Analysis of the Conformational Equilibrium of *cyclo*[Pro-NBGly$_2$] by means of Two-Dimensional NMR Spectroscopy[1]

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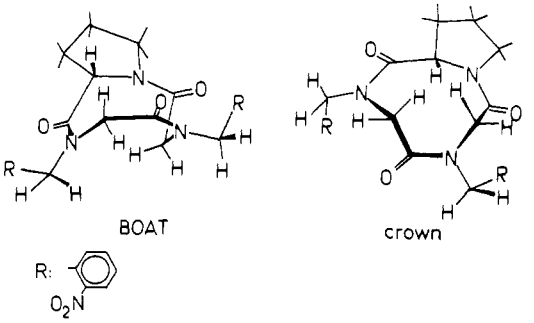
One advantage of NMR spectroscopy is the possible detection of conformational isomers when their interconversion is slow on the NMR time scale.[2] The simultaneous presence of two or more sets of signals sometimes makes spectral analysis and assignments quite difficult if not impossible. We will demonstrate that the combined use of the homonuclear two-dimensional NMR techniques spin-echo-correlated spectroscopy (SECSY)[3], and two-dimensional exchange spectroscopy[4] is extremely helpful in these cases.

Cyclic tripeptides can adopt two different backbone conformations: the "crown" and the "boat"[5]. Whether the molecule



prefers one of these conformations or both in equilibrium depends on the chirality and relative configuration of the three amino acids of the ring.[6,7] In the $CDCl_3$ solution of *cyclo*[Pro-NBGly$_2$] (NBGly = *o*-nitrobenzylglycyl) both conformations are present in a 20% (boat):80% (crown) equilibrium. The assignment of the two sets of signals to the boat and crown conformation was done by comparison of their ^1H and ^{13}C NMR spectra in the series *cyclo*[Pro$_n$-NBGly$_{3-n}$] (n = 1-3) and *cyclo*[L-Pro$_2$-D-Pro].[1,8] The characteristics shift, coupling patterns, and solvent dependence[7] allow an unambigious assignment of the conformers. The aliphatic region of the ^1H NMR spectrum exhibits two sets of proline resonances and eight different AB systems (each NBGly contains one pair of benzylic protons and another pair of α protons) which strongly overlap between 3.6 and 5.4 ppm. Hence recognition of the AB systems by difference decoupling was not possible, and the more selective double INDOR difference technique (DID)[9] turned out to be rather tedious for so many lines.

By two-dimensional spin-echo-correlated spectroscopy (SECSY) one obtains the whole scalar coupling information in one spectrum (Figure 1)—the AB systems are easy to detect by strong correlation peaks. The discrimination between benzylic and α protons was achieved via long-range coupling between aromatic protons and the diastereotopic benzyl protons (a in Figure 1). The easy detection of these couplings, which are difficult to find by conventional NMR spectroscopy, demonstrates one advantage of two-dimensional techniques.[1] Another long-range coupling in both glycine residues between one of the benzylic protons (A_1,A_2; see b in Figure 1) with the low-field α proton (C_1,C_2) in the boat conformation yields the connectivities within the amino acids.

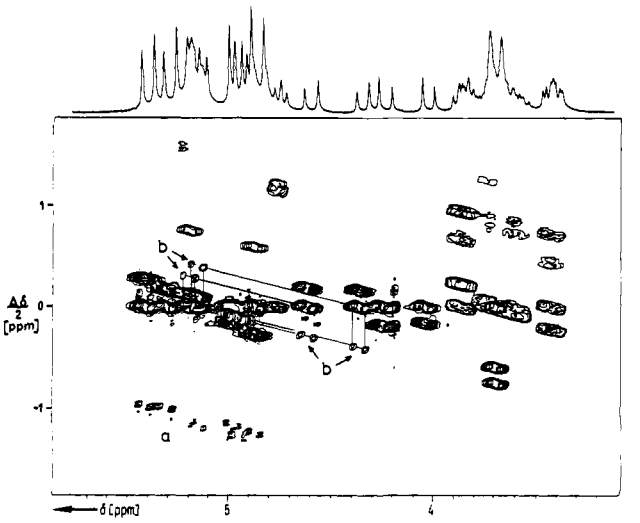


Figure 1. Part of the two-dimensional spin-echo-correlated ^1H NMR spectrum of *cyclo*[L-Pro-NBGly$_2$] at 270 MHz in $CDCl_3$ at 298 K. Only long-range couplings of the benzylic protons to the aromatic protons (a) and between one benzylic proton and one α proton (b) are indicated. The corresponding one-dimensional spectrum is shown on the top.

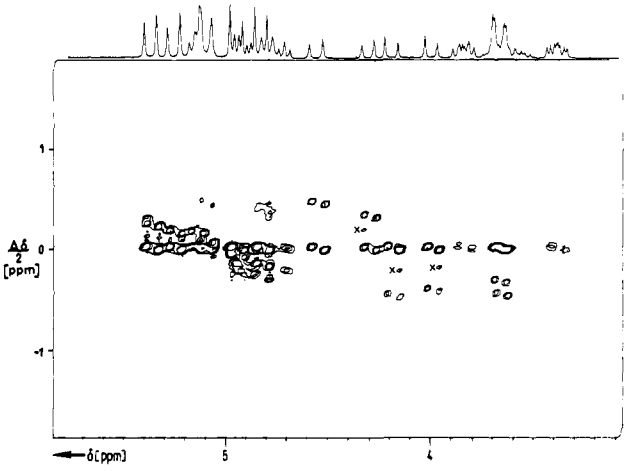


Figure 2. Part of the two-dimensional exchange ^1H NMR spectrum of *cyclo*[L-Pro-NBGly$_2$] at 270 MHz in $CDCl_3$ at 331 K. Because of the different temperatures the chemical shifts are slightly different from the SECSY spectrum in Figure 1.

It is of special interest to assign which protons exchange with each other during ring inversion. This information has previously been obtained in simpler cases by saturation transfer experiments,[7] but due to overlapping signals this is prevented for the title compound. We therefore used the two-dimensional exchange spectroscopy[4] at 58 °C in $CDCl_3$. Recording the FID's during t_2 of the pulse sequence $90_x° - t_1/2 - 90_x° - \tau_m - 90_x° - t_1/2$ (τ_m = 0.5 s) with phase cycling in order to suppress J peaks due to multiple-quantum coherences and a random τ_m delay to suppress J peaks due to zero-quantum coherence,[4] followed by complex two-dimensional Fourier transformation, results in the spectrum shown in Figure 2. Here, cross peaks do not indicate coupling but chemical exchange[4] (however, the small cross-peaks marked × in Figure 2 are probably residual unsuppressed J peaks). From this spectrum the exchange between protons in the two confor-

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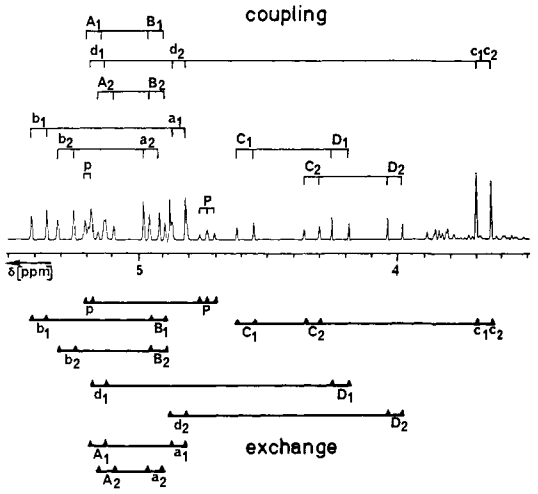

Figure 3. Coupling and exchange pattern in *cyclo*[L-Pro-NBGly$_2$] in $CDCl_3$ at 298 K. The spectrum is resolution enhanced. The signals of the boat conformations are indicted by capital letters, those of the crown in small letters.

mations can be extracted directly. It is obvious that the low-field proton of each AB system exchanges with the high-field proton of the corresponding AB system in the other conformation.

The combined analysis of both spectra yields the assignments shown in Figure 3.

The final question, which signal set belongs to which NBGly in the amino acid sequence, cannot be answered by our experiments because one does not know the population of the rapidly interconverting three boat conformations.[6,7]

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Isolation of an Iron–Nitrene Complex from the Dioxygen and Iron Porphyrin Dependent Oxidation of a Hydrazine

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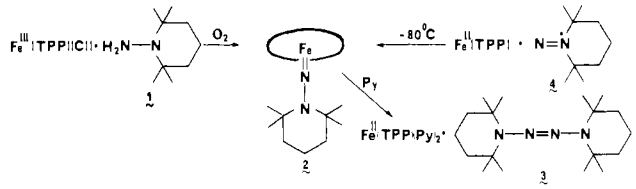
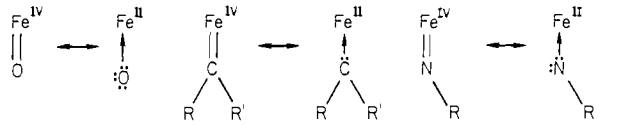
Oxo-iron porphyrin complexes have been proposed as intermediate active oxygen complexes in the hydroxylation of alkanes and other substrates by cytochromes-P-450.[1] Evidence has been provided for the formation of Fe^{IV}=O complexes upon decomposition of μ-peroxo-iron(III) porphyrin dimers in the presence of imidazoles,[2] and, of a π-cation porphyrin Fe^{IV}=O complex upon reaction of an iron(III) porphyrin with iodosoarenes.[3] Their carbon analogues, the more stable iron carbene complexes, have been prepared by reduction of polyhalogenated compounds by iron(II) porphyrins[4] and appear to be formed upon metabolic

Scheme I



reduction of polyhalogenated compounds[5] and metabolic oxidation of 1,3-benzodioxole derivatives by cytochromes P-450.[6] However,



there is so far no direct evidence for the formation of their nitrogen analogues, the nitrene- or imido-iron complexes, $[Fe^{II}\leftarrow NR] \leftrightarrow [Fe^{IV}{=}N\text{-}R]$,[7] although it has been proposed that the iron complexes formed during metabolic oxidation of 1,1-dialkylhydrazines by cytochrome P-450 could involve an iron–nitrene bond.[8]

The present communication reports the isolation and some properties of the first nitrene complex of a metalloporphyrin, which is formed by an O_2-dependent oxidation of 1-amino-2,2,6,6-tetramethylpiperidine, **1**, in the presence of an iron porphyrin.

Fe^{III}(TPP)Cl (*meso*-tetraphenylporphyrin = TPP), 10^{-2} M in aerobic CH_2Cl_2, reacts with the hydrazine **1**[9] (10^{-1} M) leading to the quantitative formation of the new complex **2**, which exhibits a characteristic visible spectrum at 437, 558, and 596 nm, within 10 min at 20 °C. As soon as complex **2** is completely formed, as shown by visible spectroscopy, dioxygen is removed from the solution by argon bubbling; the complex is then precipitated by CH_3OH addition and obtained as purple crystals (yield 90%). This complex is relatively stable toward O_2 since, being 10^{-2} M in aerated $CDCl_3$, it decomposes only slowly into the μ-oxo dimer $[Fe^{III}(TPP)]_2O$ (5% after 1 h at 20 °C).

A comparison of the ^1H NMR spectra of complex **2** and of its analogue prepared from a tetrakis(pentadeuteriophenyl)porphyrin partially deuterated on the pyrrole rings[10] allows one to assign the different signals of this paramagnetic compound (Figure 1).

In addition to the porphyrin signals (δ 66.8 (8 H, pyrrole), 9.61, 8.81, 7.53 ((20 H, phenyl)), one observes three signals for the protons of an axial ligand: δ 23.5 (12 H, CH_3), 81.5 (4 H, CH_2) −15.63 (2 H, CH_2). These data are in agreement with a paramagnetic complex with an axial symmetry and containing an axial

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