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Configurations of Morphiceptins by ^1H and ^{13}C NMR Spectroscopy

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Abstract: As part of our program to study the structure–activity relationship of peptide opioids, we have undertaken the spectroscopic examination of two proline-containing peptides: morphiceptin, Tyr-Pro-Phe-Pro-NH_2, and a highly selective morphiceptin analogue, Tyr-Pro-(NMe)Phe-D-Pro-NH_2[(NMe)Phe]. Using high-resolution ^1H and ^{13}C NMR experiments, we have assigned two of the four discernible configurational isomers observed for both morphiceptin and the (NMe)Phe analogue. The largest isomer amounting to 55% for morphiceptin and 65% for the (NMe)Phe analogue has been assigned as the all-trans isomer. The second configurational isomer accounting for 25% in both molecules studied adopts a cis conformation about the Tyr–Pro amide bond. The other isomers could not be unambiguously assigned since they are present in small amounts with overlapping and poorly resolved resonances. The exchange between the two major configurational isomers, cis/trans isomerization about the Tyr–Pro amide bond, proved to be extremely slow as established by chemical exchange measurements. Despite the two proline residues, the linear tetrapeptides do not exhibit clear conformational preferences. The measured NOE's, involving intraresidue or interresidue backbone atoms, are indicative of conformational averaging. The experimental examination of these morphiceptins is the first step in the development of structure–activity relationship for these molecules.

There have been numerous conformational studies of naturally occurring opiate peptides, most of which contain phenylalanine at the fourth position.[1,2] The result of these efforts has led to the general agreement of the necessity of tyrosine at the first position and the phenylalanine at the fourth for biological activity. Two naturally occurring opiate-active peptides with the phenylalanine at the third position are dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser)[3] and β-casomorphin (Tyr-Pro-Phe-Pro-Gly-Pro-Ile).[4,5] Dermorphin and many related analogues have been conformationally examined both experimentally and theoretically.[6,7] Recently, Schiller and co-workers reported the synthesis of a series of cyclic analogues of dermorphin.[8,9] Many of the constrained analogues displayed a high selectivity for the μ-opioid receptor. The conformational analysis of these cyclic analogues of dermorphin has been undertaken.[10,11]

The heptapeptide β-casomorphin discovered in bovine milk protein is highly selective for the μ-opioid receptor.[4,5] The amidated tetrapeptide, Tyr-Pro-Phe-Pro-NH_2, morphiceptin, is one of the most selective agonists for the μ-opioid receptor.[12] Many analogues of morphiceptin have been synthesized and biologically tested to explore the importance of specific moieties.[13,14] The ^{1}Tyr, ^{2}Pro, and ^{3}Phe are required for biological activity. The

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incorporation of a D-Pro at position 4 and methylation of the amide of phenylalanine produces an analogue, Tyr-Pro-(NMe)Phe-D-Pro-NH_2, with much greater affinity and selectivity for the μ-opioid receptor,[15]

Alkylation of the amino group of phenylalanine, (NMe)Phe, leads to some interesting conformational possibilities. The two proline residues within the native sequence allow for the possibility of cis/trans isomerization about the amide linkages resulting in four configurational isomers (trans/trans, cis/trans, trans/cis, and cis/cis). With the incorporation of the (NMe)Phe residue, which also leads to possible cis/trans isomerization about the *N*-methyl amide bond, eight configurational isomers can be envisioned. It should be noted that this analogue, Tyr-Pro-(NMe)Phe-Pro-NH_2, does not have an exchangeable, secondary amide proton.

There have been theoretical examinations of the conformational preferences of morphiceptin and related analogues.[14,16,17] Momany and co-workers examined the effects of the alkylation of the amide group on the conformational preferences of the molecule.[16] The restriction of the conformational freedom from the alkylation was mapped for model peptide sequences and the (NMe)Phe morphiceptin analogue using molecular mechanics calculations. In a study by Loew and co-workers the favorable conformations of the all-trans isomers of morphiceptin and Tyr-Pro-(NMe)Phe-Pro-NH_2 were calculated using an "aufbau" approach (i.e. a systematic optimization of the amino acids and dipeptide units with energy minimization).[17] The calculated conformations were then used to postulate the conformational requirements necessary for opiate receptor selective biological activity. However, the authors completely neglected the contributions to biological activity of cis/trans isomerization about the Tyr-Pro, Pro-(NMe)Phe, and (NMe)Phe-Pro amide linkages.

The proline residue and cis/trans isomerization is extremely important in the folding and denaturation and renaturation of polypeptides and proteins.[18] The role of cis/trans isomerization within the morphiceptins represents an important structural challenge that must be addressed. To explore the existence of the different configurational isomers and to characterize them conformationally, we initiated high-resolution ^1H and ^{13}C NMR examinations of morphiceptin and the analogue Tyr-Pro-(NMe)Phe-D-Pro-NH_2.

Experimental Section

^1H and ^{13}C NMR spectra were obtained on a General Electric GN-500 spectrometer operating at 500 and 125 MHz, respectively. The proton experiments and the reverse detection ^1H–^{13}C cross correlation were carried out at a concentration of 3.4 mg/mL in DMSO-d_6 (MSD Isotopes) or D_2O (ICN Isotopes). The 1D carbon spectra were obtained at a concentration of 20 mg/mL. There was no observed difference in the proton spectrum at this elevated concentration from that observed at 3.4 mg/mL. Samples for NOE measurements were degassed by repeated freeze–thaw cycles.[19] All the spectra were recorded at 25 °C.

The phase-sensitive correlation experiments, COSY, were run following the method of States et al.[20,21] with 512 t_1 values with 32 scans of 2K data points. Multiplication by a shifted sine bell was applied in both dimensions. Zero filling in the t_1 dimension resulted in a final matrix of 2K × 2K data points. The 2D homonuclear Hartman–Hahn experiments, HOHAHA, were run using the MLEV17 spin-locking sequence following the procedure of Bax.[22] The time proportional phase increment, TPPI, method was used to obtain the absolute phase.[23] A mixing time of 100 ms, 48 cycles of the MLEV17 sequence, with a spin-locking field of 10 kHz was employed. A recycle delay of 500 ms was used with the total time for the experiment approximately 2 h.

The NOE's were measured using the two-dimensional rotating frame experiment originally proposed by Bothner-By (CAMELSPIN, more commonly referred to as ROESY).[24,25] Spectra were obtained with mixing times varying from 75 ms to 1.5 s with a spin-locking field of 2 kHz. The experiments were carried out in the absorptive mode in the same manner as the COSY experiments (see above). Gaussian multiplication and zero filling in t_1 were applied to result in a 2K × 2K data set.

The reverse detected ^1H–^{13}C cross-correlation experiment was run following the procedure of Bax and Subramanian.[26] The 5-mm probe employed (General Electric) was specifically designed for reverse detection with the broad-band decoupler outside the proton observe coil. The ^{13}C frequency was set using the 1D carbon spectrum previously acquired. Two experiments were run for each molecule with carbon spectral widths of 10–140 ppm and 15–65 ppm. A total of 32 scans for the 512 t_1 values of 2K data points was stored and processed following the method proposed by Müller and Ernst.[21] Gaussian multiplication was used in both dimensions. The total time of the experiment was approximately 12 h.

Table I

residue	protons	morphiceptin trans	morphiceptin cis[1]	(NMe)Phe analogue trans	(NMe)Phe analogue cis[1]
		Proton Chemical Shifts			
Tyr	α	4.13	3.42	4.35	3.10
	β	2.95, 2.75	2.90, 2.80	2.85	2.80, 2.70
	ϕ	7.15, 6.70	7.10, 6.75	7.10, 6.85	6.95, 6.75
^{2}Pro	α	4.35	3.55	4.95	3.54
	β	2.00, 1.80	1.90, 1.85	2.25, 1.80	2.00, 1.85
	γ	1.75	1.70	2.0	1.60
	δ	3.60, 3.15	3.45	3.85, 3.35	3.45, 3.15
Phe	NH	8.30	8.45		
	NCH_3			2.90	2.65
	α	4.65	4.65	5.15	5.35
	β	3.10, 2.90	3.10, 2.85	3.30, 2.70	3.20, 2.85
	ϕ	7.25	7.25	7.25	7.25
^{4}Pro	α	4.20	4.20	4.25	4.10
	β	2.05, 1.85	2.00, 1.80	2.05, 1.75	2.00, 1.75
	γ	1.70	1.75	1.70	1.70
	δ	3.60, 3.50	3.55	3.20	3.25, 3.05
		Carbon Chemical Shifts			
Tyr	α	52.4	52.2	52.0	52.8
	β	35.2	36.1	34.8	36.4
	ϕ_1	124.0	124.4	124.0	124.0
	$\phi_{2,6}$	130.5	130.2	131.0	132.5
	$\phi_{3,5}$	115.3	115.5	115.0	115.5
	ϕ_4	156.5	156.8	156.5	157.0
^{2}Pro	α	59.4	59.2	56.7	57.0
	β	28.7	31.0	28.2	28.8
	γ	24.4	21.6	24.8	21.0
	δ	46.6	46.7	46.8	47.0
Phe	α	51.8	52.4	57.8	55.8
	β	36.6	35.7	34.4	33.7
	ϕ_1	137.5	137.2	138.0	137.0
	$\phi_{2,6}$	128.0	128.2	128.0	128.5
	$\phi_{3,5}$	129.3	129.0	129.0	129.5
	ϕ_4	126.5	126.8	126.5	126.8
	NCH_3			31.8	30.2
^{4}Pro	α	59.4	59.6	59.4	58.8
	β	30.0	30.2	28.8	28.8
	γ	24.3	24.5	24.3	24.2
	δ	46.8	46.8	46.2	46.2

Results and Discussion

The NMR spectra of both the analogues in DMSO show four discernible isomers with an approximate ratio of 55:25:15:5 and 65:25:5:5 for morphiceptin and the (NMe)Phe analogue, respectively. The assigned proton spectra are shown in Figure 1.

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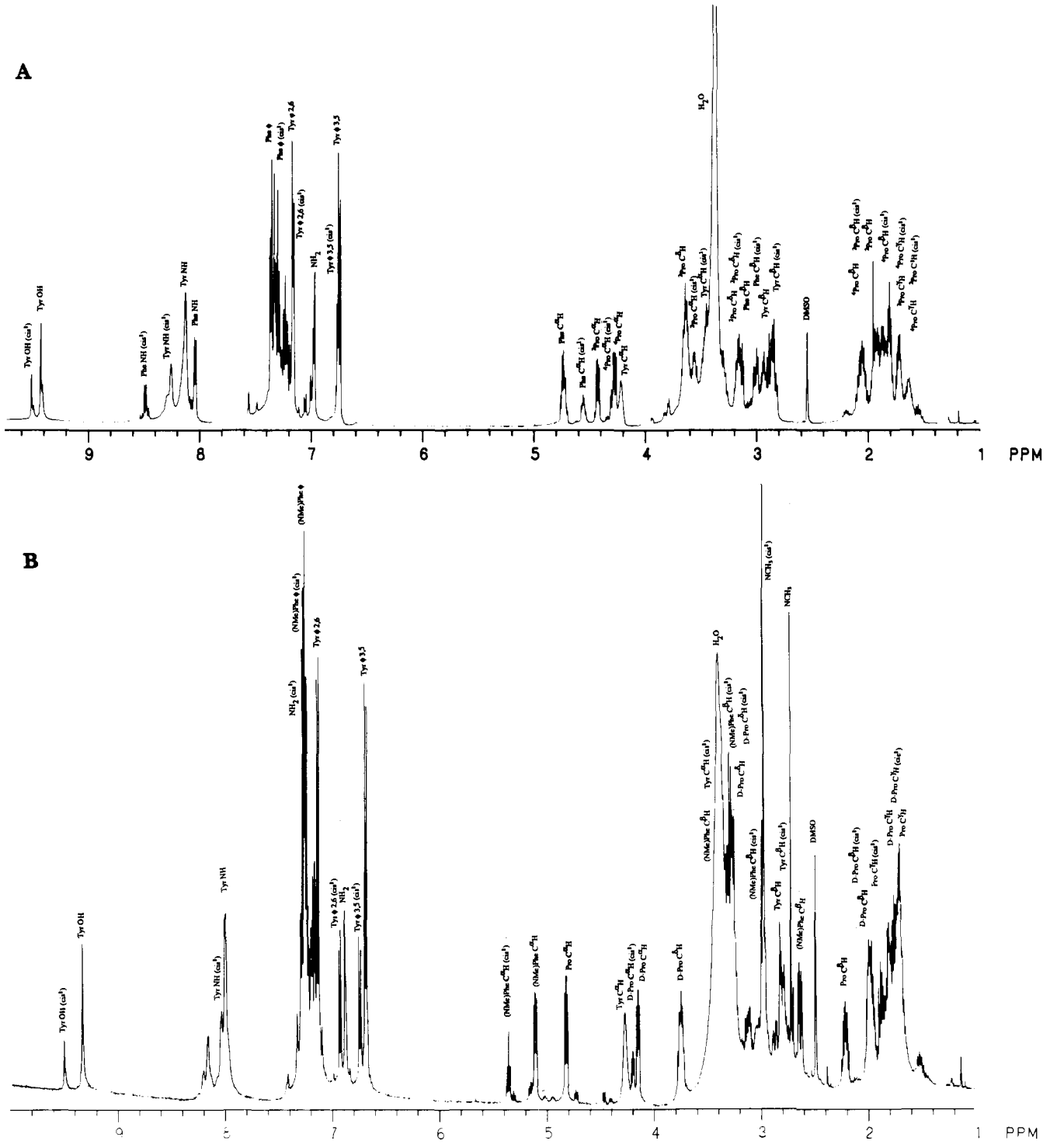

Figure 1, Assigned one-dimensional proton spectra of morphiceptin (A) and the (NMe)Phe analogue (B) in DMSO at 500 MHz.

The ratio of the isomers was determined from the integration of well-resolved peaks in proton spectra obtained with a recycle delay of 20 s. The ratio of the configurational isomers in water was 60:24:12:4 and 55:35:5:5 for morphiceptin and the (NMe)Phe analogue, respectively. There were some small differences observed in the chemical shifts of the proton resonances of morphiceptin and the (NMe)Phe analogue in DMSO and water. Following the assignment of the resonances and configurational isomers in DMSO, discussed below, the assignments in water were straightforward.

The chemical shifts of the proton and carbon resonances for the two major configurational isomers of morphiceptin and the (NMe)Phe analogue in DMSO are given in Table I. The chemical shifts for the two molecules are very similar for the different configurational isomers. The assignment of the proton resonances was carried out with COSY, HOHAHA, and ROESY experiments.

The fine structure of the cross peaks from the phase-sensitive COSY, shown for the (NMe)Phe analogue in Figure 2, allowed the assignment of the spin system of the coupled spins.[27] The complete spin system of the individual amino acids was identified using the HOHAHA experiment. The NOE's from the ROESY experiment were used to sequence the prolines (differentiate between ^{2}Pro and ^{4}Pro). In Figure 3, a ROESY experiment is shown for the (NMe)Phe analogue. The NOE's that were used for the sequencing of the prolines and the determination of the

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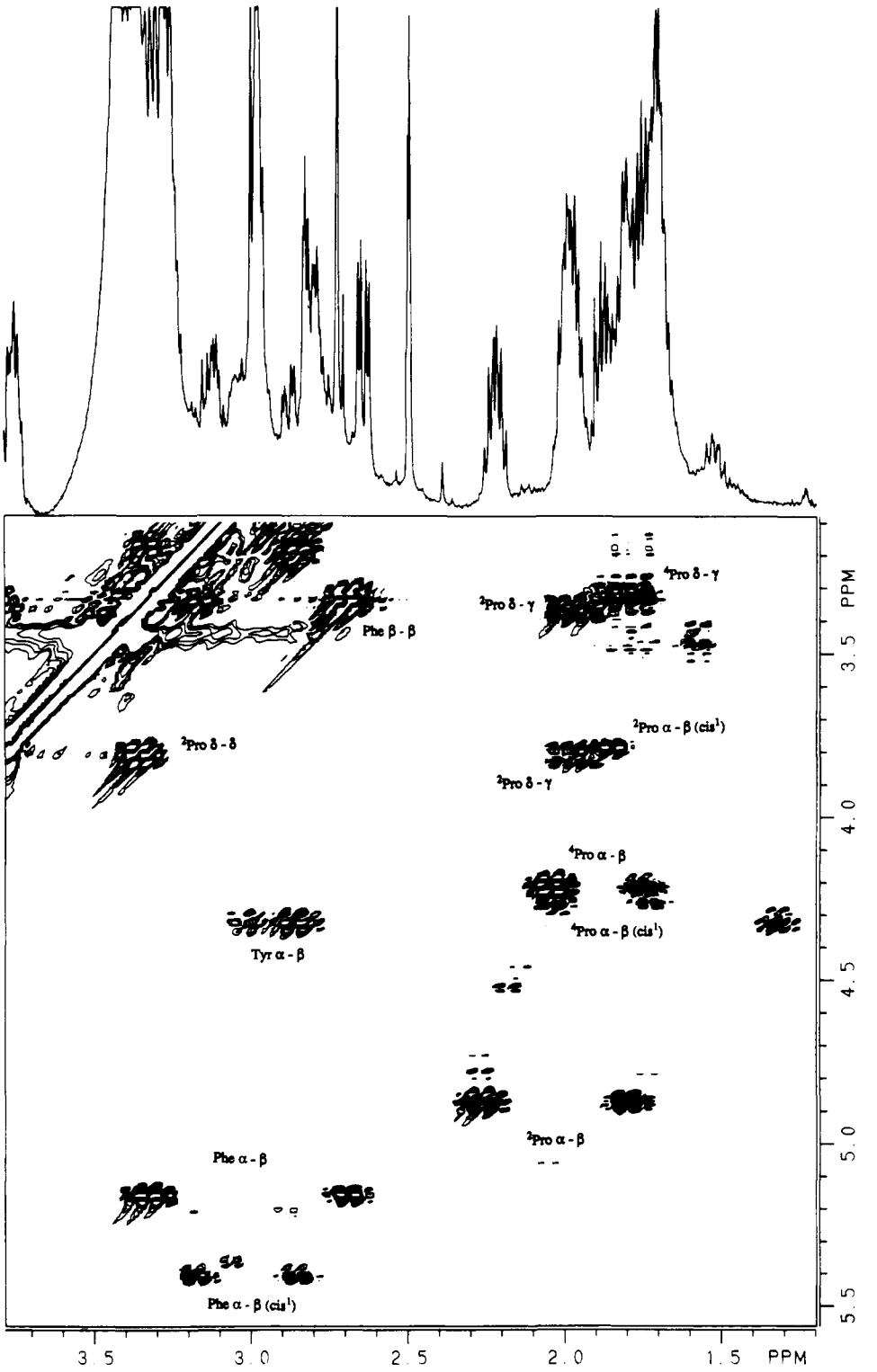

Figure 2. Contour spectrum of a phase-sensitive COSY of the (NMe)Phe analogue in DMSO at 500 MHz. Both the positive and negative contours are drawn. The assigned resonances for the major two isomers are labeled. The resonances from the second largest configurational isomer, with a *cis*-amide linkage about Tyr–^{2}Pro, are indicated as cis^1.

cis/trans configuration are illustrated. The NOE's measured for the major configurational isomers for the two molecules are shown in Figure 4. The enhancements were observed in ROESY spectra with mixing times of 75, 150, and 275 ms.

All of the proton resonances could not be unambiguously assigned with the use of two-dimensional proton techniques at 500 MHz (COSY, HOHAHA, ROESY). The assignment of the three isomers existing in smaller populations was difficult because of the extensive spectral overlap and low resolution within the aliphatic region of the spectrum. The prolines and *N*-methyl-substituted Phe, i.e. the (NMe)Phe analogue, lack amide protons so that the commonly used NH–C$^\alpha$H couplings or NOE's are not present.[28] Specifically, the assignment of the Tyr and ^{2}Pro spin systems of the second largest isomer, for both morphiceptin and the (NMe)Phe analogue, could not be unambiguously assigned using proton experiments. The assignment of these two amino acids was only possible using the rather uncrowded aliphatic region of the carbon spectrum and the ^{1}H-detected heteronuclear cross-correlation experiment.

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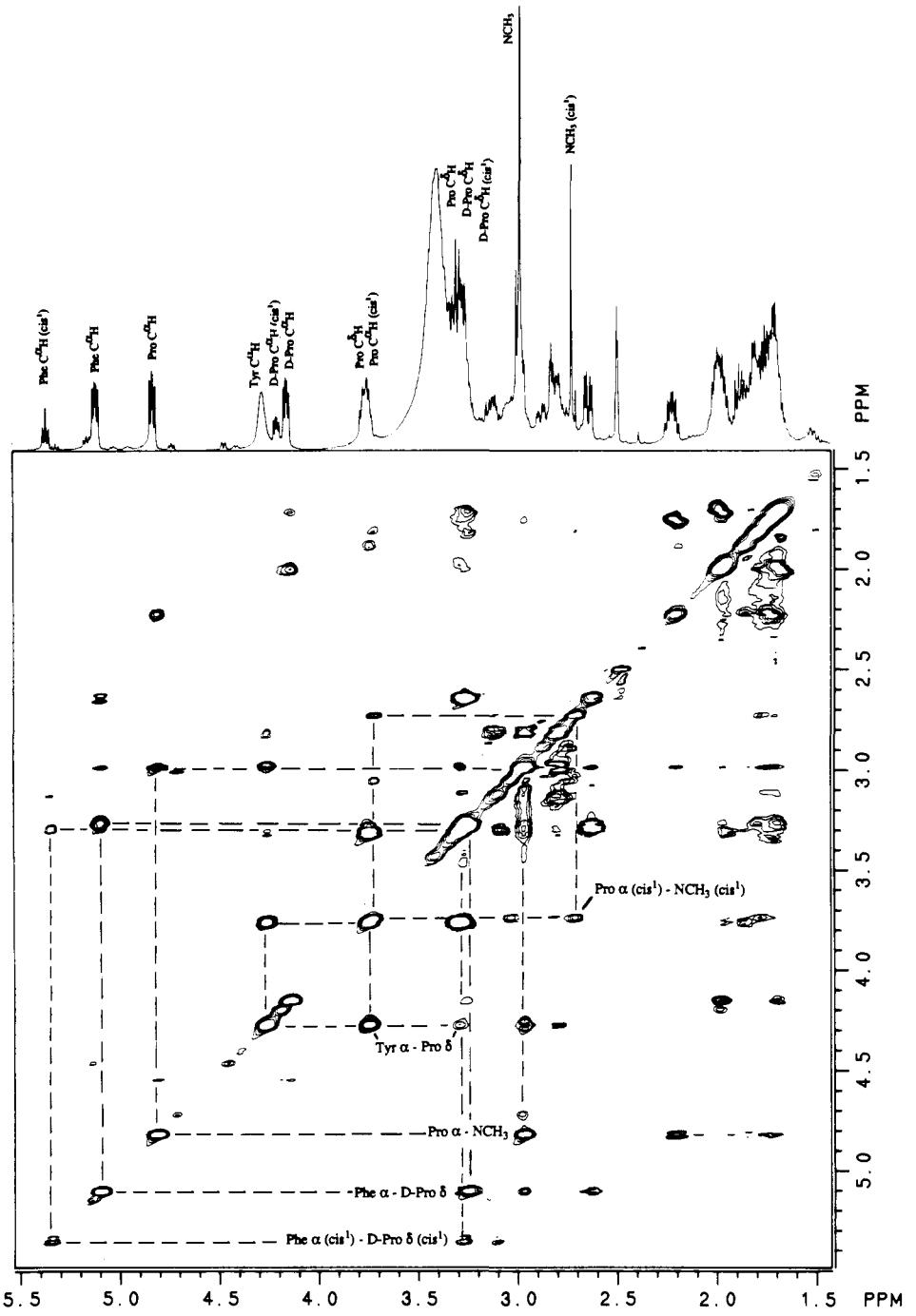

Figure 3. Contour spectrum of a ROESY with a mixing time of 175 ms of the (NMe)Phe analogue in DMSO at 500 MHz. The diagonal has positive intensity while the cross peaks from NOE's are negative, both are drawn for ease of identification. The NOE's used to indicate *trans*/*cis*-amide linkages are labeled. The second largest configurational isomer is indicated as cis[1].

In Figure 5, the aliphatic region of the assigned carbon spectrum of the (NMe)Phe analogue is shown. Most of the carbon resonances were assigned using a heteronuclear cross-correlation experiment and the proton assignments. The α carbons of the Tyr and ^{2}Pro of the second largest isomer were assigned by inspection. After the assignment of the carbon resonances, the identification of the Tyr and ^{2}Pro proton spin systems was straightforward. The assignment of the Tyr and ^{2}Pro α protons of morphiceptin is illustrated in a contour plot of the cross-correlation experiment, shown in Figure 6. These α protons are within very crowded regions of the proton spectrum and thus could not be differentiated with proton experiments. The assignment of the carbon resonances for morphiceptin and the (NMe)Phe analogue are given in Table I.

The largest configurational isomer for morphiceptin and the (NMe)Phe analogue exists with all of the amide bonds in a trans conformation. This is borne out from the proton NOE's and the small difference in the chemical shifts of the β and γ carbons of the prolines. The NOE's between the Tyr α and ^{2}Pro δ protons and the Phe α and ^{4}Pro δ protons indicate that both the Tyr–^{2}Pro and Phe–^{4}Pro amide bonds are trans. The absence of NOE's between the adjacent α protons (e.g. Tyr α–^{2}Pro α, Phe α–^{4}Pro α) expected for a cis conformation about the amide bond also supports the assignment of trans configuration. The difference in the chemical shift of the β and γ carbons of the prolines is sensitive to the configuration about the amide bond. Large differences, 8–10 ppm, are characteristic of *cis*-amide linkages while differences of 1–6 ppm are indicative of trans bonds.[29] As can be seen in Table I, the difference of chemical shift of these two

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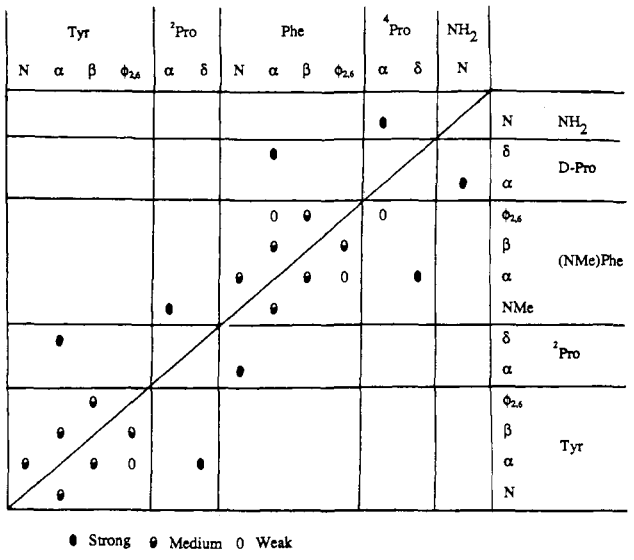

Figure 4. Observed nuclear Overhauser effects measured for morphiceptin and the (NMe)Phe analogue in DMSO at 500 MHz using the ROESY experiment.

carbon resonances for morphiceptin and the (NMe)Phe analogue fall into the trans regime. As for the ^{2}Pro–(NMe)Phe amide linkage of the (NMe)Phe analogue, the large NOE observed for the ^{2}Pro and the *N*-methyl protons, NCH_3, indicates a trans configuration.

The second isomer for both morphiceptin and the (NMe)Phe analogue, accounting for approximately 25%, has been identified as the isomer with the Tyr–^{2}Pro amide linkage in a cis arrangement. This was determined from the large difference in the chemical shifts of the β and γ carbon resonances of the ^{2}Pro. The proton NOE's indicating trans configuration about the other proline and the *N*-methyl-substituted Phe, (NMe)Phe analogue only, are still present for this second isomer. The observed NOE's for the (NMe)Phe analogue that allowed this assignment are shown in Figure 3, labeled ^{1}cis. Another indication of the *cis*-Tyr–^{2}Pro bond is the large upfield shift of the α protons of both the Tyr and ^{2}Pro. This upfield shift of the resonances is observed for both morphiceptin and the (NMe)Phe analogue (see Table I).

For morphiceptin we postulate that the third largest isomer accounting for 15% has a *cis*-amide linkage about the Phe–^{4}Pro bond. For this isomer the Tyr NH_2, Phe NH, and one of the NH_2 protons are resolved (the amide region is relatively uncrowded) and can be assigned. There are small cross peaks, from a HOHAHA experiment, indicating couplings from the Tyr NH_2 to a multiplet at 4,15 ppm and from the Phe NH to 3.50 ppm. There also is a small NOE from the NH_2 to a multiplet at 3.65 ppm. We assume that these couplings and NOE's are to the α protons of the Tyr, Phe, and ^{4}Pro, respectively, If this is the case, there is a large upfield shift in the resonances of the Phe and ^{4}Pro, which indicates a cis configuration about the Phe–^{4}Pro amide bond. The fourth isomer, ~5%, is postulated to be the remaining configurational isomer, with *cis*-amide linkages about both the Tyr–^{2}Pro and Phe–^{4}Pro bonds. These assignments are speculative and must be confirmed with more proton–proton couplings, NOE's, and carbon assignments before further conclusions can be drawn.

The (NMe)Phe analogue has the possibility of six other configurational isomers in addition to the two resolved and assigned, the all-trans and cis/trans/trans. The assignment of the two remaining is difficult because of the small proportion of these isomers, ~5% each, and the lack of the Phe NH. The only resonance that can be assigned is the Tyr NH_2. There are small couplings and NOE's, which indicate that the α proton of the Tyr for both of these isomers is at 4.20 ppm. This assignment would indicate that the Tyr–^{2}Pro amide linkage is trans for both of the remaining configurational isomers. This finding and the results

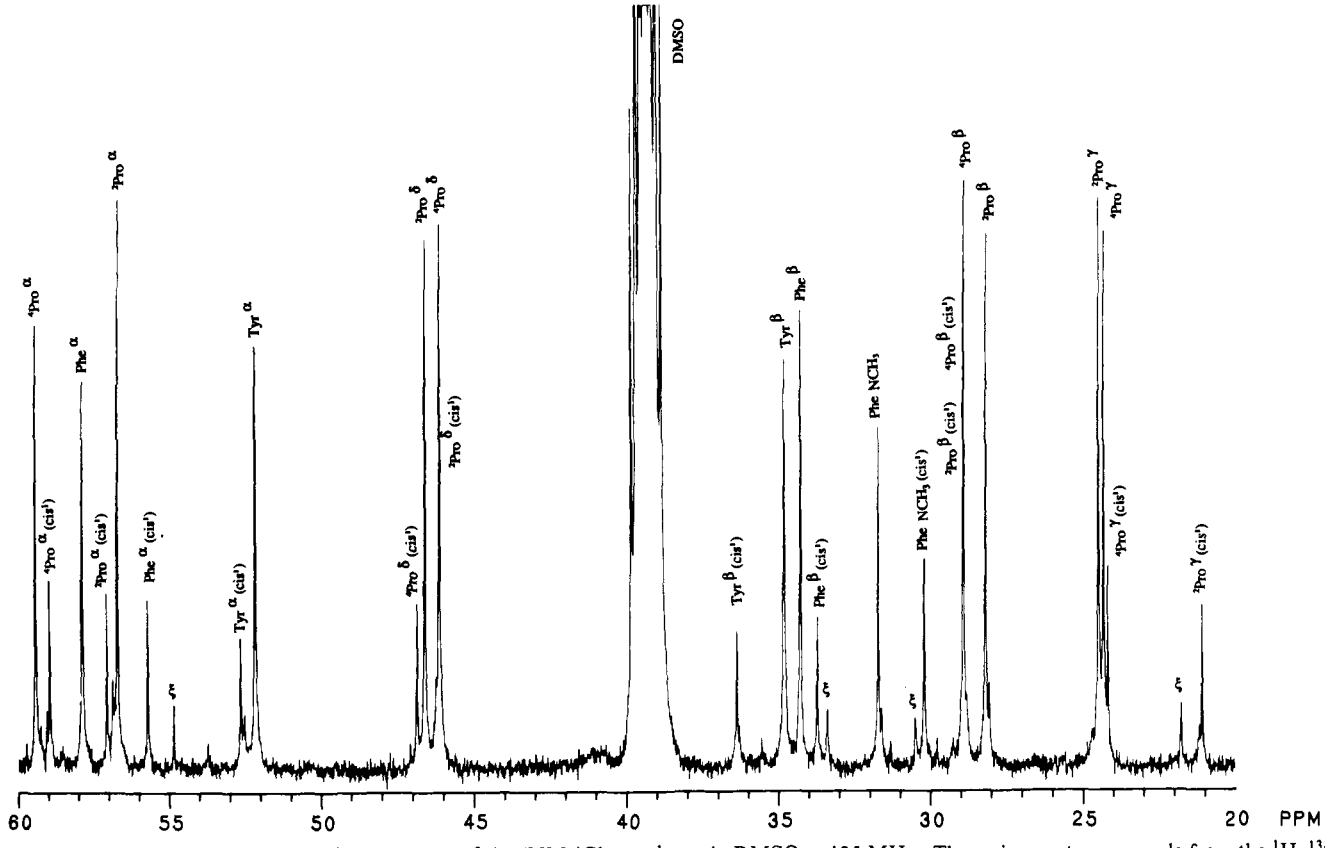

Figure 5. Aliphatic region of the carbon spectrum of the (NMe)Phe analogue in DMSO at 125 MHz. The assignments were made from the ^{1}H–^{13}C cross-correlation experiment and the assigned proton resonances. The resonances from the second largest configurational isomer are labeled with a cis^1. The few resolved resonances observed for the two isomers in smaller populations are indicated with a ξ. The extensive spectral overlap and low resolution of these isomers does not allow unambiguous configurational assignment.

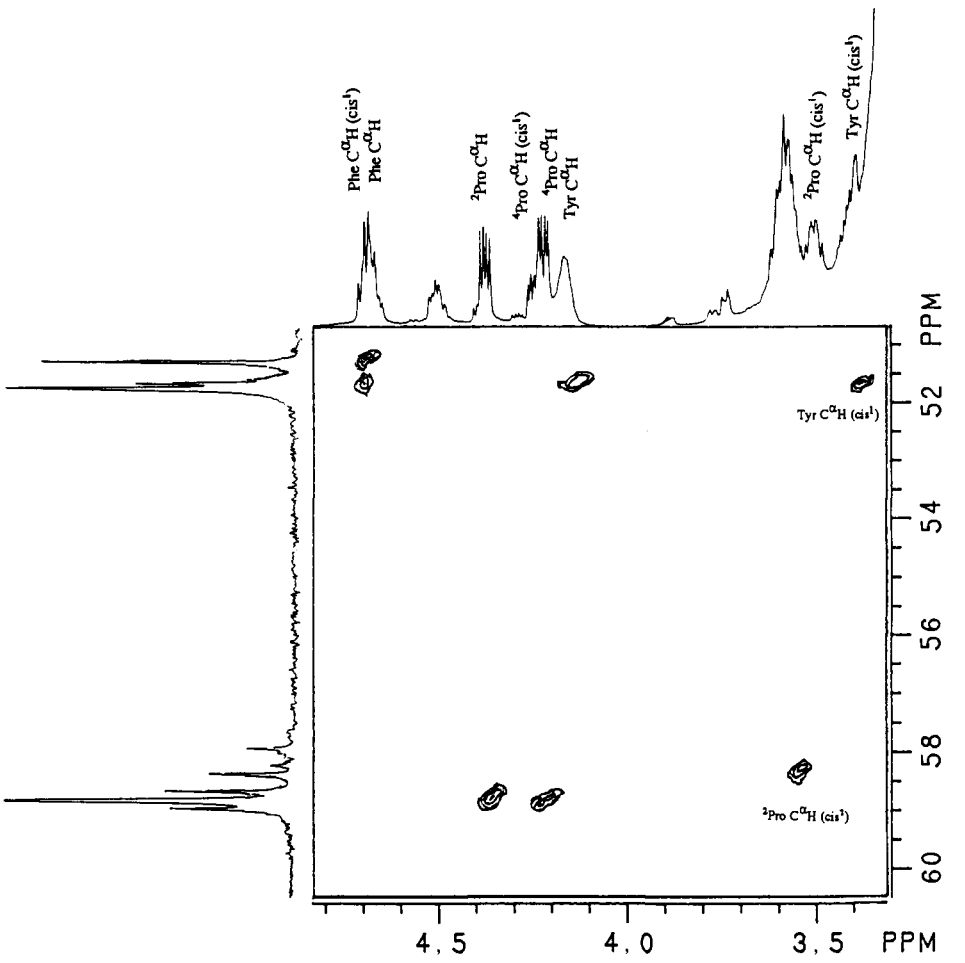

Figure 6. Contour spectrum of proton-detected $^1H-^{13}C$ cross-correlation experiment of morphiceptin in DMSO at 500 MHz. The cross sections that were used for the assignment of the α protons of the Tyr and ^{2}Pro of the second largest configurational isomer, indicated with cis^1, are shown. These proton resonances could not be assigned with proton experiments because of the large spectral overlap. The one-dimensional proton and carbon spectra are shown on the top and along the side of the contour plot, respectively.

for morphiceptin lead us to assign the remaining isomers to the "mono-cis" isomers, trans/cis/trans and trans/trans/cis, with a *cis*-amide linkage about the ^{2}Pro–(NMe)Phe and (NMe)Phe–D-^{4}Pro, respectively. Again the assignments of these isomers are speculative and require further investigation. The presence of the isomer with a cis linkage about the *N*-methyl substitution is in accord with some recent results obtained in our laboratory on sarcosine-containing analogues. In these studies, one of the analogues containing a sarcosine contained the cis isomer as the major conformation.[30]

The NOE's measured for the largest configurational isomer of morphiceptin and the (NMe)Phe analogue (shown in Figure 2) are either intraresidue NOE's or expected interresidue NOE's involving backbone atoms. This indicates that the linear tetrapeptides despite having two prolines are flexible and are undergoing conformational averaging. The side chains of the tyrosine and phenylalanine are of course quite flexible, and only intraresidue NOE's are measured. The coupling constant measured for the Phe of morphiceptin is 7.6 Hz, also suggestive of conformational averaging.

The exchange of the two major configurational isomers, isomerization about the Tyr–^{2}Pro amide bond, of morphiceptin and the (NMe)Phe analogue was investigated with ROESY experiments using extended mixing times (0.5–1.5 s). There was no evidence of exchange in the ROESY spectra, run at a temperature of 25 °C, even with mixing times of 1.5 s. Spectra obtained with longer mixing times have a significant loss in signal because of relaxation. ROESY spectra at 60 and 80 °C with mixing times of 1 s did not indicate chemical exchange between the configurational isomers.

The use of one-dimensional saturation transfer experiments is limited by the severe spectral overlap.[31,32] For the (NMe)Phe analogue, the Phe α proton of the trans and cis^1 configurational isomers are separated by 0.20 ppm, but there is significant overlap with the resonances of the two minor configurational isomers (see Figure 1). Despite this overlap one-dimensional saturation transfer experiments were carried out. The decoupler was set to the frequency of either the trans or cis^1 resonance of the Phe α proton for the first experiment and set to base line for the next experiment. The resulting free induction decays from the two experiments were subtracted after multiplication by an exponential function with a line broadening of 10 Hz. The recycle delay was set to 10 s, during which the length of saturation was varied. Exchange of the cis isomer from the saturation of the trans isomer was observed only at temperatures above 70 °C. No effects were observed with saturations up to 10 s at lower temperatures. At 70 °C saturation of at least 4 s was required before exchange was observed.

These results show that the energy barrier between the two largest configurational isomers is large. The transfer rate of the exchange even at elevated temperatures (70 °C) is still quite slow. The examination of the kinetics of the isomerization is complicated because of the overlap with the resonances of the minor configurational isomers. We are currently examining the use of mixtures of solvents and variation of pH and temperature to obtain a

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separation of resonances that will allow for the accurate measurement of peak intensities.

Conclusions

We report here the experimental observation of four different configurational isomers for both morphiceptin, Tyr-Pro-Phe-Pro-NH_2, and the (NMe)Phe analogue, Tyr-Pro-(NMe)Phe-D-Pro-NH_2. The four isomers, with small differences in ratio of configurational populations, were observed in both water and DMSO. Using ^1H and ^{13}C NMR the unambiguous assignment of the two major isomers for each compound was carried out. The results presented clearly indicate the importance of exploring all possible isomers when examining a peptide containing an internal or C-terminal proline or *N*-methyl-substituted residue. The all-trans structures for morphiceptin and the (NMe)Phe analogue, comprising 60% and 55%, respectively, may only represent a part of the biological profile for these peptidic opiates. We are continuing our studies on the conformations and possible separations of these stereoisomers in order to develop structure–biological activity relationships.

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Registry No. H-Tyr-Pro-Phe-Pro-NH_2, 74135-04-9; H-Tyr-Pro-(NMe)Phe-D-Pro-NH_2, 83397-56-2.

Vibrational Spectra and Normal Mode Analysis for [2Fe–2S] Protein Analogues Using ^{34}S, ^{54}Fe, and ^2H Substitution: Coupling of Fe–S Stretching and S–C–C Bending Modes

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Abstract: Resonance Raman (RR) and infrared (IR) spectra are reported for analogue complexes of Fe_2S_2 proteins: $[(C_2H_5)_4N]_2[Fe_2S_2(SCH_3)_4]$, $[(n\text{-}C_3H_7)_4N]_2[Fe_2S_2(SC_2H_5)_4]$, and $[(C_2H_5)_4N]_2[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]$ (S_2-o-xyl = *o*-xylene-α,α'-dithiolate) and their isotopomers with ^{34}S at the bridging positions. For the xylenedithiolate complex the effects of substituting ^{54}Fe, ^{34}S at the terminal positions and ^2H at the methylene positions were also investigated. All eight Fe–S stretching modes have been assigned via their RR and IR activities and their isotope shifts. In addition a S–C–C bending mode has been identified in RR spectra of the ethanethiolate and xylenedithiolate complexes through its interaction with a nearly coincident Fe–S stretching mode. The frequencies and isotope shifts were calculated using a $Fe_2S_2(SC_2H_5)_4$ model with point mass methyl and methylene groups and structural parameters of the *o*-xylenedithiolate complex. This model was used to gauge the sensitivity of the vibrational frequencies to the Fe–S–C–C dihedral angle, τ; as τ increases from 90°, four of the Fe–S stretching modes increase, while one decreases, owing to differential couplings with the S–C–C bending coordinates. The lowest frequency Fe–S stretching mode, at $\sim$275 cm^{-1}, is an IR-active out-of-phase breathing mode of the two linked FeS_4 tetrahedra. In the xylenedithiolate and ethanethiolate complexes, but not in the methanethiolate complex, this mode also shows significant RR activation, with an even stronger RR overtone band. This behavior is attributed to unusual sensitivity of this mode to environmental asymmetry, resulting in a significant excited-state origin shift and force constant change. RR intensities of other combination tones suggest excited-state Duschinsky rotation among the interacting S–C–C bending and Fe–S stretching modes. A band of variable intensity at $\sim$200 cm^{-1} is assigned to a mode involving mutual displacement of the Fe atoms; its large intensity for the xylenedithiolate complex suggests appreciable direct interaction of the Fe orbitals. RR bands in the 120–150-cm^{-1} region are assigned to S–Fe–S bending modes.

As part of a program of resonance Raman (RR) spectroscopic studies of iron–sulfur proteins and their small molecule analogues,[1] we present vibrational analyses of $[Fe_2S_2(SR)_4]^{2-}$ complexes, analogues of the Fe_2S_2 proteins. These complexes, first prepared in the pioneering synthetic program of Holm and co-workers,[2] have played a key role in the elucidation of the protein active sites, providing accurate structures against which various spectroscopic signatures can be compared. The first such Fe_2S_2 analogue, and the most stable one, contains the chelating ligand *o*-xylene-α,α'-dithiolate (S_2-o-xyl), used by Holm and colleagues to prevent further oligomerization of the Fe_2S_2 centers.[3]

The xylenedithiolate analogue served as the basis for a previous RR analysis of Fe_2S_2 proteins from this laboratory, using ^{34}S labeling of the labile (bridging) sulfur atoms in the proteins.[4] Isotopes were unavailable for the analogue, however, whose RR spectrum differs in significant details from those of the proteins. Moreover, the spectral quality, while significantly better than had been reported in previous studies,[5-7] left room for improvement. We now report higher quality RR, and also IR, spectra of the xylenedithiolate analogue, obtained at low temperature (77 K) in pressed KBr pellets of the tetraethylammonium salt. These data reveal new spectral features. We have also determined the effects on the spectra of isotope substitution at Fe, bridging S, terminal S, and methylene H. The isotope shifts provide secure assignments of all eight Fe–S stretching modes, some of which differ from those previously suggested. In addition there is unambiguous evidence for interaction of S–C–C bending with two of the Fe–S stretching modes.

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