CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200500215

High Resolution ^{1}H NMR Structural Studies of Sucrose Octaacetate in Supercritical Carbon Dioxide

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Abstract: High pressure (HP), high resolution (HR), proton nuclear magnetic resonance (^{1}H NMR) spectroscopy has been utilized for the first time to investigate the solution structure of a carbohydrate based system, sucrose octaacetate (SOA), in supercritical CO_2. The studies indicate that the average solution state conformation of the α-D-Glucopyranosyl ring of SOA in $scCO_2$ medium is consistent with the $^4C_1$ chair form, while the β-D-fructofuranosyl ring adopts an envelope conformation. The investigations also suggest that $scCO_2$ is a promising medium to study the solution structure and conformation of acetylated sugar systems. Spectral manifestations of a specific interaction between the acetate methyl protons and CO_2 molecules are also presented.

Keywords: carbohydrates · NMR spectroscopy · sucrose · supercritical fluids

Introduction

The use of supercritical carbon dioxide ($scCO_2$) as an alternative solvent for a wide range of applications including biomolecular separations is gaining importance in "green chemistry" since CO_2 is environmentally acceptable, nonflammable, nontoxic, and inexpensive.[1] Supercritical fluids are unique solvents for NMR studies because of their general characteristics that bridge the gap between gas-like and liquid-like properties. Additionally, the solvent properties of supercritical fluids can be tuned widely by adjusting pressure and temperature. The structural and conformational studies of biomolecules in supercritical fluids are of importance not only due to applications in analytical chemistry, but also in the correlation of their pressure-dependent structural and conformational changes to their biological activity. In the macromolecular limit, the transverse relaxation rates and the resonance line widths are directly proportional to the rotational correlation time, and thereby to the viscosity of the medium. The fast molecular tumbling and low relaxation times[2] of molecular systems in supercritical fluids can be of importance in investigating the conformational preferences of biomolecules by NMR spectroscopy, as well as in resolving the spectral overlaps often associated with systems such as glycoconjugates and other polysaccharides. Herein, we present the use of $scCO_2$ as a potential solvent for high resolution NMR studies of carbohydrate-based systems with sucrose octaacetate (abbreviated as SOA, Figure 1) as a model molecular system. The choice of SOA is primarily due to the high solubility of peracetylated sugars in $scCO_2$[3] and also inherent conformational flexibility across the interglycosidic linkage.[4]

High pressure NMR spectroscopic studies can contribute a wealth of fundamental information on the structure and dynamics of molecules in liquids under the extreme parameters of pressure and temperature. Such methods have previ-

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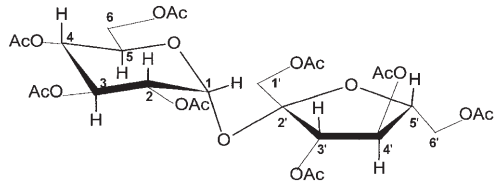

Figure 1. Sucrose octaacetate with carbons labeled. The atoms in the fructose moiety are primed, and those in the glucopyranose moiety are unprimed.

ously been used to study the solubility, media effects, van der Waals interactions, hydrogen bonding, and solute–solvent interactions of small organic molecules dissolved in liquid or supercritical CO_2.[5] In addition, over the past three decades the study of the structure and dynamics of proteins under various pressures has emerged as a promising approach to the mechanism of protein dynamics and folding.[6] Since CO_2 provides an aprotic medium, an added advantage arises due to the absence of any solvent interferences in the ^{1}H NMR spectrum. Additionally, the absence of high salt concentrations, typically associated with buffers in aqueous biomolecular systems, should allow the utilization of next-generation cryogenic probes without the loss in sensitivity due to electrical noise from high conductivity samples. However, biomolecular NMR studies in $scCO_2$ have been limited to the study of molecules dispersed in the water core of water-in-CO_2 reverse microemulsions.[7] To the best of our knowledge no attempt has been made to study carbohydrate-based systems in $scCO_2$ by using high pressure NMR spectroscopy. This is mainly due to the CO_2-phobicity of most of these systems, which are essentially polar. Recently, we demonstrated that it is possible to CO_2-philize polyhydroxy systems such as carbohydrates by substituting the hydrophilic hydroxyl groups with the CO_2-philic acetate groups.[8] We hypothesize that the NMR investigation of acetylated carbohydrate systems in $scCO_2$ will help to resolve several structural issues encountered in studies using conventional solvents. The current work presents preliminary results in that direction.

Results and Discussion

The ^{1}H NMR spectrum of the ring protons of SOA in $scCO_2$ (183 bar, 40.0 °C) by using a two-fold proton NMR capillary cell is shown in Figure 2. With gradient shimming program, 1D spectrum (32 K data points) with digital resolution of 0.189 Hz per point has been achieved without spinning the sample. The spectrum shows well-resolved signals corresponding to H-1, H-2, H-3, and H-4 of the glucopyranose moiety, and H-3′ and H-4′ of the fructofuranose moiety. As expected, the anomeric proton of the glucopyranoside ring (H-1) is the farthest downfield shifted proton and its signal appears as a doublet due to a simple first-order splitting. The H-3′ proton of the fructofuranose ring also appears as a doublet. There was considerable overlap of signals around

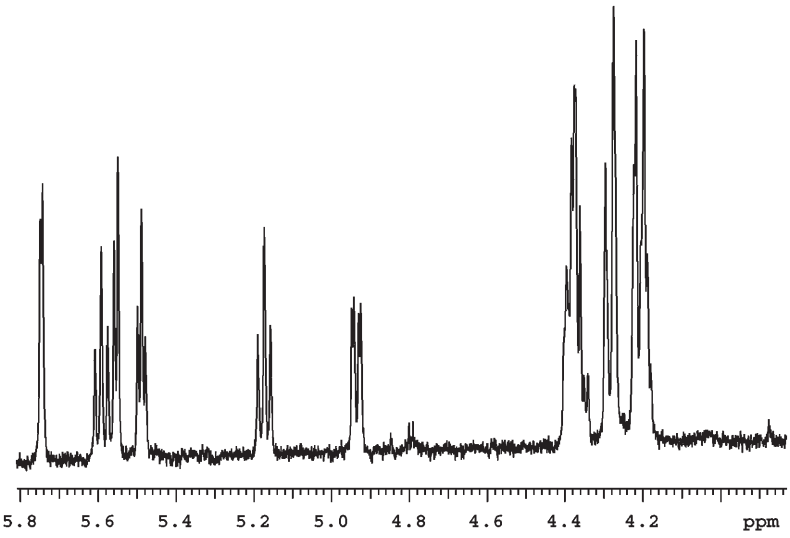

Figure 2. 600 MHz ^{1}H NMR spectrum of SOA in $scCO_2$ (183 bar, 40.0 °C) using a two-fold capillary cell.

4.25 ppm. However, the assignment of these signals were easily established from the gradient selected two-dimensional total correlation spectroscopy (gs-TOCSY)[9a] experiment. The TOCSY experiment is particularly versatile as one can establish complete connectivity among spins in a consecutive network of coupled spins.[9b] The gradient selected techniques use pulsed field gradients (PFGs) to select the desired coherence-transfer pathway which results in higher quality spectra in a much shorter time frame.[9c] The use of gradient enhanced 2D correlation techniques not only offers the above-mentioned advantage, but also markedly improves the intensity of the correlation peaks between sugar protons. This is particularly important when small coupling constants are involved.[9d]

The 600 MHz gs-TOCSY spectrum of SOA in $scCO_2$ is shown in Figure 3. The high sensitivity of the TOCSY technique together with the use of a high field (600 MHz) NMR spectrometer made it possible to record good quality HP NMR spectra with relatively low sample volume. This facilitates complete assignment of ring proton signals of SOA in the $scCO_2$ medium. The chemical shifts of ring protons of SOA in $scCO_2$ thus assigned are given in Table 1. The scalar coupling constants ($^3J_{H,H}$) of the well separated signals are also shown. The $^3J_{H,H}$ coupling constant values (Table 1) obtained demonstrate that in $scCO_2$ solution there

Table 1. 600 MHz ^{1}H NMR chemical shift data for sucrose octaacetate dissolved in $scCO_2$ (183 bar, 40.0 °C). Coupling constant values ($^3J_{H,H}$) of well separated signals are also given.

Proton type	δ [ppm]	Coupled nuclei	$^3J_{H,H}$ [Hz]
H-1	5.746	H-1,H-2	3.50
H-2	4.935	H-2,H-3	10.38
H-3	5.592	H-3,H-4	9.95
H-4	5.173	H-4,H-5	9.97
H-5	4.385		
H-6	4.282, 4.212		
H-1′	4.297, 4.271		
H-3′	5.554	H-3′,H-4′	5.90
H-4′	5.489	H-4′,H-5′	5.99
H-5′	4.374		
H-6′	4.192		

is an axial-equatorial relationship between H-1 and H-2, and *trans*-diaxial relationship between H-2/H-3, H-3/H-4, and H-4/H-5 of the glucopyranosyl ring. The H-2 coupling constants observed (3.50 and 10.38 Hz) are characteristic of an axial proton coupled to a vicinal, axial proton on one side and a vicinal equatorial proton on the other side. The observed coupling constant data suggests that the average solution state conformation of the glucopyranosyl ring of SOA in $scCO_2$ medium is consistent with the $^4C_1$ chair form. The differences in the coupling constants observed for $^3J_{H\text{-}3',H\text{-}4'}$ and $^3J_{H\text{-}4',H\text{-}5'}$ suggests that the fructofuranosyl ring of SOA adopts an envelope conformation. Also, since the J values are relatively higher, the C-3′ is *exo* rather than *endo* (in which case the $^3J_{H\text{-}3',H\text{-}4'}$ and $^3J_{H\text{-}4',H\text{-}5'}$ values are very small). Hence it is apparent that an envelope ($_3E$) conformer with an *exo* C-3′ must be responsible for decreasing the unfavorable interaction between the C-3′-acetate group and the anomeric oxygen of SOA in $scCO_2$ medium.

The acetate region of the ^{1}H NMR spectrum of SOA in $scCO_2$ is shown in Figure 4. Recent high resolution NMR studies of SOA in various conventional solvents[10] showed considerable spectral overlap of the acetate region, particularly in solvents such as $CDCl_3$, CD_3CN, C_6D_5CN and CD_3COCD_3. However, the eight acetate signals of SOA are well separated in the $scCO_2$ medium; this suggests that $scCO_2$ is an excellent solvent to study the solution structure and conformation of acetylated sugars. Nevertheless, unambiguous assignment of the eight acetate signals in $scCO_2$ is not attempted in this study, since this requires the concerted use of other relatively insensitive two dimensional techniques such as heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC). It should be noted that the spectra presented here are recorded by using a two-fold proton NMR capillary. In the future we plan more detailed studies by increasing the number of folds in the capillary or utilizing other types of high pressure cells with larger volume.[5b]

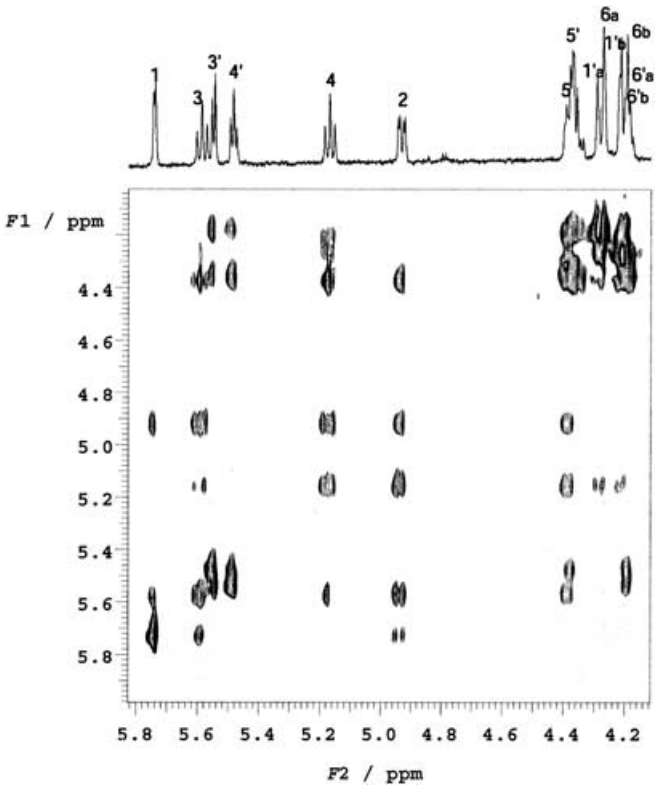

Figure 3. 600 MHz gs-TOCSY spectrum of the sugar ring protons of SOA in $scCO_2$ (183 bar, 40.0 °C). The corresponding 1D spectrum is also shown. The phase sensitive mode spectra were acquired by using MLEV-17 during the 60 ms isotropic mixing period.

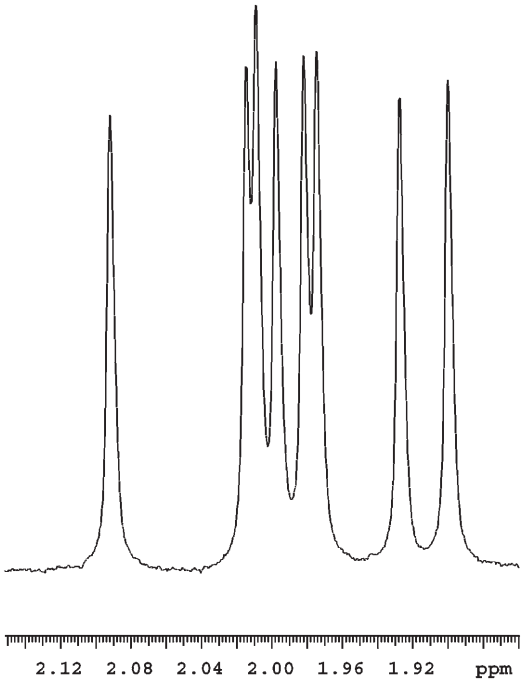

Figure 4. 600 MHz ^{1}H NMR spectra of acetate protons of SOA in $scCO_2$. The eight acetate signals are well separated in $scCO_2$ medium.

Monitoring changes in the ^{1}H NMR chemical shift as a function of decreasing pressure has been reported as a means of determining the phase behavior and density in su-

percritical fluids.[5b,11] It was also demonstrated that in the gas-like and liquid-like states the solvation structure in CO_2 varies rapidly with the bulk density, whereas near the critical point, the solvation structure remains almost unchanged or even presents an apparent increase (density augmentation) in the local solvent density.[5c,d] In order to study the effect of solvent density on the proton chemical shifts in SOA, variation of the chemical shifts of the ring protons of SOA was studied as a function of CO_2 density. Isothermal NMR spectra were first recorded at the highest pressure point. Subsequent data were then collected as a function of decreasing pressure by bleeding the sample through a fused silica restrictor and allowing for pressure and temperature equilibration. The data were collected in this manner to ensure a constant mole fraction of the solute with respect to CO_2. The chemical shift variations of the ring protons of SOA as a function of CO_2 density are presented in Figure 5.

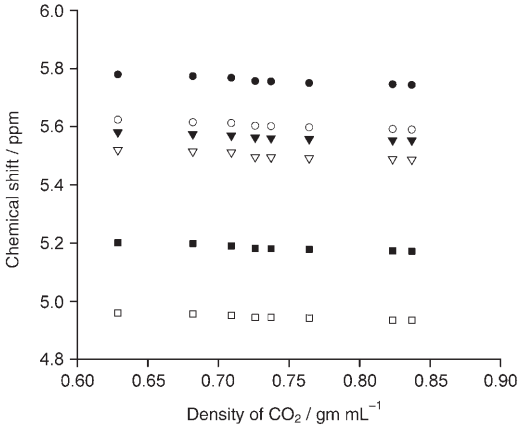

Figure 5. Change of ^{1}H NMR chemical shifts with CO_2 density (CO_2 densities of the solutions were calculated on the basis of NIST[20] data): ● H-1, ○ H-3, ▼ H-3′, ▽ H-4′, ■ H-4, □ H-2.

It is apparent that the chemical shift increases as a function of decreasing density. This trend is commonly observed for a wide variety of NMR active nuclei.[5b,c] In the absence of any specific interaction, a strong linear dependence of the chemical shift on the supercritical fluid density is generally expected. The small deviation from linearity (slope ranging from -0.183 to -0.128 ppm mL g^{-1} and correlation coefficient, R^2, ranging from 0.958 to 0.846) observed within the supercritical fluid regime in Figure 5 may be attributed to specific solute–solvent interactions.

In NMR studies of the solute–solvent interactions, the chemical shifts of the protons located at a periphery of a molecule are expected to be the most sensitive probes for the local solvent effects.[5c,d,11b] In this case, the acetate moieties on the periphery of the sugar ring are the primary sites for solute–solvent interactions.[3] Several previous studies have indicated that as far as the microscopic solvation of molecular systems in $scCO_2$ is concerned, one needs to consider CO_2 as a charge separated molecule with the carbon atom acting as a Lewis acid and the oxygen atoms as Lewis base units.[8,12] Ab initio calculations[8] of CO_2 complexes with simple, model acetate compounds indicated the possibility of a cooperative C-H···O hydrogen-bonding interaction between the methyl proton of the acetate moiety and one of the negatively polarized CO_2 oxygen atoms (in addition to the interaction of the carbonyl group and the carbon atom of CO_2), providing an additional mechanism for explaining the increased CO_2 solubility of acetate-containing compounds.[3,8] In order to gather experimental evidence for the existence of any C-H···O interaction between the methyl proton of the acetate moiety and the negatively polarized CO_2 oxygen atoms, the ^{1}H NMR chemical shift values of acetate protons of SOA (0.05 M) in $scCO_2$ medium were compared with the corresponding values in $CDCl_3$ and CD_3COCD_3 solvents and the data are presented in Table 2. Compared with the chemical shift values in $CDCl_3$ and CD_3COCD_3, the acetate proton signals experienced an upfield shift in $scCO_2$ as illustrated in Table 2 and Figure 6. These results are consistent with the earlier reports on C-H···O hydrogen bonding that CH donor with sp^3 hybridization show a bond contraction and blue shift; whereas a bond stretch and red shift are observed for sp donors.[13]

Table 2. Comparison of chemical shift values (referenced to internal TMS) of the acetate protons of 0.05 M SOA solutions for various solvents.

Solvent	Acetate signals (δ/ppm)
$scCO_2$	2.092, 2.014, 2.009, 1.998, 1.982, 1.975, 1.927, 1.900
CD_3COCD_3	2.177, 2.116, 2.116, 2.110, 2.100, 2.098, 2.045, 2.017
$CDCl_3$	2.161, 2.080, 2.080, 2.076, 2.073, 2.041, 2.016, 1.978

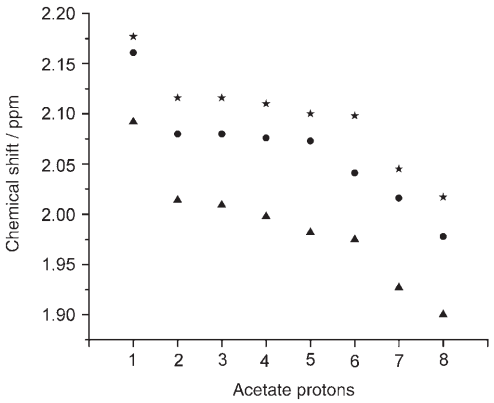

Figure 6. Comparison of ^{1}H NMR chemical shifts of acetate protons in $scCO_2$ (▲) with conventional solvents (● $CDCl_3$ and * CD_3COCD_3).

Despite a growing number of reports, the exact nature of the C-H···O interaction is still controversial.[14] While some IR studies observed the traditional red-shift in the C–H stretching mode, several other studies report a blue-shift. Some recent studies and ab initio calculations also have shown a blue-shift in the C-H stretch frequency, accompanied by a contraction of the C–H bond.[8,14,15] It was demonstrated that interactions involving sp hybridized carbon are similar to O-H···O interactions; this shows a lengthening of

the bond, high dependence of bond strength on intermolecular distance and the classical red-shift of the C–H vibrational mode.[13] However, carbons possessing either sp^2 or sp^3 hybridization show a decreased dependence of bond strength as a function of intermolecular distance. Carbons with an sp^2 hybridization can exhibit small degrees of either bond stretching or contraction, depending on the particular type of electronegative substitution. In the case of sp^3 hybridized carbons, the bond undergoes a contraction, with a subsequent blue-shift of the vibrational mode.

Consistent with the red- or blue-shift in the IR studies and ab initio calculations, corresponding deshielding or shielding of the protons were also reported, which depend on the hybridization of the carbons attached to the protons involved in hydrogen bonding.[16] Recent nuclear Overhauser enhancement spectroscopy (NOESY) studies by Donati and co-workers also provided evidence of strong CH···O bonds in solution.[17] In view of these reports and the data given in Table 2 and Figure 6, it may be plausible that the observed upfield chemical shift values of acetate protons of SOA in $scCO_2$ are spectral manifestations of a specific C-H···O interaction between the sugar acetate protons and CO_2, in agreement with the literature data.[8,18] Also, the fact that the C-H···O bond in $scCO_2$ solution observed at temperatures as high as 40.0 °C implies that this interaction should be of relevance to the solvation of these systems in $scCO_2$.

Conclusion

In this work, we have investigated the solution structure of sucrose octaaetate, as a model system for oligosaccharides, in supercritical CO_2 using high resolution ^1H NMR spectroscopy. To the best of our knowledge, this is the first NMR study of a carbohydrate-based molecular system in $scCO_2$. The individual signals of the ring protons of the ^1H NMR spectrum of SOA in $scCO_2$ medium have been assigned by using gs-TOCSY. The studies indicate that the average solution-state conformation of the glucopyranosyl ring of SOA in $scCO_2$ medium is consistent with the $^4C_1$ chair form, while the fructofuranosyl ring adopts an envelope conformation. Also, a comparison of the spectra in $scCO_2$ with those of conventional solvents suggests that $scCO_2$ is a promising solvent to study the solution structure and conformation of sugar-based systems. Spectral manifestations of specific solute–solvent interaction between the solute protons and CO_2 molecules have also been presented. The present study opens new opportunities to study the solution structure and dynamics of other acetylated polysaccharides and glycoproteins in $scCO_2$, providing new insight into their pressure dependent behavior.

Experimental Section

Materials: Sucrose octaacetate (1,3,4,6-tetra-*O*-acetyl-β-fructofuranosyl-α-D-glucopyranoside tetraacetate) was purchased from Sigma and was used as received. Supercritical fluid extraction (SFE) grade CO_2 (Scott Specialty Gases Inc., 99.999 %) and $CDCl_3$ (Aldrich, 99.98 %) were used as received.

NMR spectra: All NMR experiments were performed on a Varian Inova 600 MHz NMR spectrometer operating at 599.746 MHz for ^1H and equipped with a 5 mm triple-resonance PFG probe (90° ^1H pulse width 6 μs). The ^2H signal of $CDCl_3$ served as the reference for the field-frequency lock. The ^1H chemical shifts were referenced to internal TMS. Standard Varian pulse programs (VNMR 6.1B) were employed throughout. The 1D spectrum was acquired with 32 K data points and a digital resolution of 0.189 Hz per point. All the spectra were collected without spinning the sample. The gs-TOCSY data were acquired with a sweep width of 6000 Hz in both dimensions. 16 transients of 1024 complex points were accumulated for 128 t_1 increments and a relaxation delay of 2 s was used. The phase sensitive mode spectra were acquired using MLEV-17 during the 60 ms isotropic mixing period. The States procedure was followed for frequency discrimination in the indirect dimension. Prior to Fourier transformation, zero filling to 2 K × 2 K complex points was performed, and apodized with a weighted function (Gaussian) in both dimensions.

High-pressure setup: The high pressure setup utilized herein was based on the method of Yonker et al.[19] The high pressure NMR cell consists of a polyimide-coated, fused silica capillary tube with an internal diameter of 100 μm and an external diameter of 360 μm. This H NMR cell was directly connected to our standard high pressure equipment and placed in the triple resonance probe of a 600 MHz Varian NMR instrument. A schematic diagram of the experimental setup is shown in Figure 7.

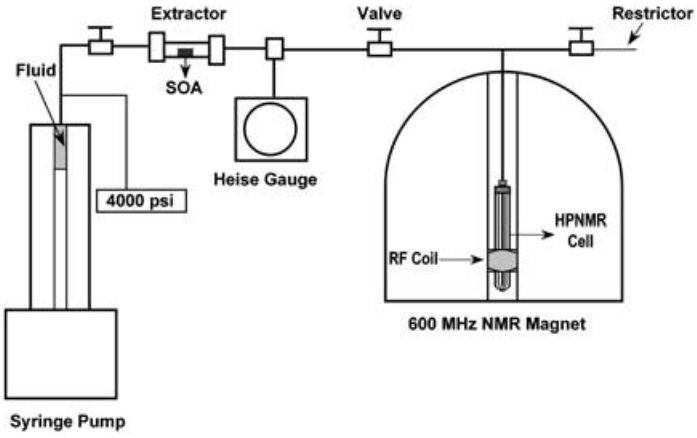

Figure 7. A schematic diagram of the HP NMR set-up.

A syringe pump (Isco 100D) was used to supply CO_2 to the HP NMR cell and to generate the experimental pressure which was measured by a Bourdon tube gauge (Heise Inc.). The two-fold capillary fits into a regular 5 mm NMR tube containing $CDCl_3$, which was used as an external lock solvent. One end of the capillary was connected to a commercial PEEK extraction vessel (15 cm length × 7.13 cm diameter, Upchurch Scientific) by using standard high pressure valves and tubings (High Pressure Equipment, HIP) and the other end was connected downstream to a fused silica restrictor with an internal diameter of 10 or 15 μm. This is important to ensure that as the carbohydrate solution was flowing through the system, the pressure drop occurred in the restrictor. This was verified with this setup through the use of a downstream pressure gauge.

The sample (0.250 g, [SOA] = 0.05 M) was first placed in the extractor, mixed with CO_2, and stirred with a magnetic stir bar. Subsequently, the system was pressurized to 207 bar and the temperature was slowly raised to 40.0 °C. Mixing and dissolution take place inside the extractor and the solution was then transferred to the capillary tube that acts as the high pressure NMR cell. The concentration was chosen by performing phase

stability studies in a view cell prior to the NMR experiments. Air in the capillary was flushed out initially by opening the valve at the end of the capillary and flushing with CO_2. The volume of the capillary tubing is essentially negligible relative to the volume of the high pressure setup.

Acknowledgements

The material presented here is based on work supported in part by the STC program of the National Science Foundation under agreement No.CHE-9876674, Merck & Co and Varian NMR. We also thank Prof. S. Subramanian (NIH) for valuable discussions.

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Received: February 25, 2005
Published online: August 3, 2005

