An Approach to Oligosaccharide Sequencing: 2D N.M.R. DEPT Experiment for Detection of Interglycosidic ^{13}C–^{1}H Spin–Spin Couplings

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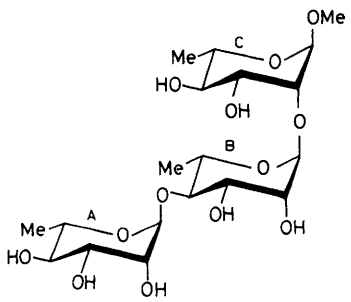
A 2D DEPT n.m.r. experiment adjusted for the detection of $^{3}J_{COCH}$ couplings is an alternative approach to the sequencing of small to medium-sized oligosaccharides.

It has recently been demonstrated that high field two-dimensional n.m.r. methods such as 2D nuclear Overhauser enhancement[1] and delayed COSY[2] can be used in the sequencing of oligosaccharides.[3,4] In these measurements, inter-residual proton–proton spin–spin interactions (through space or through bond) yield the sequence information. None of these methods seems applicable to all problems, so the need for independent sequencing methods is clear.

The existence and stereospecificity of interglycosidic $^{3}J_{COCH}$ couplings has been thoroughly investigated in the literature,[5] but it should be noted that all such couplings can rarely be obtained in one-dimensional measurements. The detection of these couplings in 2D heteronuclear chemical



(1)

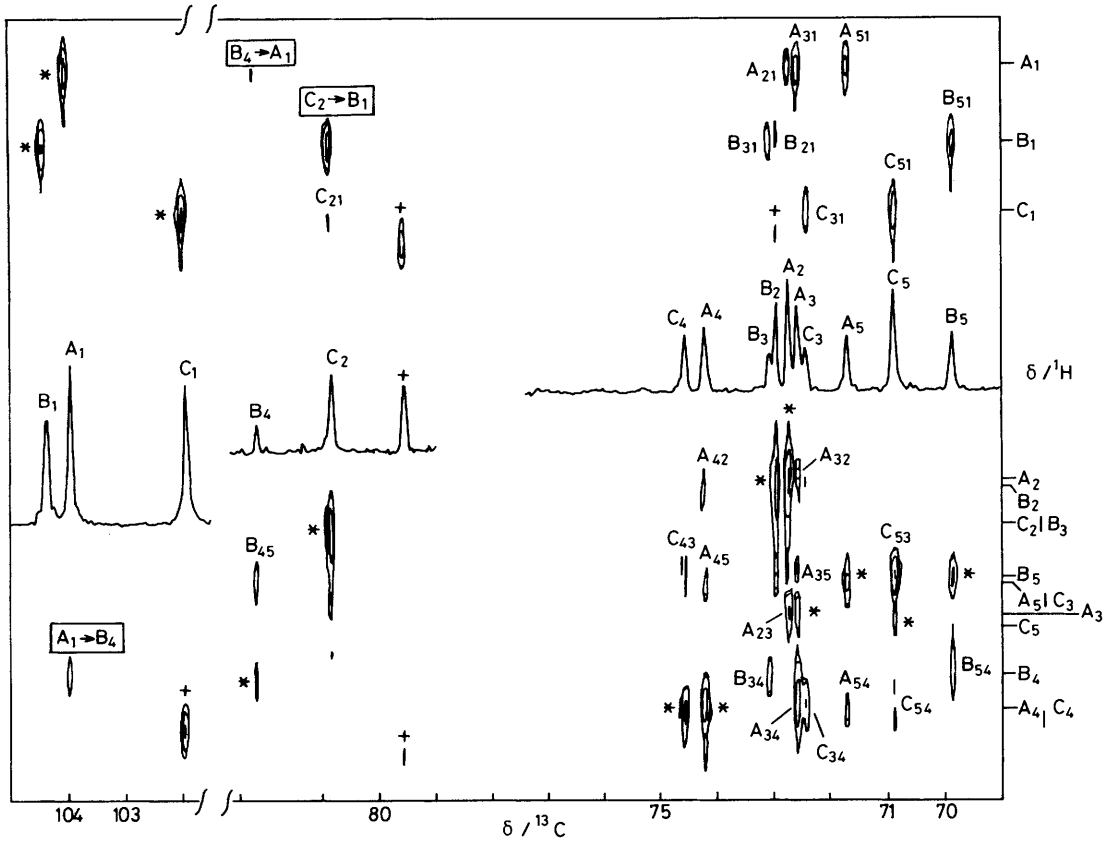


Figure 1. Contour plot of a 2D DEPT chemical shift correlation experiment (Bruker WP-200 SY instrument) on 120 mg (**1**) dissolved in 435 μl D_2O in a Wilmad 529-E-10 small volume sample bulb. The pulse sequence given by Levitt[8] was applied including a presaturation ^{13}C pulse train during the waiting time. 32 × 1000 Transients were accumulated in an overnight experiment. The size of the data matrix in the $F1$ dimension was 128 words (±250 Hz), while in F_2 2K words (2000 Hz) gave appropriate digital resolution. As a compromise, 50 ms was chosen for the $1/2J$ period in the sequence. For optimum sensitivity a waiting time of 1.3 T_1 (^1H) was allowed between consecutive pulse sequences. Before Fourier transformation, free induction decays in both dimensions were multiplied by appropriate Lorentz-to-Gauss enhancement functions.

Interglycosidic couplings are denoted by a small frame: *e.g.* $C_2 \rightarrow B_1$ means that the C_2 carbon is coupled to the B_1 proton. Intra-unit couplings: B_{51}, for example, represent a coupling between the B_5 carbon and the B_1 proton. Cross peaks arising from one bond ^{13}C–^1H couplings are indicated by asterisks, while folded signals are identified with crosses.

shift correlation[6] experiments immediately determines the linkage positions between the sugar units, presuming that correct ^1H and ^{13}C assignments are available for each of the units.

In this communication we show that a 2D DEPT experiment,[7] adjusted for the detection of $^3J_{COCH}$ couplings, may be an alternative approach for the sequencing of small to medium-sized oligosaccharides. The advantages of the 2D DEPT approach over the conventional heteronuclear chemical shift correlation experiments are apparent.[7] The former contains fewer pulses and it is less sensitive to J spreading, which is invaluable in the case of long-range ^1H–^{13}C couplings.

The trisaccharide studied in this work, Me-α-L-Rhap-(1→4)-α-L-Rhap-(1→2)-α-L-Rhap (**1**), consists of three identical, six-membered rhamnopyranosyl (Rhap) units.[9] Full assignments of the ^1H and ^{13}C n.m.r. signals can be accomplished only with difficulty using the basic chemical shift correlation methods[2,6] as described in ref. 4. Owing to some exact overlap in the 200 MHz ^1H n.m.r. spectrum (A_4 and C_4 protons), assignment of the appropriate ^{13}C n.m.r. signals was performed by means of heteronuclear-relayed coherence transfer[10] spectroscopy.

Finally, in a 2D DEPT experiment, adjusted for visualisation of long range (more than one bond) ^1H–^{13}C spin–spin couplings, the appearance of cross peaks arising from $^2J_{CH}$ and $^3J_{CH}$ couplings corroborated our ^1H and ^{13}C assignments (Figure 1). Unambiguous detection of three of the possible four interglycosidic ($^3J_{COCH}$) couplings immediately determines both the sites of glycosylation and the sequence of rhamnopyranosyl units as A(1→4)B(1→2)C.

The measured values of interglycosidic coupling constants in (**1**) are uniformly 3—5 Hz ($^3J_{B1,C2}$ 4.5, $^3J_{A1,B4}$ 5.2, $^3J_{B4,A1}$ *ca.* 3, $^3J_{C2,B1}$ 3.5 Hz, where the first index identifies the carbon atom, and the second one denotes the proton involved in the coupling path). The low intensity or lack of certain cross peaks arising from these couplings may be explained by other factors. The antiphase nature of some signals due to the proton multiplet pattern of the cross peaks in F_1 dimension may result in accidental cancellation. We further suppose that the detectability of cross peaks in a 2D DEPT experiment is governed primarily by the pertinent ^1H and ^{13}C spin–spin relaxation times. In those cases when T_2 relaxation times are comparable to $1/2J$, then τ intervals shorter than $1/2J$ should be used. All ^1H and ^{13}C T_2 relaxation times are 0.2 ± 0.05 s in

(**1**) except for the anomeric protons, in which case this value is about 0.4 s. Relatively fast (T_2 *ca.* 0.15 s) relaxation of carbon B_1 and proton C_2 at room temperature reduced the efficiency of polarisation transfer and the detectability of ^{13}C signals. Slightly raising the temperature and lowering the τ period to 40 ms allowed the detection of the missing $B_1 \rightarrow C_2$ cross peak as well.

We think that 2D DEPT sequencing of oligosaccharides may be feasible when the spin–spin relaxation times of the oligomer are longer than 0.1—0.2 s. In those cases, when relaxation times are shorter than 0.1 s there is little hope to obtain the interglycosidic ^1H–^{13}C spin–spin connectivity information under experimental conditions similar to ours, so the problem may be solved at higher fields and/or higher temperature.

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