according to ^1H NMR, and the major component is presumed to be **2** which has less steric repulsion between the substituent of the asymmetric silicon and bulky Cp′ ring than **3**. A possible mechanism of the photoreaction of **1** (eq 1) is essentially the same as that proposed previously for peralkyldisilanyliron complexes[5,6] except for the final cyclization step to form **2** and **3**.

Yellow crystals of **2** suitable for X-ray crystal structure analysis were obtained from a concentrated reaction mixture in toluene at −20 °C. The ORTEP view of **2** is shown in Figure 1.[9] The Fe–Si bond lengths (2.207 (3) and 2.222 (3) Å) are very short, and the former is the shortest Fe–Si bond length yet observed.[10] In contrast, the bond lengths of Si(1)–O(2) (1.793 (9) Å) and Si(2)–O(2) (1.799 (8) Å) are significantly longer than that of Si(2)–O(3) (1.632 (9) Å), a usual Si–O single bond, and are even longer than a dative Si–O(HMPT) bond in [(OC)$_4$Fe=Si(O-*t*-Bu)$_2$·HMPT] (1.730 (3) Å).[3] Each silicon atom and the atoms directly attached to this except O(2) are almost coplanar: Si(1) and Si(2) lie only 0.32 and 0.30 Å above the planes defined by Fe, C(5), and C(6) and Fe, O(3), and C(4), respectively. Interestingly, the trivalent oxygen O(2) and three atoms bonded to this, Si(1), Si(2), and C(2), are also coplanar: the deviation of O(2) from the plane defined by Si(1), Si(2), and C(2) is only 0.14 Å.

In consideration of the nearly planar arrangement of atoms at each silicon except O(2), the short Fe–Si and long Si–O(2) bond lengths, and the equivalence of two Fe–Si bonds and two Si–O(2) bonds, we propose a bonding model which is schematically represented by the structure B in Chart I. Structure B lies in the midst of two canonical structures A and C. Charge separation seems to be very little because **2** is very soluble in typical nonpolar organic solvents. Therefore, it is considered that two Fe–Si bonds take on the partial double bond character, whereas two Si–O(2) bonds are regarded as a mixture of dative bonding and covalent bonding. By using classical bonding description, structure B could be described as a combination of two resonance forms D and E (Chart II).

The sp^2 character of silicon atoms is further supported by the ^{29}Si NMR data: A mixture of **2** and **3** in the ratio of ca. 2:1 shows four signals at remarkably low field (δ 127.4, 121.1, 101.9, and 93.9 ppm) with ca. 1:2:2:1 intensity.

The CO stretching frequency for the terminal carbonyl ligand of **2** (and also **3**) (1875 cm^{-1}) is appreciably lower than those of **1** (1920 and 1972 cm^{-1}) and that of a structurally similar complex CpFeH(SiCl$_3$)$_2$(CO) (2025 cm^{-1}).[11] This result indicates the strong back donation of electron density from iron to carbonyl ligand and therefore suggests the poor electron-accepting ability of the two silylene ligands, which may be due to the partial occupancy of vacant 3p orbitals of silicon atoms by the lone pairs of methoxy oxygen O(2). The strong back donation to the carbonyl ligand is also reflected in the short Fe–C(1) bond (1.68 Å) and the long C(1)–O(1) bond (1.20 Å) compared to those of CpFeH(SiCl$_3$)$_2$(CO) (1.75 and 1.13 Å, respectively).[12]

The complexes **2** and **3** are extremely air and moisture sensitive, and the light yellow color of those crystals or solutions immediately turns to dark brown when they are exposed to air or moisture.

The similar photochemical behavior was observed for Cp′Fe(CO)$_2$SiMe$_2$SiMe$_2$OMe, Cp′Fe(CO)$_2$SiMe$_2$SiMe$_2$O-*t*-Bu, CpFe(CO)$_2$SiMe$_2$SiMe(O-*t*-Bu)$_2$, and CpFe(CO)$_2$SiMe$_2$SiMe$_2$O-*t*-Bu which will be reported elsewhere in detail.

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Supplementary Material Available: Tables of atomic positional and thermal parameters and bond distances and bond angles for **2** (6 pages); listings of observed and calculated structure factors for **2** (9 pages). Ordering information is given on any current masthead page.

(9) Crystal data of **2**: orthorhombic, space group $P2_12_12_1$, a = 10.547 (2) Å, b = 21.932 (4) Å, c = 8.742 (3) Å, V = 2022.2 (8) Å^3, d_{calcd} = 1.26 g cm^{-3}, Z = 4. X-ray diffraction data were collected at 15 °C on a Rigaku AFC-6A diffractometer with graphite-monochromated Mo Kα radiation. Reflections (3488) with $3° < 2\theta < 60°$ were collected by the ω-2θ scan technique. The crystal structure was solved by the heavy atom method and refined with block-diagonal least-squares by using individual anisotropic thermal parameters. None of the hydrogen atoms was found. As a result of refinement, the structure drawn in Figure 1 was slightly more favored than that with inverse chirality, applying anomalous dispersion correction. For 1900 unique reflections ($|F_o| > 3\sigma(F_o)$) R = 0.076 (R_w = 0.114) was obtained.

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Two-Dimensional NMR Strategies for Carbon–Carbon Correlations and Sequence-Specific Assignments in Carbon-13 Labeled Proteins

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Applications of carbon NMR spectroscopy to proteins in the past have been limited to one-dimensional spectroscopy[1] with a few explorations into two-dimensional heteronuclear chemical shift correlation.[2-7] Carbon–carbon scalar couplings have been totally unexploited in NMR spectroscopy of proteins. Uniform or selective carbon-13 enrichment can be used to increase sensitivity of heteronuclear shift correlation experiments as well as to introduce a readily observable level of ^{13}C–^{13}C coupling. Although a prototype ^{13}C–^{13}C correlation experiment was carried out with an amino acid ([85% U1-^{13}C]lysine) several years ago,[8] protein experiments have been slow to follow. We report here that the NMR spin system assignments of the nine leucine residues in [85% U1-^{13}C]leucine streptomyces subtilisin inhibitor ([85% U1-^{13}C]leucine SSI)[9,10] have been extended from the carbonyl carbons[11-13] to the intraresidue α carbons by means of two-dimen-

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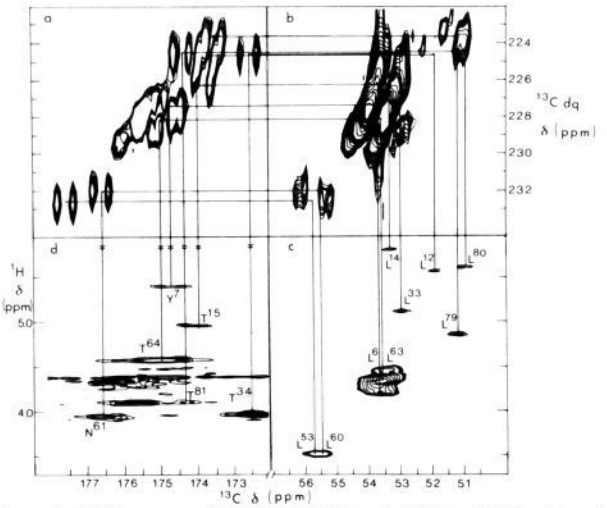

Figure 1. NMR spectra of ([85% U1-^{13}C]leucine)SSI at 61 °C collected on a Bruker AM-500 NMR spectrometer (500 MHz for ^{1}H; 125.77 MHz for ^{13}C). The sample was 100 mg of the labeled protein in 2 mL of 0.05 M phosphate buffer, pH 7.3, in $^{2}H_2O$. Dioxane in $^{2}H_2O$ at 61 °C was used as an external reference and was assigned a chemical shift of 67.8 ppm with respect to $(CH_3)_4Si$. Parts (a) and (b) show, respectively, the $^{13}C_o/(^{13}C_o+^{13}C_\alpha)$ and $^{13}C_\alpha/(^{13}C_o+^{13}C_\alpha)$ regions of the 2D $^{13}C\{^{13}C\}$DQC NMR spectrum. A resonance-offset-compensated INADEQUATE[16,17] pulse sequence was modified by the incorporation of a time proportional 45° phase incrementation[18,19] of the double-quantum propagator to give two-dimensional pure-absorption phase spectra. The total delay time for the double-quantum propagator was 9.08 ms. The recycle time was 1 s, and 256 blocks of 4096 data points were collected. The probe size was 10 mm, and the ^{13}C 90° pulse width was 14.5 μs. (c) $C_\alpha{}^1H/^{13}C_\alpha$ region of the $^{1}H\{^{13}C\}$HSBC spectrum obtained by a ^{1}H-detected HETCOR pulse sequence with broadband ^{13}C decoupling during acquisition.[6] The sample volume was 0.5 mL in a 5 mm ^{1}H NMR probe equipped with a broadband decoupling coil that was tuned to the ^{13}C frequency. (d) $C_\alpha{}^1H/^{13}C_\alpha$ region of the 2D $^{1}H\{^{13}C\}$HMBC spectrum obtained by the heteronuclear multiple-bond correlation pulse sequence.[20] Conditions were as in (c), but 1024 blocks of 2048 data points were collected, and the ^{13}C 90° pulse width was 11 μs. The delay time for buildup of anti-phase multiple-bond coherence was 60 ms.

sional (2D) ^{13}C double-quantum correlation [$^{13}C\{^{13}C\}$DQC], to intraresidue α protons by ^{1}H–^{13}C heteronuclear single-bond correlation [$^{1}H\{^{13}C\}$HSBC], and to the α protons of the next sequential amino acid by $^{1}H\{^{13}C\}$ heteronuclear multiple-bond correlation [$^{1}H\{^{13}C\}$HMBC] NMR experiments.

The $^{13}C_o/(^{13}C_o+^{13}C_\alpha)$ region of the 2D $^{13}C\{^{13}C\}$DQC experiment of [85% U1-^{13}C]leucine SSI (Figure 1a) contains simple antiphase doublets. The same region of the pure-phase 2D broadband proton-decoupled $^{13}C\{^{13}C\}$ correlated spectrum (not shown) shows severe overlap of active and passive couplings. Similar overlap is seen in the $^{13}C_\alpha/(^{13}C_o+^{13}C_\alpha)$ region (Figure 1b) of the $^{13}C\{^{13}C\}$DQC spectrum where the active coupling from the $^{13}C_o$ overlaps with passive coupling to the 85% ^{13}C enriched nuclei in the leucine side chain. This complication can be avoided by using a lower level of ^{13}C enrichment.[14,15] Peaks in the $C_\alpha{}^1H/^{13}C_\alpha$ region of the 2D $^{1}H\{^{13}C\}$HSBC spectrum (Figure 1c) arise from one-bond coupling between the $C_\alpha{}^1H$ and the $^{13}C_\alpha$ of ^{13}C enriched leucines in the protein. Table I contains the assignments for leucine $^{13}C_o$, $^{13}C_\alpha$, and $C_\alpha{}^1H$ resonances in [85% U1-^{13}C]leucine SSI.

Peaks in the $C_\alpha{}^1H/^{13}C_\alpha$ region of the 2D $^{1}H\{^{13}C\}$HMBC spectrum of [85% U1-^{13}C]leucine SSI (Figure 1d) arise from protons that are two or three bonds removed from a ^{13}C nucleus.

Table I. Chemical Shifts (ppm) for the Carbonyl Carbons, α Carbons, and α Protons of the Leucine Residues and the α Protons of Residues Following Leucine in the Sequence of *Streptomyces* Subtilisin Inhibitor

leucine position[a]	carbonyl carbon[b]	α carbon	α proton	(i + 1) residue[a]	(i + 1) proton
6	175.0	53.6	4.32	Tyr^{7}	5.39
12	173.8	51.9	5.53	Val^{13}	*c*
14	173.9	53.2	5.78	Thr^{15}	4.96
33	172.7	52.9	5.10	Thr^{34}	3.95
53	177.6	55.7	3.52	Ala^{54}	*c*
60	176.6	55.3	3.52	Asn^{61}	3.93
63	175.2	53.6	4.20	Thr^{64}	4.57
79	173.6	51.2	4.84	Leu^{80}	*d*
80	174.5	50.9	5.53	Thr^{81}	4.09

[a] From ref 9. [b] The carbonyl signals were assigned by a ^{13}C, ^{15}N labeling strategy;[11-13] however, a possible ambiguity remained in the assignments to Leu^{33} and Leu^{80}.[12] The present results remove this ambiguity. [c] Peak absent or too weak to be assigned. [d] Peak not expected because of the tandem repeat of [85% U1-^{13}C]Leu in the amino acid sequence (Leu^{79}-Leu^{80}) (see text).

The pulse sequence contains a ^{13}C pulse whose function is to suppress peaks arising from protons that have a one-bond coupling to a ^{13}C nucleus. With the 85% ^{13}C enrichment used here, the suppression pulse eliminates proton signals from carbon-bound protons in the [85% U1-^{13}C]leucine spin systems, and, therefore, there are no peaks corresponding to leucine $^{13}C_\alpha$–^{1}H in Figure 1d. The $C_\alpha{}^1H/^{13}C_o$ cross peaks in Figure 1d arise from three-bond coupling of the ^{13}C enriched leucine carbonyl carbon to the backbone α proton of the following residue (leucine $(^{13}C_o)_i$–$(N-C_\alpha{}^1H)_{i+1}$. Note that the tandem pair of $leucine^{79}$–$leucine^{80}$ does not show a cross peak in Figure 1d since the ^{13}C–^{1}H of 85% ^{13}C $leucine^{80}$ is suppressed. Small three-bond coupling constants, as a consequence of an unfavorable dihedral angle ϕ,[21] may explain why the cross peaks from residues 13 and 54 are very weak or absent in Figure 1d.

Our results show that scalar coupling between the $^{13}C_o$ of residue i and the $C_\alpha{}^1H$ of residue i + 1 is sufficient to make sequence-specific assignments in a protein labeled only with ^{13}C. In proteins labeled uniformly with ^{13}C (optimal level, 20–30%),[14,15] a possible sequence-specific assignment strategy would traverse the peptide bond via three-bond coupling from the $^{13}C_o$ of residue i to the $C_\alpha{}^1H$ of residue i + 1. Extension of the assignment within residue i + 1 from the $C_\alpha{}^1H$ to the $^{13}C_o$ proceeds by correlating the ^{1}H–$^{13}C_\alpha$ connectivity in a $^{1}H\{^{13}C\}$HSBC experiment and then correlating the $^{13}C_\alpha$–$^{13}C_o$ connectivity by $^{13}C\{^{13}C\}$DQC. The feasibility of this approach is demonstrated by Figure 1.[22] This method should provide sequential assignments of dipeptides having dihedral angles ϕ_i that provide coupling constants that are sufficiently large; when the coupling is small, alternative scalar coupling approaches[11-13] or the usual NOE-based approaches[23] must be employed. With the use of this or any other sequential assignment strategy, the identification of amino acid types can be obtained in uniformly ^{13}C-enriched proteins by analysis of the carbon spin systems[14,15] in the $^{13}C\{^{13}C\}$DQC spectrum. Assignments of side chain proton resonances could then be obtained by means of the $^{1}H\{^{13}C\}$HSBC experiment performed on the same ^{13}C-enriched sample.

The novel techniques described in this paper should assist in extending the molecular weight limit for sequential assignments

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beyond 20 000. They may be useful in cases where homonuclear ^1H-^1H methods fail because of overlap or spin diffusion problems. Selective ^{13}C or ^{15}N labeling coupled with isotope filtering can alleviate spectral overlap problems associated with homonuclear ^1H-^1H approaches;[24] however, these methods still rely on the nuclear Overhauser effect for sequential assignments, and it will be useful to have alternative methods such as this that are immune to spin diffusion effects.

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Detailed Analysis of Carbon-13 NMR Spin Systems in a Uniformly Carbon-13 Enriched Protein: Flavodoxin from *Anabaena 7120*

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The two-dimensional (2D) resonance-offset-compensated ^{13}C-$\{^{13}C\}$ double quantum correlation (DQC) NMR experiment discussed in the previous paper[1] has been used to obtain one-bond carbon-carbon correlations in oxidized flavodoxin, a protein of molecular weight ~21 000 (Figure 1). The flavodoxin was purified from a cyanobacterium, *Anabaena 7120*, that had been grown on [26% ^{13}C]CO_2 as the sole carbon source. Carbon spin systems of an alanine and tyrosine residue have been outlined in Figure 1, beginning at the carbonyl carbon and following through to the end of the side chain.[2] Although not obvious from the scale of Figure 1, many additional spin systems can be resolved in spectral expansions. The resolution and sensitivity are exemplified by one row of the spectrum (shown at the bottom of Figure 1) containing the $^{13}C_\zeta$-$^{13}C_\epsilon$ correlation of the outlined tyrosine residue. This type of information, when combined with proton-carbon correlations,[3-5] will be useful in the assignment of the proton spin systems of amino acid residues.

The ^{13}C-^{13}C correlation results have been used to assign several of the carbon resonances of the noncovalently bound flavin mononucleotide cofactor of the flavodoxin. Table I shows assignments resulting from observed correlations between $^{13}C_{7_a}$-$^{13}C_7$, $^{13}C_7$-$^{13}C_6$, $^{13}C_6$-$^{13}C_{5_a}$, and $^{13}C_{8_a}$-$^{13}C_8$ of the isoalloxazine ring as well as from

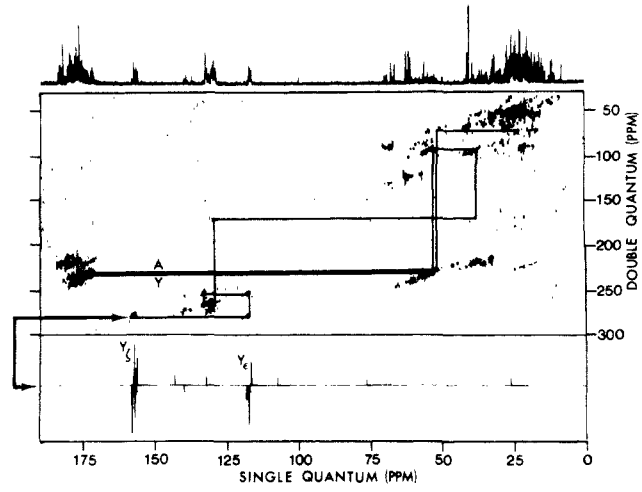

Figure 1. ^{13}C-^{13}C correlations in oxidized *Anabaena 7120* flavodoxin obtained by two-dimensional $^{13}C\{^{13}C\}$DQC NMR. The sample was 0.4 mL of 3.5 mM [26% Ul-^{13}C]flavodoxin in ^2H_2O containing 100 mM phosphate buffer at pH 7.5. A 5 mm (OD) NMR tube was used. Data were collected on a Bruker AM-500 spectrometer, with a ^{13}C frequency of 125.76 MHz. The total acquisition (time, 84 h) consisted of 256 blocks, each containing the average of 1792 free induction decays (8192 data points each). The 90° carbon pulse was 7 μs. WALTZ-16[13] decoupling (500 MHz proton) was used during acquisition to collapse ^{13}C-^1H splittings. The experiment was optimized for $^{13}C_\alpha$-$^{13}C_o$ couplings (~55 Hz) by setting the total delay in the double-quantum propagator to 9.26 ms. The raw data were multiplied by a Lorentzian-to-Gaussian function and zero-filled to 32 768 data points in ω_2 and multiplied by a cosine function and zero-filled to 1024 data points in ω_1 before Fourier transformation in each dimension. Noise was reduced in the spectrum by using the program "MAKEUP".[14] ^{13}C chemical shifts are referenced to tetramethylsilane. Single- and double-quantum chemical shifts are plotted along the horizontal and vertical axes, respectively. Each correlation is represented at the double-quantum frequency by two antiphase doublets, one at the single-quantum frequency of each component. The one-dimensional projection in the ω_2 dimension is plotted at the top. Connectivities are outlined beginning at the carbonyl carbons for an alanine (A) and tyrosine (Y) residue (see Table I for chemical shifts). The row of the spectrum with a double-quantum chemical shift of 272.3 ppm is shown at the bottom of the figure. The antiphase doublets in this row representing the $^{13}C_\zeta$-$^{13}C_\epsilon$ correlation of the outlined tyrosine residue are indicated.

correlations between the ribityl chain carbons. Flavin carbon resonances have been assigned previously in other flavoproteins by incorporating ^{13}C-labeled flavin into unlabeled apo-protein.[6-8] Because of the presence of several closely spaced resonances, it was necessary to incorporate two or more selectively enriched flavins into each protein, either independently or as mixtures with varying levels of enrichment. The present results show that such assignments can be made directly with a single uniformly labeled sample without the necessity of organic syntheses and cofactor reconstitution. Three of the ribityl carbon assignments have been extended to the ribityl protons via a proton-carbon correlation experiment[5] (see Table I).

The outlined tyrosine carbon spin system demonstrates the potential of this experiment for assigning aromatic spin systems. Ring carbon assignments can be extended via proton-carbon correlations to ring protons.[3,5] This through-bond technique circumvents ambiguities that may arise by using through-space (NOESY) connectivities between C_β-H's and aromatic ring side chain protons to assign aromatic ring protons.[9] Additional quaternary carbon resonances (such as tyrosine $^{13}C_\zeta$) also can be assigned directly in this manner (Table I).

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