more complex group of peptide-like antibiotics, exemplified by vancomycin,[15-18] ristocetin,[19,20] and avoparcin,[21,22] form complexes selectively with *N*-acyl-D-Ala-D-Ala derivatives, and complexation has been studied by NMR techniques.

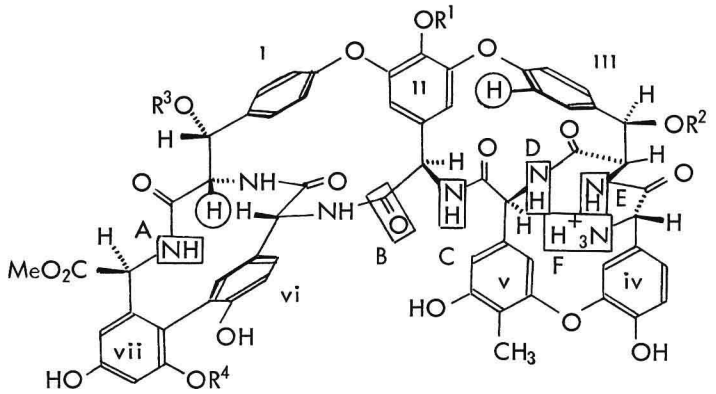
The study of the binding site of vancomycin is a particularly interesting illustration of the power of NMR methods. The binding site originally deduced

from the crystal structure[23] of a vancomycin degradation product was subsequently revised on the basis of NMR spectroscopy and further chemical studies.[16,17,18] In the original proposal[23] the NH and CO groups enclosed in rectangles in **1** were used as the basis for hydrogen bonding to an *N*-acetyl-D-Ala-D-Ala guest. A subsequent examination of the ^{1}H NMR spectrum of the vancomycin–acetyl-D-Ala-D-Ala complex under conditions of slow exchange between the complex and its free components [-1°C in $(CD_3)_2SO$–CCl_4] showed that the proton of the circled NH group in **1** moved downfield 3.7 ppm in the complex as compared with free vancomycin. Therefore, this NH group is involved in hydrogen bonding to the free carboxylate group of the substrate. From a study of molecular models it was recognized that this implied a major conformational change of the right-hand side of structure **1** in the vancomycin complex to provide a carboxylate anion "receptor pocket" in the vancomycin structure. This receptor pocket was found from the models to resemble closely the receptor pocket found for the related antibiotic ristocetin **2**. Therefore, the



R = sugars

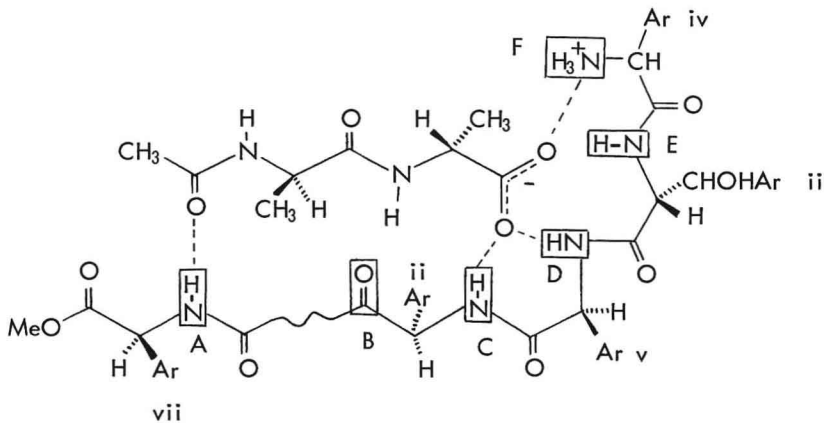
1



R^1, R^2, R^3, R^4 = sugars

2

NH, CO, and $\overset{+}{N}H_3$ groups in ristocetin enclosed in rectangles (see **2**) have been shown,[20] on the basis of molecular models and *intermolecular* nuclear Overhauser enhancements (NOEs) between the encircled protons of the host antibiotic (see **2**) and the C*H* and CH_3 protons of the D-Ala residues of the guest, to be placed correctly for the formation of intermolecular hydrogen bonds. The diagrammatic representation (**3**, where the binding sites are labeled A–F and the aromatic rings i–vii to correspond with the labels in **2**) of this hydrogen bonding between host and guest shows all of the postulated binding interactions. A similar set of hydrogen bonds can be found for an appropriate conformation of vancomycin. The *intramolecular* NOEs found for both vancomycin and ristocetin and their complexes support these views regarding the conformational similarities of the macrotricyclic host, vancomycin **1**, and the macrotetracyclic host, ristocetin **2**. The related antibiotic avoparcin[21,22] has a macrotricyclic structure **4** that presumably adopts a conformation similar to the conformations of vancomycin and ristocetin.



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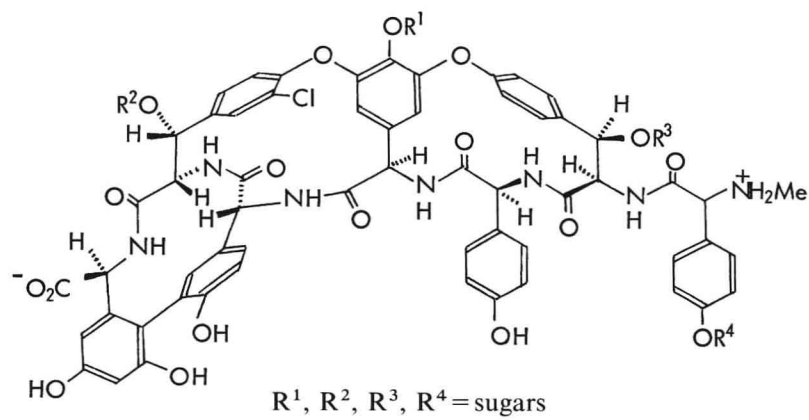

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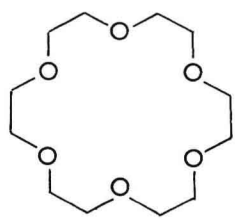
These studies of conformation and intermolecular relationships using observations of intramolecular and intermolecular NOEs are of great interest and are likely to serve as models for other investigations of guest–host complexes involving organic guest molecules. They are most applicable, however, to asymmetrical systems having well-dispersed ^{1}H NMR spectra, whereas many synthetic host molecules are symmetrical; and in general, the NOE has not yet been applied for the study of complexes of such symmetrical host molecules.

Complexes of Crown Ethers and Analogous Hosts

The discovery by Pedersen[24,25] that crown ethers, such as 18-crown-6 **5**, form strongly bound complexes with alkali metal and other metal cations and alkylammonium cations has generated an impressive volume of research. The complexation of metal cations by crown ethers is an important area of research, but in many cases the structures of such complexes have been studied by x-ray crystallography, rather than by NMR spectroscopy. The study of metal ion complexation by cryptands, crown ethers, and ionophoric antibiotics by a variety of techniques has been reviewed;[26] it is not discussed further in this chapter. The complexation of alkylammonium cations has proved to be particularly suitable for study by NMR techniques, and an account of this work constitutes the bulk of this section.

Monocyclic Systems

Methods. Early studies of crown ether complexes by ^{1}H NMR spectroscopy were limited to the recognition of the changed chemical shifts for both guest and host molecules upon complexation[27] and to the measurement of the relative amounts of guest and host species present in solution in order to determine values for association constants.[27–29] It was noted in this early work that guest–host exchange was fast on the NMR time scale at normal probe temperatures, resulting in, for example, the averaging of signals for diastereoisomeric complexes simultaneously present in solution. At that time (1973), although slow exchange had been recognized for complexes of macromolecular



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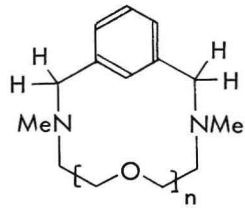
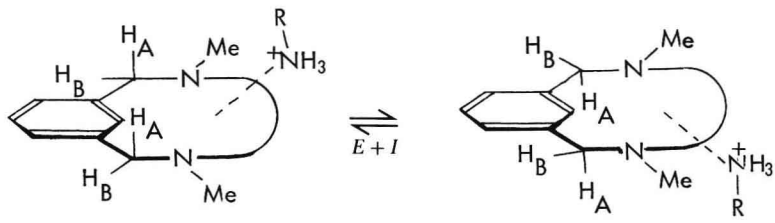
systems, there was no well-defined example of a hydrogen-bonded complex between molecules of relatively low molecular weight in which exchange was slow on the NMR time scale, even at very low temperatures. It was, therefore, surprising to find that complexes of the metacyclophanes **6** with benzyl-ammonium thiocyanate in CD_2Cl_2 showed temperature dependence of their ^1H NMR spectra that was not matched by that found for the free host molecules.[30] The indicated protons of the benzylic CH_2 groups of the hosts **6** complexed with benzyl-ammonium thiocyanate gave singlet signals, at ambient temperatures, which changed to AB systems at low temperatures (for **6**, $n=2$, at $< -45°C$ and for **6**, $n=3$, at $< -65°C$). This change was consistent with the formation of a complex (in **7** and a number of other formula of this type the crown ether system is represented by a circle or part of a circle for simplicity) in which exchange of the guest cation between the two faces of the host **7a**⇋**7b** became slow on the NMR time scale at low temperatures. The free energy of activation associated with this process could be obtained in the usual way[31–33] from the coalescence data for the spectroscopic change. The process **7a**⇋**7b** is clearly associated with both face to face exchange of the guest molecule (*E*) and an appropriate conformational change (*I*) of the host molecule. This conformational change must involve the interconversion of conformers related by a horizontal plane of symmetry; it is conveniently described as "conformational inversion" (*I*) and has an obvious similarity to the conformational inversion of, for example, six-membered ring systems. The overall process $E+I$, **7a**⇋**7b**, may or may not involve complete dissociation of the complex.

These early results were extended in later work, and a number of different

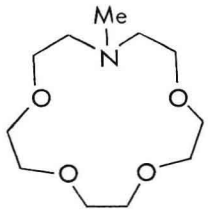


$n=2$ or 3

6



7a **7b**



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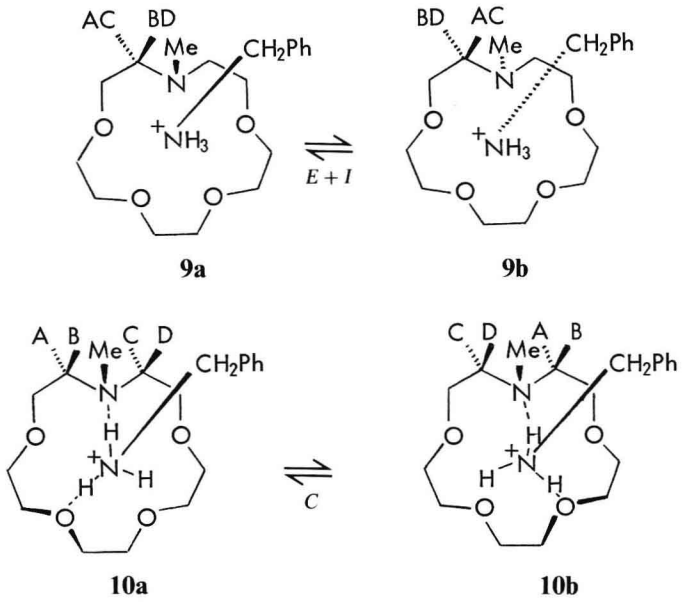
types of dynamic behavior were recognized for crown ether–alkylammonium cation complexes for which structural evidence relevant to the complexation could be derived.[34] This is best exemplified by a detailed study[35] of the complexes of the monoaza-15-crown-5 derivative **8**. The ^{1}H NMR spectrum of the host **8** is unexceptional at 25°C and does not show any evidence for slow conformational changes in the temperature range +25° to −110°C. The NMR spectrum of the 1:1 complex of **8** and benzyl-ammonium thiocyanate in CD_2Cl_2 shows changed chemical shifts for the host protons, and the NCH_2 signal, observable as a triplet at 25°C, changes below −22°C to two broad multiplets that are assignable to H_A and H_B of the four-spin system $NCH_AH_BCH_2O$. This change is consistent with face to face exchange of the guest cation (**9a**⇌**9b**, where the labels A, B, C, D, etc, are used to denote hydrogen atoms in environments A, B, C, D, etc) becoming slow on the NMR



9a **9b**

10a **10b**

Scheme I

time scale. Finally, at very low temperatures ($< -102°C$) the NCH_2 multiplets separate to give two pairs of signals assignable to H_A, H_B, H_C, and H_D of the slowly interconverting species **10a** and **10b**. The process **10a**⇌**10b** (*C*) involves reorganization of the hydrogen bonding and, probably more importantly, rotation about an OCH_2—CH_2O bond of the macrocycle from one gauche conformation to the other. The site exchanges associated with these interconversions $E+I$ **9a**⇌**9b** and C **10a**⇌**10b** are summarized in Scheme I.

These assignments of NMR temperature dependence to rate processes were checked by studying the complex of the host **8** with a chiral guest, 1-phenylethylammonium thiocyanate. The complex with the (*R*)-guest (1:1 ratio) gave a multiplet for the NCH_2 protons at 30°C, corresponding to a fast rate for the processes $E+I$ and C. At lower temperatures ($< -20°C$) the process $E+I$