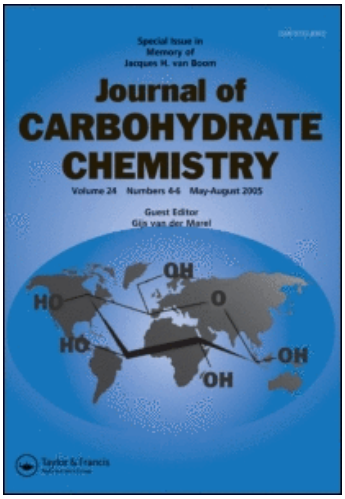


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# DOSY OF SILYLATED SACCHARIDES

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COMMUNICATION

# DOSY OF SILYLATED SACCHARIDES

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With steadily improving NMR hardware and software, diffusion ordered spectroscopy (DOSY) is becoming increasingly popular for analysis of mixtures (for a review and leading references, see ref. 1). In principle, DOSY enables separation of NMR signals from molecules that differ in their diffusion rates (or diffusion coefficients, D). In contrast to LC-NMR, DOSY achieves signal separation without physical separation of compounds by a suitably chosen pulse sequence that employs pulsed (magnetic) field gradients (PFG). The gradients attenuate NMR signals from slowly moving molecules less than the signals from fast moving molecules. Using a series of different gradient strengths, the signal attenuation is measured and analysis yields a diffusion coefficient for each resolved line in the spectrum. Theoretically, signals coming from the same molecule should have the same diffusion coefficient; hence on an appropriate 2D plot the NMR signals from the same molecule should appear on a line parallel to the NMR chemical shift axis.

DOSY is an attractive option for analysis of mixtures of oligosaccharides differing in molecular size.[2] This straightforward analysis is based on the generally accepted idea that, the larger the molecule, the slower it diffuses. It has recently been reported that hydrogen bonding can substantially affect diffusion rates.[3] Although this effect was observed in a study of phenol and cyclohexanol,[3] variation in the number of hydroxyl groups present in different carbohydrate molecules will complicate the interpretation of DOSY spectra in terms of molecular size. An obvious remedy would be to block the hydroxyl groups by suitable substituents.

In this communication we want to demonstrate that trimethylsilylation offers such a possibility and, in addition, that DOSY methodology can be applied to $^{29}$Si

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NMR spectra by utilizing the recognized dispersion of $^{29}$Si NMR signals of trimethylsilyl groups in different environments.[4] Moreover, when a DOSY sequence based on INEPT polarization transfer (INEPT-DOSY)[5] is used, $^{29}$Si NMR signals are intense and; at the same time, attenuated by PFG as strongly as are $^{1}$H NMR signals.

For this demonstration, we have chosen a simple mixture consisting of methyl β-D-xylopyranoside (**1**) and methyl 4-*O*-β-D-xylopyranosyl-β-D-xylopyranoside (**2**). Their pertrimethylsilylation was carried out as described earlier in the study of the $^{29}$Si NMR spectra.[6] According to that study, the pertrimethylsilyl derivative of **1** (methyl 2,3,4-tri-*O*-trimethylsilyl-β-D-xylopyranoside, **3**) exhibits three lines at d 19.14 (Si-2), 18.85 (Si-4), and 18.57 (Si-3) in its $^{29}$Si NMR spectrum, while the derivative of **2** (methyl 2,3-di-*O*-trimethylsilyl-4-*O*-(2,3,4-tri-*O*-trimethylsilyl-β-D-xylopyranosyl)-β-D-xylopyranoside, **4**) shows five lines at 19.59 (Si-2), 19.15 (Si-3), 19.08 (Si-2′), 18.60 (Si-3′), and 18.44 (Si-4′). The mixture was dissolved in chloroform-*d* (approx. 0.05 M equimolar solution) containing 1% (V/V) of hexamethyldisilane (HMDSS), which served as a secondary chemical shift reference (δ = -19.79).

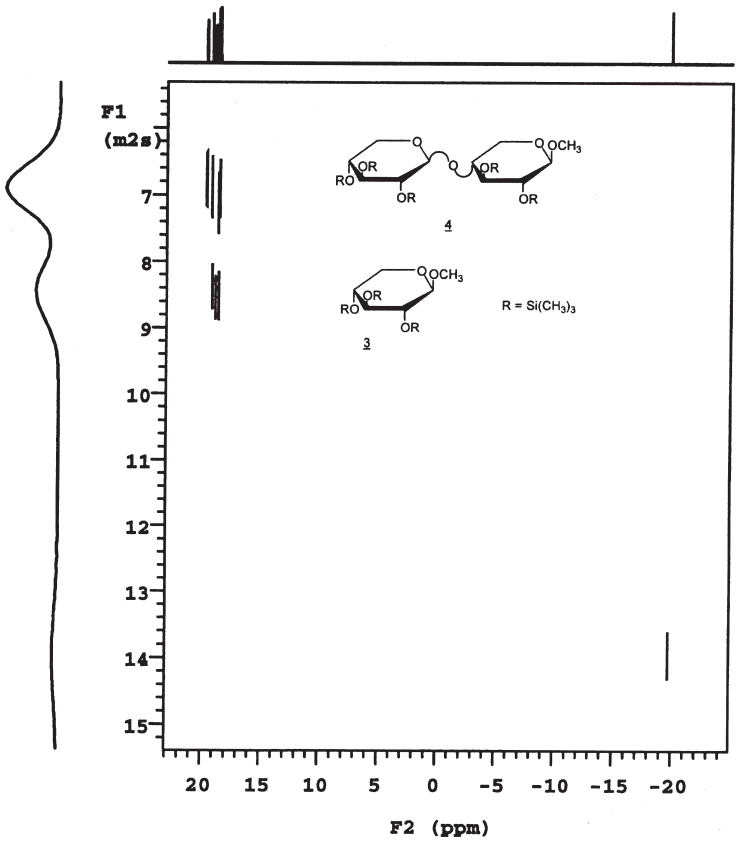


***Figure 1.*** Complete $^{29}$Si-INEPT-DOSY spectrum of the mixture of **3**, **4**, and HMDSS at 293 °C. This figure plots diffusion coefficients D ($10^{-10}$m$^2$s$^{-1}$) against $^{29}$Si chemical shifts.





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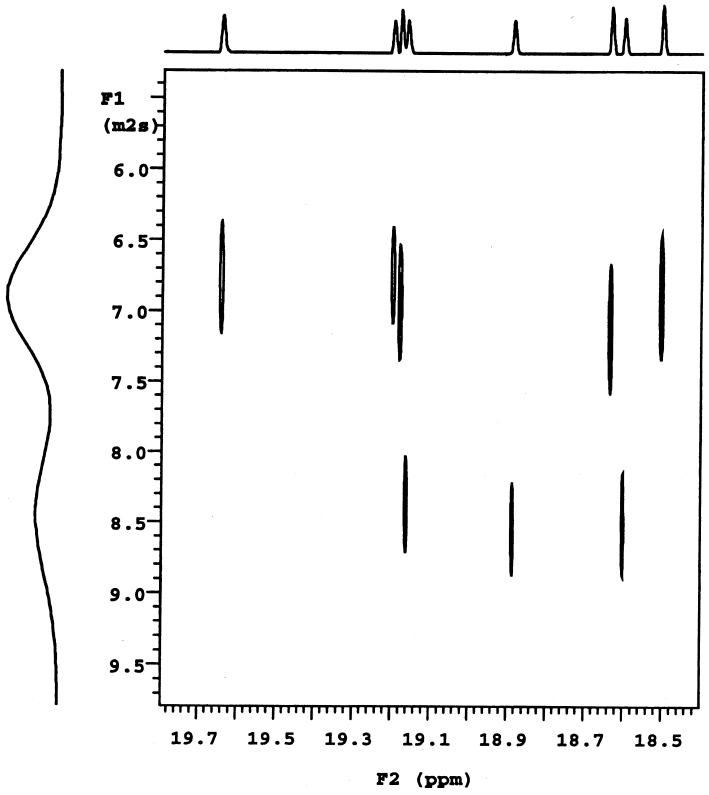


***Figure 2.*** Expansion of part of the $^{29}$Si-INEPT-DOSY spectrum of Fig. 1 showing the $^{29}$Si NMR lines of **3** and **4**. The line-width of each peak along the D axis is proportional to the experimental error in its D value. Note that the lines from the same molecule have diffusion coefficients that are identical within the experimental error.

The DOSY spectra were measured on a Varian Unity 500 spectrometer equipped with a Performa II source of PFG and a 5 mm inverse detection PFG probe. The pulse sequence used was INEPT-DOSY described in ref. 5 as LED-INEPT-DOSY but the delay LED was omitted together with its two proton pulses. The software employed was VNMR 6.1B with the added DOSY package. The spectra shown in Figs 1 and 2 were obtained using 768 transients for each of ten PFG levels (in the range of 3.9 – 12.5 gauss/cm) in an overnight run. Routine INEPT parameters for trimethylsilylated derivatives were utilized[7] with a 5 s relaxation delay between transients; rectangular gradient pulses lasted 2 ms, and the molecules were allowed to diffuse for 80 ms. The DOSY software analyzed the decay of each of the $^{29}$Si lines in the series of ten 1D spectra. The analysis yielded diffusion coefficients (and their errors) for each line. The results were presented as a 2D plot. The diffusion coefficients (D) were calibrated using a neat sample of HMDSS and value of its diffusion coefficient reported by Aksnes and Kimtys.[8]

The full $^{29}$Si-INEPT-DOSY spectrum is shown in Fig. 1; its expansion is

shown in Fig. 2. The traces shown on top and along the diffusion axis are projections (sums) of the DOSY 2D plot on the corresponding axes.

Obviously, the proposed method works well; the signals of the two mixture components are separated correctly (and so is the signal of HMDSS, as apparent from Fig. 1). Minor differences between the chemical shift values found here and in the literature[6] are due to the lower concentration used in the present work.

To be fair, a certain warning against over optimistic expectations for the power of DOSY is appropriate here. First of all, at present DOSY requires superb performance from the whole NMR system and very careful setup of the experiment and the data processing. Certainly this is not yet trivial or routine. Secondly, it is often incorrectly assumed that DOSY can always resolve overlapping lines when they originate from molecules with different diffusion coefficients. This might be true if multiexponential analysis[9] can be applied to excellent experimental data. Since this is rarely the case, an NMR method must be sought that resolves the lines (e.g., the 2D COSY experiment), and this method is then combined with DOSY (e.g., the 3D variant COSY-DOSY[1,10]). The use of $^{29}$Si-INEPT-DOSY on pertrimethylsilylated products appears to be a good alternative because of the narrow$^{29}$Si NMR lines, the sufficiently large spectral dispersion, and the good NMR sensitivity combined with a high sensitivity to magnetic field gradients. Application of $^{29}$Si-INEPT-DOSY to pertrimethylsilylated derivatives obviates the necessity of using much more demanding 3D DOSY methods and, at the same time, eliminates the uncertain effects of hydrogen bonding on the diffusion order.



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