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Applying Excitation Sculpting to Construct Singly and Doubly Selective 1D NMR Experiments

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The ^{1}H NMR spectra of biological macromolecules frequently exhibit regions of severe overlap, making it necessary to acquire spectra with the highest possible resolution and to use two-dimensional (2D) or three-dimensional (3D) techniques to obtain unambiguous information. Several groups have shown that in this situation substantial savings of spectrometer time can be made by replacing the 2D and 3D experiments with their selective, one-dimensional (1D) counterparts (*1–8*). Such experiments use selective excitation sequences in place of the indirect frequency dimensions of the analogous multidimensional experiments, giving a reduction in the minimum recording time. This approach is attractive when only a restricted amount of spectral information is required, and when the sample gives adequate signal-to-noise ratio with recording times that are less than the minimum recording time of the full-dimensionality experiment, a situation which is more likely to occur when very high resolution is required in the indirect dimensions.

NMR studies of oligosaccharides often meet these conditions, and in recent years, selective 1D equivalents of homonuclear (^{1}H) multidimensional experiments have been developed and widely used in structural studies of oligosaccharides (*1, 4, 5, 8, 9–13*). For example, it has become common to obtain intraresidue assignments from selective 1D TOCSY (*1, 9, 10, 12*) experiments, usually by recording spectra with long mixing times (about 140 ms) starting with selective excitation of an anomeric proton, resulting in coherence transfer to several of its scalar-coupled partners. Both intra- and interglycosidic connectivities can be determined using 1D ROESY (*10, 13*) experiments with appropriate mixing times. Doubly selective 1D pulse sequences, such as TOCSY–ROESY and TOCSY–NOESY, have also been used (*4, 11, 13*). In all of these selective experiments the first transfer is typically from a well-separated anomeric proton.

Recently, the so-called ''excitation sculpting'' technique (*14*) has been applied to 1D TOCSY experiments (*15*), as well as 1D COSY (*15*), NOESY (*16*), and RELAY (*15*) sequences. Excitation sculpting consists of applying the double-pulsed field-gradient spin-echo (DPFGSE) (*14*) sequence $-G_1-S-G_1-G_2-S-G_2-$ to a system with transverse magnetization, where S is some pulse or train of pulses and G_1 and G_2 are gradient pulses with unrelated amplitudes. In the original analysis of excitation sculpting (*14*), it was elegantly demonstrated that the net effect of this sequence is simply to return the transverse magnetization to its starting place with its intensity scaled by a factor P^2, where the frequency-dependent factor P is the degree to which longitudinal magnetization is inverted by the sequence S. Thus, the selectivity of the method depends solely on the inversion properties of S, and no frequency-dependent phase distortions are introduced, regardless of the specific choice of S. It is therefore an extremely versatile technique and has already been used for solvent suppression (*14, 17*) and 2D heteronuclear experiments (*18–21*), as well as the above-mentioned selective 1D experiments.

This article describes the use of excitation sculpting in the construction of 1D ROESY (Fig. 1b), TOCSY–ROESY (Fig. 1c), and ROESY–TOCSY (Fig. 1d) experiments, as well as the previously reported 1D TOCSY (Fig. 1a) experiment, referred to here as XSROESY, XSTR, XSRT, and XSTOCSY, respectively. In the singly selective XSTOCSY and XSROESY experiments, one multiplet is selected by a single DPFGSE element. The doubly selective XSTR and XSRT experiments use two DPFGSE sequences and are thus 1D analogues of 3D experiments. Since the important property of the selective pulses used in the DPFGSE sequences is their inversion characteristics, there need not be any specific phase relationship between one pair of DPFGSE pulses and another nor between either pair and the hard pulses. It is thus possible to use different frequency sources for each pair of selective pulses and the hard pulses, making implementation particularly straightforward.

These experiments were used in the NMR analysis of an about 0.4 mM D_2O solution of a trisaccharide fragment (Fig. 2), obtained from digestion and subsequent oxymercuration of chondroitin sulfate C, a glycosaminoglycan polysaccharide. The primary structure of this molecule had been de-

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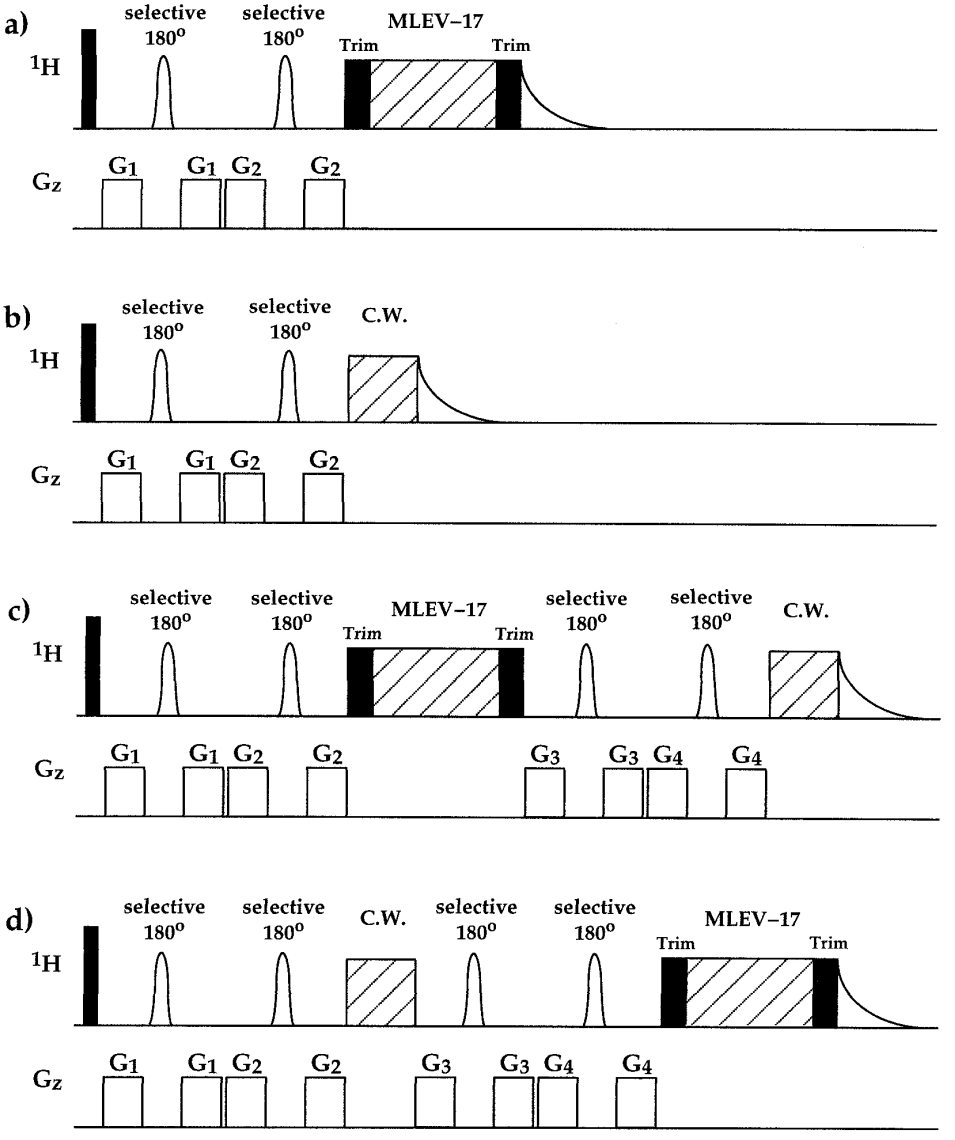

FIG. 1. Pulse schemes used for the singly and doubly selective experiments. The hard 90° excitation pulses are represented by narrow, black rectangles. Thick open rectangles represent the gradient pulses. Solid rectangles annotated with ''Trim'' indicate trim pulses 2 ms in length. Mixing sequences are represented by cross-hatched blocks. ''C.W.'' represents a CW spin lock for ROESY transfer. (a) Excitation-sculpted singly selective 1D TOCSY (''XSTOCSY''). (b) Excitation-sculpted singly selective 1D ROESY (''XSROESY''). (c) Excitation-sculpted doubly selective 1D TOCSY-ROESY (''XSTR''). (d) Excitation-sculpted doubly selective 1D ROESY-TOCSY (''XSRT''). In all experiments, the phase of the initial 90° pulse and the receiver were inverted on alternated scans. In addition, all pulses were cycled through the CYCLOPS scheme to minimize quadrature artifacts.

duced from joint analysis of NMR and mass spectroscopic data (*12*). New NMR assignments and structural confirmation were obtained with the series of experiments described here. The results of a 140 ms XSTOCSY and a 300 ms XSROESY obtained by selecting the H1β proton of the GalNAc I residue are shown in Figs. 2b and 2c, respectively. TOCSY transfer (Fig. 2b) is achieved as far as the H4β proton, but there are no further relayed responses, consistent with there being a very small H4β–H5β scalar coupling. Intraresidue ROEs (Fig. 2c) are detected between H1β and the H3β and H5β protons.

These experiments were used to set the excitation frequencies of the second DPFGSE elements in the doubly selective XSTR and XSRT sequences. Figure 2d shows the results from the XSTR pulse sequence of Fig. 1c, obtained by setting the second DPFGSE element to the frequency of H3β and truncating the sequence after the second DPFGSE element. This demonstrates the extremely effective ''sculpting out'' of H3β after TOCSY transfer from H1β. Similarly, H5β can be sculpted out after the ROESY transfer from the anomeric proton, as shown in Fig. 2f. The TOCSY–ROESY and ROESY–TOCSY experiments are

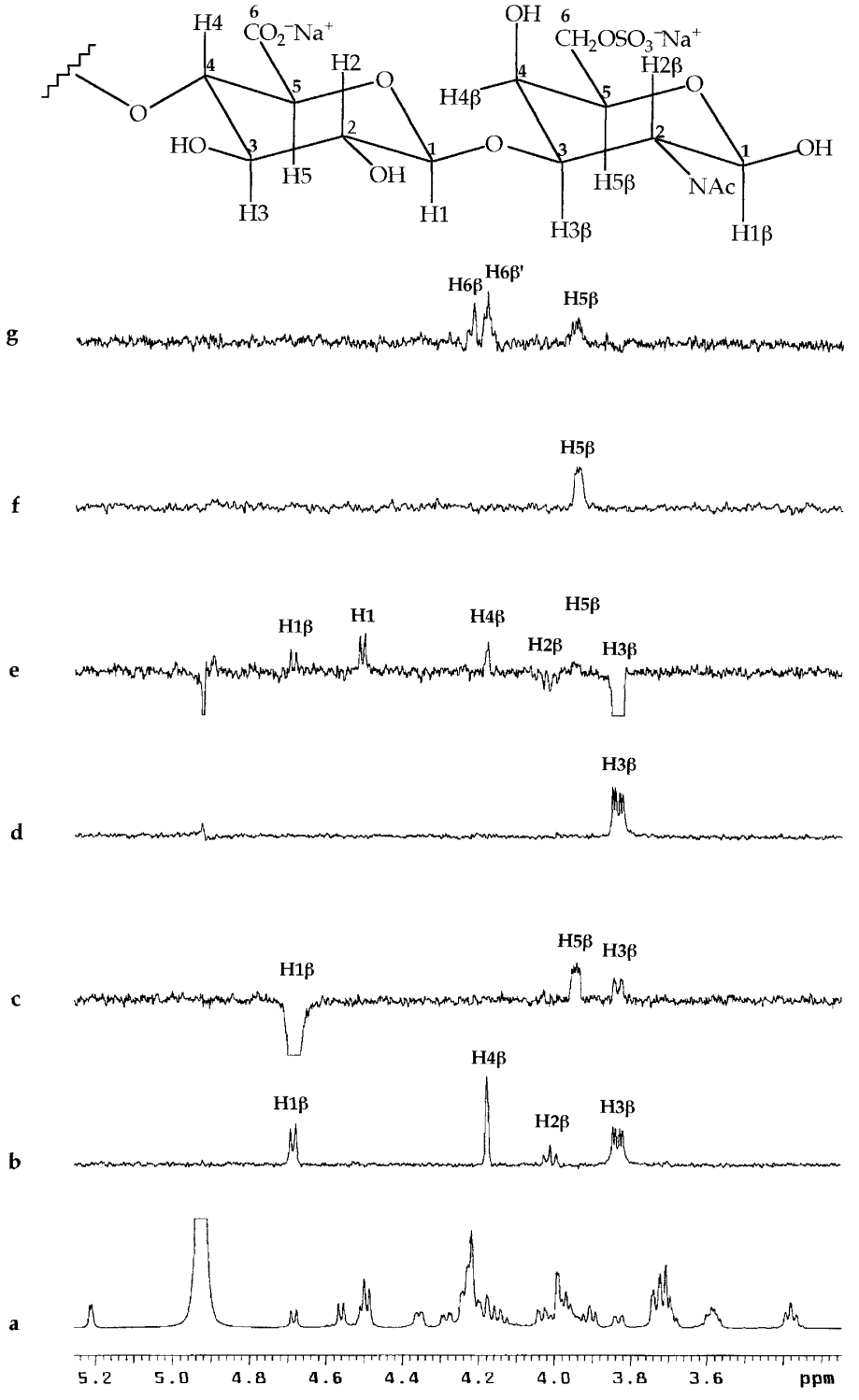

FIG. 2. ^{1}H NMR spectra recorded on a 0.4 m*M* solution in D_2O of GalNAc(6S)β1−4GlcAβ1−3GalNAc(6S)α/β at 12°C on a Varian Unity 600 spectrometer. The labeling of the protons of the residues GlcA and GalNAc Iβ are indicated at the top of the figure. TOCSY and ROESY mixing times were 140 and 300 ms, respectively. Selective 180° pulses were 25 ms in length and Gaussian shaped. Gradient pulses were along the *z* axis and were 1 ms in length. Delays of 500 μs were inserted after these pulses for hardware recovery. The amplitudes of G_1, G_2, G_3, and G_4 were about 19, 5, 13, and 10 G/cm, respectively. All FIDs were apodized with 1Hz exponential line broadening. (a) Reference region of a one-dimensional ^{1}H spectrum obtained with 256 transients. (b) XSTOCSY spectrum recorded with selection of the H1β proton of GalNAc I. The total number of transients was 256. (c) XSROESY spectrum obtained with 1024 transients, selecting the above proton. (d) The result of ''sculpting'' the H3β proton of GalNAc I out by truncating the XSTR sequence (Fig. 1c) after the second DPFGSE element. Initially, H1β was selected, with selection of H3β after the TOCSY transfer. A total of 1024 transients were recorded. (e) Final XSTR obtained, with 8192 transients, by initial selection of H1β with subsequent selection of H3β, as in (d). (f) Sculpting of the H5β resonance by truncating the XSRT sequence (Fig. 1d) before the isotropic mixing, in analogy with (d), starting with ROESY transfer from H1β. A total of 1024 transients were recorded. (g) Final XSRT spectrum, with H1β initially selected for the ROESY stage, followed by TOCSY transfer after selection of H5β. The number of transients was 8192.

then constructed by addition of further periods of mixing, as shown in Figs. 1c and 1d. The results of the final XSTR and XSRT experiments are presented in Figs. 2e and 2g, respectively. The TOCSY–ROESY shows intraresidue ROEs from H3β to H1β, H4β, and H5β. In addition, an interesidue ROE is detected to H1 of the neighboring GlcA residue, confirming the primary structural assignment. A small TOCSY transfer to H2β is also observed. TOCSY peaks can be minimized if desired by moving the transmitter offset further away from the signals of interest: this would allow accurate quantification of ROEs [after appropriate corrections for offset effects have been made (*22, 23*)], making the XSTR experiment a valuable tool for conformational studies. The ROESY–TOCSY spectrum (Fig. 2g) shows transfer from H5β to the two H6β methylene protons. These are new assignments. The shifts for these newly assigned protons unambiguously confirm that the 6 position of the reducing GalNAc residue is sulfated (*12*).

Since the gradients merely serve to suppress unwanted signals, they are not subject to the sensitivity losses associated with gradient coherence pathway selection (*24, 25*). Transverse relaxation during the experiments and self-diffusion in the sample during the gradient spin echos do however contribute to signal losses. The reduction in signal intensity caused by diffusion can be calculated (*26, 27*) using estimated values of diffusion coefficients (*28*) and was found to be $<5\%$ for a single DPFGSE element with the trisaccharide sample and under conditions used in this work: with larger molecules or lower temperatures, the loss would be lower. The loss in signal intensity due to T_2 relaxation for small- to medium-sized molecules such as this trisaccharide, although still fairly small, is approximately double that obtained with a conventional nongradient implementation of these experiments. However, the suppression of unwanted resonances is excellent, allowing a high setting of the spectrometer receiver gain, which compensates to some extent for the greater relaxation loss in the excitation-sculpted sequences.

The ease of setup and the excellent spectra obtained with these excitation-sculpted pulse sequences represents an improvement over existing methods. The spectra presented in this paper were obtained for a relatively dilute sample of a trisaccharide, since this is a typical application for experiments of this type (*1, 4, 9–13*). In addition the XSTOCSY, XSROESY, XSTR, and XSRT sequences should also be of value in the study of other kinds of biomolecules or organic compounds, although applications should generally be restricted to small- to medium-sized molecules on account of the intensity losses associated with transverse relaxation. Larger molecules are also less likely to have well-dispersed signals available for selective inversion. Nevertheless, excitation sculpting lends itself well to the construction of the 1D equivalents of 2D and 3D experiments shown here, and could therefore be applied successfully to the creation of further reduced dimensionality experiments.

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