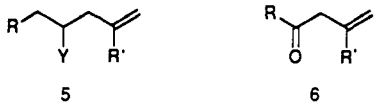


5 6

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 $\omega_2$-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 $\omega_1$($^1$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 $\epsilon CH_2$, arginyl $\delta CH_2$, and threonyl $\gamma CH_3$ 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 $\epsilon$NH and $\eta NH_2$ 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 $\beta$CH and $\gamma CH_3$ of Ile 47 were thus unambiguously identified as NOEs with hydration water molecules, while, for example, cross peaks with Ile 47 $\delta CH_3$ and Met 54 $\epsilon CH_3$ 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

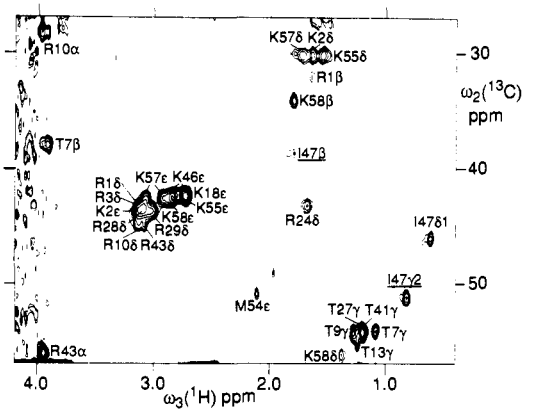



Figure 1. $\omega_2$($^{13}$C)–$\omega_3$($^1$H) cross plane taken at the $\omega_1$($^1$H) frequency of the water signal through a 3D $^{13}$C-correlated [$^1$H,$^1$H]-NOESY spectrum of the 1:1 complex of the uniformly $^{13}$C-enriched *Antp(C39S)* homeodomain with the DNA duplex d(GAAAGCCATTAGAG)·(CTCTAATGGCTTTC). (Concentration of complex = 2.3 mM, solvent 90% $H_2O$/10% $^2H_2O$, pH = 6.0, $T$ = 36 °C, $^1$H frequency = 600 MHz, mixing time = 60 ms, $t_{1max}$ = 52.8 ms, $t_{2max}$ = 13.3 ms, $t_{3max}$ = 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 $\omega_2$($^{13}$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 

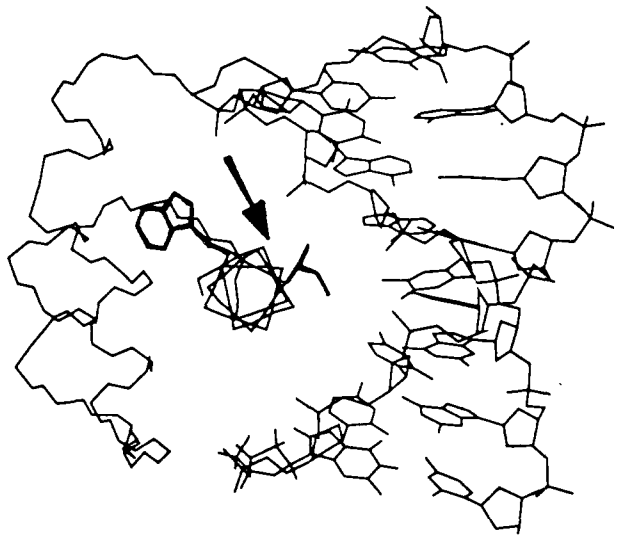


Figure 2. View along the axis of the recognition helix of one of the 16 NMR conformers representing the solution structure of the *Antp(C39S)* homeodomain-DNA complex (Billeter, M.; Qian, Y. Q.; Otting, G.; Müller, M.; Gehring, W. J.; Wüthrich, K., to be submitted). The presentation includes all heavy atoms of base pairs 5-12 of the 14-base-pair DNA, the polypeptide backbone of residues 8-56 of the *Antp(C39S)* homeodomain, and with bold lines, the side chains of Ile 47 and Trp 48 of the homeodomain. The arrow indicates the location of the hydration water molecules detected by NMR.

stringent requirements, it is likely that additional cross peaks representing intermolecular NOEs with hydration water were not identified as such. On the other hand, since several intermolecular NOEs were observed between the side chain of Ile 47 and the DNA (Billeter, M.; Qian, Y. Q.; Otting, G.; Müller, M.; Gehring, W. J.; Wüthrich, K., to be submitted), and since the amide proton of Trp 48 is next to Ile 47, we have well established that the few NOEs attributed to hydration waters are with hydrogen atoms in the interface between the protein and the DNA (Figure 2).

Only very few of the water molecules hydrating the homeodomain-DNA complex could be observed by NOEs. Apparently, most of the other solvent-solute NOEs are very weak, indicating that the residence times of water molecules in the surface hydration sites are shorter than about 0.5 ns, as was found in other peptides and proteins.[4] The negative sign and the higher intensities of the NOEs with the water molecules in the protein-DNA interface show that the lifetimes of these hydration water molecules are longer than 1 ns, and since these water molecules have the same chemical shift as the bulk water, we can also establish an upper limit for the lifetimes of about 20 ms.[12] This coincides with the behavior of hydration water in interior cavities of globular proteins, where the water molecules constitute integral parts of the molecular architecture.[4,6,12,13] The present studies thus indicate an important structural role of the hydration water in the protein-DNA interface. Since the lifetimes of these waters are much shorter than the lifetime of the complex,[14] they also show that the structure of the complex undergoes time fluctuations with similar frequencies and amplitudes as observed for globular proteins.[12]

**Acknowledgment.** We would like to thank Dr. W. Gehring and Dr. M. Müller, Biocenter of the University of Basel, for providing the homeodomain polypeptides used in this study, and Dr. M. Billeter for helpful discussions. Financial support was obtained from the Schweizerischer Nationalfonds (Project 31.32033.91).

(12) Otting, G.; Liepinsh, E.; Wüthrich, K. *J. Am. Chem. Soc.* **1991**, *113*, 4363-4364.
(13) Clore, G. M.; Bax, A.; Wingfield, P. T.; Gronenborn, A. M. *Biochemistry* **1990**, *29*, 5671-5676.
(14) Affolter, M.; Percival-Smith, A.; Müller, M.; Leupin, W.; Gehring, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4093-4097.
(15) (a) Marion, D.; Ikura, M.; Tschudin, R.; Bax, A. *J. Magn. Reson.* **1989**, *85*, 393-399. (b) Bax, A.; Ikura, M.; Kay, L. E.; Zhu, G. *J. Magn. Reson.* **1991**, *91*, 174-178.

## Reaction of Alkoxysilane Coupling Agents with Dehydroxylated Silica Surfaces

Lawrence H. Dubois* and Bernard R. Zegarski

*AT&T Bell Laboratories*
*Murray Hill, New Jersey 07974*

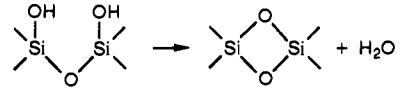
*Received October 22, 1992*

Alkoxysilane coupling agents are commonly used to bond polymers to glass surfaces by the following reaction:[1]

$$R'Si(OR)_3 + HOSi\equiv \rightarrow R'Si(OR)_2OSi\equiv + ROH \quad (1)$$

R is typically a methyl or ethyl group, and R′ contains a functionality that can react readily with the polymer (e.g., mercaptan or epoxide). HOSi≡ can be either an isolated surface silanol or a hydrogen-bonded hydroxyl group. The rate of the coupling reaction can be further increased in the presence of water by first hydrolyzing the silane.[1] Until now, it was thought that reactive hydroxyl groups *must* be present on the surface in order for bonding to take place. Here we demonstrate that alkoxysilane coupling agents can react directly with highly strained siloxane bonds present on *dehydroxylated* silica, thus yielding a new mechanism for polymer-silica surface adhesion.

Strained siloxane bonds can be formed readily on high surface area silica samples by heating to temperatures in excess of 900 K.[2-4] The most reactive of these sites are edge-shared tetrahedra formed by the dehydroxylation of adjacent, isolated silanol groups.[2,3,5] The strain in these four-membered-ring structures




(2)

(estimated to be 23 kcal/mol of Si-O bonds[6]) is due to the large distortion of both the O-Si-O and Si-O-Si bond angles[4,7] and results in infrared active Si-O stretching modes at 888 and 908 $cm^{-1}$.[2-4]

The adsorption of methyltrimethoxysilane (MTMSi) on a dehydroxylated silica surface at 330 K is followed using infrared spectroscopy (Figure 1a).[8] From this data it is clear that the intensity of the $CH_3$ stretching vibrations from both the methyl (asym: 2978; sym: ~2920 $cm^{-1}$) and methoxy (asym: 2957; sym: 2851 $cm^{-1}$) groups increases as a function of time.[10] A corresponding decrease in the intensity of the bands due to the Si-O stretching modes of the strained siloxane rings is also observed.[10] These data, normalized to a constant peak height, are summarized in Figure 1b.

The intensity of the isolated (i.e., non-hydrogen-bonded) O-H stretching vibration (3750 $cm^{-1}$) remains constant at 0.104 ± 0.001

(1) Plueddemann, E. P. *Silane Coupling Agents*, 2nd ed.; Plenum: New York, 1991.
(2) Morrow, B. A.; Devi, A. *Trans. Faraday Soc.* **1972**, *68*, 403.
(3) Morrow, B. A.; Cody, I. A. *J. Phys. Chem.* **1976**, *80*, 1995, 1998.
(4) Bunker, B. C.; Haaland, D. M.; Ward, K. J.; Michalske, T. A.; Smith, W. L.; Binkley, J. S.; Melius, C. F.; Balfe, C. A. *Surface Sci.* **1989**, *210*, 406.
(5) Bunker, B. C.; Haaland, D. M.; Michalske, T. A.; Smith, W. L. *Surface Sci.* **1989**, *222*, 95.
(6) O'Keeffe, M.; Gibbs, G. V. *J. Chem. Phys.* **1984**, *81*, 876.
(7) Molecular analogs of these species are known and their structures well-characterized. See, for example: Fink, M. J.; Haller, K. J.; West, R.; Michl, J. *J. Am. Chem. Soc.* **1984**, *106*, 822.
(8) Silica substrates were prepared using the technique described in refs 9 and 10. Approximately 12 mg of Cab-O-Sil M5 was pressed into a fine tantalum screen to make a disk ~1.2 cm in diameter and ~0.3 mm thick. Samples were then mounted in a small, turbomolecular pumped vacuum cell (base pressure of $<1 \times 10^{-8}$ Torr) and dehydroxylated for 3-6 h at ≳1250 K by resistively heating the tantalum mesh. Spectra were collected in transmission mode using a Fourier transform infrared spectrometer (Mattson Instruments) and a liquid nitrogen cooled, wide band MCT detector. Typically 256 scans were averaged at 4 $cm^{-1}$ resolution (~1 min acquisition time).
(9) Ballinger, T. H.; Wong, J. C. S.; Yates, J. T., Jr. *Langmuir* **1992**, *8*, 1676.
(10) Dubois, L. H.; Zegarski, B. R. *J. Phys. Chem.*, in press.

0002-7863/93/1515-1190$04.00/0 