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Development of a ^{13}C -Optimized 1.5-mm High Temperature Superconducting NMR Probe

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Abstract

We report a 1.5-mm NMR probe based on high temperature superconductors operating at 14.1 T optimized for ^{13}C detection. The probe has a total sample volume of about 35 microliters (μL) with an active volume of 20 μL and provides exceptional mass sensitivity for ^{13}C detection. The probe also has excellent ^1H sensitivity and employs a ^2H channel lock; ^{15}N irradiation capability can be added in the future. The coils are cooled to about 20 K using a standard Agilent cryogenic refrigeration system, and the sample temperature is regulated near room temperature. The coil design considerations are discussed in detail. This probe is ideal for directly detected ^{13}C NMR experiments for natural products chemistry and metabolomics applications, for which 35 μL is an optimal sample volume. The outstanding ^{13}C sensitivity of this probe allowed us to directly determine the ^{13}C connectivity on 1.1 mg of natural abundance histidine using an INADEQUATE experiment. We demonstrated the utility of this probe for ^{13}C -based metabolomics using a synthetic mixture of common natural abundance metabolites whose concentrations ranged from 1 to 5 mM (40 to 200 nmol).

Introduction

Nuclear magnetic resonance (NMR) suffers from low intrinsic sensitivity because of the small nuclear Zeeman splitting and the resulting small Boltzmann polarization. ^{13}C sensitivity is especially low because of its low gyromagnetic ratio and low natural

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Author contributions

VR and JH ran electromagnetic simulations for the components of the probe; WB, RW, and RN designed coils, with significant input from VR and JH; RN, RW, VR, and WB constructed the probe and laser trimmed coils; RW did all the NMR testing at Agilent; VR, WB, and ASE installed the probe in the AMRIS facility; VR collected the data in Table 1; ASE suggested the initial general design, obtained funding for the project, and wrote the paper with significant input from all authors.

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abundance. The observed signal may be enhanced by using more material, but this is often not possible for naturally occurring compounds available in limited quantities. NMR sensitivity may be improved by relatively costly approaches such as using higher magnetic field strengths and hyperpolarization techniques. The current work focuses on improving the detection efficiency through the use of high sensitivity NMR probes, which improve sensitivity on their own but also provide a multiplicative improvement when used with other sensitivity enhancement methods.

¹³C NMR

Because of its high sensitivity and natural abundance, ¹H detection is the standard for almost all biomolecular NMR experiments in widespread use today. Over several decades of research, NMR spectroscopists have developed many elegant methods to correlate protons with protons or heteronuclei in compound identification and structural studies [1]. In contrast, ¹³C detection has been much less common because of relatively low sensitivity and only 1.1 % natural abundance. However, ¹³C detection has several advantages over ¹H detection for biomolecular studies. First, whereas the diamagnetic chemical shift range for ¹H is only about 12 ppm, for ¹³C it is over 200 ppm, providing much greater chemical shift dispersion and less overlap. Second, for compounds at natural abundance, with ¹H decoupling, ¹³C resonances are narrow singlets, in contrast to the rather complicated multiplet patterns seen in ¹H spectra. Third, biological molecules are based predominantly on carbon scaffolds [2], and correlations between ¹³C atoms provide much more direct information for small molecule structure elucidation than the many combinations of ¹H-based correlations that are more commonly used. Several natural products are rich in quaternary carbons, which pose difficulties for indirect ¹H detection. Many macromolecular studies rely on partial deuteration to remove the line-broadening influences of protons, and in this situation direct ¹³C detection provides the only way to detect a signal since the protons are not there [3]. Large macromolecular systems with long correlation times are also challenging with indirect ¹H detection because of loss in coherence transfer, making ¹³C detection even more advantageous.

Even with all of the advantages that direct ¹³C detection provides, it remains rarely used in biomolecular studies because of its low sensitivity at natural abundance or the need for isotopic labeling, which can be very expensive for some types of samples or impossible with organisms that cannot be cultured. Dynamic nuclear polarization (DNP) is one attractive method for significantly enhancing ¹³C sensitivity [4]. Although promising, DNP is not yet a generally applicable technique, and the sensitivity gain from improved NMR probes, such as the one reported here, will be multiplicative with gains from polarization enhancement techniques.

It is well known that RF sample losses limit the sensitivity gain which can be achieved with cryogenic probes [5], and the dependence of this loss on the choice of buffer has been previously studied [6]. We note that RF loss increases strongly with frequency, so species such as ¹³C with lower gyromagnetic ratio have greater potential for sensitivity improvement than ¹H. Sample loading scales as the 4th power of sample diameter and so should be minimal with the 1.5-mm samples [7].

We report here the design, construction, and initial validation of a novel 1.5-mm NMR probe operating at 14.1 T based on high temperature superconducting (HTS) coils that is optimized for ¹³C detection, but still has outstanding ¹H detection capabilities. An introduction to the technology of microcryogenic HTS NMR probes is available in a recent review [8]. Briefly, the coils are fabricated from a thin (~ 300 nm) layer of superconducting oxide—generally yttrium barium copper oxide (YBCO)—deposited onto a sapphire

substrate. The coils are photolithographically patterned and laser-trimmed to be self-resonant at the desired frequency. Microscopically, each conductor is patterned as a number of thin parallel wires, and closed loops are avoided to reduce persistent current effects in the superconductor, which would degrade B_0 homogeneity. As shown in Figure 1A, a Helmholtz-like pair of coils is mounted in a cryogenic probe body parallel with the B_0 field to produce a uniform B_1 field across the sample. The coils are cooled to around 20 K using a standard Agilent closed-cycle cryogenic system. A coaxial cable terminated into a loop is used to inductively couple energy into the HTS coil pair. Impedance matching is adjusted by varying the position of this loop. A second shorted loop is included, the position of which can be moved to provide fine-tuning of the resonant frequency. Both the loops are fashioned out of susceptibility-compensated wire to minimize their effect on B_0 homogeneity. The coil space is evacuated to provide thermal insulation from the sample, which is placed inside a center tube that is fabricated with RF transparent material. The sample is maintained near room temperature using a standard Agilent heater/thermocouple unit, which regulates the temperature of the airflow through the center tube.

Design goals

The design goals of the project were to achieve the highest possible ^{13}C sensitivity without significantly compromising ^1H sensitivity. We needed a ^2H channel for lock, and we added a fourth set of coils that in the future could be trimmed to add ^{15}N -decoupling capabilities. The coil dimensions were chosen to provide a uniform B_1 field over an active sample length of 18 mm and sample tube inner diameter of 1.2 mm. The overall coil layout shown in cross-section in Figure 1B is dictated by these design goals.

The coil designs were optimized through electromagnetic simulation using the commercial EM solver HyperLynx 3D by Mentor Graphics (formerly I3ED, by Zeland Inc.). Due to the high cost and lengthy lead times involved in HTS coil fabrication, coil features were initially fine-tuned using simulation to accurately predict the resonance frequencies. At NMR frequencies, HTS materials have a finite surface resistance that is dependent on the physical properties of the film, the temperature, the orientation of the static magnetic field, and the frequency [9]. In our simulations the superconductor was modeled as a normal conductor with low surface resistivity. Similarly, we used simulation to predict the change in coil frequency upon laser trimming, enabling us to reduce the number of iterations in this personnel-intensive step. Normalized simulations of candidate coil designs were used to choose designs that produced uniform B_1 fields with low electric fields in the sample region. The ‘method of moments’ technique employed by HyperLynx allows very accurate and rapid simulation of planar conducting structures on one or more parallel dielectric substrates. However, it is not capable of handling arbitrary dielectrics. So for this design process, we neglected interactions between orthogonal coils. We did simulate all four parallel coils in each orientation, and we expect interactions between parallel coils to be much stronger than between orthogonal coils.

^1H HTS coils generally utilize a “racetrack” design, such as shown in Figure 1C, which produces a minimal fringing electrical field [10]. The racetrack coil achieves this by using a number of interdigital capacitors, typically 2 or 4, which are arranged in series and are distributed along the length of the coil. Increasing the number of capacitors N on a racetrack coil without changing the resonance frequency requires increasing the interdigital capacitance per unit length by a factor N^2 (Figure 1D). Voltages around the coil are reduced, leading to smaller electric fields and less sample loading. Smaller interdigital gaps also yield better electric field confinement and hence less sample loading and detuning. The large interdigital capacitance required is achieved by the use of fine feature size for the conducting fingers and gaps between the fingers. The capacitance density may be

maximized by using equal finger and gap widths [11]. However, a finger to gap ratio of approximately 3:1 is generally employed in order to accommodate the high current carrying capacity needed to achieve the required B_1 field. The upper limit on the number of capacitors that may be used in a coil is determined by the availability of high-quality film deposition and the resolution of photolithography processes. The other constraint is the minimum gap width required between the digits of the capacitors to avoid arcing between the fingers. The ^1H coils in this probe have been designed as 4-capacitor racetrack coils (Figures 1C and 1D).

The ^{13}C coils were placed closest to the sample to maximize sensitivity. Therefore, it was important to design a coil that resonates at the relatively low frequency of 150 MHz and produces minimal fringing electric field. Racetrack coil designs are typically used for the innermost detection coils in ^1H optimized probes because of their superior electric field confinement. However, they are not generally used for lower resonance frequency channels because the interdigital capacitance required is too high to achieve in practice. At the dimensions of the ^{13}C coils, and using a finger to gap ratio of 3:1, our simulations predict that the minimum resonance of a 1-capacitor racetrack coil would be approximately 250 MHz. This is not low enough for use in the ^{13}C channel, so it is clear that a different resonant structure will be required. The other structure commonly used is a spiral resonator. Spiral resonators such as shown in Figure 1E are generally used for decoupling coils since they can be conveniently tuned to resonate at low frequencies [8]. These coils are far from the sample and not used for detection. However, spirals generate a large fringing electric field because of the large potential difference developed across the ends of the conductor (Figure 2). This electric field may be neglected for decoupling coils, but for the innermost coil it would degrade the sensitivity due to power dissipation in the sample [7].

In order to reduce the electric field of the inner ^{13}C spiral coils, we utilized an innovative counterwound spiral design [12]. The counterwound design utilizes both sides of a sapphire substrate, with spirals on either side of the substrate having opposite helicity/handedness when viewed from the same remote point. At the operating frequency, the RF current in both the spirals has the same helicity, thus developing opposing voltages across both spirals. Consequently, most of the electric field from the spirals is confined within the substrate between the two spirals. Figure 3 shows simulations of the magnetic and electric fields produced across the sample for both standard spirals (top panels) and counterwound spirals (bottom panels). A counterwound spiral was placed on either side of the sample in a Helmholtz-like arrangement to produce a homogenous B_1 field. Compared to a pair of single spirals, a pair of counterwound spirals produces approximately 10 times lower electric field over the sample volume.

Single-sided spiral designs were used for the ^2H and ^{15}N coils. Since these coils were placed farther from the sample, their fringing electric field is not significant. The single-sided spirals sustain their fundamental resonance mode with a current distribution resembling a standing wave on a vibrating string, as shown in Figure 2B. At this fundamental mode, the spiral has current nulls only at the ends of conductor. These spirals also sustain higher order resonance modes much like the harmonic resonances on a vibrating string. At these higher modes, the current distribution has additional nulls within the length of the conductor with the current changing direction at each of these nulls. Consequently, the magnetic field produced by these modes is nowhere near the uniformity desired for NMR excitation or detection. If the frequency of one of the higher modes is close to the frequency of a different nucleus, the mode will produce undesired interactions. To avoid these interactions, it is required to accurately simulate the higher order resonance modes. Simulations were used to predict the behavior of the fundamental as well as higher order modes as a result of the laser trimming. The ^{15}N channel is still ~2 MHz below the desired target, and another iteration of

laser trimming is necessary before full functionality of the triple-resonance probe can be achieved.

NMR specifications

Table 1 lists performance specifications of the 1.5-mm ^{13}C HTS probe. The ^{13}C signal-to-noise (S/N) was measured to be 396:1 using a sealed standard of 40% dioxane in C_6D_6 (ASTM sealed standard provided by Cambridge Isotope Labs using tubes provided by Norell). ^1H S/N of 490:1 was measured using a sealed standard of 0.1% ethylbenzene in CDCl_3 (Cambridge Isotope Labs/Norell). To compare these values to more standard 5-mm probes, we can scale them by the ratio of total volumes (35 μL for the 1.5-mm and 600 μL for 5-mm sample tubes). This leads to mass normalized S/N values of 6788:1 and 8400:1 for ^{13}C and ^1H , respectively. Alternatively, the S/N values may be scaled by the ratio of active volumes (20 μL for 1.5-mm and 250 μL for 5-mm sample tubes). This leads to mass normalized S/N values of 4950:1 and 6125:1 for ^{13}C and ^1H respectively. Specifications for commercial 500 MHz Agilent probes scaled to 600 MHz are 2860:1 for ^{13}C using a 5-mm XSENS probe and 5400:1 for ^1H using a 5-mm ^1H -optimized cryogenic probe. Similar values are reported by Bruker. Thus, the new 1.5-mm ^{13}C HTS probe reported here has more than 2x the mass sensitivity for ^{13}C than the most sensitive commercial ^{13}C optimized cryogenic probe.

The 90° pulse lengths were 15.5 μs and 11.6 μs for ^{13}C and ^1H , respectively. Pulse length in HTS probes is not a linear function of the applied transmitter voltage. Instead, the coil RF dissipation increases as the coil approaches critical current density [13]. Therefore it is not possible to draw conclusions about receiving performance based on transmitter power levels needed to reach a given pulse length. We operate the probe at relatively low powers to avoid damaging the coils, so we have not tested shorter 90° pulse lengths since these are acceptable for most applications. The B_1 homogeneity values were 86.3% and 94.3% for ^{13}C and ^1H 450/90 measurements, respectively. The ^{13}C homogeneity corresponds well to the simulated value of 85.4%. The ^1H homogeneity was simulated to be 86.9% neglecting the effect of the orthogonal ^{13}C coil. We conclude that the magnetic screening effect from the inner ^{13}C coil improves the homogeneity of the ^1H magnetic field.

NMR Results

The outstanding ^{13}C sensitivity provided by our probe suggests that it can be used for many different types of experiments that require ^{13}C detection, including ones that are not routinely used in today's applications due to limited sensitivity of available NMR instruments. We specifically anticipate two immediate application areas: metabolomics and natural products. When we add ^{15}N decoupling—planned in the near future—the 1.5-mm probe should also provide unique ^{13}C detection capabilities in proteins [3,14].

The excellent ^{13}C sensitivity of this probe with small volume samples makes ^{13}C -based metabolomics studies feasible. Figure 4A shows a 1D ^{13}C spectrum of a mixture of 20 common metabolites dissolved in 40 μL D_2O at concentrations ranging from 1 to 5 mM (40 to 200 nmols) (Table 2). The spectrum was collected for 2 hours with a 60° ^{13}C pulse, 0.8 second acquisition time, and 0.1 second relaxation delay. ^1H decoupling was used for the entire experiment for NOE enhancement. Figure 4B shows the corresponding ^1H spectrum of the same mixture sample and the same probe. Even with the relatively simple mixture shown here, it is clear that the chemical shift dispersion and narrow singlet resonances in the ^{13}C spectrum (Figure 4A) provide a much clearer view of the mixture than the traditionally used ^1H spectrum (Figure 4B). Moreover, a 2 h 1D ^{13}C spectrum is possible with as little as about 40 nmol of material, making analysis of large numbers of samples in a metabolomics workflow feasible.

In the field of natural product studies, a large number of 2D NMR techniques have been developed over the decades to determine the covalent structure of unknown compounds [15]. Natural products analysis is in many ways the same as biomarker discovery in metabolomics [16]. The vast majority of NMR natural product studies utilize ^1H detection with indirect detection of ^{13}C . ^1H detected experiments provide for good sensitivity but have limitations around quaternary carbons. The ^{13}C -HMBC experiment [17], which correlates ^1H to ^{13}C separated by 2 or 3 bonds, can help with this problem, but HMBC can be ambiguous with respect to number of chemical bonds being correlated. Similar ambiguities are even present in dqf-COSY experiments [18], which provide correlations across 2, 3, or sometimes more covalent bonds.

Perhaps the perfect experiment for the identification of organic compounds is the 2D INADEQUATE [19]. This experiment has a double quantum ^{13}C evolution period in t_1 and detects ^{13}C . Thus, it provides a directly bonded carbon correlation network, which can be traced without ambiguity as long as the carbon chain is continuous. The problems with the INADEQUATE experiment are low intrinsic sensitivity of ^{13}C detection combined with a low probability (1 in 8264) of 2 directly-bonded ^{13}C nuclei at natural abundance. These limitations have largely limited the INADEQUATE to academic developments, because it is almost always too insensitive to apply to real unknown compounds.

Figure 5 shows a 2D INADEQUATE spectrum of 1.1 mg of natural abundance histidine dissolved in 35 μL of D_2O (200 mM solution). The spectrum was collected using adiabatic ^{13}C 180° pulses with the Agilent INADEQUATEAD experiment. The acquisition time was 0.134 s using 4096 complex points and a spectral width of 30487.8 Hz, collected with 256 t_1 increments with a double quantum spectral width of 60975.6 Hz, and 108 scans for a total experiment time of 48 h and 24 m. Based on comparisons of sensitivity with a 5-mm XSENS probe at the same field strength, this same experiment would require nearly 2 weeks with the same sample on the most sensitive existing ^{13}C -optimized commercial probes.

Discussion

We have shown that it is possible to significantly improve ^{13}C sensitivity using HTS probe technology. The problems with a large electric field generated by spiral coils are largely overcome by the use of counterwound spirals [12], making it possible to place the ^{13}C coils close to the sample. Our 1.5-mm probe uses a standard vertical loading system that is completely compatible with existing instrumentation with the use of adapter inserts (Norell Inc.) to hold the sample tube in standard 10-mm rotors. The 1.5-mm tubes with a 1.2-mm ID have a total fill volume of about 35 μL , making these convenient for many samples and well matched to chromatographic fractions. Unlike 1-mm tubes that require centrifugation to load high surface tension aqueous samples to the bottom of the tube (Edison, unpublished results), 1.5-mm tubes are easily loaded with a syringe, and aqueous samples can be shaken by hand to the bottom of the tube.

The 1.5-mm ^{13}C HTS probe offers outstanding ^{13}C detection sensitivity, while maintaining good ^1H sensitivity. The $> 2\times$ sensitivity improvement beyond existing commercially available options makes experiments such as ^{13}C 1D metabolomics and 2D INADEQUATE feasible on many samples. The extra resolution afforded by ^{13}C 1D spectra in metabolomics will allow more complex samples to be statistically analyzed and matched to experimental databases. The uniformly narrow singlets in natural abundance ^{13}C spectra simplify analysis and database matching. Using covariance techniques developed by the Brüscheiler laboratory [20], it will be possible to efficiently generate nD correlation matrices with conventional NMR ^1H and ^{13}C detected datasets collected with this probe. Other approaches

such as statistical heterospectroscopy (SHY) [21] could also be used to obtain correlations between 1D ^1H and ^{13}C spectra.

Our 1.5-mm ^{13}C HTS probe also makes the 2D INADEQUATE a practical experiment for unknown organic compounds. With about 7 μmol of sample, our probe can obtain an interpretable INADEQUATE spectrum in 48 hours. The drawback is that the material needs to be soluble in 35 μL of solvent, making this appropriate for mass-limited but not concentration-limited samples.

Despite the outstanding ^{13}C sensitivity performance of our new 1.5-mm probe, some things still are not optimal. Shimming the probe for optimal B_0 homogeneity is not routine, and although we can achieve reasonable ^1H lineshape values (Table 1), the ^1H and ^{13}C lineshapes are not as good as we can achieve with a standard 5-mm non-superconductive probe. As a result, ^1H detected experiments with our 1.5-mm HTS probe are difficult with significant amounts of H_2O .

Although the ^1H S/N is good, it is not great and is less than about half of the mass sensitivity of our previous ^1H optimized 1-mm HTS probe [22]. In the future we will be exploring ways to move the ^1H coils closer to the sample in a ^{13}C -optimized probe in order to try to capture more of the sensitivity that these coils are capable of detecting. We are quite pleased with the ^{13}C sensitivity of this probe but think that there is room for improvement. After construction of the probe, the Q of the ^{13}C channel is only 1300, a value much lower than the Q of almost 10,000 that is possible for HTS coils in probes. This could be a combination of effects due to interactions with all the other coils and loops, and other components in the final probe. Some loss in Q is also expected due to operation in extremely high magnetic fields [9].

In conclusion, we have designed, built, and characterized a 1.5-mm HTS probe that is optimized for ^{13}C detection. The ^{13}C coils required a counterwound spiral design [12] to reduce the large electric field that would result with standard spiral coils. The resulting mass sensitivity ^{13}C S/N of this probe is more than 2x greater than the best currently available commercial ^{13}C optimized probe. As a result, new applications are possible that previously would be unfeasible including natural abundance ^{13}C -based metabolomics on compounds with as little material as 40 nmols and INADEQUATE experiments with as little as 7 μmol of sample. This probe is available to external users through the National High Magnetic Field Laboratory.

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Figure 1.

Overall design of the 1.5-mm ^{13}C -optimized HTS probe. The details and dimensions in this figure are simplified or distorted for illustrative purposes and are not exact reproductions of the design. A) The arrangement of a single pair of HTS coils supported by a coldhead and surrounding the inner tube, into which the sample is loaded. The copper-colored loops on the outside are for inductive coupling and matching (closest) and tuning (in back). B) Cross sectional schematic of coil layout. The sample chamber (light grey) accommodates a 1.5-mm tube and is regulated near room temperature. The coils are maintained under vacuum near 20 K using a standard Agilent cryogenic system (white background). The 4 Helmholtz-like pairs of coils are arranged around the sample as indicated. C) Schematic of a 4-capacitor racetrack design like the ^1H coils used in this probe. D) RF equivalent circuit model using lumped elements for a 4-capacitor racetrack shown in C. The resonance frequency of the model is $f = 1/2\pi \sqrt{LC}$. E) Schematic of a spiral resonator design, which is commonly used for all non- ^1H nuclei.

Figure 2. Spiral coils consist of a single long conductor in multiple turns, and (A) produce a relatively large potential difference across the ends of the spiral. (B) When represented linearly, the current along the length of the conductor at the fundamental resonance frequency is a standing wave with current nulls at the ends of the spirals.

Figure 3. Simulations of radio frequency magnetic (B and E) and electric (C and F) fields generated by a standard spiral resonator (A, B, C) or a counterwound spiral (D, E, F). The scale bar on the right applies to all simulations. The white circles in the middle indicate the location of the sample, and the thin horizontal lines on the top and bottom of each field plot represent the coil locations.

Figure 4.

Mixtures of synthetic metabolites from Table 2 with concentrations ranging from approximately 1 to 5 mM (40–200 nmol). The ^{13}C 1D spectrum (A) was collected in 2 hours using a 60° ^{13}C pulse, an acquisition time of 0.8 sec, a recycle delay time of 0.1 sec, and ^1H decoupling during the entire experiment to build up an NOE. Under these conditions, the slowly relaxing quaternary carbons are attenuated, relative to aliphatic carbons. The ^1H 1D spectrum (B) was collected in 3.5 min using a 90° ^1H pulse, acquisition time of 2.28 sec, a recycle delay of 2 sec, and 32 scans. The insets show expansions of the comparable regions of each spectrum, demonstrating the much greater chemical shift dispersion in the ^{13}C spectrum. Resonances from one of the components (isoleucine at about 3 mM) are indicated.

Figure 5.

^{13}C - ^{13}C INADEQUATE spectrum of 1.1 mg natural abundance ^{13}C histidine. The 1D spectrum on the top is a ^{13}C spectrum collected for 27 minutes under conditions described in Figure 4. The INADEQUATE spectrum was collected for 48 hours using the Agilent “INADQUATEAD” pulse sequence, which utilizes an adiabatic ^{13}C 180 pulse. The dotted lines and circles indicate the correlations in the INADEQUATE spectrum, which allows a complete assignment of the ^{13}C backbone. The traces below are single slices from the maxima of the INADQUATE spectrum.

Table 1

Measurements of 1.5-mm HTS version 1 probe

	^1H	^{13}C	^2H
90° Pulse Length	11.6 μs @ 47dB	15.5 μs @ 45dB	280 μs @ 37dB
B ₁ Homogeneity	94.3% (450/90) 84.4% (810/90)	86.3% (450/90) 61.0% (810/90)	
S/N	490 ^a	396 ^b	
Non-spinning lineshape ^c	0.94/9.1/14.3Hz		

^a0.1% ethylbenzene in CDCl₃ standard. Mean of two measurements each on duplicate samples.

^b40% dioxane in C₆D₆ ASTM standard. Mean of two measurements each on duplicate samples.

^c1% CDCl₃ in acetone-D₆ standard.

Table 2

Composition and approximate concentrations of the mixtures used in Figure 4.

Compound	[mM]
Alanine	1.5
Trehalose	1.0
Phenylalanine	1.0
Malate	2.5
Aspartic Acid	3.0
Asparagine	2.5
Threonine	3.5
Fructose	1.5
Glutamine	2.0
Valine	3.0
Methionine	2.0
Proline	3.0
Glutamate	5.0
Glucose	2.5
Isoleucine	3.0
Arginine	2.0
Nicotinamide	2.0
Carnitine	1.0
Lysine	1.0
Tryptophan	1.5