quence. Conversion of the primary alcohol to its *tert*-butyldimethylsilyl ether **4** (90%)[11] completed the introduction of the protected C9 appendage.

Attachment of the remaining ring carbons of the NCS nucleus to C1 was then achieved in two ways, both based on palladium-mediated coupling. Addition of pent-2-en-4-ynol[12] (1 equiv) to an excess of bromide **4** (4 equiv) directly produced diyne **7** (97%). Unreacted **4** was readily recovered. Reactions conducted without an excess of **4** resulted in alkyne dimerization. As an alternative, bromide **4** was coupled first to (trimethylsilyl)acetylene to produce **5** (88%) which was then deprotected (**6**, 86%) and coupled to *cis*-3-iodo-2-propen-1-ol[13] to afford **7** (65%).

Modification of **7** as a prelude to B-ring formation entailed initially the conversion of its allylic alcohol functionality to an allylic bromide through mesylation and lithium bromide displacement. Without isolation, the resultant bromide was then desilylated (HOAc, THF, H_2O) to afford compound **8** in a combined yield of 75% for the three-step process. Oxidation of **8** with manganese dioxide provided aldehyde **9** (76%), possessing terminal functionalities required for an internal metal-mediated ring closure.

When aldehyde **9** was added to a suspension of chromium(II) chloride in THF, intramolecular closure producing the desired nine-membered ring occurred smoothly and efficiently (77–88%).[14] Three diastereomers were generated: a 1:1 ratio of two inseparable compounds epimeric at C9 and possessing a cis-substitution pattern about the newly formed bond (**10a** and **10b**, 67–76%) and a compound with a trans-substitution pattern about the C4–C5 bond (**10c**, 10–12%). **10** was also produced through the Wittig rearrangement of **13**,[15] although this process resulted in lower yields and products that were difficult to purify.

The efficacy of the above chromium-mediated closure for strained-medium-ring synthesis[9] is remarkable and unique among the reactions that we screened. Presumably, chromium first inserts into the carbon–bromine bond; coordinative activation of the aldehyde would then generate a 13-membered macrometallocyclic chelate.[16] Effective ring contraction would proceed through a six-centered transition state, producing the nine-membered carbocycle.

In order to establish the nucleofugal potential of the C5 substituent as required for diyl formation and to protect the secondary alcohol from the ensuing dehydration conditions, **10a** and **10b** were acetylated to give **11a** and **11b** (52%). This mixture was then treated with 4-(dimethylamino)pyridine, methanesulfonyl chloride, and triethylamine to afford the functionalized NCS Chr I carbocycle (**12**: 25%).[17] As expected, this functionalized analogue was even more labile than the parent hydrocarbon.[7] The half-life of **12** at room temperature was ~8 h as compared to ~48 h for the desacetoxy compound.[7] The instability of these compounds in the absence of activating reagents is noteworthy, suggesting that unimolecular activation mechanisms may be available for dilyl generation in these systems and others such as NCS Chr II. This possibility and the chemistry and cleavage reactions of **12** and its analogues are currently under investigation.

In summary, this study has resulted in the first synthesis of a *functionalized* bicyclo[7.3.0]dodeca-1,8-diene-2,6-diyne system, suitably equipped for conversion to an NCS diyl analogue. This strategy, by virtue of its convergent nature and its potential to accommodate DNA recognition elements at C10 and C11 and various leaving groups at C5, offers much flexibility for NCS analogue design, as needed to explore and exploit systematically the chemistry of the biologically active bicyclic subunit of NCS. In addition to its effective service for NCS analogue synthesis, the mild and efficient internal chromium-mediated closure represents a potentially general solution to the problem of strained-medium-ring synthesis presented by other DNA cleaving agents. These studies are in progress.

Acknowledgment. This research was supported by a grant (CA31845) from the National Institutes of Health. Fellowship support for J.A.McK. and C.M. from the National Institutes of Health and the Ministry of Education, Science, and Culture of Japan, respectively, is gratefully acknowledged.

Supplementary Material Available: IR, ^1H NMR, ^{13}C NMR, and mass spectroscopic data for compounds **3**, **6–9**, and **11a,b**, ^1H NMR data for compounds **10a,b**, and ^1H and partial ^{13}C NMR data for compound **12** (5 pages). Ordering information is given on any current masthead page.

(11) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(12) (a) This compound was obtained through the desilylation of 5-(trimethylsilyl)pent-2-en-4-ynol (*n*-BuN_4F, 74%). For a preparation of the silyl compound, see ref 13. (b) For further information on palladium cross-coupling reactions of this type, see ref 7 and the following references: Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4467. Stephans, R. D.; Castro, C. E. *J. Org. Chem.* **1963**, *28*, 3313. Cassar, L. *J. Organomet. Chem.* **1975**, *93*, 253. Dieck, H. A.; Heck, F. R. *J. Organomet. Chem.* **1975**, *93*, 259.
(13) Feldman, K. S. *Tetrahedron Lett.* **1982**, *23*, 3031.
(14) Beginning with **5**, compounds were stored in solution at −20 °C to avoid decomposition. Compounds **10a,b** were separated from **10c** by flash chromatography (silica gel; diethyl ether/hexane (3:1)).
(15) Compound **15** was obtained through the palladium-mediated coupling of compound **3** and the protected pent-2-en-4-ynol (67%). For a review of the [2,3] Wittig rearrangement, see: Nakai, T.; Mikami, K. *Chem. Rev.* **1986**, *86*, 885. For recent applications, see: Marshall, J. A.; Robinson, E. D.; Zapata, A. *J. Org. Chem.* **1989**, *54*, 5854.
(16) For a discussion of the proposed cyclic transition state, see: Buse, C. T.; Heathcock, C. H. *Tetrahedron Lett.* **1978**, 1685.
(17) This yield was obtained by the concentration of purified **12** in solution. Due to the fact that **12** decomposes in the absence of solvent, 25% represents a maximum figure.

Pseudo-Four-Dimensional Nuclear Magnetic Resonance by Off-Resonance Decoupling. An Approach for Distinguishing Coupled Proton Pairs by the Frequencies of Their Attached Heteronuclei

Stephen W. Fesik,* Hugh L. Eaton, Edward T. Olejniczak, and Robert T. Gampe, Jr.

Pharmaceutical Discovery Division
Abbott Laboratories, D-47G, AP9
Abbott Park, Illinois 60064

Received February 27, 1990

For large molecules (>10 kDa), many of the proton NMR signals overlap, hindering an assignment process based on the analysis of proton–proton *J*-correlated and NOE data.[1] By combination of a heteronuclear shift correlation (e.g., HMQC) and homonuclear 2D NMR experiment (e.g., COSY, NOESY) in a [$X-H_A-H_B$] 3D NMR experiment,[2] many of the proton–proton correlations can be resolved by editing with respect to the chemical shift of a heteronucleus (X) attached to one of the coupled protons (H_A). Although H_A may be uniquely defined by the chemical shift of X in this 3D experiment, the coupling partner, H_B, may be difficult to identify. In principle, H_B could be uniquely defined in a [$X-H_A-H_B-Y$] 4D NMR experiment by the chemical shift of the heteronucleus (Y) attached to H_B. However, a true 4D NMR experiment may be impractical due to the requirements for three independent, incrementable time periods and the large number of pulses and delays necessary to effect all of the coherence transfers.

In this communication, we describe an approach for identifying scalar or dipolar coupled proton pairs (H_A, H_B) by the chemical shifts of *both* of their attached heteronuclei (X, Y). The two frequencies of the coupled protons (ν_{H_A}, ν_{H_B}) and one of the heteronuclear frequencies (ν_X) are determined in a heteronuclear 3D NMR experiment (e.g., HMQC–NOESY, HMQC–COSY).[2] The other heteronuclear frequency (ν_Y) which is used to characterize the H_B spin is obtained by applying an off-resonance

(1) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
(2) Fesik, S. W.; Zuiderweg, E. R. P. *J. Magn. Reson.* **1988**, *78*, 588–593.

0002-7863/90/1512-5370$02.50/0

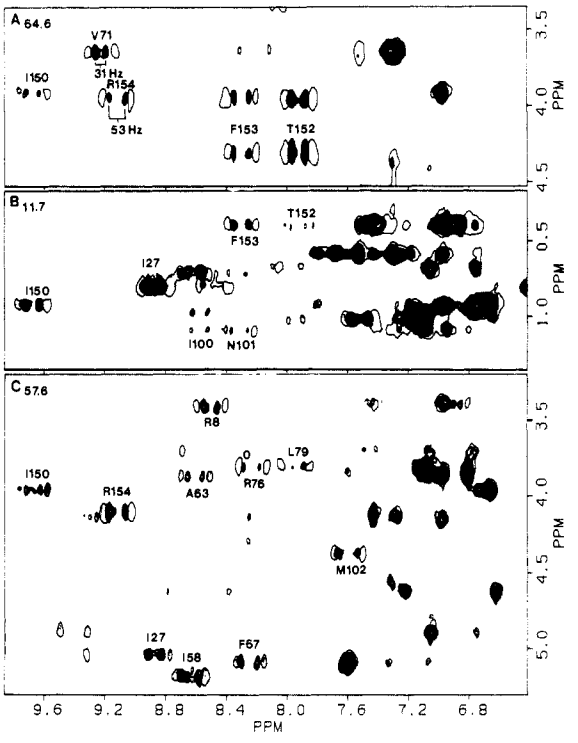

Figure 1. ^1H–^1H (ω_2, vertical; ω_3, horizontal) cross sections of a ^{15}N-coupled (one contour level) and off-resonance ^{15}N-decoupled (multiple contour levels) 3D [^{13}C–^1H–^1H] HSMQC–NOESY spectrum of ^{13}C- and ^{15}N-labeled T4 lysozyme in ^2H_2O at the ^{13}C chemical shifts (ω_1) given at the top of the spectra. The HSMQC–NOESY spectra were collected as previously described[5] on a Bruker AM 500 NMR spectrometer as a series (t_1) of 89 real ^{13}C-filtered NOESY experiments of 160 complex t_2 values and 2K complex t_3 points with eight scans and four dummy scans per increment. The ^{13}C nuclei were decoupled during t_2 and t_3 by using a Waltz-16[9] and a GARP sequence,[10] respectively. Off-resonance ^{15}N decoupling was accomplished with a Bruker BSV3 CW amplifier ($\gamma H_3/2\pi$ = 1110 Hz). The residual H_2O resonance was saturated[11] during the 1-s relaxation delay and 101-ms NOE mixing time. The final size of the data matrices was 128 × 512 × 1024 real points with digital resolutions of 67, 22.2, and 5.5 Hz/point in ω_1, ω_2, and ω_3, respectively. Interpolation was used to define the peak positions to a precision better than the resolution of the data set. The ^{15}N chemical shifts (ppm) of the amides labeled in Figure 1 as determined from an HMQC experiment and the pseudo-4D experiment are as follows: R8, 123.8, 124.0; I27, 122.4, 121.7; I58, 117.8, 118.8; A63, 122.5, 121.9; F67, 120.7, 119.2; V71, 126.3, 127.2; R76, 119.0, 120.4; L79, 115.2, 116.1; I100, 119.7, 120.4; N101, 119.9, 119.5; M102, 116.4, 116.8; I150, 121.4, 122.1; T152, 123.3, 122.5; F153, 121.2, 120.9; R154, 120.0, 120.4. The standard deviation between ^{15}N chemical shifts determined by the two methods is 0.81 ppm.

decoupling field during the acquisition time (t_3) in a 3D experiment. The heteronuclear frequency (ν_Y) is calculated[3] from the one-bond heteronuclear–proton coupling constant (J), residual coupling (J_R), and decoupling field strength ($\gamma H_3/2\pi$) by using the equation

$$\delta = J_R(\gamma H_3/2\pi)/(J^2 - J_R^2)^{1/2}$$

in which δ is the difference between the heteronuclear frequency and the decoupler offset. Since four frequencies (ν_X, ν_{H_A}, ν_{H_B}, ν_Y) are obtained in these 3D experiments, they may be referred to as pseudo-four-dimensional NMR experiments.

Although off-resonance decoupling has been effectively used in the past[4] as a method to correlate the ^1H and ^{13}C chemical shifts of small molecules, this approach has been largely superseded by 2D heteronuclear correlation experiments due the difficulties in analyzing crowded 1D NMR spectra. However, in heteronuclear 3D NMR spectra, the peaks are generally well-resolved, allowing the residual couplings to be extracted and analyzed to yield the heteronuclear frequencies.

The approach is illustrated in the case of a 3.8 mM sample of T4 lysozyme (MW = 19.7 kDa, 164 amino acids) that was uniformly labeled (>97%) with ^{13}C and ^{15}N[5] dissolved in ^2H_2O. In a 3D [^{13}C–^1H–^1H] heteronuclear single multiple quantum correlation–NOESY (HSMQC–NOESY) experiment,[5] the aliphatic protons (ω_2) can be distinguished from one another by the different ^{13}C chemical shifts of their attached carbons. The identity of their NOE partners (amide protons), on the other hand, may still be ambiguous. To distinguish between the different amide protons of T4 lysozyme, off-resonance ^{15}N decoupling was applied at a chemical shift of 135.1 ppm during the acquisition (t_3) period in a 3D HSMQC–NOESY experiment. Figure 1, parts A–C, depicts three ^1H–^1H NOESY planes extracted at the ^{13}C frequencies given at the top of the spectra from a ^{15}N-coupled (one contour level) and off-resonance ^{15}N-decoupled (multiple contour levels) 3D [^{13}C–^1H–^1H] HSMQC–NOESY experiment. The cross peaks correspond to NOEs between aliphatic (ω_2) and amide/aromatic protons (ω_3). In the ^{15}N-coupled HSMQC–NOESY spectrum, the slowly exchanging amides that are still present in ^2H_2O after several months appear as doublets of ~92 Hz due to the ^1H–^{15}N J coupling, whereas, in the off-resonance decoupled spectrum, the amide proton doublets are partially collapsed by an amount that is dependent on the ^{15}N frequency of the amides relative to the ^{15}N-decoupling frequency. For example, in Figure 1a, the amide of Val 71 whose ^{15}N frequency (126.3 ppm) is relatively close to the decoupler offset (135.1 ppm) has a smaller residual ^1H–^{15}N coupling (31 Hz) compared to R154 (53 Hz), which has an ^{15}N frequency of 120.0 ppm. From the ^{15}N chemical shifts calculated from the residual $^1J_{^{15}N-^1H}$ couplings (see figure caption), NOEs involving the amide protons are uniquely distinguished in the [^{13}C–^1H–^1H–^{15}N] pseudo-4D NMR experiment as illustrated in Figure 1A–C for the nearly degenerate amide protons of T152/L79, F153/N101, A63/I58, and R76/F67.[6]

In summary, an approach is described for distinguishing scalar or dipolar coupled protons by the frequencies of their attached heteronuclei, removing ambiguities in the assignment of NMR spectra. The method is especially useful in correlating data from ^{15}N-resolved[2,7] and ^{13}C-resolved[7a,8] 3D NMR experiments.

Acknowledgment. We thank Lawrence McIntosh for preparing the sample of ^{15}N- and ^{13}C-labeled T4 lysozyme and providing the ^1H and ^{15}N T4 lysozyme assignments before publication, Dr. Erik R. P. Zuiderweg for the software used in processing the data, and Bruker Instruments (Billerica, MA) for the additional PTS synthesizer required in the experiment. H. L. Eaton is a postdoctoral associate supported by NIH Grant U01 AI27220-01.

(3) Freeman, R. *A Handbook of Nuclear Magnetic Resonance*; Wiley: New York, 1988; pp 148–150.

(4) (a) Birdsall, B.; Birdsall, N. J. M.; Feeney, *J. Chem. Soc., Chem. Commun.* **1972**, 316–317. (b) Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon-13 NMR Spectra*; Heyden: New York, 1976.

(5) Zuiderweg, E. R. P.; McIntosh, L. P.; Dahlquist, F. W.; Fesik, S. W. *J. Magn. Reson.* **1990**, *86*, 210–216.

(6) McIntosh, L. P.; Wand, A. J.; Lowry, D. F.; Redfield, A. G.; Dahlquist, F. W. *Biochemistry*, in press.

(7) (a) Fesik, S. W.; Zuiderweg, E. R. P. *Q. Rev. Biophys.* **1990**, *23*, 97–131. (b) Marion, D.; Kay, L. E.; Sparks, S. W.; Torchia, D. A.; Bax, A. *J. Am. Chem. Soc.* **1989**, *111*, 1515–1517. (c) Zuiderweg, E. R. P.; Fesik, S. W. *Biochemistry* **1989**, *28*, 2387–2391. (d) Marion, D.; Driscoll, P. C.; Kay, L. E.; Wingfield, P. T.; Bax, A.; Gronenborn, A. M.; Clore, G. M. *Biochemistry* **1989**, *28*, 6150–6156.

(8) (a) Fesik, S. W.; Gampe, R. T., Jr.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **1989**, *111*, 770–772. (b) Ikura, M.; Kay, L. E.; Tschudin, R.; Bax, A. *J. Magn. Reson.* **1990**, *86*, 204–209. (c) Zuiderweg, E. R. P.; McIntosh, L. P.; Dahlquist, F. W.; Fesik, S. W. *J. Magn. Reson.* **1990**, *86*, 210–216. (d) Fesik, S. W.; Eaton, H. L.; Olejniczak, E. T.; Zuiderweg, E. R. P.; McIntosh, L. P.; Dahlquist, F. W. *J. Am. Chem. Soc.* **1990**, *112*, 886–888. (e) Kay, L. E.; Ikura, M.; Bax, A. *J. Am. Chem. Soc.* **1990**, *112*, 888–889.

(9) Shaka, A. J.; Keeler, J.; Frenkiel, T.; Freeman, R. *J. Magn. Reson.* **1983**, *52*, 335–338.

(10) Shaka, A. J.; Barker, P. B.; Freeman, R. *J. Magn. Reson.* **1985**, *64*, 547–552.

(11) Zuiderweg, E. R. P.; Hallenga, K.; Olejniczak, E. T. *J. Magn. Reson.* **1986**, *70*, 336–343.