Ultrahigh Resolution NMR. 2. Measurements of Secondary Isotope Effects on Chemical Shifts, Long-Range Carbon–Carbon Scalar Coupling Constants, and Carbon-13 Spin–Spin Relaxation Times Directly from Line Widths

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Abstract: Representative applications of ultrahigh resolution ^{13}C NMR are presented. We arbitrarily define "ultrahigh resolution" NMR as having been achieved when there is less than 20-mHz instrumental contribution to the line width. First, we show that ultrahigh resolution NMR greatly extends the range and precision of measurements of secondary isotope effects on chemical shifts. For example, the ^{18}O (0.2%) satellite is fully resolved from the main carbonyl resonance in the ^{13}C NMR spectrum of acetone of natural isotopic composition. In the spectrum of $(CH_3)_4Si$ of natural isotopic composition, satellites are observed not only for ^{29}Si (spin $^1/_2$, 4.67%) but also for ^{30}Si (spin 0, 3.10%) and the doubly ^{13}C-labeled molecules. The separation between the latter two minor resonances is only 0.000 46 ppm (23 mHz), and yet they are resolved. Then, we show that a single ultrahigh resolution ^{13}C NMR spectrum of toluene yields all satellites caused by ^{13}C–^{13}C scalar splittings, including all long-range splittings. The resulting values of J_{CC} have an accuracy of ±0.003 Hz. With the use of ultrahigh resolution NMR, the instrument time required to determine long-range J_{CC} values is greatly diminished relative to double-quantum coherence methods. Finally, we show that the observed line widths in a single ultrahigh resolution NMR spectrum of a molecule large enough to have natural line widths of about 0.5 Hz or more yield very accurate values of the natural line widths (equal to $1/\pi T_2$). Experimental verification comes from a comparison of the measured line widths and $1/\pi T_1$ values of the ^{13}C resonances of an aqueous disaccharide (sucrose) and tetrasaccharide (stachyose). The resulting accurate and easy to measure T_2 values are an attractive alternative to T_1 measurements in studies of molecular motions and other applications of relaxation times.

In a preceding communication,[1] we demonstrated the feasibility of achieving an instrumental contribution to the line width of as little as 7 mHz in proton-decoupled ^{13}C NMR spectra on standard commercial NMR instruments, with the use of standard large (12-mm diameter) sample tubes and large (4 mL) sample volumes and standard single-pulse excitation. Our studies suggest that the resolution performance of typical commercially available high-resolution superconducting magnets (with ^1H resonance frequencies of 200 or 300 MHz and perhaps even 400 MHz) is much better than is generally believed and that the ultimate available resolution has been generally masked by broadening from chemical shift gradients caused by *temperature gradients in the sample*.[2] This phenomenon is not surprising if one examines available reports of the temperature dependence of chemical shifts (Table I or ref 1). We initially found large temperature gradients in our probe,[2] which we eliminated as described.[1,2] In this report, we present three general types of applications of ultrahigh resolution NMR.

The first type of application deals with isotope effects on the chemical shifts of nucleus A caused by the presence of more than one isotope of nucleus B (secondary isotope effects), which have useful applications to chemical[3] and biochemical[4] problems and are also of interest for theoretical calculations of chemical shifts.[5] However, often these isotope effects are hard to measure because they are small. The difficulty is greatest when the isotopic composition of nucleus B is very lop-sided and the minor isotope has a zero spin and therefore produces no scalar splitting of the resonance under observation, as in the case of ^{18}O (0.20% natural abundance). We show that ultrahigh resolution NMR greatly improves our ability to detect isotopically shifted minor resonances. For example, we can resolve the ^{13}C resonance of the carbonyl of $(CH_3)_2{}^{13}C{}^{18}O$ from that of $(CH_3)_2{}^{13}C{}^{16}O$ in a sample of acetone of natural isotopic composition, even though the chemical shift difference is only 2.5 Hz at 50 MHz. Please note that the capacity to resolve two closely spaced lines diminishes rapidly as the ratio of the two intensities deviates from unity. For example, if the width at half-height of the major peak is 0.200 Hz, its width at 1% height is 1.99 Hz, and its width at 0.1% height is 6.32 Hz, for a Lorentzian line shape. Although nucleus A is ^{13}C in this report, our ultrahigh resolution methodology is easily extrapolated to other spin $-^1/_2$ nuclei.

The second type of application deals with measurements of long-range ^{13}C–^{13}C scalar coupling constants directly from resolved satellites in ordinary (single-pulse excitation) natural-abundance ^{13}C NMR spectra. Long-range ^{13}C–^{13}C scalar coupling constants can be very useful in determinations of structure and conformation.[6,7] For compounds of natural isotopic composition, the detection of ^{13}C–^{13}C satellites is now generally done by suppressing the strong signal of the main resonances with the use of double-quantum coherence methods.[8,9] However, the pulse spacings for each double-quantum coherence spectrum can only be targeted for a small range of J_{CC} values, so that this methodology is most useful for observing the relatively invariant $^1J_{CC}$, typically in the range 35–50 Hz.[10] Because long-range J_{CC} values are quite variable,[6] the pulse spacings become a variable parameter, and many spectra per sample may be required.[9] The resulting total accumulation time may be prohibitive for low concentrations. Therefore, it is of great interest to establish if a single spectrum may yield all long-range J_{CC} values. We show that one ordinary (single-pulse excitation) ultrahigh resolution spectrum may yield most of the long-range ^{13}C–^{13}C scalar coupling constants, as well as all the $^1J_{CC}$ values.

In the above two types of examples, we focus on applications of ultrahigh resolution NMR to molecules small enough to yield

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natural line widths of less than 30 mHz. In the third type of application, we show that ultrahigh resolution conditions are useful for studying relaxation behavior of large molecules. Specifically, we show that the observed line widths (W) from a single ultrahigh resolution spectrum yield very accurate spin–spin relaxation times (T_2) of each resolved resonance of a spin $-^1/_2$ nucleus, in contrast to the need for many inversion–recovery spectra to measure spin–lattice relaxation times (T_1). In the past, T_2 values of quadrupolar nuclei of molecules in solution have been extracted routinely from the line widths of the broad resonances of such nuclei. The dwindling value of the instrumental contribution to the line width made possible by ultrahigh resolution[1] should expand the range of such measurements to spin $-^1/_2$ nuclei of molecules with line widths of about 0.5 Hz or more. Here we test this expectation experimentally by doing detailed measurements of T_1 and W of aqueous sucrose (**1**) and stachyose (**2**). The latter is β-D-fructofuranosyl *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-α-D-glucopyranoside. As expected,[11] the T_1 value of C-2 of the fructofuranosyl residue of **1** yields a very narrow estimated natural line width (0.055 Hz under our sample conditions). Therefore, no attempt was made to extract the T_2 of this resonance of **1** and **2** from observed line widths. The results and discussion presented below refer only to the methine and methylene carbon resonances of **1** and **2**.

Experimental Section

Proton-decoupled natural-abundance ^{13}C NMR spectra were recorded on a slightly modified Nicolet NT-200 NMR spectrometer at a frequency of 50.3 MHz in a standard Nicolet 12-mm probe and standard 12-mm NMR tubes (Wilmad 514A-7PP, flat bottom, 11.05-mm inside diameter). The sample volume was 4.2 mL. The spectrometer was modified as follows: (i) WALTZ-16 proton decoupling[12] was incorporated with the use of a digital control box purchased from Bio-Magnetic Instruments of West Layfayette, IN, which created the required 180° phase shifts in the ^1H decoupler circuit of the NT-200 spectrometer. Decoupler power was in the range 0.9–1.7 W, which yielded $\gamma H_2/2\pi$ of 2700–3800 Hz. (ii) The original digital pyrometer which had a resolution of 1 °C was replaced by one with a resolution of 0.1 °C (Newport Electronics 268-TC2.05.D2). (iii) The temperature stability was improved by first running the compressed air used for sample temperature control through a copper coil in a large water reservoir. Since this work was completed, the water reservoir has been replaced by a Neslab RTE-8DD constant temperature bath, which has a temperature stability of ±0.02 °C. (iv) Temperature gradients in the sample were minimized by raising the air flow to about 20 L/min and by keeping the ^1H decoupling power to 1.7 W or less.[2]

Spin–lattice relaxation times were measured by the inversion–recovery method[13] with composite 180° pulses to correct for H_1 inhomogeneity[14] and 180° phase alternation in order to correct for T_2 relaxation effects,[15] even though the interval between successive 180° pulses was always $\gtrsim 6T_1$. Other experimental details are given in the figure legends and footnote a of Table II.

Secondary Isotope Effects on Chemical Shifts

Figure 1 shows the carbonyl resonance of acetone of natural isotopic composition, after 92 scans, processed with a 10-mHz digital exponential broadening to improve the signal-to-noise ratio. The resulting width at half-height of the main peak is 36.8 mHz. If we subtract the above 10 mHz and an estimated natural line width (based on $1/\pi T_1$) of 9.4 mHz, the remaining instrumental broadening is 17.4 mHz, considerably greater than in a single-scan experiment,[1] probably as a result of the large temperature dependence of the carbonyl chemical shift: a temperature drift of 0.01 °C during spectral accumulation would cause a 7-mHz

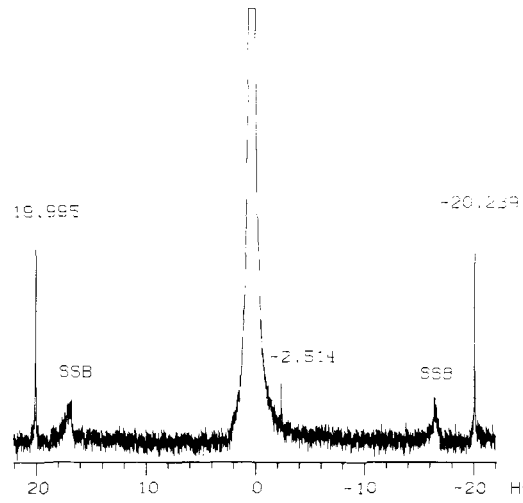

Figure 1. Proton-decoupled carbonyl resonance of air-free acetone (with 20% v/v CD_3OD) under argon at 22.5 °C and 50.3 MHz, after 92 scans with an acquisition time per scan of 81.92 s, a spectral width of ±100 Hz, and 32K data points. The data were processed with 10-mHz Lorentzian broadening to improve the signal-to-noise ratio and with an additional 32K "zero fill" data points, which yielded a simulated acquisition time of 163.84 s and 6.1-mHz digital resolution. The main peak is truncated at 2% of its full height. The peaks labeled SSB are spinning sidebands. Zero frequency has been set at the chemical shift of the main peak.

broadening.[1] There seems to be a widely held belief that very small peaks near very large ones are difficult to detect not only because of resolution problems but also because of interference from spurious signals such as spinning sidebands and "artifacts" at the base line of large peaks. Figure 1 is evidence to the contrary, at least for ultrahigh resolution NMR. Spinning sidebands, labeled SSB in Figure 1, are broad because the spinning speed is not constant enough relative to our line width of 37 mHz. In any case, it is easy to modulate the spinning speed on purpose and thus smear out the spinning sidebands.[16] There are no other artifacts in Figure 1. The peak at −2.514 Hz has an intensity about 1/500 that of the main resonance and can be readily assigned to $^{13}C^{18}O$ on the basis of published results for ^{18}O-enriched acetone.[17] The peaks at −20.239 and 19.995 Hz clearly arise from the carbonyl of $^{13}CH_3$–^{13}CO–CH_3. Although $^1J_{CC}$ values can be readily obtained without ultrahigh resolution by suppressing the major resonance with double-quantum coherence methods,[8] the ultrahigh resolution spectrum of Figure 1 not only makes double-quantum coherence unnecessary for $^1J_{CC}$ measurements but it also allows very precise measurements of the peak positions of the satellites, thus improving the precision of $^1J_{CC}$ *and* of the ^{13}C isotope effect on the ^{13}C chemical shift. From Figure 1, we get $^1J_{CC}$ = 40.234 ± 0.003 Hz and a $^{13}CH_3$ isotope effect on the carbonyl chemical shift of −122 ± 3 mHz (−0.00242 ± 0.00006 ppm). Experimental evidence for this unprecedented level of accuracy is presented in the following section.

Figure 2A shows the proton-decoupled ^{13}C NMR spectrum of $(CH_3)_4Si$ of natural isotopic composition, after 40 scans, processed with no digital broadening or narrowing. The width at half-height of the main peak is 36.6 mHz. When we subtract the estimated natural line width (based on $1/\pi T_1$) of 20.1 mHz, the remaining instrumental broadening is 16.5 mHz. The natural isotopic composition of silicon is 92.23% ^{28}Si (spin 0), 4.67% ^{29}Si (spin $^1/_2$), and 3.10% ^{30}Si (spin 0).[18] The peaks at −25.465 and 25.380 Hz in Figure 2A are the ^{29}Si satellites, which yield a ^{13}C–^{29}Si scalar coupling constant of 50.845 Hz. Reported values are 52 ± 2 Hz,[19]

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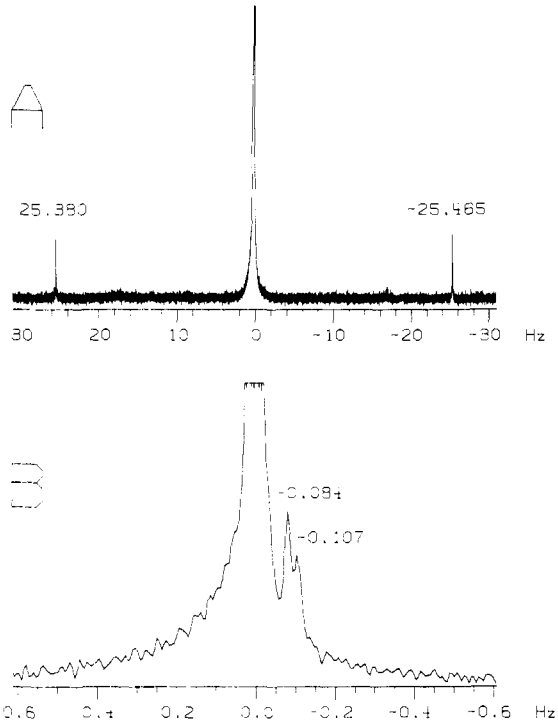

Figure 2. Proton-decoupled ^{13}C NMR spectrum of air-free Me_4Si (15% v/v in a 2:1 v/v mixture of acetone and acetone-d_6) under argon at 22.2 °C, after 40 scans. Other acquisition and processing conditions were as for acetone (Figure 1), except as indicated below. The main peak is truncated at 10% of its full height. (A) Region of −31 to 31 Hz (from the main peak), processed with no digital broadening or narrowing of the resonances. Spinning sidebands at about ±18.5 Hz are essentially buried in noise. (B) Region of −0.61 to 0.61 Hz, processed with 30-mHz Lorentzian narrowing plus 20-mHz Gaussian broadening.

50.3 ± 1.0 Hz,[20] and 51.0 ± 0.5 Hz.[21] We believe that our value has an accuracy of ±0.003 Hz. Evidence is presented in the following section.

Figure 2A also yields a ^{29}Si isotope effect (relative to ^{28}Si) on the ^{13}C chemical shift of Me_4Si of −43 ± 3 mHz (−0.000 85 ± 0.000 06 ppm). As far as we know, this is the first report of a ^{29}Si isotope effect on a ^{13}C chemical shift. A 43-mHz effect is easily detectable here because the resonances are very narrow. Additional minor peaks are visible slightly upfield of the strong resonance of $^{13}CH_3$–^{28}Si–$(^{12}CH_3)_3$. In order to improve the resolution of these peaks, the data processing for Figure 2B included 30 mHz of Lorentzian narrowing plus 20 mHz of Gaussian broadening.[22] The resulting width at half-height of the main peak was reduced to 21 mHz, and also the wings of the main peak tapered off more rapidly than in Figure 2A. The intensity of the peak at −107 mHz is greater in the spectrum of specifically (34%) ^{13}C-enriched $^{13}CH_3$–Si–$(CH_3)_3$ than in Figure 2B and is therefore assigned to the expected 3.3% of $(^{13}CH_3)_2$–^{28}Si–$(^{12}CH_3)_2$, relative to $^{13}CH_3$–^{28}Si–$(^{12}CH_3)_3$, in the sample of natural isotopic composition. The resulting ^{13}C isotope effect on the ^{13}C chemical shift of Me_4Si is −0.0021 ± 0.0001 ppm. By elimination, the peak at −84 mHz in Figure 2B must arise from the $^{13}CH_3$–^{30}Si–$(^{12}CH_3)_3$ molecules. The ^{30}Si isotope effect is −0.0017 ± 0.0001 ppm, which is, within experimental error, twice the value we obtained from Figure 1 for the ^{29}Si isotope effect.

It is noteworthy that the resonances of $^{13}CH_3$–^{30}Si–$(^{12}CH_3)_3$ and $(^{13}CH_3)_2$–^{28}Si–$(^{12}CH_3)_2$ are separated by only 0.000 46 ppm (23 mHz). Ultrahigh resolution NMR of small molecules provides an opportunity to resolve chemical shifts that differ by less than one part per *billion*, perhaps even as little as 0.2 ppb at higher frequencies than our 200 MHz (^1H) value.

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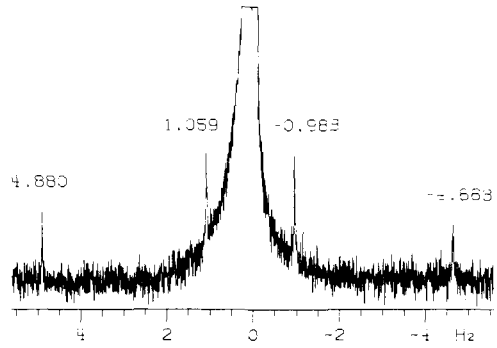

Figure 3. Proton-decoupled C-1 resonance of air-saturated toluene (with 20% v/v CD_3OD) at 35 °C and 50.3 MHz, after 40 scans with an acquisition time of 81.92 s per scan, a spectral width of ±50 Hz, and 16K data points. The data were processed without exponential broadening or narrowing but with an additional 16K time-domain "zero fill" points, which yielded a final digital resolution of 6.1 mHz. Zero frequency is set at the chemical shift of the main peak, which is truncated at 2% of its full height. The chemical shifts (in hertz) displayed above the peaks were obtained with the use of a Lorentzian line-shape-fitting routine.

Table I. Long-Range ^{13}C–^{13}C Scalar Coupling Constants (Hertz) in Toluene[a]

	coupled carbon[c]				
obsd C[b]	1	2, 6	3, 5	4	7
1			2.042	9.543	
2, 6			9.003[d]	2.555	3.168
3, 5	2.042	9.004[d]			3.852
4	9.540	2.555			0.920
7		3.170	3.854	0.921	

[a] Obtained from Figures 3 and 4 and a similar spectrum of C-7 (see text). [b] The carbon whose main resonance was in between the observed satellites. [c] The second carbon involved in the ^{13}C–^{13}C scalar interaction. [d] Coupling of C-2 to C-5 and C-3 to C-6.

Long-Range ^{13}C–^{13}C Scalar Coupling Constants

Figure 3 shows the proton-decoupled C-1 resonance of air-saturated toluene after 40 scans, processed with no digital broadening or narrowing. The resulting width at half-height of the main resonance is 16 mHz, which yields an instrumental broadening of 9 mHz after subtraction of an estimated natural line width (based on $1/\pi T_1$) of 6.8 mHz. The expected two long-range ^{13}C–^{13}C satellites are fully resolved. The $^1J_{CC}$ satellites are far outside the spectral range shown in Figure 3. Each inner satellite of Figure 3 has about 1% of the intensity of the main peak (instead of 0.5%) because they arise from the sum of the equivalent 1–3 and 1–5 carbon pairs (see below).

At the time of these experiments, our Nicolet NT-200 NMR instrument was equipped with 64K data memory, which was inadequate for recording the full range of ^{13}C chemical shifts of toluene (about 6000 Hz). Therefore, as a temporary measure, we recorded one spectrum for C-1, one for the methine aromatic region, and one for the methyl group. When the spectra were processed without digital broadening or narrowing, the observed line widths for C-2, C-3, C-4, and C-7 (methyl) were 41, 37, 45, and 60 mHz, respectively, and the corresponding estimated natural line widths, based on $1/\pi T_1$ values, were 18, 19, 23, and 28 mHz, respectively. In order to enhance the observation of very small J_{CC} values, the methine carbon spectrum (Figure 4) was processed with 40-mHz Lorentzian narrowing plus 30-mHz Gaussian broadening, and the methyl carbon spectrum was processed with 50-mHz Lorentzian narrowing plus 50-mHz Gaussian broadening.[22] It is clear from Figure 4 that satellites even as close as 0.5 Hz from the main resonance are resolvable.

Figures 3 and 4 demonstrate the feasibility of observing *all* $J_{CC} \gtrsim 1$ Hz in a single ultrahigh resolution ^{13}C NMR spectrum, without the use of double-quantum coherence methods. Furthermore, the combined use of ultrahigh resolution and double-quantum coherence may permit the observation of J_{CC} values as small as 0.1 Hz. In addition, with or without double-quantum coherence,

Table II. Values (Hertz) of W and $1/\pi T_1$ of **1** and **2**[a]

residue[b]	relaxation rate	carbon[c] 1	2	3	4	5	6
1F	W	1.16[d]	*e*	0.76	0.72	0.77	0.97[d]
	$1/\pi T_1$	1.13[d]	*e*	0.70	0.69	0.71	0.96[d]
1G	W	0.72	0.70	0.70	0.69	0.70	1.07[d]
	$1/\pi T_1$	0.69	0.68	0.66	0.65	0.66	1.08[d]
2F	W	1.46[d]	*e*	1.09	1.03	1.02	1.19[d]
	$1/\pi T_1$	1.46[d]	*e*	0.99	0.93	0.98	1.20[d]
2G	W	1.09	1.18	1.14	1.21	1.18	1.71[d]
	$1/\pi T_1$	1.05	1.08	1.08	1.11	1.13	1.74[d]
2I	W	1.06	1.22	1.22	1.16	1.21	1.91[d]
	$1/\pi T_1$	1.05	1.14	1.15	1.04	1.17	1.95[d]
2T	W	0.73	0.93	0.86	0.80	0.87	0.81[d]
	$1/\pi T_1$	0.69	0.85	0.85	0.70	0.83	0.77[d]

[a] W values have an estimated precision of ±3%, on the basis of repeated measurements. W values of **2** were obtained from the spectrum shown in part in Figure 6. Those of **1** (0.5 M in H_2O with 10% v/v CD_3OD and 0.5% v/v $^{13}CH_3OH$, pH 4.0, 42 °C) are the averages of values from six spectra obtained as described for **2** in the caption of Figure 6, except that each spectrum was recorded with 400 scans, an acquisition time of 9.08 s per scan, a spectral width of ±902.527 Hz (enough to cover the CH and CH_2 resonances), and a final digital resolution of 0.0551 Hz. The T_1 values of **1** and **2** were measured on the same samples and at the same temperatures as were used for getting the values of W, and they have an estimated precision of ±5%. Each T_1 value is the arithmetic average of two values from two independent sets of 18–28 inversion–recovery spectra. The number of scans per inversion–recovery spectrum was 160 and 500 for **1** and **2**, respectively. Other information about the measurements of W and T_1 is given in the text. [b] The letters F, G, I, and T after the compound designations **1** and **2** refer to fructose, glucose, internal galactose, and terminal galactose, respectively. [c] Methine carbon unless otherwise indicated. [d] Methylene carbon. [e] Nonprotonated carbon.

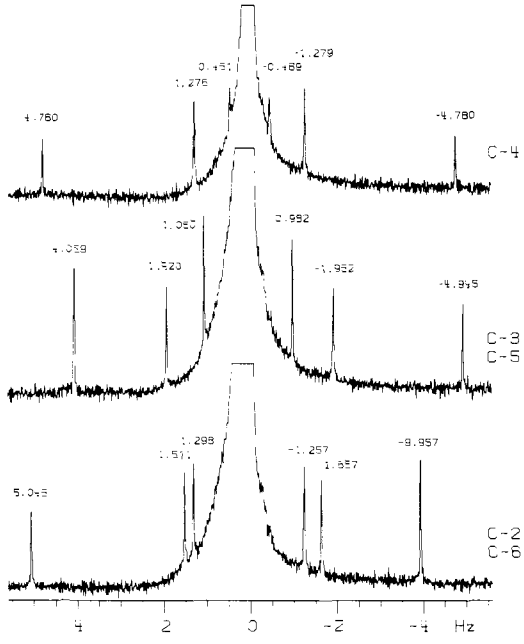

Figure 4. Proton-decoupled ^{13}C NMR spectrum of the methine carbons of air-saturated toluene (with 20% v/v CD_3OD) at 35 °C and 50.3 MHz, after 160 scans with an acquisition time of 54.61 s per scan, a spectral width of ±150.015 Hz, and 32K data points. All three spectral regions come from a single spectrum, processed with 40-mHz Lorentzian line narrowing and 30-mHz Gaussian line broadening, and an additional 32K "zero fill" time-domain points, which yielded 9.2-mHz digital resolution. The same vertical scale is used for all three spectral regions. The main peak of C-4 is truncated at 2% of its full height. The other two main peaks are truncated at 1%. Zero frequency is set at the chemical shift of each main peak. The chemical shifts (in hertz) displayed above the peaks were obtained with the use of a Lorentzian line-shape-fitting routine.

ultrahigh resolution ^{13}C NMR of small molecules (with small natural line widths) permits the resolution of two or more very closely spaced ^{13}C–^{13}C satellites *and* the measurement of very accurate J_{CC} values. The available accuracy is dramatically demonstrated in Table I, which tabulates the J_{CC} values for toluene.[23-25] Because we have detected *all* possible long-range

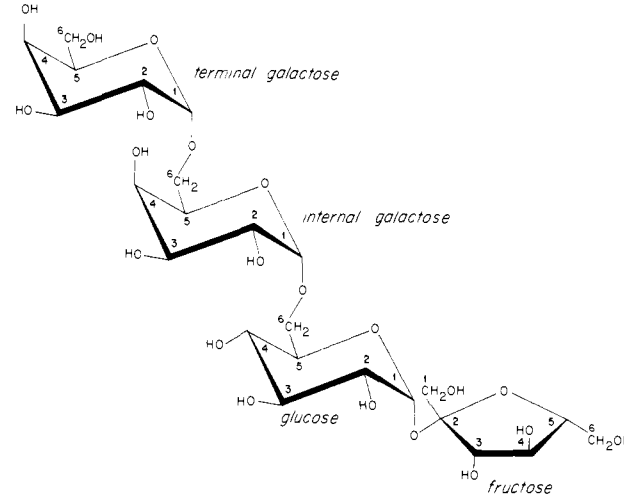

Figure 5. Structure of **2**.

^{13}C–^{13}C satellites, Table I lists two independently measured values for each J_{CC}, so that we can assign *every* satellite to a specific carbon–carbon pair, and we can also estimate the precision of the measurements. For each pair of theoretically identical J_{CC} values, Table I yields a discrepancy no greater than 0.003 Hz. Even greater precision is feasible, because our digital resolution was 0.009 Hz for the methine carbon resonances (see caption of Figure 4), which can be improved with the use of more than 64K data memory.

As a bonus, ultrahigh resolution spectra such as Figures 3 and 4 also yield detectable long-range ^{13}C isotope effects on ^{13}C chemical shifts.[26] Because most of these isotope effects are very small, it is desirable to determine the chemical shifts of the ^{13}C–^{13}C pairs with the use of the expressions for the strongly coupled AB system, even for pairs whose chemical shifts differ by much more than the value of J_{CC}.

Measurement of ^{13}C Spin–Spin Relaxation Times Directly from Line Widths

For measurements of the line widths (W) of sucrose (**1**), six spectra were recorded under identical conditions, except that the

(23) For early reports of some long-range J_{CC} values of toluene, see ref 24. A nearly complete set has been reported in ref 25. Within experimental error (which is ±0.15 Hz in ref 24b and ±0.03 Hz in ref 25), our values are in agreement with the reported ones, except for the 1–3 coupling constant in ref 24b, which was reported as 0.78 Hz (but 2.05 Hz in ref 25).

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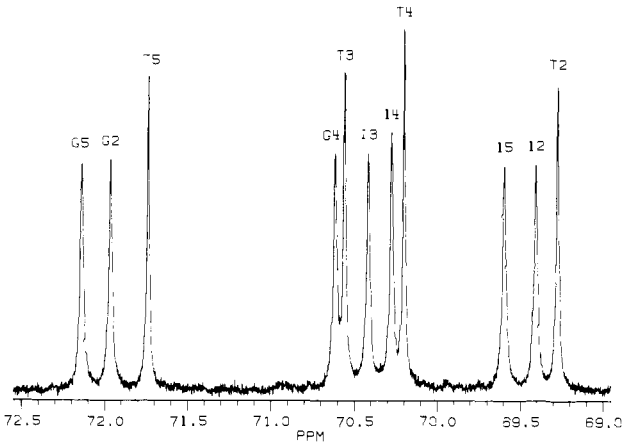

Figure 6. Only crowded region in the proton-decoupled ^{13}C NMR spectrum of **2** (0.2 M in H_2O with 20% v/v CD_3OD) at 55 °C and 50.3 MHz, after 4000 scans with an acquisition time of 4.83 s per scan, a spectral width of ±1694.91 Hz, and 32K data points. The data were processed without exponential broadening or narrowing but with an additional 32K "zero-fill" time-domain points, which yielded a final digital resolution of 0.103 Hz. The chemical shift scale was set by assuming that the value for C-6 of the glucose residue is the same as reported for **2** in D_2O at 22 °C.[30] F, G, I, and T designate fructose, glucose, internal galactose, and terminal galactose, respectively (see Figure 5), and the numbers are standard carbon designations. The full spectrum covers the range 62.0–104.6 ppm, with chemical shifts similar to those reported by Christofides and Davies,[30] when corrected for the effect of deuteration of hydroxyls in D_2O.[30]

WALTZ-16 proton-decoupler center frequency was 3 ppm from Me_4Si in one spectrum, 5 ppm in another, and 4 ppm in the remaining ones. As expected,[27] no broadening caused by decoupler frequency offsets could be detected. All resonances of **1** and all but two resonances of stachyose (**2**, see structure in Figure 5) were resolved well enough for a least-square fit to a single Lorentzian line. The exceptions were the two peaks of **2** at about 70.6 ppm (see Figure 6), whose line widths were obtained by a least-squares fit to two Lorentzian peaks. Assignments of the resonances of **1** are the reported ones[28,29] based on independent evidence from selective proton-decoupling[28] and double-quantum coherence.[29] The assignments for **2** are the reported ones based on deuterium isotope effects,[30] which did not distinguish the resonances of carbons 1–4 of the terminal galactose from the corresponding ones of the internal galactose (Figure 5). We used the T_1 values measured here to distinguish these resonances, as described earlier for a few of them under much lower resolution.[31]

There is excellent agreement between each measured W and the corresponding $1/\pi T_1$ value (Table II), as should happen if there is negligible instrumental broadening *and* if the molecular tumbling rate is much greater than the resonance frequency, a condition that is satisfied here.[11] The instrumental contribution to the line width ($\lesssim$0.05 Hz) is difficult to pinpoint precisely because it is masked by the experimental errors for W and T_1. In any case, the comparisons of W and $1/\pi T_1$ (Table II) clearly show that measurements of T_2 from a single ultrahigh resolution spectrum can replace the more time-consuming T_1 measurements when the natural line widths are about 0.5 Hz or greater. Furthermore, small but important differences in relaxation times can be verified by measuring W and T_1, because measurements of W and T_1 are affected by very different systematic errors. For example, the glucose and fructose rings of **1** seem to have slightly different T_1 values, but the difference is very small, and it is reassuring to observe an analogous trend in the values of W. An earlier measurement of the ^{13}C T_1 values of **1** was seen as indicative of equal values for the two rings and, therefore, as evidence of isotropic tumbling of the sucrose molecule in aqueous solution.[32] Table II suggests a slight anisotropy.

There is a wealth of information and potential applications in a detailed analysis of the data for **2** (Table II), but this will be discussed in a future report. Finally it should be noted that the easy and accurate method for measuring T_2 values of spin $-^1/_2$ resonances presented here is also of interest in situations when one wishes to detect important contributions to $1/T_2$ which do not affect $1/T_1$, such as chemical exchange and various types of scalar relaxation.

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Registry No. 1, 57-50-1; **2**, 470-55-3; $(CH_3)_4Si$, 75-76-3; acetone, 67-64-1; toluene, 108-88-3.

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