gomers were calculated for different mole fractions of methanol and *tert*-butyl alcohol. Equation 6, which does not contain any adjustable parameters, was solved under the restrictions X_1 real, $K_3X_1 < 1$, $X_1 \leq X_A$.

The experimental rate constants, k_{obsd} were plotted against the total concentration of methanol oligomers higher than dimer while for *tert*-butyl alcohol they were plotted against the free monomer concentration (Figures 2 and 3). Despite the limitations of the two-constant model, straight lines of excellent quality were obtained. Thus to a good approximation the reactions of **2a** and **2b** with methanol were first order with respect to methanol oligomers, which were far more reactive than monomer or dimer, i.e. $k_1 \sim k_2 \ll k_3 \sim k_4 \ldots k_n$. By contrast, the reactions were first order with respect to *tert*-butyl alcohol monomer, which was more reactive than dimer or oligomer, i.e., $k_1 \gg k_2 \sim k_3 \ldots k_n$.

The rate constants for the reactions of **2a** and **2b** with methanol oligomers were $(2.9 \pm 0.2) \times 10^9$ and $(4.3 \pm 0.4) \times 10^9$ M^{-1} s^{-1} (per methanol unit), respectively, which implies that the rate constant for the reaction was close to diffusion controlled. By contrast, the rate constants for the reaction of **2b** with methanol monomer were ca. 2×10^7 M^{-1} s^{-1} (extrapolated to $[CH_3OH] \rightarrow 0$) in isooctane and 6.5×10^6 M^{-1} s^{-1} in acetonitrile. For *tert*-butyl alcohol monomer reacting with **2b** in isooctane, the rate constant was found to be $(2.52 \pm 0.15) \times 10^6$ M^{-1} s^{-1}.

If the above interpretation is correct, it follows that k_{obsd} for methanol quenching should decrease while that for *tert*-butyl alcohol quenching should increase as the temperature is increased. This is because the association energies[16,17] for dimer and oligomers are such that the concentrations of these species decrease substantially with increasing temperature. Thus, the lifetime of **2b** in acetonitrile containing 0.23 M methanol gave a good Arrhenius plot with $E_a = -4.7 \pm 0.3$ kcal mol^{-1} for data in the 245–323 K range, this value being in the range of the hydrogen bond strength in methanol.[16,17,20] In the case of *tert*-butyl alcohol, the reactivity was low enough that **2b** could be easily monitored in *tert*-butyl alcohol as solvent, where the lifetime at 300 K was 650 ns. An Arrhenius plot between 300 and 348 K led to $E_a = +3.23 \pm 0.60$ kcal mol^{-1}.[21]

The formation of **3** as a product raises the interesting question of whether it is formed in a rapid reaction involving two methanol molecules contained in the oligomer chain or whether $PhCH(OCH_3)Cl$ diffuses away from the reaction site to react with a second molecule of methanol at a later stage. This question does not have a bearing on the interpretation of the reaction kinetics, but it clearly warrants further investigation.

To summarize, it was found that the reactions of carbenes **2a** and **2b** with methanol and *tert*-butyl alcohol were not first order with respect to the bulk concentrations of those substrates. The results were thought to suggest that oligomers of methanol reacted more readily with these carbenes than its monomer while the converse was true for *tert*-butyl alcohol, where steric effects play a dominant role. Analysis of the distributions of monomers, dimers, and oligomers in the alcohols showed that while methanol monomer was about 2 orders of magnitude less reactive that its oligomers, it was nevertheless somewhat more reactive than *tert*-butyl alcohol monomer. Methanol oligomers reacted at the diffusion-controlled rate on a per methanol unit basis, while *tert*-butyl alcohol oligomers were essentially inert in the time scale of these experiments. At this point it is not clear whether these properties are characteristic of chlorocarbenes or whether they would also apply to other systems.

Registry No. **1a**, 4460-46-2; **1b**, 4222-26-8; **2a**, 19807-41-1; **2b**, 82849-42-1; methanol, 67-56-1; *tert*-butyl alcohol, 75-65-0.

(20) Joesten, M. D.; Schaad, L. J. "Hydrogen Bonding"; Marcel Dekker: New York, 1974; Chapter 5.

(21) A few experiments were carried out to examine the possibility of traces of water leading to complications in the measurements reported herein. It was concluded that in the concentrations in which it may be present as an impurity in carefully dried solvents (≪0.2%) water cannot account for the effects observed. Preliminary experiments suggest that water quenching also leads to curvature. UV spectroscopy of diazirine–methanol samples shows no evidence of complexation at concentrations of methanol three times higher than those used in our experiments.

Total Synthesis of the Complement Inhibitor K-76 in Racemic Form. Structural Assignment to "K-76 Monocarboxylic Acid"

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Received July 14, 1982

Activation of the complement system contributes to the inflammatory processes associated with diseases such as rheumatoid arthritis, lupus erythematosus, and glomerulonephritis, and in consequence great interest is attached to the discovery of agents that are potent inhibitors of complement.[1] Among the most exciting developments in this area is the recent finding by the Otsuka group of a fungal metabolite from *Stachybotrys complementi* nov. sp. K-76, which inhibits the crucial complement C_5 step at concentrations of ca. 10 μg/mL.[2,3] The active substance, termed K-76, was formulated as the tetracyclic dialdehyde **1** (Chart I) on the basis of X-ray crystallographic studies of a derivative.[4] We report herein a total synthesis of (±)-K-76 (**1**) by a route that allows access to a wide variety of structural analogues as well. The general course of this synthesis follows from methodology developed recently for the total synthesis of (±)-aphidicolin[5] and (±)-stemodin,[6] which makes the bicylic keto ester **2**[6] readily available in five steps from geranyl bromide.

Treatment of **2**, mp 76–80 °C, with sodium hydride in ether at 0 °C followed by triflic anhydride afforded the vinyl triflate **3**[7] (90%), which by reaction with 3 equiv of lithium dimethylcopper in ether (−78 °C, 2 h, −50 °C, 2 h, and −30 °C, 1 h) gave the β-methylated α,β-unsaturated ester **4** (90%). Reduction of ester **4** (lithium aluminum hydride in ether at 23 °C for 4 h) provided the corresponding primary alcohol, mp 87–88.5 °C (95%), which was quantitatively converted to the allylic bromide **5** in one flask by (1) mesylation (1.2 equiv. of mesyl chloride, 2 equiv of triethylamine in methylene chloride at −50 °C for 10 min) and (2) bromide displacement (addition of excess lithium bromide in tetrahydrofuran (THF) and reaction at −50 to 0 °C for 1 h).

The aromatic unit was derived from the symmetrical acetal **6**, which was synthesized from methyl 3,5-dihydroxybenzoate by the sequence (1) conversion to the bis(methoxymethyl) (MOM) derivative (77%) by treatment with sodium hydroxide (4 equiv) in methanol and subsequent reaction with 4 equiv of chloromethyl methyl ether at 0 °C, (2) reduction to the primary alcohol (96%) (lithium aluminum hydride in ether at 23 °C), (3) oxidation to the aldehyde (95%) (1.2 equiv of pyridinium chlorochromate and sodium acetate in methylene chloride at 23 °C for 6 h), and (4) acetalization with tosic acid in methanol at 0 °C for 1 h (90%). Reaction of **6** with 1 equiv of *n*-butyllithium in 2:1 THF–tetramethylethylenediamine at −20 to 0 °C for 1 h afforded the lithio derivative **7**, which was coupled to the bromide **5** at −78 °C in the presence of added hexamethylphosphoric triamide (HMPA) (one-third by volume) for 1 h (−78 to 0 °C) to give **8** in 75% yield. Treatment of **8** with 1:3 1 N hydrochloric acid–THF at 23 °C for 1 h produced the aldehyde **9**, mp 76–78 °C (93%), which upon exposure to 15:1:3 THF–ethylene glycol–6 N hydrochloric acid at 45–50 °C for 6 h resulted in cleavage of the MOM protecting

(1) See: Patrick, R. A.; Johnson, R. E. *Annu. Rep. Med. Chem.* **1980**, *15*, 193.

(2) Miyazaki, W.; Tamaoka, H.; Shinohara, M.; Kaise, H.; Izawa, T.; Nakano, Y.; Kinoshita, T.; Hong, K.; Inoue, K. *Microbiol. Immunol.* **1980**, *24*, 1091.

(3) Hong, K.; Kinoshita, T.; Miyazaki, W.; Izawa, T.; Inoue, K. *J. Immunol.* **1979**, *122*, 2418.

(4) Kaise, H.; Shinohara, M.; Miyazaki, W.; Izawa, T.; Nakano, Y.; Sugawara, M.; Sugiura, K. *J. Chem. Soc., Chem. Commun.* **1979**, 726.

(5) Corey, E. J.; Tius, M. A.; Das, J. *J. Am. Chem. Soc.* **1980**, *102*, 1742.

(6) Corey, E. J.; Tius, M. A.; Das, J. *J. Am. Chem. Soc.* **1980**, *102*, 7612.

(7) Satisfactory proton magnetic resonance and infrared spectroscopic data were obtaned for each synthetic intermediate. In addition, high-resolution mass spectra, obtained on chromatographically purified and homogeneous samples, confirmed the molecular formula of each thermally stable intermediate.

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Chart I

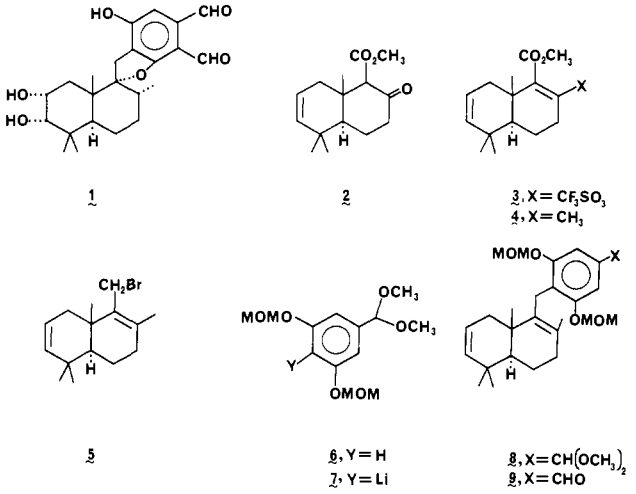
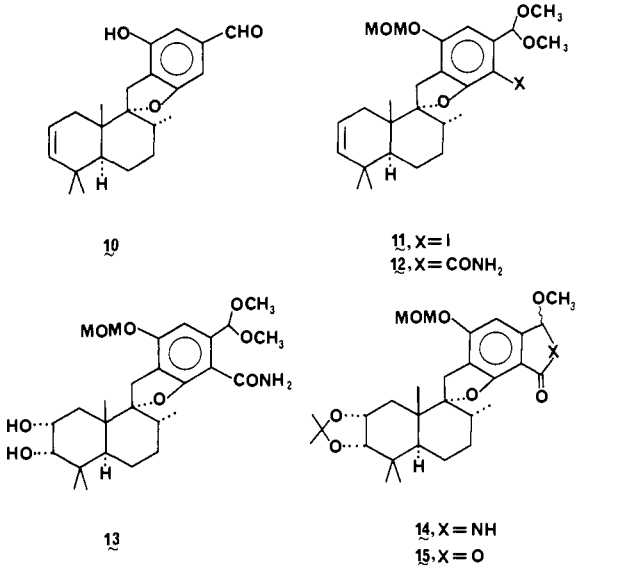


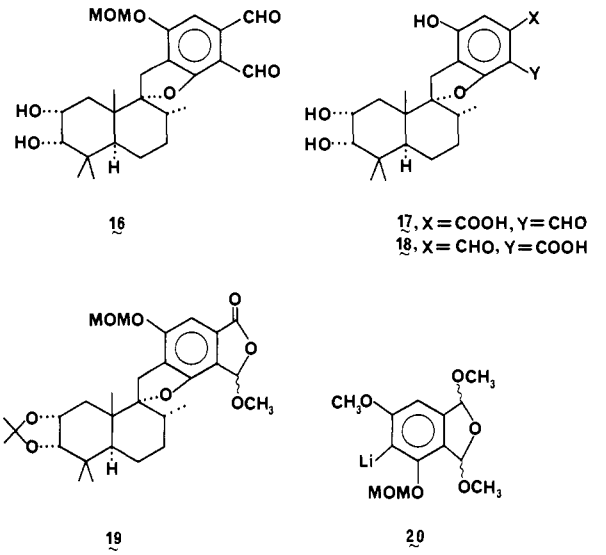
Chart II



groups with partial cyclization. Completion of the reaction was carried out by using 4:1:2 THF–ethylene glycol–2 N hydrochloric acid at 23 °C for 48 h to afford the desired tetrahydrofuran derivative **10** (Chart II) in 50–70% yield and, in addition, ca. 20% of the isomeric tetrahydropyran (R_f values on silica gel plates using 30% THF in hexane 0.42 and 0.48, respectively). The tetrahydrofuran structure for **10** was clearly indicated by ^{1}H NMR data, including a doublet at δ 0.745 (J = 5.5 Hz) for the methyl group at C_8 (steroid numbering). The stereochemistry of **10** at C_8 and C_9, which was expected on the basis of a trans-diaxial course of addition to the double bond, was confirmed by further conversion to K-76.

Reaction of **10** with methanol and a catalytic amount of tosic acid at 23 °C for 1 h generated the corresponding dimethyl acetal (97%), which was iodinated by treatment of the tetra-*n*-butylammonium phenoxide in methylene chloride–methanol with 1 equiv of tetra-*n*-butylammonium triiodide (at 23 °C for 10 min) and further transformed in situ to the corresponding methoxymethyl ether **11** (85% from **10**) by the addition of chloromethyl methyl ether and stirring at 0 °C for 15 min. Replacement of iodine by a cyano group (10 equiv of cuprous cyanide in HMPA at 85 °C for 24 h, 90% yield)[8] and basic hydrolysis (potassium hydroxide in *tert*-butyl alcohol at reflux for 48 h[9]) produced the amide **12**, mp 180–182 °C, in 92% yield.

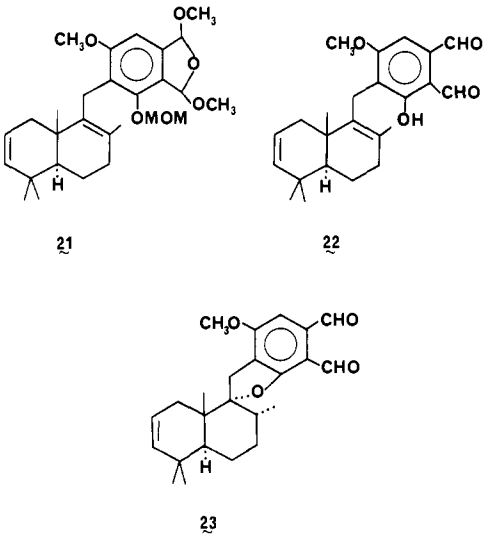
Chart III



The next step, cis hydroxylation of the 2,3-double bond, turned out to be the most troublesome of the synthetic sequence. Reaction of **12** with osmium tetraoxide in pyridine at 23 °C for 16 h proceeded to form 2,3-diol in 80% yield, but surprisingly a 1:1 mixture of 2α,3α- and 2β,3β-diols was obtained. Nor did modification of the reaction conditions lead to a greater proportion of the desired α diol. Furthermore, it was found that the osmium tetraoxide–pyridine hydroxylation of intermediates further along in the sequence (e.g., the 2,3-olefin corresponding to **14**) produced mainly (>90%) the 2β,3β-diol. Evidently the 2,3-double bond of the chair conformer of the A ring (expected to undergo α hydroxylation) is sufficiently hindered against attack by osmium tetraoxide that reaction via higher energy conformers having A or A and B rings in twist boat form is competitive, accounting for β hydroxylation. This unusual result is reminiescent of Barton's findings on the preferred 2α-bromination of the Δ^2-enol acetate of lanostenone.[10] The 2α,3α-diol **13** could be separated from the 2β,3β-isomer by chromatography (R_f values 0.14 and 0.25, respectively, on silica gel plates with ethyl acetate), but it was found more convenient to separate at a later stage. Diol **13** in the mixture was converted to methoxy lactam **14** (90% overall) by (1) treatment with 1 N hydrochloric acid in THF (1 h at 23 °C) to form a trihydroxy lactam (99%), (2) reaction with 0.02% tosic acid in methanol (1 h at 23 °C) to form γ-methoxy γ-lactam,[11] and (3) reaction with acetone, 2,2-dimethoxypropane, and tosic acid at 23 °C for 1 h. Treatment of **14** with 2 equv of dinitrogen tetraoxide–sodium acetate in carbon tetrachloride at 0 °C for 30 min[12] afforded after aqueous workup and chromatography on silica gel the methoxy lactones **15** (R_f values for **15** and the 2β,3β-diastereomer on silica gel with 30% THF in hexane, 0.21 and 0.28, respectively). Reduction of the lactone carbonyl of **15** with 2 equiv of diisobutylaluminum hydride in toluene at −78 °C for 10 min followed by extractive workup and deketalization (5:1 THF–1 N hydrochloric acid at 23 °C for 30 min) gave the phenolic MOM ether of (±)-K-76 (**16**) (Chart III), which was identical with a sample of the phenolic MOM derivative of K-76 by the following measurements: 270 MHz ^{1}H NMR, infrared, and mass spectra; high-performance liquid chromatography (HPLC) both normal-phase on silica gel and reversed-phase with C_{18} silanized silica with 65:35 methanol–water containing 0.1% acetic acid for elution. Cleavage of the MOM protecting group in **16** using THF–6 N

(8) Suzuki, H.; Hanafusa, T. *Synthesis* **1974**, 53.
(9) (a) Gassman, P. G.; Hodgson, P. K. G.; Balchunis, R. J. *J. Am. Chem. Soc.* **1976**, *98*, 1275. (b) Hall, J. H.; Gisler, M. *J. Org. Chem.* **1976**, *41*, 3769.
(10) Barton, D. H. R.; Lewis, D. A.; McGhie, J. F. *J. Chem. Soc.* **1957**, 2907.
(11) This and subsequent γ-methoxy lactams and lactones in the sequence were obtained as a 1:1 mixture of diastereomers differing at the γ carbon as evidenced by the appearance of two methoxy peaks in the ^{1}H NMR spectra.
(12) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6008, 6011, 6015.

Chart IV



21 22 23

hydrochloric acid (1:1) at 23 °C for 4 h afforded 87% of (±)-K-76 (**1**) identical with an authentic sample of K-76 by ^{1}H NMR, infrared, and reversed-phase HPLC comparison.[13]

Oxidation of (±)-K-76 in 1 N sodium hydroxide with 12 equiv of silver oxide at 23 °C for 30 min afforded cleanly after acidification and workup (±)-K-76 monocarboxylic acid, which was found to be identical with an authentic sample[13] by infrared, 270-MHz ^{1}H NMR, and HPLC comparison. We have been able to show that the structure of this product is **17** rather than the isomeric **18**, which has previously been supposed.[3,4,14] The synthetic evidence for our assignment is as follows. Treatment of K-76 monocarboxylic acid with tosic acid in methanol (23 °C, 30 min) afforded the γ-methoxy lactone (97%), which was then transformed via the 2,3-acetonide (acetone, 2,2-dimethoxypropane, tosic acid at 0 °C for 1 h) into the two diastereomeric MOM ethers **19**[11] in 70% yield. These two diastereomers **19** were clearly different by ^{1}H NMR and TLC comparison with the diastereomeric pair (±)-**15** obtained by total synthesis. Structure **17**, which is firmly established for K-76 monocarboxylic acid by these results, was also favored by us a priori on mechanistic grounds. Considering that the oxidation of formyl by silver oxide under strongly basic conditions probably occurs by hydrogen atom abstraction from a hydroxide ion formyl group adduct, in the oxidation of the phenoxide ion of K-76, the formyl meta to the phenoxy oxygen should be the more reactive.

In addition to the synthetic route to K-76 described herein, we have also investigated a modification in which the aromatic–alicyclic coupling step employed the lithio derivative **20**[15] and bromide **5**. The coupling product **21** (Chart IV) (75% yield) could be converted to the dialdehyde **22** (95% with aqueous hydrochloric acid–THF). However, cyclization of **22** to **23** (tosic acid–methylene chloride at 45 °C for 30 min) afforded only 26% yield of the desired product **23**, and other reaction conditions were even less effective.

This synthesis of (±)-K-76 provides as well a useful route to a host of possibly useful structural analogues. This synthetic capability should be of value in determining the structural basis for anti-complement activity of K-76. The work has produced a number of interesting chemical findings as well as a revision of the structure of K-76 monocarboxylic acid, which has anti-complement activity at least comparable to K-76 itself.[16]

Supplementary Material Available: ^{1}H NMR and IR spectral information for **1–6**, **8–13**, **15–17**, and **19–23** (3 pages). Ordering information is given on any current mastehead page.

(13) We are indebted to the Otsuka Pharmaceutical Co. for their cooperation and for providing samples of K-76 and K-76 monocarboxylic acid.

(14) The same conclusion regarding the formulation of K-76 monocarboxylic acid has been reached in a reinvestigation by Dr. H. Kaise of the Otsuka Pharamaceutical Co. (personal communication) on the basis of ^{1}H NMR studies of various K-76 derivatives.

(15) The lithio derivative **20** was prepared by the hydrogen–lithium exchange reaction as indicated above for the conversion of **6** to **7**. The requisite phthalaldehyde derivative (**20** with H replacing Li) was synthesized by the sequence (1) formylation of methyl 3,5-dihydroxybenzoate (50% yield with zinc cyanide–aluminum chloride–hydrogen chloride ether at 0 °C for 30 min and 23 °C for 16 h), (2) selective etherification para to formyl (93% yield with potassium carbonate–methyl iodide in acetone at 23 °C for 20 h) to give methyl 2-formyl-3-hydroxy-5-methoxybenzoate, (3) MOM ether formation (100% with chloromethyl methyl ether–potassium carbonate–triethylamine in acetone at 0 to 23 °C for 1 h), (4) conversion to a γ-hydroxy γ-lactone (95% yield with aqueous potassium hydroxide–THF), (5) conversion to a γ-methoxy γ-lactone (100% yield with tosic acid in methanol), (6) conversion to the 3-methoxymethyl, 5-methyl ether of σ-phthalaldehyde (85% with diisobutyl aluminum hydride at −78 °C in toluene), and (7) formation of the cyclic dimethyl acetal (90% with tosic acid in methanol).

(16) We are pleased to acknowledge financial support from the National Institutes of Health and the National Science Foundation. Thanks are also due to the Otsuka Pharmaceutical Co. and in particular Drs. H. Kaise and T. Kawaguchi for their generous help.

De Novo Sequencing of Oligosaccharides by Proton NMR Spectroscopy

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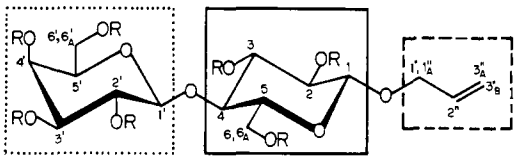
Received December 21, 1981

We report a method based on proton nuclear magnetic resonance (^{1}H NMR) for the structural elucidation of oligomeric organic molecules; we focus here on oligosaccharides[1] but believe the protocol to be generally applicable. The overall objective of the approach is to identify the monomeric units and establish their sequence in the chain; its success depends critically on the fact that the protons of each monosaccharide constitute a separate, relatively small, spin system. Identification of each monosaccharide is achieved by measurement of all the coupling constants[2] and assignment of each resonance; sequencing follows from measurement of the specific interring nuclear Overhauser enhancements.[3] All these data are obtained by a highly efficient combination of one- and two-dimensional (2-D) experiments. Results for the simple glycoside allyl β-D-galactopyranosyl-1β→4-D-glucopyranoside **1** illustrates the approach.



1 : R = H

2 : R = CO.CD$_3$

The first stage of the protocol requires complete resolution of all proton resonances, which is best achieved by use of the proton 2-D *J*-resolved[5] experiment. Because the spectrum of **1** in D_2O

(1) A review of Aspinall and Stephen (Aspinall, G. O.; Stephen, a. M. In "MTP International Reviews of Science. Organic Chemistry, Series One"; Aspinall, G. O., Ed.; University Park Press: Baltimore, MD, 1973; Vol. 7, pp 285–317) covers polysaccharide methodology, including analytical methods.

(2) An extensive tabulation of carbohydrate vicinal coupling constants has been compiled by Altona and Haasnoot (Altona, C.; Haasnoot, C. A. G. *Org. Magn. Reson.* **1980**, *13*, 417–429).

(3) Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect"; Academic Press: New York, 1971.

(4) The area has been reviewed by Freeman (Freeman, R. *Proc. R. Soc. London, Ser. A* **1980**, *373*, 149–178), Nagayama (Nagayama, K. *Adv. Biophys.* **1981**, *14*, 139–204), Morris (Morris, G. A. In "Fourier, Hadamard and Hilbert Transfers in Chemistry"; Marshall, A. G., Ed.; Plenum Press: New York, 1982), and Bax (Bax, A. "Two Dimensional Nuclear Magnetic Resonance in Liquids"; D. Reidel Publishing Co.; Hintham, Mass., 1982).

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