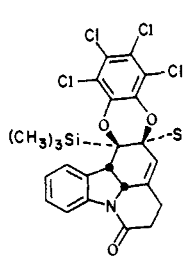
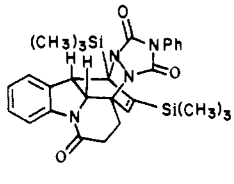


11



12

The complexes **2a–c** and **4e–g** exhibit hindered rotation of the trimethylsilyl group on the ^{1}H NMR time scale (300 MHz), only the second observation of such a phenomenon.[17] In contrast, neither the isomer of **2a**, free ligands of **2a**, **4e**, and **4e′**, nor the corresponding carbazole derived from **2a** have this property. (9) 3,3-Dialkylindole systems as observed in the ligands of **2c**, **4e–g**, **5e–g**, **6h**, **7h**, and **10j** may be of particular importance in medicinal applications.[18]

In short, the described chemistry opens up the way to utilizing the indole 2,3 and perhaps other aromatic double bonds in cobalt-mediated cyclizations, providing novel synthetic flexibility in polyheterocycle construction.

Acknowledgment. This work was supported by NIH-GM22479. K.P.C.V. is a Miller Professor in Residence (1985–1986).

Supplementary Material Available: Melting point, boiling point, spectral, and analytical data on 37 new compounds reported (21 pages). Ordering information is given on any current masthead page.

---

(16) Woodward, R. B.; Cava, M. P.; Ollis, W. D.; Hunger, A.; Daeniker, H. U.; Schenker, K. *Tetrahedron* **1963**, *19*, 247.

(17) Clinet, J. C.; Duñach, E.; Vollhardt, K. P. C. *J. Am. Chem. Soc.* **1983**, *105*, 6710. Butenschön, H.; Winkler, M.; Vollhardt, K. P. C. *J. Chem. Soc., Chem. Commun.*, in press. For **2a** in acetone-d_6, $\Delta H^* = 9.77 \pm 0.20$ kcal mol^{-1}, $\Delta S^* = -5.6 \pm 0.8$ eu; for **2c** in CDCl$_3$, $\Delta H^* = 11.91 \pm 0.55$ kcal mol^{-1}, $\Delta S^* = -10.6 \pm 1.7$ eu; for **4e′** in acetone-d_6, $\Delta H^* = 12.68 \pm 0.40$ kcal mol^{-1}, $\Delta S^* = -4.08 \pm 1.30$ eu; for **4e** in acetone-d_6, $\Delta H^* = 8.86 \pm 0.12$ kcal mol^{-1}, $\Delta S^* = -8.3 \pm 0.5$ eu. Broadening of the trimethylsilyl resonances could be observed for **2b** but at temperatures too low to permit a reasonable line-shape analysis.

(18) Joshi, K. C.; Jain, R.; Chand, P. *Heterocycles* **1985**, *23*, 957.

---

^{1}H and ^{13}C Assignments from Sensitivity-Enhanced Detection of Heteronuclear Multiple-Bond Connectivity by 2D Multiple Quantum NMR

Ad Bax*[†] and Michael F. Summers[‡]

Laboratory of Chemical Physics
National Institute of Arthritis, Diabetes
and Digestive and Kidney Diseases
National Institutes of Health
Bethesda, Maryland 20892
Biophysics Laboratory, Division of Biochemistry
and Biophysics, Food and Drug Administration
Bethesda, Maryland 20892

Received December 6, 1985

We present a new and sensitive method for determining long-range (two- and three-bond) ^{1}H–^{13}C connectivity. The method is a modified version of the ^{1}H-detected heteronuclear multiple quantum experiment,[1-3] previously used for obtaining high-sensitivity ^{1}H–^{15}N shift correlation spectra.[4-7]

Table I. Phases, ϕ and ψ, of the First Two 90°(^{13}C) Pulses and the Receiver Phase in the Eight Steps of the Long-Range Multiple Quantum Shift Correlation Experiment

step	ϕ	ψ	receiver	step	ϕ	ψ	receiver
1	x	x	x	5	x	y	y
2	x	$-x$	$-x$	6	x	$-y$	$-y$
3	$-x$	x	x	7	$-x$	y	y
4	$-x$	$-x$	$-x$	8	$-x$	$-y$	$-y$

Recently, it has been demonstrated convincingly that detection of long-range ^{1}H–^{13}C connectivity provides a wealth of structural and assignment information.[8-12] Unfortunately, the 2D COLOC experiment[8,9] proposed for this purpose suffers from low sensitivity and yields spectral intensities that are modulated by the size of both the one-bond J_{CH} coupling and the homonuclear proton couplings. The other possible method, the one-dimensional selective INEPT experiment,[10] has the disadvantage of requiring exact adjustment of pulse widths and being time consuming if a large number of connectivities are to be investigated.

Here, we demonstrate that a simple extension of the ^{1}H-detected heteronuclear multiple quantum experiment can be used successfully to circumvent the problems mentioned above. The sequence we propose is

$$\begin{array}{llllllll} {}^{1}\mathrm{H} & 90^\circ_x-\Delta_1- & & -\Delta_2- & & -t_1/2-180^\circ_x-t_1/2- & & -\mathrm{Acq}(t_2) \\ {}^{13}\mathrm{C} & & 90^\circ_\phi & & 90^\circ_\psi & & 90^\circ_x & \end{array}$$

where $\Delta_1 = 1/2\,{}^1J_{\mathrm{CH}}$, and the duration of Δ_2 is about 60 ms. The phase cycling employed is given in Table I. The first 90°(^{13}C) pulse serves as a low-pass J filter[11] and suppresses one-bond correlations in the 2D spectrum. This pulse creates heteronuclear multiple quantum coherence for protons that are directly coupled to a ^{13}C nucleus, which is removed from the 2D spectrum by alternating the phase of the ^{13}C pulse along the $\pm x$ axis without changing the receiver phase. Removal of these direct connectivities from the 2D spectrum is not essential but it simplifies the final spectrum at a very small cost in sensitivity. The second 90°(^{13}C) pulse creates the ^{1}H–^{13}C multiple (zero and double) quantum coherence of interest. The 180°(^{1}H) pulse interchanges the zero and double quantum components and thus removes the effect of ^{1}H chemical shift from the t_1 modulation frequency. Consequently, after the final 90°(^{13}C) pulse, the ^{1}H signals that originate from ^{1}H–^{13}C multiple quantum coherence are modulated by ^{13}C chemical shifts and homonuclear proton couplings. Signals from protons that do not have a long-range coupling to ^{13}C are removed by phase cycling of the second 90°(^{13}C) pulse.

Because the detected signal is also phase-modulated by the homonuclear scalar coupling, absorptive 2D spectra cannot be recorded and the spectra are presented most conveniently in the absolute value mode. Very recently, Frey et al.[12] proposed the use of purge pulses and z filters to allow the recording of absorptive ^{1}H–^{113}Cd shift correlation spectra. Unfortunately, in the application to ^{13}C these modifications degrade the sensitivity unacceptably and they also make the suppression of signals not coupled to ^{13}C more difficult.

As an example, we illustrate the multiple-bond shift correlation method for a 4-mg sample of (5′-deoxyadenosyl)cobalamin (coenzyme B$_{12}$, MW 1580), dissolved in 0.35 mL of phosphate-

---

[†] National Institutes of Health.

[‡] Food and Drug Administration.

(1) Müller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481.

(2) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301.

(3) Bendall, M. R.; Pegg, D. T.; Doddrell, D. M. *J. Magn. Reson.* **1983**, *52*, 81.

(4) Griffey, R. H.; Poulter, C. D.; Bax, A.; Hawkins, B. L.; Yamaizumi, Z.; Nishimura, S. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5895.

(5) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Am. Chem. Soc.* **1983**, *105*, 7188.

(6) Live, D. H.; Davis, D. G.; Agosta, W. C.; Cowburn, D. *J. Am. Chem. Soc.* **1984**, *106*, 6104.

(7) Griffey, R. H.; Davis, D.; Yamaizumi, Z.; Nishimura, S.; Bax, A.; Hawkins, B. L.; Poulter, C. D. *J. Biol. Chem.* **1985**, *260*, 9734.

(8) Kessler, H.; Bermel, W.; Griesinger, C. *J. Am. Chem. Soc.* **1985**, *107*, 1083.

(9) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331.

(10) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314. Bax, A.; Niu, C.-H.; Live, D. *J. Am. Chem. Soc.* **1984**, *106*, 1150. Bax, A.; Ferretti, J. A.; Nashed, N.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 3029.

(11) Kogler, H.; Sørensen, O. W.; Bodenhausen, G.; Ernst, R. R. *J. Magn. Reson.* **1983**, *55*, 157.

(12) Frey, M. H.; Wagner, G.; Vasak, M.; Sørensen, O. W.; Neuhaus, D.; Wörgötter, E.; Kägi, J. H. R.; Ernst, R. R.; Wüthrich, K. *J. Am. Chem. Soc.* **1985**, *107*, 6847.

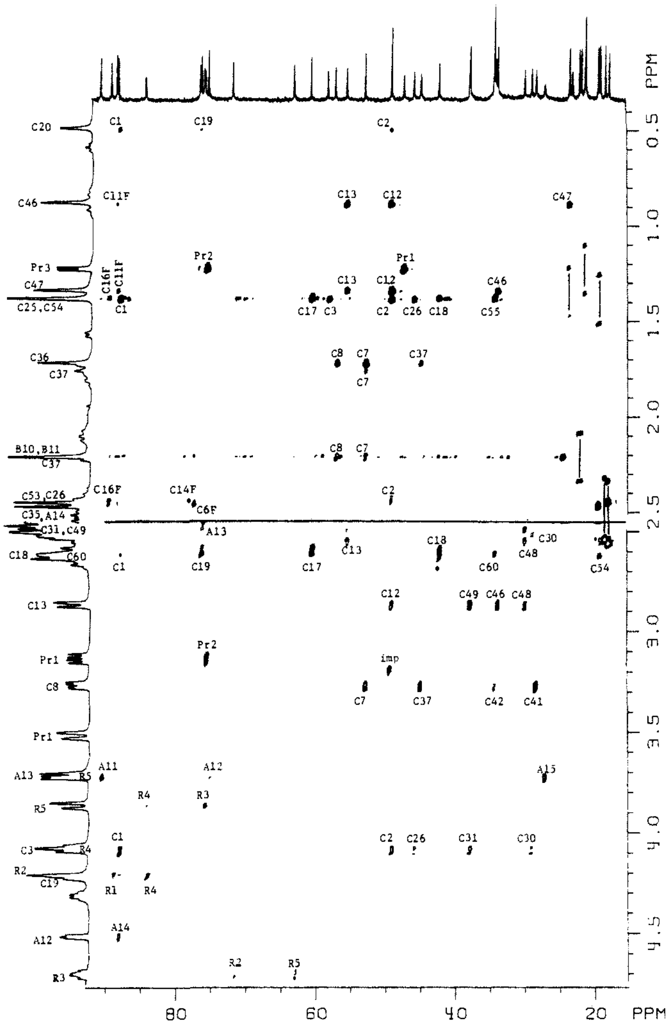


Figure 1. High-field region of the 500-MHz absolute value mode $^1H-^{13}C$ long-range correlation spectrum of a sample of 4 mg of coenzyme B_{12}, dissolved in 0.35 mL of D_2O. The measuring time was 15 h. The lowest contour level in the upper half of the spectrum (above the drawn line) has been chosen 3 times higher than for the lower half, because, at lower contour levels, t_1 noise from the intense methyl signals starts obscuring the connectivities of interest. At the top of the spectrum, the conventional ^1H-decoupled ^{13}C spectrum recorded on a JEOL GX400 spectrometer (using 50 mg of sample) is shown. Incompletely suppressed direct correlations, marked by vertical bars, are observed for the methyl groups C53, C35, B10, B11, C54, C25, C47, and Pr3.[15] Resonances that are folded in the ^{13}C dimension are labeled "F".

buffered D_2O (pH 7.0), in a 5-mm sample tube. Spectra were recorded on a modified NT-500 spectrometer, equipped with a Cryomagnet Systems ^1H probe[13] with a heteronuclear decoupling coil. The high-field part of the spectrum obtained with this method is shown in Figure 1. The entire spectrum contains well over 100 correlations. Connectivities to methyl groups are particularly intense when observed with this method since three methyl protons are used to detect the presence of a single ^{13}C nucleus. Also, both the two-bond and three-bond J_{CH} couplings to methyl protons are usually rather large (4–5 Hz[14]), sufficient to provide an efficient transfer mechanism. For example, Figure 1 shows connectivity between the protons of methyl group 46[15] and carbons C12, C47, and C13. Similarly, the C47 methyl protons show connectivity to C46, C12, and C13. This confirms that the two methyl groups C46 and C47 are attached to the same carbon, C12. A 2D NOE spectrum confirmed the original ^1H assignments[16] of the two methyl groups. However, it follows from Figure 1 that the ^{13}C assignment of the two methyl carbons was incorrect in earlier work.[17] The resonance assignments of many other protonated and nonprotonated carbon resonances follow in a straightforward manner from such a long-range $^1H-^{13}C$ shift correlation spectrum. By use of this method in combination with other recently developed techniques,[18,19] complete and unambiguous ^1H and ^{13}C assignments have been made. In a forthcoming publication[20] this reassignment will be reported, together with conformational information derived from 2D NOE data.

The spectrum of coenzyme B_{12} clearly demonstrates that with the new method determination of long-range $^1H-^{13}C$ connectivity is now feasible for relatively large molecules, using small sample quantities. In addition, the ability to suppress direct connectivity is helpful for minimizing the complexity of the long-range CH connectivity map. If more than one long-range CH connectivity is detected for one particular proton, the relative intensities of the corresponding resonances are directly related to the magnitude of the coupling constant. For example, the presence of an intense correlation between proton C8 and carbon C42 indicates that this coupling is significantly larger than the coupling between proton C8 and carbon C36, for which no connectivity is observed. This information may be used for distinguishing gauche (small coupling) and trans (larger coupling) conformations.[14] In combination with other 2D experiments, the long-range multiple quantum method provides a direct method for determining both the structure and the complete and unambiguous ^1H and ^{13}C assignments of molecules of up to at least 1600 daltons.

Acknowledgment. We thank Rolf Tschudin for continuous technical support and professor Luigi G. Marzilli (Emory University) for providing the sample of coenzyme B_{12}.

Registry No. (5'-Deoxyadenosyl)cobalamin, 13870-90-1.

---

(13) Cryomagnet Systems Inc., Indianapolis, IN 46203.

(14) Hansen, P. E. *Prog. Nucl. Magn. Reson. Spectrosc.* **1981**, *14*, 175.

(15) For the structure of coenzyme B_{12} and the IUPAC numbering system, see ref 17.

(16) Hensens, O. D.; Hill, H. A. O., McClelland, C. E. *in B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, p 463.

(17) Bratt, G. T.; Hogenkamp, P. C. *Biochemistry* **1984**, *23*, 5653 and references therein.

(18) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355.

(19) Bothner-By, A. A.; Stephens, R. L.; Lee, J. T.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.

(20) Summers, M. F.; Marzilli, L. G.; Bax, A., submitted for publication in *J. Am. Chem. Soc.*

Synthesis of Alternating Hydroxy- and Methyl-Substituted Hydrocarbons by Oxymercuration of Cyclopropylcarbinols

David B. Collum

Department of Chemistry, Cornell University
Ithaca, New York 14853

W. Clark Still and Fariborz Mohamadi*

Department of Chemistry, Columbia University
New York, New York 10027

Received December 16, 1985

In addition to the Corey,[1a] Woodward,[1b] and Stork[1c] ring-disconnection methods used for the formation of poly-

---

(1) (a) Corey, E. J.; Trybulski, E. J.; Melvin, L. S., Jr.; Nicolaou, K. C.; Secrist, J. A.; Lett, R.; Sheldrake, P. W.; Falck, J. R.; Brunelle, D. J.; Haslanger, M. F.; Kim, S.; Yoo, S. *J. Am. Chem. Soc.* **1978**, *100*, 4619. Corey, E. J.; Trybulski, E. J.; Melvin, L. S.; Jr.; Nicolaou, K. C.; Lett, R.; Falck, J. R.; Brunelle, D. J.; Haslanger, M. F.; Kim, S.; Yoo, S. *J. Am. Chem. Soc.* **1978**, *100*, 4620. (b) Woodward, R. B.; Logusch, E.; Nambiar, K. P.; Sakan, K.; Ward, D. E.; et al. *J. Am. Chem. Soc.* **1981**, *103*, 3210, 3213, 3215. (c) Stork, G.; Paterson, I.; Lee, F. K. C. *J. Am. Chem. Soc.* **1982**, *104*, 4686. (d) Heathcock, C. H. *Science (Washington*, D.C.) **1981**, *214*, 395. Masamune, S.; Choy, W. *Aldrichimica Acta* **1982**, *15*, 47. Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *13*, 1-115. (e) Kishi, Y. *Pure Appl. Chem.* **1981**, *53*, 1163. Still, W. C.; Barrish, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 1487. (f) Oishi, T.; Nakata, T. *Acc. Chem. Res.* **1984**, *17*, 338. Narasaka, K.; Pai, F.-C. *Tetrahedron* **1984**, *40*, 2233. Masamune, S.; Imperiali, B.; Garvey, D. S. *J. Am. Chem. Soc.* **1982**, *104*, 5528. (g) Nagaoka, H.; Kishi, Y. *Tetrahedron* **1981**, *37*, 3873. Roush, W. R.; Adam, M. A.; Peseckis, S. M. *Tetrahedron Lett.* **1983**, *24*, 1377. (h) Danishefsky, S.; Harvey, D. F. *J. Am. Chem. Soc.* **1985**, *107*, 6647.

0002-7863/86/1508-2094$01.50/0 © 1986 American Chemical Society