ESR spectral features in various samples of our PPN·C_{60} in different time frames were quite similar to the reported spectrum. They suggested that there might be a facile electron exchange as follows:

$$C_{60}^- + C_{60} \rightleftarrows C_{60} + C_{60}^- \qquad (1)$$

$$C_{60}^- + Bu_4N^+ \rightleftarrows Bu_4N^+C_{60}^- \qquad (2)$$

$$Bu_4N^+C_{60}^- + C_{60} \rightleftarrows C_{60} + Bu_4N^+C_{60}^- \qquad (3)$$

They also assigned a broad and a sharp signal to ion-paired $C_{60}^{\bullet -}$ and the free $C_{60}^{\bullet -}$, respectively.

However, on closer inspection it was noted that this sharp signal was due not to a single species but to two species, B1 and B2. Line widths of these sharp signals were nearly constant in the measured temperature range (293–77 K). The sharp signal (B1) may be tentatively assigned to the free $C_{60}^{\bullet -}$ ($g = 2.000_7$) on the basis of Greaney's report.[12] The other signal (B2) ($g = 1.999_5$) is not so amenable to saturation by microwave power as B1 and gradually disappeared with time. This species seems to be an intermediate one. The broad signal (A) is thus assignable to $PPN^+C_{60}^{\bullet -}$.

Dubois et al.[13] observed a concomitant sharp signal superimposed on the $C_{60}^{\bullet -}$ signal corresponding to A in a frozen electrolyte solution of C_{60}. Furthermore, they proposed the occurrence of a disproportionation as follows:

$$2C_{60}^{\bullet -} \rightleftarrows C_{60} + C_{60}^{2-} \qquad (4)$$

In our case, we also cannot rule out the existence of triplet C_{60}^{2-} (Figure 1e, T), to which Dubois et al.[13] referred for the first time. Although we failed to observe a half-field resonance for C_{60}^{2-}, which would be conclusive evidence of the existence of a triplet state ($S = 1$), this fact is also consistent with the absence of the half-field signal in the experiment of Dubois et al.[13] After the broad signal (A) and the (B2) sharp signal disappeared completely, we invariably observed the signals due to C_{60}^{2-} (T). The C_{60}^{2-} species generated on photoexcitation[14] was also observed. As yet, we are not sure whether C_{60}^{2-} is present in the electrolyte solution from the beginning of crystal formation as a result of the disproportionation of $C_{60}^{\bullet -}$ or appears later in the solid state. Thus, taking the disproportionation into account, $C_{60}^{\bullet -}$ (PPN$^+$ adduct (A) and free (B1)) and C_{60}^{2-} generated due to the electron exchange in the neighborhood of the Pt electrode might randomly grow crystals of PPN·C_{60} on the electrode. Alternatively the electron exchange process (eqs 1 and 3) and the disproportionation (eq 4) might occur even in the solid state.

We also propose the occurrence of concurrent crystal lattice degradation, because the ESR spectral changes should not be interpreted merely by the decomposition of $PPN^+C_{60}^{\bullet -}$. In samples left at room temperature for several weeks, we no longer observed the broad signal for $PPN^+C_{60}^{\bullet -}$, but instead, only sharp signals assignable to free $C_{60}^{\bullet -}$ (B1). The sharp signal (B2) superimposed on the free $C_{60}^{\bullet -}$ signal also disappeared. The total spin concentration also decreased to ca. 1% of the original content within 2 months. Irreversible degradation (the reverse reaction of ion pairing, e.g., eq 2) of the lattice of $PPN^+C_{60}^{\bullet -}$, as follows,

$$\underset{\text{A}}{PPN^+C_{60}^{\bullet -}} \rightarrow \underset{\text{B2}}{[PPN^+\cdots C_{60}^{\bullet -}]} \rightarrow PPN^+ + \underset{\text{B1}}{C_{60}^{\bullet -}} \qquad (5)$$

may even occur in the solid state.

Preliminary results of X-ray diffraction studies on the single crystal revealed that the structure demonstrated a zigzag arrangement composed of a discrete 1:1 PPN$^+$ salt of $C_{60}^{\bullet -}$ radical anion.[15] Surprisingly, the monoclinic lattice of PPN$^+$ and C_{60} appears to persist for a long time in spite of the gradual decrease of the X-ray peak intensities. Presumably this corresponds to the above mentioned degradation (eq 5).

However, for a comprehensive understanding of the intrinsic electronic "metastable" state of the crystal, it would be necessary to investigate the ESR spectra in more detail. This analysis is in progress right now.[16]

Acknowledgment. We thank Dr. M. Takayama (Toho University) for EIMS and Mr. H. Nakagawa (Newly Instruments Ltd.) for FTMS of pure C_{60} and PPN·C_{60}. We are indebted to Mr. H. Sakamura (Institute of Industrial Science, The University of Tokyo) for his assistance in obtaining a SEM micrograph of the crystal.

---

(13) Dubois, D.; Jones, M. T.; Kadish, K. M. *J. Am. Chem. Soc.* **1992**, *114*, 6446-6451.

(14) Wasielewski, M. R.; O'Neil, M. P.; Lykke, K. R.; Pellin, M. J.; Gruen, D. M. *J. Am. Chem. Soc.* **1991**, *113*, 2774-2776.

(15) Crystal data for PPN·C_{60}: formula $C_{96}H_{30}NP_2$ (FW = 1259.15), crystal dimensions 0.45 × 0.3 × 0.3 (mm); crystal system, monoclinic, space group *Cc* (or *C*2/*c*), lattice constants, $a = 12.429(4)$ Å, $b = 27.433(6)$ Å, $c = 17.850(4)$ Å, $\beta = 90.45(2)°$, $V = 6086(3)$ Å^3, $Z = 4$. The preliminary *R* factor was 0.20 for 1880 observed unique reflections (at room temperature). Lattice constants designate the packing structure of this molecule with four molecules of PPN·C_{60} in one unit cell. The diameter of C_{60} (7.0 Å) and the separation of neighboring C_{60} molecules (10.5 Å, center-to-center) are well consistent with those of the crystal composed of a free C_{60} molecule (7.065 Å)[8a] and a center-to-center distance for adjacent C_{60} molecules (9.96 Å).[8b] C_{60} moieties seem to be tightly packed in the crystal structure. A detailed X-ray crystal structure analysis at low temperatures will be published elsewhere.

(16) Watanabe, T.; et al. To be submitted. We have found recently that these changes of ESR spectra were significantly facilitated by a gradual increase of the sample temperature up to 150 °C.

Four Carbon Component Coupling Reaction Based on Free-Radical Carbonylation: An Easy Access to β-Functionalized δ,ε-Unsaturated Ketones

Ilhyong Ryu,* Hiroshi Yamazaki, Akiya Ogawa,
Nobuaki Kambe, and Noboru Sonoda*

Department of Applied Chemistry, Faculty of Engineering
Osaka University, Suita, Osaka 565, Japan

Received October 19, 1992

Free-radical carbonylation is now experiencing a renaissance.[1] Despite the large body of knowledge accumulated in the past three decades concerning transition-metal-catalyzed carbonylations,[2] the carbonylation of *alkyl* moieties remains a challenge.[3] Obviously, one of the most noteworthy aspects of free-radical carbonylation is that this conceptually different approach provides a simple solution to this long-standing problem. In this paper, we are pleased to report the accomplishment of a four-carbon connection process, where carbon monoxide is incorporated as one of the components. This new type of free-radical transformation couples the following four-carbon units in the sequence: R-X **1**, carbon monoxide, functionalized alkene **2**, and allylstannane **3** (Scheme I). In terms of the versatility in substrates and the importance of the products, the procedure leading to β-functionalized δ,ε-unsaturated ketones **4** has considerable synthetic potential.

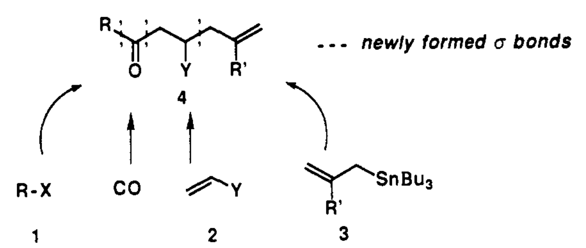
---

(1) (a) Ryu, I.; Kusano, K.; Ogawa, A.; Kambe, N.; Sonoda, N. *J. Am. Chem. Soc.* **1990**, *112*, 1295. (b) Ryu, I.; Kusano, K.; Masumi, N.; Yamazaki, H.; Ogawa, A.; Sonoda, N. *Tetrahedron Lett.* **1990**, *31*, 6887. (c) Ryu, I.; Kusano, K.; Hasegawa, M.; Kambe, N.; Sonoda, N. *J. Chem. Soc., Chem. Commun.* **1991**, 1018. (d) Ryu, I.; Kusano, K.; Yamazaki, H.; Sonoda, N. *J. Org. Chem.* **1991**, *56*, 5003. (e) Ryu, I.; Yamazaki, H.; Kusano, K.; Ogawa, A.; Sonoda, N. *J. Am. Chem. Soc.* **1991**, *113*, 8558. Also, see: (f) Nakatani, S.; Yoshida, J.-I.; Isoe, S. *J. Chem. Soc., Chem. Commun.* **1992**, 880.

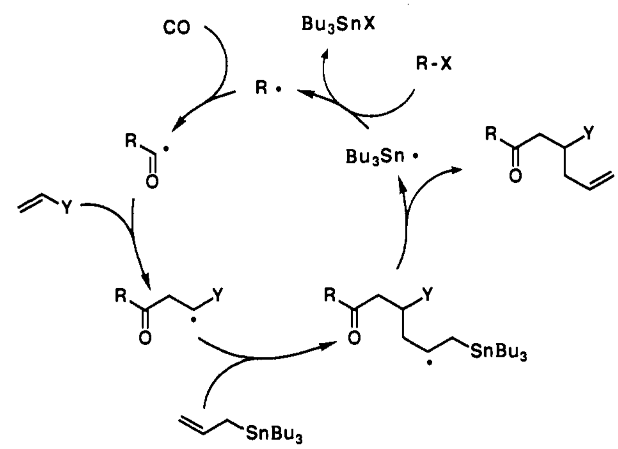
(2) For a recent review, see: Colquhoun, H. M.; Thompson, D. J.; Twigg, M. V. *Carbonylation: Direct Synthesis of Carbonyl Compounds*; Plenum Press: New York, 1991.

(3) For recent efforts by transition-metal methods, see: (a) Takeuchi, R.; Tsuji, Y.; Fujita, M.; Kondo, T.; Watanabe, Y. *J. Org. Chem.* **1989**, *54*, 1831. (b) Ishiyama, T.; Miyaura, N.; Suzuki, A. *Tetrahedron Lett.* **1991**, *32*, 6923. (c) Urata, H.; Maekawa, H.; Takahashi, S.; Fuchikami, T. *J. Org. Chem.* **1991**, *56*, 4320. (d) Boese, W. T.; Goldman, A. S. *J. Am. Chem. Soc.* **1992**, *114*, 350.

Scheme I



Scheme II

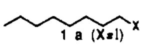
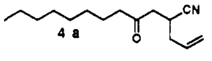
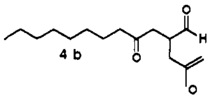
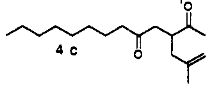
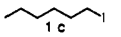
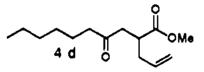
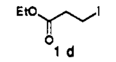
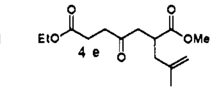
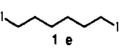
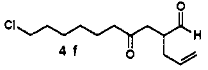
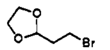
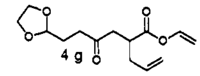
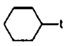
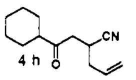
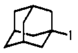
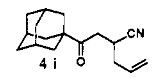
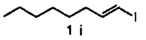
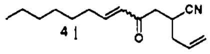
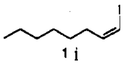
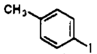
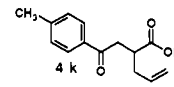


The four-component coupling procedure simply requires heating a mixture of **1**, **2**, **3**, and a catalytic amount of AIBN in benzene under CO pressure in an autoclave. For example, the AIBN-initiated reaction of octyl iodide (**1a**) with carbon monoxide in the presence of acrylonitrile (**2a**) (1.2 equiv) and allyltributyltin (**3a**) (2.0 equiv) (benzene, [**1a**] = 0,025 M, 80 °C, 8 h, 10 atm) gave 4-cyano-1-tetradecen-6-one (**4a**) in 72% yield after purification by flash column chromatography.[4] The formation of **4a** can be explained by the free-radical chain propagation mechanism outlined in Scheme II: (1) the stannyl free radical abstracts an iodine atom from octyl iodide to give the octyl free radical, (2) trapping of the octyl free radical by carbon monoxide to form the acyl free radical, (3) the addition of the acyl free radical to alkene **2a** to give an alkyl free radical, (4) S_H2' type reaction of the resulting radical with allylstannane to give desired ketone **4a** and, coincidentally, regenerate tributyltin radical (Scheme II).

Table I contains some representative results of the four carbon component coupling. This synthetic method is flexible in terms of the versatility of R–X (R = primary, secondary, and tertiary alkyls, aryl, and vinyl) and activated alkenes ($CH_2{=}CHY$: Y = CN (**2a**), (CO)H (**2b**), COMe (**2c**), CO_2Me (**2d**), CO_2CHCH_2 (**2e**), $CO_2CH_2CH_3$ (**2f**)). Thus, besides 3-cyano ketones (**4a**, **4h**, **4i**, **4j**), 1,4-diketones (**4c**), 4-keto esters (**4d**, **4e**, **4g**, **4k**), and 4-keto aldehydes (**4b**, **4f**) tethered by allylic moieties β to the carbonyl, were synthesized conveniently in a single-operation. Yields are generally good. These products are not easily accessible by other methods, and each functionality would be eligible for further elaboration. Also, the alkyl chain can easily tolerate functionality (runs 6–8).

Initial screening of the reaction conditions indicated the importance of the stoichiometric use (ca. 1.2 equiv) of alkenes **2** to minimize the undesired direct addition of alkyl free radical to **2**, which resulted in the formation of **5**.[5] This is in sharp contrast to our earlier study on tin hydride-mediated three-component coupling of R–X, CO, and activated alkenes,[1d] wherein a large excess (3–4 equiv) of an activated alkene is necessary to compete with premature quenching of acyl radical by tin hydride.[1d] On the other hand, the three-component coupling[1e] consisting of R–X, CO, and allylstannane to give β,γ-enone **6** is negligible under the dilute conditions employed.[6] The preference for a four-component coupling rather than this three-component process is a result of kinetics: (i) the addition of acyl free radicals to **2** takes place faster than to less reactive allylstannane **3**,[7] and (ii) S_H2' reaction of allylstannane is more rapid with the resulting radicals tethered to an electron-withdrawing substituent, which should have a lower SOMO energy[8] than the acyl radicals.

Table I. Synthesis of β-Functionalized δ,ε-Unsaturated Ketones from R–X, CO, an Alkene, and an Allylstannane[a]

run	R-X	alkenes, conditions	product	yield[b]
1	 1 a (X=I)	2a, 3a [1] = 0.025 M CO 10 atm	 4 a	72 %
2	1 b (X=Br)	CO 20 atm	4 a	66 %[c]
3	1 a	2b, 3b [1] = 0.025 M CO 20 atm	 4 b	74 %
4	1 a	2c, 3b [1] = 0.025 M CO 20 atm	 4 c	77 %
5	 1 c	2d, 3a [1] = 0.025 M CO 20 atm	 4 d	72 %
6	 1 d	2d, 3b [1] = 0.025 M CO 20 atm	 4 e	67 %
7	 1 e	2b, 3a [1] = 0.025 M CO 20 atm	 4 f	70 %
8	 1 1	2e, 3a [1] = 0.033 M CO 50 atm	 4 g	60 %
9	 1 g	2a, 3a [1] = 0.050 M CO 50 atm	 4 h	67 %
10	 1 h	2a, 3a [1] = 0.050 M CO 80 atm	 4 i	61 %
11	 1 i	2a, 3a[d] [1] = 0.100 M CO 30 atm	 4 j	66 %[e]
12	 1 j	2a, 3a[d] [1] = 0.100 M CO 50 atm	4 j	70 %[f]
13	 1 k	21, 3a [1] = 0.100 M CO 50 atm	 4 k	65 %

[a] Conditions: **1** (0.5–1.0 mmol), **2** (1.2 equiv), **3** (**a**: R′ = H, **b**: R′ = Me, 1.5–2.0 equiv), AIBN (0.2–0.4 equiv), C_6H_6 (10–40 mL), CO (10–80 atm), 80 °C, 8 h. For details, see footnote 4 and the supplementary material. [b] Isolated yields by column chromatography on silica gel. [c] **1b** was recovered in 10% yield. [d] Three equivalents. [e] E/Z = 89/11. [f] E/Z = 90/10.

---

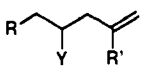
[4] The following procedure is typical for the synthesis of 4-cyano-1-tetradecen-6-one (**4a**): Benzene (20 mL), 1-iodooctane (**1a**; 120 mg, 0.50 mmol, 0.025 M), allyltributylstannane (**3a**; 330 mg, 1.00 mmol), acrylonitrile (**2a**; 31 mg, 0.59 mmol), and AIBN (33 mg, 0.20 mmol) were placed in a 50-mL stainless steel autoclave lined with a round-bottomed glass tube. The autoclave was then pressurized with 10 atm of CO and was heated, with stirring, at 80 °C for 8 h. After excess CO was discharged at room temperature, the benzene was evaporated. The residue was purified by flash chromatography on silica gel (hexane, then 10% Et_2O–hexane eluant). The major fraction (R_f = 0.2, 10% Et_2O–hexane eluant) eluted from the column contained 84 mg (72%) of 4-cyano-1-tetradecen-6-one (**4a**).

[5] (a) Mizuno, K.; Ikeda, M.; Toda, S.; Otsuji, Y. *J. Am. Chem. Soc.* **1988**, *110*, 1288. (b) Curran, D. P.; Shen, W.; Zhang, J.; Heffner, T. A. *J. Am. Chem. Soc.* **1990**, *112*, 6738. (c) Stack, J. G.; Curran, D. P.; Geib, S. V.; Rebek, J., Jr.; Ballester, P. *J. Am. Chem. Soc.* **1992**, *114*, 7007. (d) Porter, N. A.; Rosenstein, I. J.; Breyer, R. A.; Bruhnke, J. D.; Wu, W.-X.; McPhail, A. T. *J. Am. Chem. Soc.* **1992**, *114*, 7664.

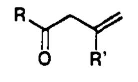
[6] Usually, successful three-component reaction leading to **6** requires a higher concentration ([R–X] = 0.1 M). See ref 1e.

[7] Curran, D. P.; van Elburg, P. A.; Giese, B.; Gilges, S. *Tetrahedron Lett.* **1990**, *31*, 2861.

[8] Giese, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 753.



5



6

In summary, this paper describes a unique general method for the synthesis of β-cyano-, formyl-, acyl-, and alkoxycarbonyl-substituted δ,ϵ-unsaturated ketones, which demonstrates the power of free-radical carbonylation. To the best of our knowledge, this is the first example of a successful intermolecular four carbon component coupling reaction by a free-radical process except for polymerization. Application to carbonylative cyclizations will be the subject of a forthcoming paper.

Supplementary Material Available: Detailed experimental procedures and physical characteristics of **4a–k** (5 pages). Ordering information is given on any current masthead page.

NMR[1] Detection of Hydration Water in the Intermolecular Interface of a Protein–DNA Complex

Yan Qiu Qian, Gottfried Otting, and Kurt Wüthrich*

Institut für Molekularbiologie und Biophysik
Eidgenössische Technische Hochschule-Hönggerberg
CH-8093 Zürich, Switzerland

Received October 26, 1992

Hydration water molecules associated with protein–DNA complexes have been observed by X-ray diffraction in single crystals diffracting to a higher resolution than 2.5 Å.[2,3] On the basis of these observations, important roles in sequence-specific DNA recognition have been attributed to water molecules located in the protein–DNA interface which are hydrogen-bonded to polar groups of both the protein and the DNA bases.[2] Hydration water molecules in aqueous solution have been detected by NMR for both proteins[4] and DNA,[5] using the NOEs between water protons and protons of the solute.[6] The present communication reports the NOE detection of hydration water molecules bound with residence times longer than 1 ns in the protein–DNA interface of a 1:1 complex formed between the *Antp(C39S)* homeodomain and the 14-base-pair DNA duplex d(GAAAGCCATTAGAG)·(CTCTAATGGCTTTC).

Individual cross peaks among the numerous overlapping signals in the ^{1}H NMR spectrum of the homeodomain–DNA complex were resolved using 3D ^{15}N-correlated [^{1}H,^{1}H]-NOESY with a complex containing the uniformly ^{15}N-enriched *Antp(C39S)* homeodomain bound to the unlabeled 14-base-pair DNA duplex, and 3D ^{13}C-correlated [^{1}H,^{1}H]-NOESY with a similar complex containing the uniformly ^{13}C-labeled homeodomain. The data were recorded using the experimental scheme of Messerle et al.[7] In these 3D NMR spectra the cross peaks are separated in the ω_2-dimension by the different ^{13}C or ^{15}N chemical shifts, respectively, of the individual ^{13}C–^{1}H and ^{15}N–^{1}H fragments involved in the ^{1}H–^{1}H NOEs with the water.[8] Figure 1 shows the two-dimensional cross section through the 3D ^{13}C-correlated [^{1}H,^{1}H]-NOESY spectrum taken at the ω_1(^{1}H) chemical shift of the water resonance. For an analysis of the NOEs with hydration water, one has to account for the fact that most of the cross peaks in Figure 1 correspond to intramolecular NOEs with solvent-exchangeable protons of NH and OH groups, which are relayed to the water line by chemical exchange.[6,9] This situation prevails quite generally for all nonlabile hydrogen atoms that are spatially close to protons in rapid chemical exchange with the solvent, e.g., the lysyl ϵCH$_2$, arginyl δCH$_2$, and threonyl γCH$_3$ groups (Figure 1). Correspondingly, in the 3D ^{15}N-correlated [^{1}H,^{1}H]-NOESY spectrum, intense exchange peaks with the water resonance were observed, for example, for the ϵNH and ηNH$_2$ groups of the arginyl side chains. To unambiguously identify intermolecular NOEs between protons of the complex and protons of hydration water molecules against this background of exchange peaks, NOE identification was accepted only for nonlabile hydrogen atoms and for potentially labile protons[10] that had been shown to exchange slowly in the *Antp(C39S)* homeodomain–DNA complex. Furthermore, these hydrogen atoms had to be more than 4.0 Å away from the nearest rapidly exchanging proton in any of the 16 conformers representing the refined solution structure of the complex (Billeter, M.; Qian, Y. Q.; Otting, G.; Müller, M.; Gehring, W. J.; Wüthrich, K., to be submitted). In Figure 1, the cross peaks involving βCH and γCH$_3$ of Ile 47 were thus unambiguously identified as NOEs with hydration water molecules, while, for example, cross peaks with Ile 47 δCH$_3$ and Met 54 ϵCH$_3$ were dismissed because these methyl groups are near the labile side chain protons of Lys 46 and Arg 53, respectively, in about half of the 16 conformers. In the 3D ^{15}N-correlated [^{1}H,^{1}H]-NOESY experiment, a NOE with hydration water was similarly identified for the amide proton of Trp 48.[11] Clearly, with these

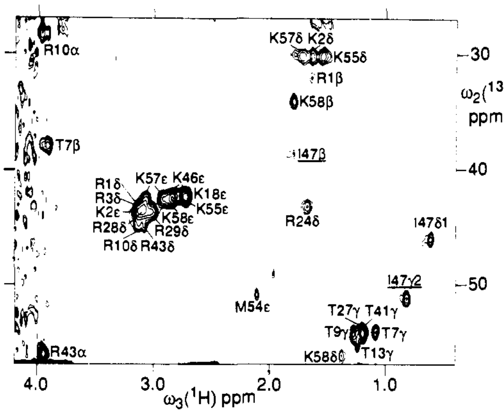


Figure 1. ω_2(^{13}C)–ω_3(^{1}H) cross plane taken at the ω_1(^{1}H) frequency of the water signal through a 3D ^{13}C-correlated [^{1}H,^{1}H]-NOESY spectrum of the 1:1 complex of the uniformly ^{13}C-enriched *Antp(C39S)* homeodomain with the DNA duplex d(GAAAGCCATTAGAG)·(CTCTAATGGCTTTC). (Concentration of complex = 2.3 mM, solvent 90% H$_2$O/10% ^{2}H$_2$O, pH = 6.0, T = 36 °C, ^{1}H frequency = 600 MHz, mixing time = 60 ms, $t_{1\max}$ = 52.8 ms, $t_{2\max}$ = 13.3 ms, $t_{3\max}$ = 67.6 ms, time domain data size 400 × 64 × 1024 points, total recording time about 130 h, States-TPPI with delayed acquisition[15] in the t_2(^{13}C) dimension.) The peak identification is by one-letter amino acid symbols, sequence positions, Greek letters, and where applicable, numerals for the type of hydrogen atoms of the *Antp(C39S)* homeodomain that are involved in the cross peaks with the water signal. Underlined symbols identify intermolecular NOE cross peaks with water molecules (see text). Because of spectral folding along ω_2(^{13}C), the chemical shift positions 30, 40, and 50 ppm coincide with, respectively, chemical shifts of –1.9 and 61.9 ppm, 8.1 and 71.9 ppm, and 18.1 and 81.9 ppm.

---

[1] Abbreviations used: NMR, nuclear magnetic resonance; 2D, two-dimensional; 3D, three-dimensional; NOE, nuclear Overhauser enhancement; NOESY, two-dimensional nuclear Overhauser enhancement spectroscopy; TPPI, time-proportional phase incrementation; *Antp(C39S)* homeodomain, 68-residue fragment from the *Antennapedia* protein containing Met in position 0, the homeodomain in positions 1–60, with Cys 39 replaced by Ser, and seven residues following the homeodomain in the *Antp* protein in positions 61–67.

[2] Otwinowski, Z.; Schevitz, R. W.; Zhang, R.-G.; Lawson, C. L.; Joachimiak, A.; Marmorstein, R. Q.; Luisi, B. F.; Sigler, P. B. *Nature* **1988**, *335*, 321–329.

[3] Aggarwal, A. K.; Rodgers, D. W.; Drottar, M.; Ptashne, M.; Harrison, S. C. *Science* **1988**, *242*, 899–907.

[4] Otting, G.; Liepinsh, E.; Wüthrich, K. *Science* **1991**, *254*, 974–980.

[5] (a) Kubinec, M. G.; Wemmer, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 8739–8740. (b) Liepinsh, E.; Otting, G.; Wüthrich, K. *Nucleic Acids Res.*, in press.

[6] Otting, G.; Wüthrich, K. *J. Am. Chem. Soc.* **1989**, *111*, 1871–1875.

[7] Messerle, B. A.; Wider, G.; Otting, G.; Weber, C.; Wüthrich, K. *J. Magn. Reson.* **1989**, *85*, 608–613.

[8] Wüthrich, K.; Otting, G. *Int. J. Quantum Chem.* **1992**, *42*, 1553–1561.

[9] Liepinsh, E.; Otting, G.; Wüthrich, K. *J. Biomol. NMR* **1992**, *2*, 447–465.

[10] Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.

[11] Otting, G.; Qian, Y. Q.; Billeter, M.; Müller, M.; Affolter, M.; Gehring, W. J.; Wüthrich, K. *EMBO J.* **1990**, *9*, 3085–3092.

0002-7863/93/1515-1189$04.00/0