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2D NMR spectra of oligosaccharides enhanced by band-selective suppression of unwanted signals

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Abstract

A general scheme is proposed for eliminating disturbing signals of some protecting groups from the 2D NMR spectra of oligosaccharides by making use of a band-selective suppression–restoration sequence that serves as a preparation step to various 2D NMR experiments. © 2000 Elsevier Science Ltd. All rights reserved.

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Signal overlap in 1D NMR spectroscopy is a common obstacle to the analysis of all but the simplest spectra. ^{1}H NMR spectra of oligosaccharides notoriously feature narrow ranges of chemical shifts for the ring protons (with the exception of the anomeric ones) even at lower molecular masses (e.g., di- or trisaccharides). Anomeric proton signals tend to stand out downfield of the bulk of resonances and can therefore be utilized as structure 'reporter' signals in cases where no detailed assignments are available.[1] They are, on the other hand, natural starting points for resonance assignments by 2D methods.

Difficulties arise, however, when anomeric signals get buried by other, low-field shifted resonances arising, e.g., from protecting groups, such as benzylic or acetalic methylenes or others used in oligosaccharide synthesis.[2,3] It was shown recently that signals from protons not J-coupled to the spin system of interest, e.g. those constituted by sugar backbone protons, can be selectively purged from 1D spectra even in the case of severe overlap.[4] The method is based on band-selective suppression of all signals, including the 'useful' ones, from one region of the spectrum, followed by TOCSY transfer to recover resonances of protons J-coupled to the nonsuppressed ones. For instance, signals of anomeric protons buried under those from OCH_2's in carbohydrate benzyl ethers could be cleanly recovered while eliminating the latter disturbing resonances.[4]

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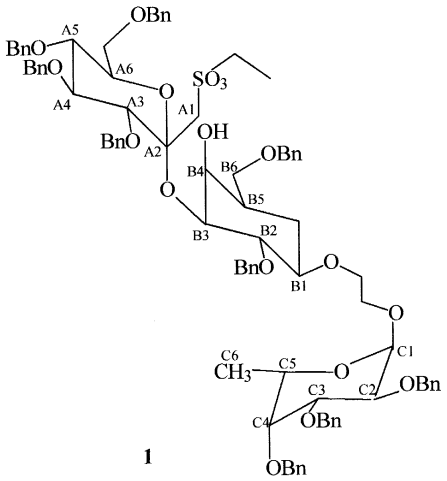

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Overlap is much less of a problem with 2D NMR but not uncommon even with smaller molecules having unfavorable spectral properties (such as compressed chemical shift range). This is exemplified by trisaccharide **1,** bearing eight benzylic groups whose CH_2 signals overlap with anomeric resonances (B1, C1) and with quite a few of the backbone proton signals (A3, A4, A6, C3). In the course of the assignments of ^{1}H and ^{13}C spectra of this molecule it turned out, furthermore, that one of the backbone cross peaks (later identified as A6) got completely buried by those from benzylic CH_2's in the ^{1}H, ^{13}C HSQC map (Fig. 1a) as well. In order to selectively eliminate disturbing cross peaks arising from the latter we have introduced a modification to the original HSQC sequence.[5] This consists of a preparation step whereby band-selective suppression of signals in the 'benzylic' region (ca. 4.1 to 5.2 ppm, including the shift of A6) is followed by restoration of the suppressed backbone resonances using MLEV17[6] transfer

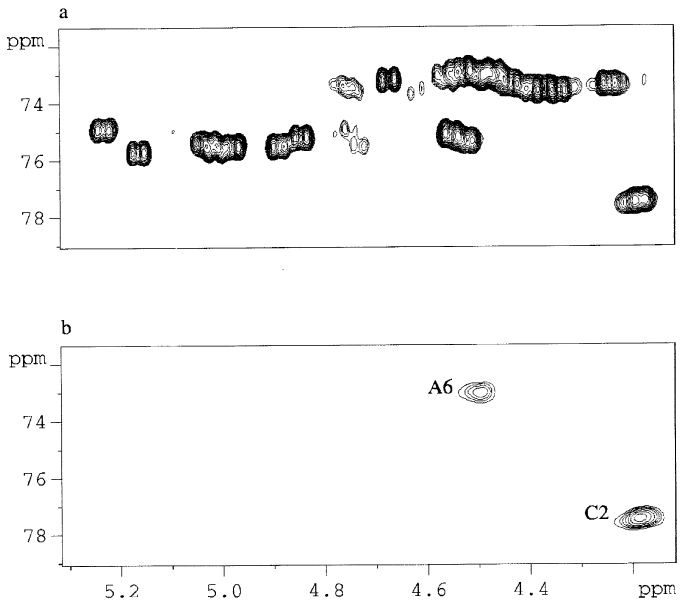

Fig. 1. Partial plots of gradient-enhanced ^{1}H, ^{13}C HSQC spectra of **1** displaying the spectral region characteristic for benzylic CH_2 resonances; without (a) and with (b) insertion of the band-selective suppression element of Fig. 2 before the HSQC sequence

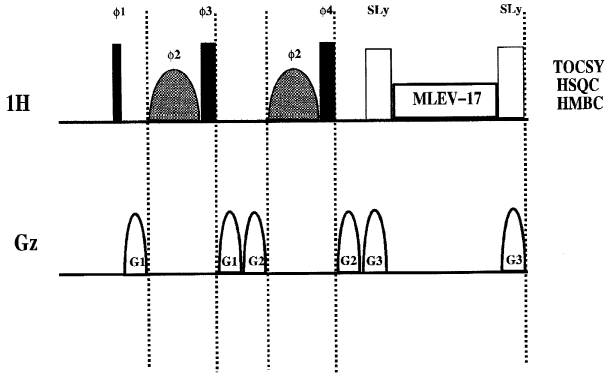

Fig. 2. Band-suppressive excitation scheme serving as a preparation pulse in regular 2D experiments. Thin and thick bars represent ^{1}H 90° and 180° pulses, respectively. Grey colored shaped bars indicate band-selective inversion pulses. The MLEV17 scheme is flanked by simultaneously switched gradient (G3) and spin-lock (Sly) pulses in order to achieve pure phase excitation. The strengths of sine-bell shaped gradient pulses of 1 ms duration were: G1=5 G/cm, G2=9 G/cm and G3=10 G/cm. The phase cycling was set as ϕ1=x, −x; ϕ2=x, −x; ϕ3=x, −x; and ϕ4=x, −x, y, −y

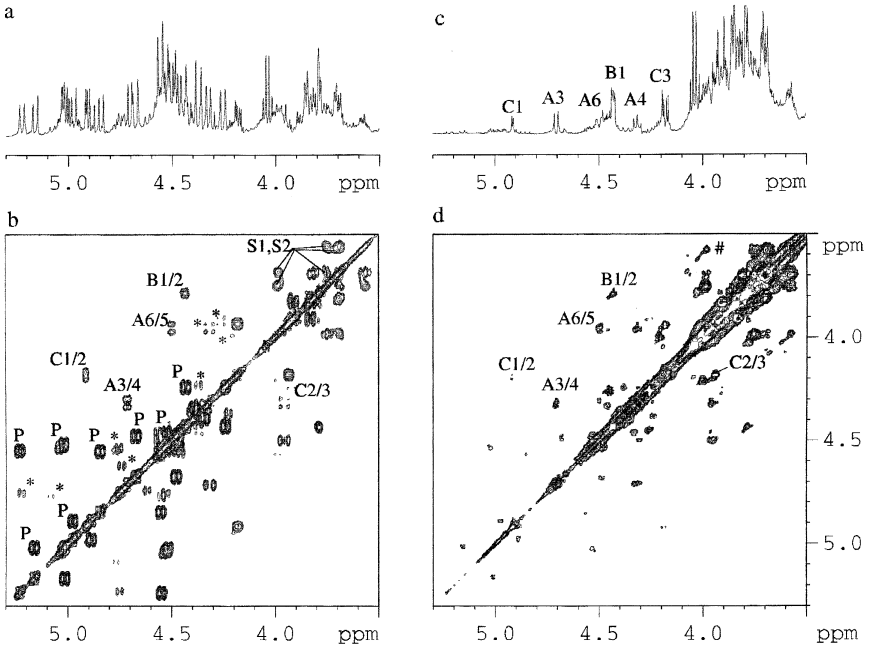

Fig. 3. Partial TOCSY map of **1** showing (d) selective suppression of the cross peaks from benzylic CH_2 protons (denoted by P) achieved with the modified sequence (Fig. 2) as compared with the result obtained using regular, gradient COSY (b). Signals denoted by stars belong to an unidentified impurity in the sample. The mixing time in the TOCSY experiment was 15 ms resulting in cross peaks arising from one step magnetization transfer, whereas the one denoted by # originates from a two step transfer due to the large value of the corresponding coupling constant. Shown above the 2D maps is the regular 1D proton spectrum (a) vs 1D TOCSY (c) obtained with the pulse sequence in Ref. 4

(Fig. 2). Suppression is effected by the DPFGSE sequence or 'excitation sculpting'[7,8] wherein the band-selective 180° pulses are implemented as pulse width modulated DANTE trains.[9] We find this method a robust one, and more convenient in terms of calibration and bandwidth adjustment than using shaped pulses[10] or RE-BURP.[11] The result of the modified HSQC experiment, evident from Fig. 1b, to clear all benzylic CH_2 cross peaks while retaining that of A6 (with somewhat decreased intensity).

The proposed purging scheme is not limited to the application shown; it is, in fact, quite general and can be utilized as a preparation step preceding other homo- or heterocorrelated 2D sequences like HMBC or TOCSY. As an example, Fig. 3 shows COSY and CH_2-suppressed 2D TOCSY maps of **1**. Finally we note that, as another extension, the MLEV17 restoration scheme can, in principle, be replaced by other sequences, like NOESY or ROESY, to recover signals in the suppressed spectral region. NOESY or ROESY will, however, revive resonances that are coupled by dipolar (or through-space), rather than by scalar-J (or through-bond), mechanism to the spins in the unsuppressed region(s). This may offer a viable alternative when through-bond transfer (by MLEV17) becomes inefficient because the relevant scalar couplings are small.

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