Journal of Magnetic Resonance 198 (2009) 209–214

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Stable isotope-enhanced two- and three-dimensional diffusion ordered ^{13}C NMR spectroscopy (SIE-DOSY ^{13}C NMR) ☆

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ARTICLE INFO

Article history:
Received 22 December 2008
Revised 20 February 2009
Available online 25 February 2009

Keywords:
Stable isotope-enhanced diffusion-ordered spectroscopy
DOSY
Peracetylation
Diffusion
Carbohydrate

ABSTRACT

The feasibility of obtaining high quality homonuclear or heteronuclear diffusion-ordered ^{13}C NMR data is shown to be greatly improved by using ^{13}C isotopically-enriched samples. Stable isotope-enhanced diffusion ordered (SIE-DOSY) ^{13}C NMR has been applied to ^{13}C-enriched carbohydrates, and has been used to determine diffusion coefficients for pentose and hexose monosaccharides, and a disaccharide and trisaccharide. These 2D spectra were obtained with as little as 8 min of acquisition time. Fully resolved 3D DOSY-HMQC NMR spectra of [U-^{13}C]xylose, [U-^{13}C]glucose, and [1-$^{13}C^{gal}$]lactose were obtained in 5 h. Sample derivatization with [*carbonyl*-^{13}C]acetate (peracetylation) extends the usefulness of the technique to included non-labeled sugars; the ^{13}C-carbonyl – carbohydrate ring proton ^{1}H–^{13}C correlations also provide additional structural information, as shown for the 3-D DOSY-HMQC analysis of a mixture of maltotriose and lactose per-[*carbonyl*-^{13}C]acetates.

1. Introduction

Diffusion-ordered NMR spectroscopy (DOSY NMR) is a powerful but as yet under-utilized NMR technique for the resolution of component molecules based on differences in translational diffusion rates [1,2]. Individual diffusion constants are derived for molecules with differing hydrodynamic radii that may arise as a consequence of molecular mass, complexation or even as a consequence of conformational variation [3–5]. Diffusion rates are calculated from the exponential decay of the NMR signal due to the molecular motion between sets of gradient pulses. Typically, a conventional spectrum is obtained with chemical shift in one dimension, resolved by diffusion constant (expressed as log ($D/m^2\ s^{-1}$)) in the second dimension [3]. This approach has been successfully applied to the analysis of a variety of systems including glycans in glycoproteins [6], oligosaccharide mixtures of various degrees of polymerization [7,8], proteins [9], and for quantitative screening of ligand-substrate interactions [10,11].

Because of its intrinsic sensitivity, to date ^{1}H has been the nucleus of choice for the DOSY experiment. In the analysis of mixtures of structurally related compounds, such as molecules of biological interest, the overlap of signals in the ^{1}H NMR domain results in apparent averaging of the diffusion constants. The combination of DOSY with homo- (COSY, TOCSY) and heteronuclear (HMQC) two-dimensional NMR experiments to give a three-dimensional spectrum greatly enhances the versatility of the technique [12–14], but suffers from two difficulties. First, the addition of a third dimension necessitates excessively long experimental times to give usable resolution. To some extent this can be mitigated by the spectral aliasing technique of Vitorge and Jeannerat [15]. Second, for sensitivity reasons, DOSY NMR methods based on proton nuclei have been generally preferable, limiting these methods to ^{1}H-detected spectra.

In many respects, because of the narrow peak widths and large chemical shift range and, hence, improved spectral resolution, ^{13}C is an attractive nucleus for DOSY NMR. Other nuclei, including ^{19}F, ^{31}P, and even ^{195}Pt, have been used [16–18], but there are as yet very few reports of DOSY ^{13}C NMR [19]. We presume this is because of the poor sensitivity of ^{13}C NMR due to the low abundance of the ^{13}C isotope in natural carbon-based samples, along with ^{13}C's weak sensitivity relative to ^{1}H NMR. ^{13}C-enriched samples are often used to increase the sensitivity of the ^{13}C NMR experiment, leading to the idea that isotopic enrichment might also enable us to use the desirable properties of ^{13}C to obtain DOSY ^{13}C NMR spectra in a timely manner. This report describes how the components in a mixture of ^{13}C-enriched sugars can be characterized

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doi:10.1016/j.jmr.2009.02.008

by DOSY ^{13}C NMR. 3D spectra were obtained in 5 h and 2D spectra in 8 min. For those samples not amenable to enrichment by growth on a ^{13}C-labeled substrate, derivatization of sugars with ^{13}C-labeled acetate is shown to provide similar utility.

2. Experimental

All NMR experiments were performed on a Bruker Avance spectrometer (Bruker BioSpin Corp., Billerica, MA) operating at 500.11 Mhz using a standard 5 mm z-gradient BBI probe at 27 °C. Chemical shifts are reported as ppm from tetramethylsilane calculated from the lock solvent. The deuterated solvents used were obtained from Cambridge Isotope Labs (Andover, MA). The sugars were obtained from Omicron Biochemicals, Inc. (South Bend, IN) and dissolved at a concentration of 10 mg/mL.

2.1. Diffusion experiments

The pulse sequences used were Bruker programs ledbpgp2s and stebpgpin1s for ^{1}H-detected and ^{13}C-detected DOSY respectively, and the 3D heteronuclear sequence DOSY-HMQC [13]. Adiabatic decoupling (Bruker sequence p5m4sp180 with smoothed chirp pulse) was used to reduce convection caused by sample heating in the 3D experiments. The gradient strength was incremented in (typically) 16 steps from 2% to 95% of the maximum gradient strength. Diffusion times were varied between 30 and 150 ms and gradient pulse lengths ranged from 1.7 to 6 ms in order to attenuate the NMR signals of interest to ~10% of original intensity. All processing was done with the Bruker TOPSPIN software package (version 1.3).

2.2. Isotopic labeling with [carbonyl-^{13}C]acetate

Carbohydrate samples (10 mg) were peracetylated in a 2:1 (vol/vol) mixture of trifluoroacetic anhydride/glacial [*carbonyl*-^{13}C] acetic acid (200:100 μL) at room temperature (25 °C) for 30 min [20,21]. The completeness of the reaction was monitored by MALDI-TOF mass spectrometry. The sample generally dissolved within 5 min and, at the end of the reaction time the solution was evaporated to dryness. The acetylated product was dissolved in $CDCl_3$/DMSO-d_6 (20/80 v/v) for NMR spectroscopy.

3. Results and discussion

A comparison of the DOSY NMR spectra obtained on representative hexose sugars (the aldose glucose and the 2-ketose fructose) is shown in Fig. 1. The ^{1}H NMR spectrum (not shown) of unlabeled D-glucopyranose in deuterated water is characterized by alpha- and beta-anomeric proton resonances at 5.15 ppm ($J_{1\alpha,2\alpha}$ = 4 Hz) and 4.55 ppm ($J_{1\beta,2\beta}$ = 8 Hz) respectively, and pyranosyl ring protons between 3 and 4 ppm. For the DOSY ^{1}H NMR spectrum of universally-^{13}C-labeled [U-^{13}C]glucopyranose, these resonances are split by ^{1}H–^{13}C coupling (~170 Hz) to adjacent ^{13}C nuclei. Hence, the alpha proton doublet is observed at 5.00 and 5.35 ppm, and the beta proton doublet at 4.40 and 4.75 ppm. This ^{1}H–^{13}C coupling, and the sugar ring protons, can be seen in the DOSY spectrum (Fig. 1, panel A) to give a diffusion coefficient (log (D/m^2 s^{-1})) of −9.21 (Fig. 1, panel A). A small signal due to residual water is apparent at 4.65 ppm, with log (D/m^2 s^{-1}) = −8.67. Comparable results were obtained for the ^{1}H DOSY of [1-^{13}C]glucose (Supplemental Fig. S1, panel A) and assigned log (D/m^2 s^{-1}) = −9.23 (Table 1).

The SIE-DOSY[1] ^{13}C NMR spectrum of [U-^{13}C]glucose shown in Fig. 1, panel B, was obtained with just 11 min of acquisition time. The expected ^{13}C resonances for the alpha and beta-anomeric carbons are evident at 92.1 and 95.9 ppm, respectively, and are split by ^{13}C–^{13}C coupling to the ^{13}C-labeled C-2′s ($J_{1,2}$ = 46 Hz). Other signals, due to labeled glucose ring carbons, are evident at 61 ppm (C-6, methylenes) and 70–78 ppm (C-2 to C-5, methines). Comparable with the ^{1}H DOSY result, the ^{13}C DOSY data extrapolated to log (D/m^2 s^{-1}) = −9.24 (Fig. 1, panel B). The ring carbon resonances and ^{13}C–^{13}C coupling are absent for selectively labeled [1-^{13}C]glucose, but there was sufficient spectral information to assign the diffusion coefficient (log (D/m^2 s^{-1})) as −9.23 (Supplemental Fig. S1, panel B; Table 1).

The data for the ^{13}C-labeled ketose sugar, [1-^{13}C]fructose, is shown in the lower panels of Fig. 1. Fructose has no anomeric proton signal because the anomeric carbon is quaternary. Hence, all of the proton resonances for fructose are in the range 3.3–4.1 ppm (Fig. 1, panel C), where they overlap with the ring proton signals for aldose sugars such as glucose. Although the diffusion coefficient for fructose is apparent from the ^{1}H DOSY spectrum (log (D/m^2 s^{-1}) = −9.21, Fig. 1, panel C), without a unique anomeric signal, this overlap of the ring proton signals limits the usefulness of DOSY ^{1}H NMR for resolving fructose in carbohydrate mixtures. In the SIE-DOSY ^{13}C NMR experiment, the isotopically-labeled –CH_2OH group at C-1 is apparent from resonances at 62.7, 62.9 (minor), and 63.9 ppm, and these signals were clearly resolved in the ^{13}C DOSY spectrum (Fig. 1, panel D). Extrapolation of these ^{13}C signals in the diffusion domain assigned the diffusion coefficient log (D/m^2 s^{-1}) for fructose as −9.22 (Fig. 1, panel D), comparable to that estimated from the ^{1}H DOSY (Table 1).

Because fructose and glucose are both hexose sugars with equivalent molecular masses, similar diffusion coefficients are expected and the results are shown in Table 1. A disaccharide such as lactose, Gal-β-(1 → 4)-Glc, has a slower diffusion, as evident from the ^{1}H DOSY spectrum (Fig. 2, panel A); the diffusion coefficient log (D/m^2 s^{-1}) obtained in this spectrum for [1-$^{13}C^{gal}$]lactose is −9.35. In the ^{13}C DOSY experiment, the single [1-^{13}C]-labeled beta-anomeric carbon of the galactose residue is observed at 103.0 ppm (Fig. 2, panel B), and is well resolved from the anomeric carbons for the monosaccharides, glucose or xylose. The diffusion constant obtained from this ^{13}C DOSY data correlates well with that from the comparable ^{1}H DOSY experiment (Table 1).

We chose to examine the DOSY ^{1}H NMR spectrum and the SIE-DOSY ^{13}C NMR spectrum of a 5-carbon pentose sugar, D-xylose, the relative molecular mass of which differs from that of glucose and fructose by just 30 mass units. The ^{1}H DOSY spectrum for universally-labeled [U-^{13}C]xylose is shown in Fig. 2, panel C, and the comparable ^{13}C DOSY spectrum is shown in Fig. 2, panel D. As with the U-^{13}C-labeled glucose, the xylose alpha- and beta-anomeric proton signals are further split by ^{1}H–^{13}C coupling and, in this case, one of the beta signals is partially overlapped by the residual water peak. However, the xylose and water signals are well resolved in the diffusion domain to reveal the anomeric signals at 4.35 and 4.65 ppm (H-1, beta) and 4.96 and 5.30 ppm (H-1, alpha), and the diffusion coefficient log (D/m^2 s^{-1}) = −9.18. In the SIE-DOSY ^{13}C NMR experiment on [U-^{13}C]xylose the anomeric carbon resonances were sufficiently resolved to observe ^{13}C–^{13}C coupling (Fig. 2, panel D), as was also observed for the U-labeled glucose experiment (Fig. 1, panel B). The diffusion ^{13}C NMR spectra are therefore obtained rapidly but without significant degradation of the spectral quality. Noticeably two of the ^{13}C signals, at 65.5 and 94.0 ppm, were specific to [U-^{13}C]xylose and do not overlap the ^{13}C signals from either glucose or lactose. The diffusion coefficient for [U-^{13}C]xylose was extrapolated from this data (log (D/m^2 s^{-1}) = −9.20) and compares favorably with that obtained from the ^{1}H DOSY experiment (Table 1).

[1] *Abbeviations used:* SIE-DOSY, stable isotope-enhanced diffusion-ordered spectroscopy; DOSY, diffusion-ordered spectroscopy; HMQC, heteronuclear multiple quantum correlation.

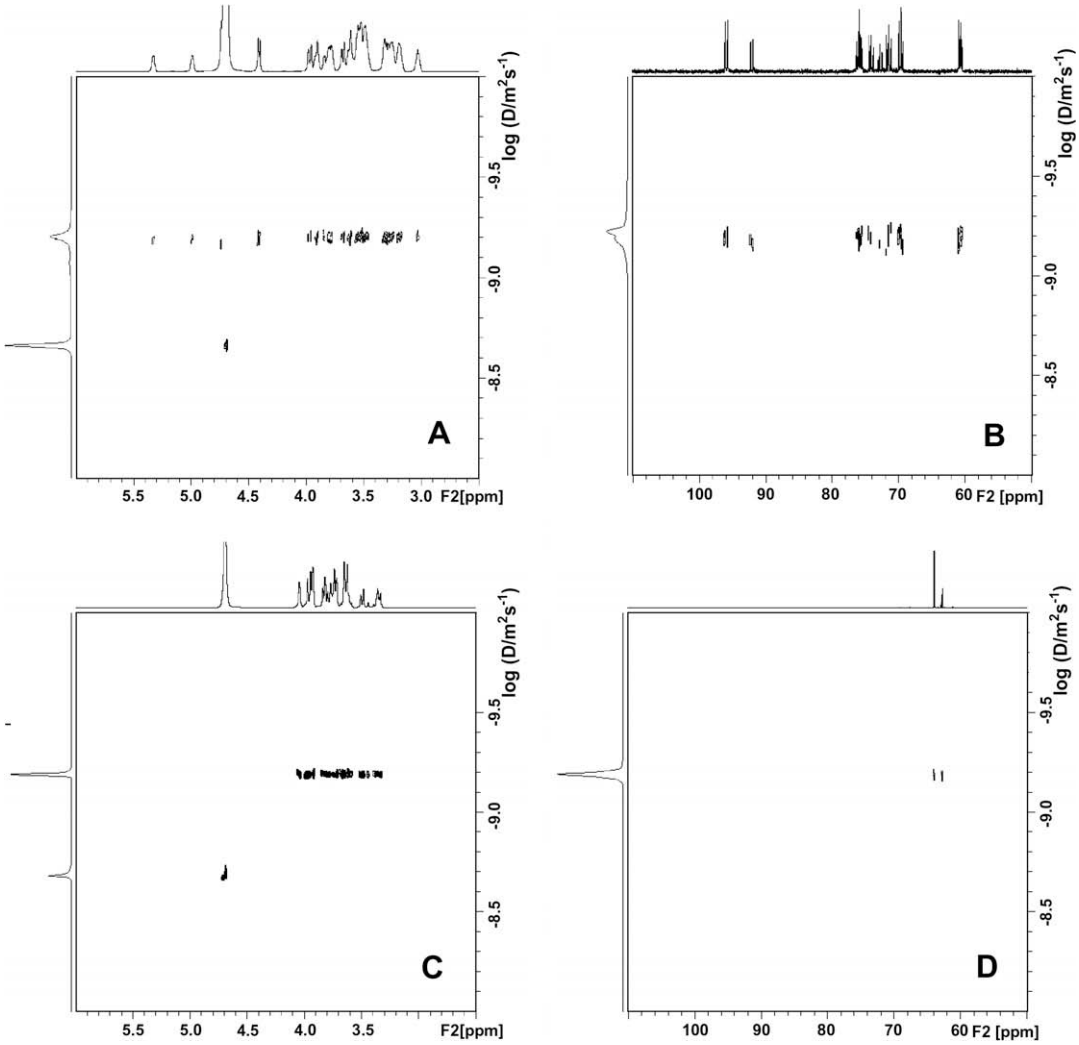

Fig. 1. DOSY NMR spectra of isotopically-enriched hexose sugars: aldohexose D-[U-^{13}C]glucose (TOP) and ketohexose D-[1-^{13}C]fructose (LOWER). (A) and (C) show DOSY ^{1}H NMR spectra, and (B) and (D) are DOSY ^{13}C NMR spectra. Chemical shifts (ppm) are on the *x*-axis and diffusion data (log (D/m^2 s^{-1})) on the *y*-axis. Comparable data for anomerically-labeled D-[1-^{13}C]glucose are included as on-line Supplementary Fig. S1.

Table 1
Compiled diffusion coefficients obtained from DOSY ^{1}H NMR and corresponding stable isotope-enhanced DOSY ^{13}C NMR spectra.

[a]Carbohydrate	Nuclei	[b]log (D/m^2 s^{-1}) (water)	log (D/m^2 s^{-1}) (sugars)
D-[1-^{13}C]glucose	^{1}H	−8.688	−9.230
	^{13}C		−9.234
D-[U-^{13}C]glucose	^{1}H	−8.670	−9.212
	^{13}C		−9.237
	^{1}H/^{13}C		−9.215[d]
D-[1-^{13}C]fructose	^{1}H	−8.686	−9.209
	^{13}C		−9.222
D-[1-^{13}C^{gal}]lactose	^{1}H	−8.685	−9.350
	^{13}C		−9.378
	^{1}H/^{13}C		−9.330[d]
D-[U-^{13}C]xylose	^{1}H	−8.691	−9.182
	^{13}C		−9.203
	^{1}H/^{13}C		−9.160[d]
maltotriose per-[*carbonyl*-^{13}C]acetate	^{1}H/^{13}C		−9.765[c]
lactose per-[*carbonyl*-^{13}C]acetate	^{1}H/^{13}C		−9.694[c]

[a] Concentrations are 10 mg/mL.
[b] Diffusion coefficient (D) has units of m^2 s. Log (D/m^2 s^{-1}) is dimensionless.
[c] log (D/m^2 s^{-1}) values for solutions in $CDCl_3$/DMSO-d_6.
[d] Taken from 3D DOSY-HMQC data.

We sought to resolve a mixture of structurally similar carbohydrates containing isotopically-labeled [U-^{13}C]xylose, [U-^{13}C]glucose and [1-^{13}C^{gal}]lactose using stable isotope-enhanced 3D DOSY-HMQC NMR. Two-dimensional planes in the diffusion domain from the 3D DOSY-HMQC spectrum are shown in Fig. 3. DOSY-HMQC has been used previously for the analysis of complex mixtures [13], but suffers from low sensitivity and associated long acquisition times for non-enriched samples. By contrast, the 3D DOSY spectrum shown in Fig. 3 was obtained in about 5 h of acquisition time. The alpha- and beta-anomeric signals are attributed to the [U-^{13}C]xylose and [U-^{13}C]glucose, and the majority of sugar ring proton signals are comparable to those obtained in a simple 2D-HMQC experiment (Fig. 3, Panels B1–B4). For [U-^{13}C]xylose, the anomeric signals are apparent at 4.5/96.3 ppm (beta) and 5.1/91.1 ppm (alpha), with six other signals observed in the 3.0–4.0 ppm region (Fig. 3, Panel A2). Comparable data were apparent for the [U-^{13}C]glucose (Fig. 3, Panel A3) and, as expected, one signal at 4.4/103.0 ppm is attributed to the isotopically-enriched H^{gal}-1/C^{gal}-1 for [1-^{13}C^{gal}]lactose (Fig. 3, Panel A4). All of these signals were also discernible from the spectra summed over the diffusion dimension (Fig. 3, Panel A1). Diffusion coefficients were obtained for the component sugars and were compatible with ^{1}H and ^{13}C DOSY data (Table 1.)

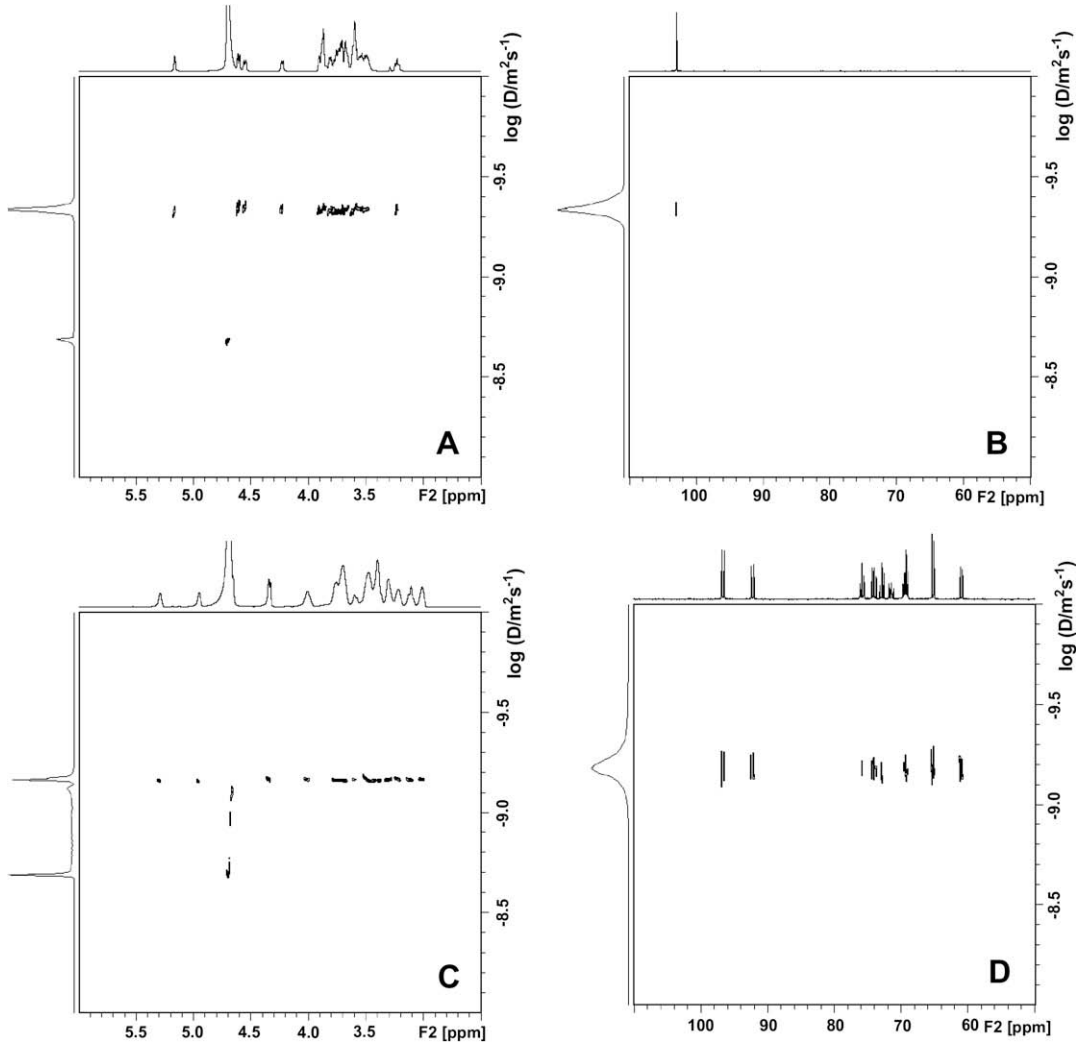

Fig. 2. DOSY NMR spectra of isotopically-enriched disaccharide, D-[1-$^{13}C^{gal}$]lactose (TOP), and aldopentose D-[U-^{13}C]xylose (LOWER). (A and C) show DOSY ^{1}H NMR spectra. (B and D) are DOSY ^{13}C NMR spectra. Chemical shifts (ppm) are on the *x*-axis and diffusion data (log (D/m^2 s^{-1})) on the *y*-axis.

The preceding experiments show the advantages of using ^{13}C isotope-enrichment for 2-D and 3-D DOSY ^{13}C NMR which might find use in applications such as metabolic labeling with stable isotopes. Because ^{13}C-labeled sugars are not always available, we reasoned that it would be beneficial to prepare labeled carbohydrate derivatives using ^{13}C-enriched derivatizing reagents. Fully acetylated carbohydrate esters (peracetylation) are in general use for sugar analysis [21] and the carbonyl carbon chemical shifts of per-[*carbonyl*-^{13}C]acetylated oligosaccharides are sensitive to carbohydrate structure [22,23]. Previously, Bendiak used the ^{1}H–^{13}C splittings to determine linkage substitution of per-[*carbonyl*-^{13}C]acetylated oligosaccharides by COSY and HMBC [22–24], but did not utilize diffusion NMR. We made use of a novel mixed anhydride method using economical [1-^{13}C]acetic acid to prepare fully ^{13}C-acetylated derivatives of maltotriose, lactose and xylose [20,21]. The completeness of the peracetylation was assessed by MALDI-TOF mass spectrometry and the isotopically-labeled derivatives were sufficiently pure for DOSY NMR without the need for chromatography. Prior carbonyl-^{13}C peracetylations have involved using [*carbonyl*-^{13}C]-labeled acetic anhydride in excess pyridine [22], which is inefficient in terms of ^{13}C-acetylation equivalents, or [1-^{13}C]acetic-pivalic anhydride in dry pyridine which resulted in extraneous resonances due to residual labeling reagent [25].

The 3-D DOSY-HMQC spectrum and corresponding 2-D HMQC for a single component per-[*carbonyl*-^{13}C]acetylated trisaccharide (maltotriose) is shown in Fig. 4. Carbohydrate ring protons showing two-bond coupling to the acetyl ^{13}C-enriched carbonyls were observed in the region 3.8–6.2 ppm. The resolution of carbonyl carbon resonances was satisfactory and observed at 168.8–170.7 ppm (Fig. 4 and Supplemental Fig. S2). The more intense signals are due to spectral overlap of the three glucose residues. Assuming overlap at C-2, C-3 and C-6, five ^{13}C-enhanced resonances are expected for each anomeric form of maltotriose. Good agreement was observed between the 2-D slice through the diffusion domain of the DOSY-HMQC spectrum (Fig. 4, panel A) and the HMQC spectrum (Fig. 4, panel B), except for the signals at 5.25/169.8 ppm. Literature values assign these as due to the α-3 CH nuclei [26]. Two-bond couplings from the carbonyl-^{13}C's to the acetyl methyl protons were apparent in the 2 ppm region, but were insufficiently resolved to provide further structural information.

The 3-D DOSY-HMQC for a mixture of maltotriose and lactose per-[*carbonyl*-^{13}C]acetates is shown as 2-D slices in Supplemental Fig. S2. Maltotriose per-[*carbonyl*-^{13}C]acetate was apparent in spectral slices 44–56, with median slice 48 corresponding to log (D/m^2 s^{-1}) = −9.765. The alpha-anomeric proton is assigned at 6.10 ppm, with signals due to the per-[*carbonyl*-^{13}C]acetylated maltotriose ring protons between 3.9 and 5.1 ppm (Fig. 4, panel

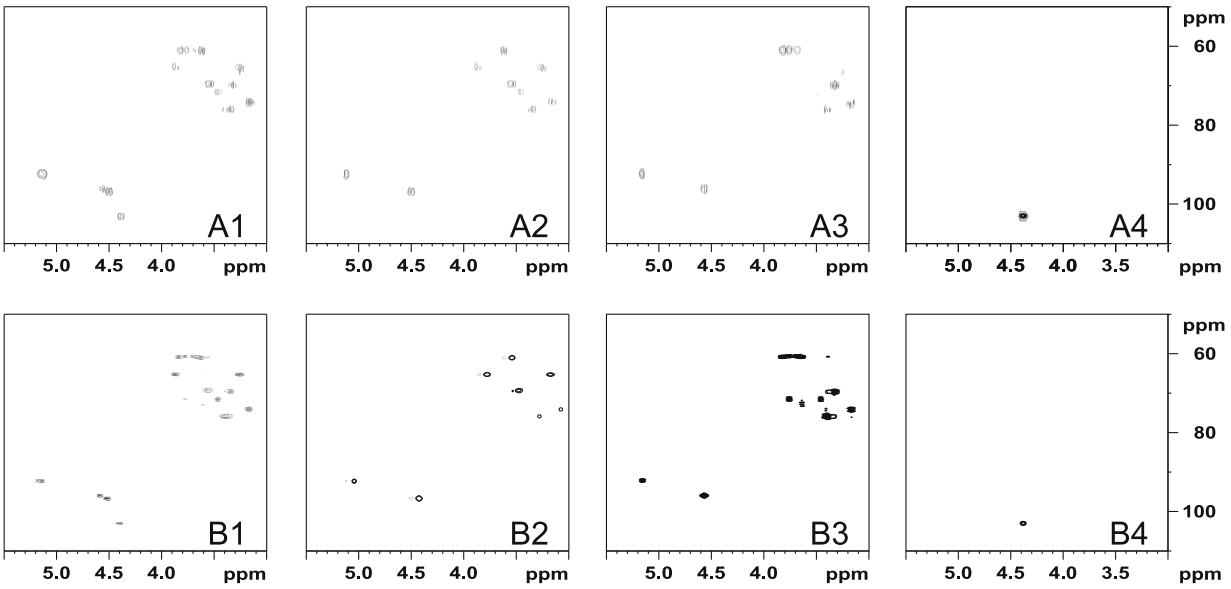

Fig. 3. Resolved planes from a 3-D DOSY-HMQC spectrum of a mixture containing D-[U-^{13}C]xylose (panel A2, log (D/m^2 s^{-1}) = −9.13 to −9.19), D-[U-^{13}C]glucose (panel A3, log (D/m^2 s^{-1}) = −9.20 to −9.23), and D-[1-$^{13}C^{gal}$]lactose (panel A4, log (D/m^2 s^{-1}) = −9.31 to −9.35). The summed projections of the diffusion dimension are shown in panel A1 (log (D/m^2 s^{-1}) = −9.00 to −9.50). The lower B panels are the corresponding HMQC spectra for the mixture (panel B1), and individual component sugars; D-[U-^{13}C]xylose (panel B2), D-[U-^{13}C]glucose (panel B3), and D-[1-$^{13}C^{gal}$]lactose (panel B4).

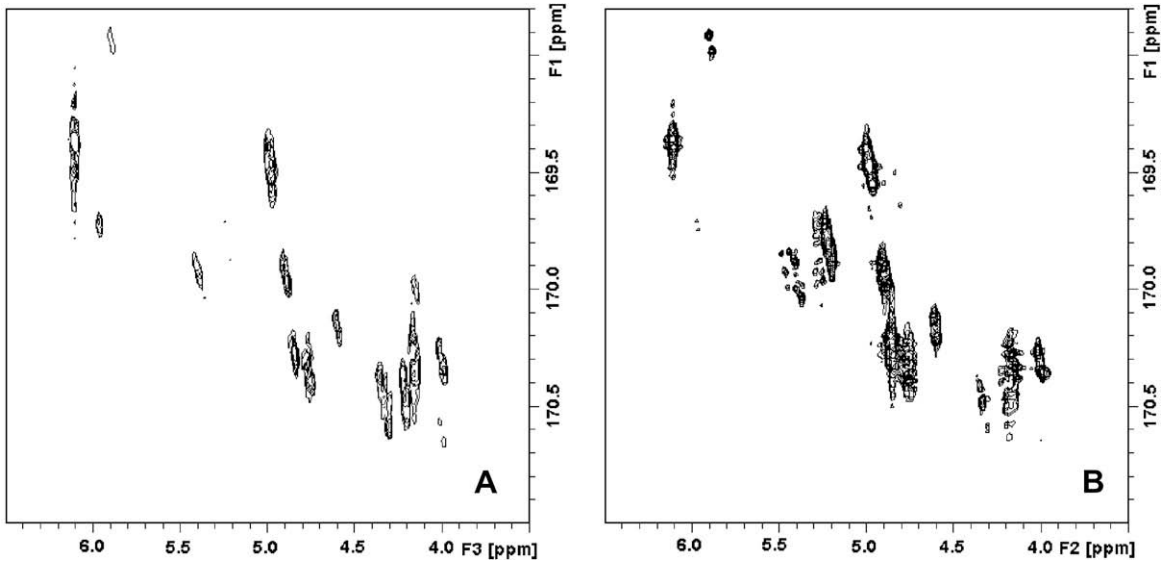

Fig. 4. Stable isotope-enhanced DOSY NMR spectra of per-[*carbonyl*-^{13}C]acetyl-derivatized maltotriose. Panel A: Two-dimensional slice from the 3-D DOSY-HMQC spectrum; panel B: 2-D HMQC spectrum.

A). The alpha-anomeric proton for lactose per-[*carbonyl*-^{13}C]acetate overlapped with the equivalent maltotriose signal, with the beta-anomeric at 5.95 ppm and ring sugar resonances at 4.0–5.2 ppm (Fig. 4, panel B). In the diffusion domain the lactose per-[*carbonyl*-^{13}C]acetate was apparent in spectral slices 26–43, with median slice 35 corresponding to log (D/m^2 s^{-1}) = −9.694 (Table 1). These latter experiments demonstrate the feasibility of heteronuclear 3D DOSY NMR enabled by isotopic enhancement with ^{13}C-labeled carbohydrate derivatives.

In conclusion, we have demonstrated a novel method (SIE-DOSY ^{13}C NMR) for the rapid analysis of mixtures by diffusion NMR spectroscopy that incorporates the resolution advantage of ^{13}C acquisition. The problem of long acquisition time arising from samples with low (natural) isotopic abundance are overcome with a simple, efficient peracetylation using [*carbonyl*-^{13}C] acetic acid. It is anticipated that the techniques described will be of value for diffusion studies such as molecular size estimations, ion pairing, ligand-protein binding, complexation and micelle formation.

Acknowledgments

We thank our colleagues, Drs. Joe Rich, Mark Berhow, and Terry Isbell, for careful reading of the manuscript prior to submission.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmr.2009.02.008.

References

[1] K.F. Morris, C.S. Johnson Jr., Diffusion-ordered two-dimensional nuclear magnetic resonance spectroscopy, J. Am. Chem. Soc. 114 (1992) 3139–3141.
[2] Y. Cohen, L. Avram, L. Frish, Diffusion NMR spectroscopy in supramolecular and combinatorial chemistry: an old parameter – new insights, Angew. Chem. Int. Ed. Engl. 44 (2005) 520–554.
[3] C.S. Johnson Jr., Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications, Prog. Nucl. Magn. Reson. Spectrosc. 34 (1999) 203–256.
[4] K.F. Morris, C.S. Johnson Jr., Resolution of discrete and continuous molecular size distributions by means of diffusion-ordered 2D NMR spectroscopy, J. Am. Chem. Soc. 115 (1993) 4291–4299.
[5] T.S. Derrick, C.K. Larive, Use of PFG-NMR for mixture analysis: measurement of diffusion coefficients of cis and trans isomers of proline-containing peptides, Appl. Spectrosc. 53 (1999) 1595–1600.
[6] K. Ortner, V.N. Sivanandam, W. Buchberger, N. Muller, Analysis of glycans in glycoproteins by diffusion-ordered nuclear magnetic resonance spectroscopy, Anal. Bioanal. Chem. 388 (2007) 173–177.
[7] P. Groves, M.O. Rasmussen, M.D. Molero, E. Samain, F.J. Cañada, H. Driguez, J. Jiménez-Barbero, Diffusion ordered spectroscopy as a complement to size exclusion chromatography in oligosaccharide analysis, Glycobiology 14 (2004) 451–456.
[8] M.D. Díaz, S. Berger, Studies of the complexation of sugars by diffusion-ordered NMR spectroscopy, Carbohydr. Res. 329 (2000) 1–5.
[9] P. Groves, M. Palczewska, M. Dolores Molero, G. Batta, F. Javier Cañada, J. Jiménez-Barbero,, Protein molecular weight standards can compensate systematic errors in diffusion-ordered spectroscopy, Anal. Biochem. 331 (2004) (2004) 395–397.
[10] C. Anselmi, F. Bernardi, M. Centini, E. Gaggelli, N. Gaggelli, D. Valensin, G. Valensin, Interaction of ferulic acid derivatives with human erythrocytes monitored by pulse field gradient NMR diffusion and NMR relaxation studies, Chem. Phys. Lipids 134 (2005) 109–117.
[11] K. Bleicher, M. Lin, M.J. Shapiro, J.R. Wareing, Diffusion edited NMR: screening compound mixtures by affinity NMR to detect binding ligands to vancomycin, J. Org. Chem. 63 (1998) 8486–8490.
[12] J.M. Newman, A. Jerschow, Improvements in complex mixture analysis by NMR: DQF-COSY iDOSY, Anal. Chem. 79 (2007) 2957–2960.
[13] H. Barjat, G.A. Morris, A.G. Swanson, A three-dimensional DOSY-HMQC experiment for the high-resolution analysis of complex mixtures, J. Mag. Res. 131 (1998) 131–138.
[14] E.K. Gozansky, D.G. Gorenstein, DOSY-NOESY: diffusion-ordered NOESY, J. Mag. Res. Ser. B 111 (1996) 94–96.
[15] B. Vitorge, D. Jeannerat, NMR diffusion measurements in complex mixtures using constant-time-HSQC-IDOSY and computer-optimized spectral aliasing for high resolution in the carbon dimension, Anal. Chem. 78 (2006) 5601–5606.
[16] T.S. Derrick, L.H. Lucas, J.-L. Dimicoli, C.K. Larive, ^{19}F diffusion NMR analysis of enzyme-inhibitor binding, Mag. Res. Chem. 40 (2002) 98–105.
[17] O. Segev, I. Columbus, Y. Ashani, Y. Cohen Y, Probing the molecular interaction of chymotrypsin with organophosphorus compounds by ^{31}P diffusion NMR in aqueous solutions, J. Org. Chem. 70 (2005) 309–314.
[18] D. Nama, P.G.A. Kumar, P.S. Pregosin, ^{195}Pt, ^{1}H and ^{31}P PGSE diffusion studies on platinum complexes, Mag. Res. Chem. 43 (2005) 246–250.
[19] D. Li, R. Hopson, W. Li, J. Liu, P.G. Williard, ^{13}C INEPT diffusion-ordered NMR spectroscopy (DOSY) with internal references, Org. Lett. 10 (2008) 909–911.
[20] E.J. Bourne, M. Stacey, J.C. Tatlow, J.M. Tedder, Studies on trifluoroacetic acid. Part I: trifluoroacetic anhydride as a promoter of ester formation between hydroxy-compounds and carboxylic acids, J. Chem. Soc. (1949) 2976–2979.
[21] P.-K. Tsai, A. Dell, C.E. Ballou, Characterization of acetylated and acetolyzed glycoprotein high-mannose core oligosaccharides by fast-atom-bombardment mass spectrometry, Proc. Natl. Acad. Sci. USA 82 (1986) 4119–4123.
[22] B. Bendiak, Nuclear magnetic resonance spectroscopy of peracetylated oligosaccharides having ^{13}C-labeled carbonyl groups in lieu of permethylation analysis for establishing linkage substitutions of sugars, Carbohydr. Res. 315 (1999) 206–221.
[23] B. Bendiak, T.T. Fang, D.N.M. Jones, An effective strategy for structural elucidation of oligosaccharides through NMR spectroscopy combined with peracetylation using doubly ^{13}C-labeled acetyl groups, Can. J. Chem. 80 (2002) 1032–1050.
[24] J.N. Jones, B. Bendiak, Novel multi-dimensional heteronuclear NMR techniques for the study of ^{13}C–O–acetylated oligosaccharides: expanding the dimensions for carbohydrate structures, J. Biomol. NMR 15 (1999) 157–168.
[25] W.J. Goux, The determination of complex carbohydrate structure by using carbonyl carbon resonances of peracetylated derivatives, Carbohydr. Res. 184 (1988) 47–65.
[26] W.J. Goux, C.J. Unkefer, The assignment of carbonyl resonances in ^{13}C NMR spectra of peracetylated mono- and oligo-saccharides containing D-glucose and D-mannose: an alternative method for structural determination of complex carbohydrates, Carbohydr. Res. 159 (1987) 191–210.