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NEW NMR TECHNIQUES FOR STRUCTURE DETERMINATION AND RESONANCE ASSIGNMENTS OF COMPLEX CARBOHYDRATES

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ABSTRACT

This paper describes several new NMR techniques for structure determination and spectral assignment of polysaccharides. Positions of linkages between sugar units can be determined unambigously and with high sensitivity using a modified version of the well known INEPT experiment. A new two-dimensional experiment is shown to provide excellent resolution and sensitivity in correlating ^1H and ^{13}C chemical shifts.

INTRODUCTION

NMR spectroscopy, and in particular ^1H and ^{13}C NMR spectroscopy, has become an increasingly important tool for the chemical and physical characterization of carbohydrates and their derivatives. The usefulness of the NMR method follows from the assignments of individual proton and carbon resonances to particular hydrogens or

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carbon atoms.[1] The ability to make these assignments has been enormously facilitated by the introduction of two-dimensional (2-D) NMR techniques.[2] A variety of 2-D ^1H experiments[3-11] and, especially, homonuclear chemical shift correlation (COSY),[3-7] has aided signal assignments in ^1H spectra. When proton assignments have been made, ^{13}C assignments follow, straightforwardly, from the construction of a ^1H-^{13}C heteronuclear, chemical shift correlation map[12-15] or, as will be demonstrated in this paper, by a recently introduced "totally decoupled" CSCM experiment.[16,17] In those instances in which only partial assignment of the proton spectrum can be made, a two-dimensional, heteronuclear RELAY experiment can be used to identify adjacent, protonated ^{13}C nuclei;[18-24] alternatively, a ^{13}C-^{13}C INADEQUATE experiment[25-26] can be used to trace the entire carbon skeleton of the molecule. Recently, the two-dimensional NOE experiment[8] and the COSY experiment were used for determining oligosaccharide interglycosidic linkages.[27,28] Both of these approaches to establishing the interglycosidic linkage rely on proton NMR, and are often complicated because of severe spectral overlap. Additionally, the effects on which those methods are based, NOE effect across the glycosidic linkage and the $^4J_{HCOCH}$ scalar coupling, are strongly conformation dependent, and therefore not always unambiguous. Alternatively, the linkage site can be determined via the ^1H-^{13}C three-bond scalar coupling from the anomeric proton across the oxygen atom to the aglycon carbon atom. The presence of such a coupling, as well as its magnitude, can be gotten from either a selective proton flip experiment[29] or a selective heteronuclear decoupling experiment.[30-32] Both of these experiments, however, are time-consuming, and the selective decoupling experiment suffers especially from poor sensitivity.

We now wish to show that the recently introduced selective INEPT experiment[33,34] can be used to effectively detect the presence of long-range ^1H-^{13}C couplings, and thus can be used as a method for determining the position of linkages between sugar units. We will also show that the selective INEPT experiment provides excellent sensitivity.

Spectral assignment of a trisaccharide

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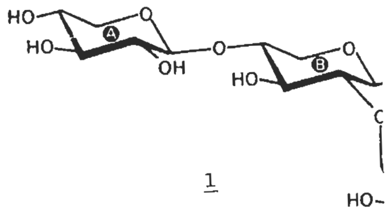

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Spectral assignment of the proton resonance
"C" follows immediately from the COSY spect
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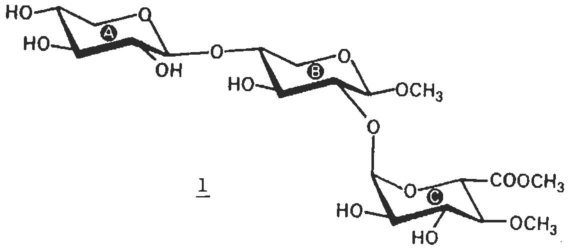
refocusing the dephasing that normally occ
period due to the proton-proton coupling.

Spectral assignment of a trisaccharide

While spectral assignments of a saccharide are usually accomplished by comparison with related compounds, they can also be done in a direct manner. As an example, we describe here the ^1H and ^{13}C assignment of the aldotriuronic acid derivative (1).



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Spectral assignment of the proton resonances of the ring designated "C" follows immediately from the COSY spectrum. First, the anomeric proton, C1, is identified on the basis of the small homonuclear coupling and the downfield shift. From this (see Figure 1), the C2 proton is then measured to be at 3.60 ppm, and this proton is in turn coupled to C3 (3.80 ppm) (broken lines in Fig. 1). Protons C4 (3.34 ppm) and C5 (4.68 ppm) are then also straight forwardly assigned. Protons of the remaining two sugar rings, A and B, are assigned in an identical manner; however, distinguishing which protons correspond to which ring is not readily possible but requires correlation with the ^{13}C spectrum and use of the selective INEPT experiment, as will be described later. Several proton resonances are close together, and correlation with ^{13}C chemical shifts[12-17] requires very high resolution in the proton dimension (F_1) of the CSCM spectrum. This high F_1 resolution dramatically decreases the sensitivity of the experiment, because signal energy is distributed over all proton-proton multiplet components in the F_1 dimension.[35,36] A recently introduced modification of the chemical shift correlation experiment avoids this loss in sensitivity by refocusing the dephasing that normally occurs during the evolution period due to the proton-proton coupling. The signal of all proton

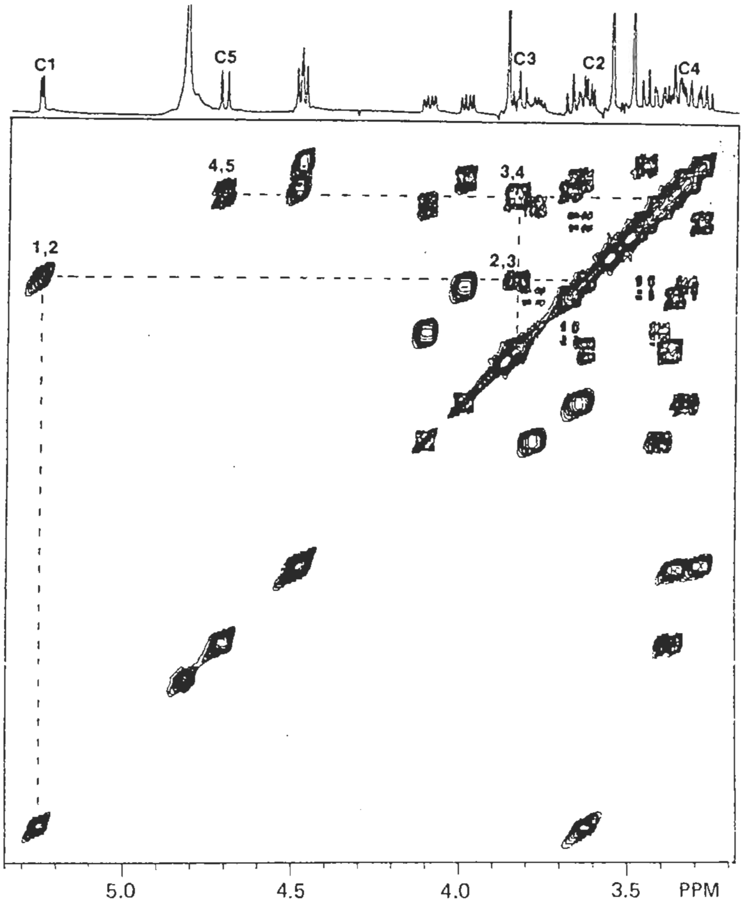

Figure 1. Two-dimensional COSY spectrum of trisaccharide, 1, recorded at 500 MHz. The spectrum results from a 256x512 data matrix, which corresponds to data acquisition times (t_{1max} and t_{2max}) of 230 ms in both dimensions. A non-shifted sine bell filtering function was used in both dimensions. In order to reduce spectrometer instabilities that generate "t_1-noise," the sample was not spun. The coupling network can easily be traced out from this spectrum, as discussed in the text.

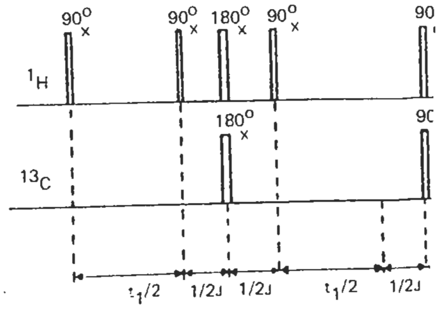

Figure 2. Pulse scheme of the homonu
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average proton longitudinal relaxati
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experiment is depicted in Fig. 2. The
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pulse[37] that refocuses dephasing due
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methylene protons. A section of the
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spectrum was recorded on a NT-270 spe
^{13}C frequency. A 128x2048 data matri
measuring time was 2 hrs. The proto
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assignment of the ^{13}C shifts is dire
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Determination of linkages

A modification of the INEPT exp
introduced that allows magnetizatio

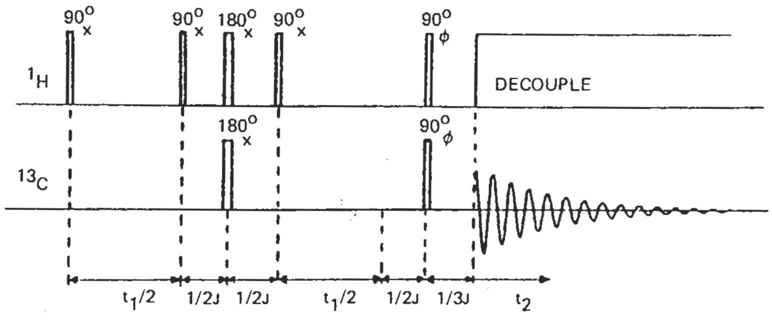

Figure 2. Pulse scheme of the homonuclear broad-band decoupled heteronuclear shift correlation experiment. The phases of the rf pulses and receiver are cycled according to Table 1. For optimum sensitivity, the delay time between the end of data acquisition (and proton decoupling) and the first 90° ^1H pulse of the next experiment should be on the order of 1.5 T_1, where T_1 is the average proton longitudinal relaxation time. This experimental scheme yields a heteronuclear shift correlation map from which the vicinal $^1H-^1H$ couplings are effectively removed. Splittings due to geminal proton coupling will still be present in the F_1 dimension.

multiplet components will then be concentrated into one narrow line at the proton chemical shift frequency.[16,17] The pulse sequence of this totally decoupled heteronuclear chemical shift correlation experiment is depicted in Fig. 2. The difference between this sequence and the regular CSCM experiment is that the 180° ^{13}C pulse at the mid-point of the evolution period is replaced by a bilinear pulse[37] that refocuses dephasing due to all proton-proton scalar couplings, apart from the geminal coupling between non-equivalent methylene protons. A section of the CSCM spectrum, displaying the most crowded area of the 2D spectrum, is shown in Figure 3. The spectrum was recorded on a NT-270 spectrometer, operating at 68 MHz ^{13}C frequency. A 128x2048 data matrix was acquired and the total measuring time was 2 hrs. The proton chemical shifts can be measured accurately from this decoupled shift correlation map, and assignment of the ^{13}C shifts is directly possible by comparison with the proton shifts determined from Figure 1.

Determination of linkages

A modification of the INEPT experiment[39-41] has recently been introduced that allows magnetization transfer from a pre-selected

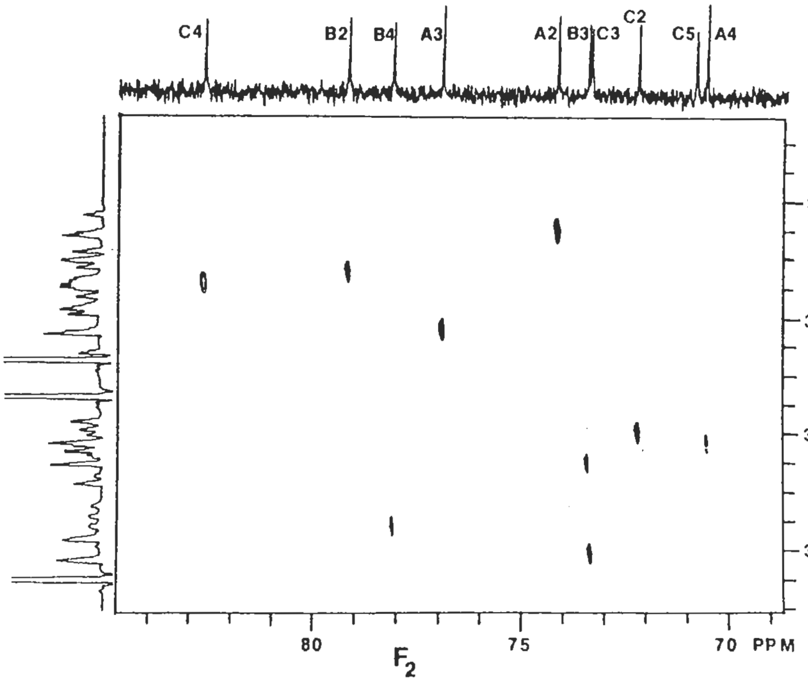

Figure 3. The most crowded section of the homonuclear-decoupled, heteronuclear shift correlation spectrum of trisaccharide, <u>1</u>, recorded at 270 MHz proton frequency. The full spectrum resulted from a 128x2048 data matrix (t_{1max}=300 ms, t_{2max}=250 ms). The total measuring time was 2 hrs. Moderate Lorentzian to Gaussian resolution enhancement is used in both dimensions. The proton-proton coupling in the F_1 dimension has been effectively removed.

TABLE 1. The rf phases of the final proton pulse and ^{13}C pulse (ϕ), and the way data are added to or substracted from memory in the various steps of the experiment.

Step No.	ϕ	ACQ
1	x	+
2	y	−
3	−x	+
4	−y	−

TABLE 2. ^1H and ^{13}C Chemical

Ring		1	2	3	4	5
A	^1H	4.46	3.25	3.42	3.62	3.32 3.98
	^{13}C	103.3	74.1	76.9	70.5	66.5
B	^1H	4.47	3.32	3.65	3.76	3.39 4.09
	^{13}C	105.4	79.2	73.4	78.1	64.0
C	^1H	5.23	3.60	3.80	3.34	4
	^{13}C	99.5	72.2	73.3	82.6	

proton to a nucleus (<u>e</u>.<u>g</u>., ^{15}N or ^{13}C) th
interaction of several or more Hertz with
been demonstrated that this modified, se
be used for sensitivity enhancement of n
and for assignment of ^1H and ^{13}C spectra
that the coupling across the interglycos
oligosaccharide can be used, in a direct
establish the presence of this linkage.
modified INEPT experiment is schematize
functions basically in the same way as
experiment;[41] there are several importa
pulses are soft pulses ($\gamma H_2 \approx 20$ Hz), a
magnetization of one preselected proto
coupling magnetization vectors precess
^1H-^{13}C interaction (provided that a ^{13}
bonds removed from this proton) and al
shift, homonuclear proton coupling, an
inhomogeneity) are removed by the <u>soft</u>
mid-point of the interval Δ_1. In analo
experiment, the delay, Δ_1, is set to a

TABLE 2. ^{1}H and ^{13}C Chemical Shifts of Trisaccharide **1**.

Ring		1	2	3	4	5	6	Me(1)	Me(4)	Me(6)
	^{1}H	4.46	3.25	3.42	3.62	3.32				
A						3.98				
	^{13}C	103.3	74.1	76.9	70.5	66.5				
	^{1}H	4.47	3.32	3.65	3.76	3.39		3.53		
B						4.09				
	^{13}C	105.4	79.2	73.4	78.1	64.0		58.6		
	^{1}H	5.23	3.60	3.80	3.34	4.68			3.46	3.84
C										
	^{13}C	99.5	72.2	73.3	82.6	70.8	173.3		61.1	54.5

proton to a nucleus (*e.g.*, ^{15}N or ^{13}C) that has a long-range scalar interaction of several or more Hertz with this proton.[33,34] It has been demonstrated that this modified, selective INEPT experiment can be used for sensitivity enhancement of non-protonated ^{15}N nuclei,[33] and for assignment of ^{1}H and ^{13}C spectra.[34] We herein demonstrate that the coupling across the interglycosidic linkage in an oligosaccharide can be used, in a direct and unambiguous way, to establish the presence of this linkage. The pulse sequence of the modified INEPT experiment is schematized in Figure 4. The experiment functions basically in the same way as the usual, refocused INEPT experiment;[41] there are several important differences: all proton pulses are soft pulses ($\gamma H_2 \approx 20$ Hz), affecting only or predominantly magnetization of one preselected proton. During the delay Δ_1, proton coupling magnetization vectors precess under influence of long-range ^{1}H-^{13}C interaction (provided that a ^{13}C nucleus is present at two or three bonds removed from this proton) and all other effects (proton chemical shift, homonuclear proton coupling, and static magnetic field inhomogeneity) are removed by the *soft* 180° ^{1}H pulse, applied at the mid-point of the interval Δ_1. In analogy with the regular INEPT experiment, the delay, Δ_1, is set to a duration on the order of

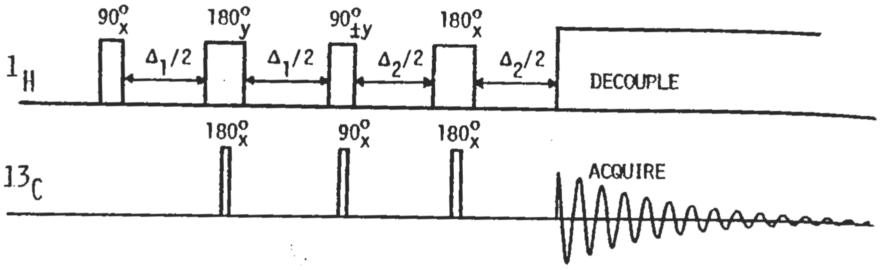

Figure 4. Pulse scheme of the selective INEPT experiment. All proton pulses are soft pulses ($\gamma H_2 \approx 20$ Hz), applied to a preselected proton resonance. The phase of the second 90° ^1H pulse is alternated along the ± y axis in successive experiments, and data are accordingly added and subtracted. For optimum polarization transfer from one proton to one ^{13}C nucleus with a scalar coupling J, the optimum condition for transfer is $\Delta_1 + 2\tau_{90} = \Delta_2 + \tau_{90} = 1/2J$, where τ_{90} is the duration of the soft 90° proton pulse (≈ 12 ms).

$1/(2\,^{1r}J_{CH})$, where $^{1r}J_{CH}$ is the expected value for the three-bond long-range J_{CH} coupling. To minimize the effects of ^1H transverse relaxation, a shorter value for Δ_1 is often selected (on the order of 50 msec). At the end of the interval, Δ_1, the 90°(^1H)/90°(^{13}C) pulse-pair transfers the proton polarization to the ^{13}C nucleus.[39,41] During the delay, Δ_2, the average precession of the transferred ^{13}C magnetization is determined solely by the long-range coupling $^{1r}J_{CH}$, to the preselected proton, since all other interactions are refocused by the ^{13}C pulse. After a time, $\Delta_2 = 1/(2\,^{1r}J_{CH})$, all transferred magnetization is in phase along the y axis, when high power broad-band proton decoupling is begun. To limit relaxation effects, the delay, Δ_2, is also chosen to be slightly (20-40%) shorter than $1/(2\,^{1r}J_{CH})$. For transfer of magnetization from the three equivalent protons of a methyl group to a ^{13}C nucleus that has a long-range coupling with those protons, the optimum Δ_2 value is approximately $1/(5\,^{1r}J_{CH})$.[41]

It has been shown[34] that the selective INEPT experiment is very sensitive and, like the regular INEPT experiment, can give a ^{13}C signal enhancement of approximately a factor of four. Because of a miss set of the delays, Δ_1 and Δ_2, for the generally unknown values

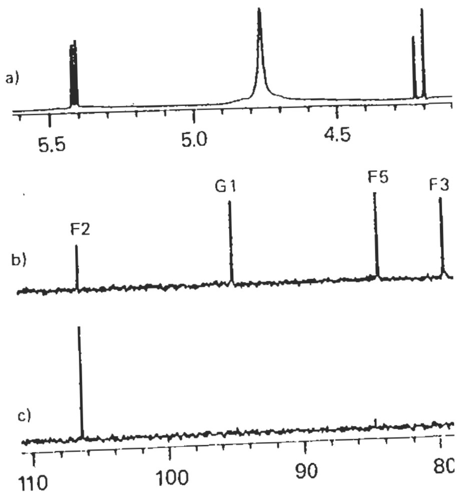

Figure 5. (a) Proton spectrum of suc
0.6 M solution in a 5 mm sample tube.
8 scans (c) Selective INEPT spectrum,
from 32 scans. All ^{13}C resonances th
range coupling with proton G-1 appear
spectrum.

of the long-range couplings, sensitivit
be better than, for example, selective
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transfer via long range couplings.[42]

As a first example, Fig. 5 shows t
of a 0.6 M solution of sucrose (2) in 2
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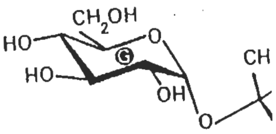

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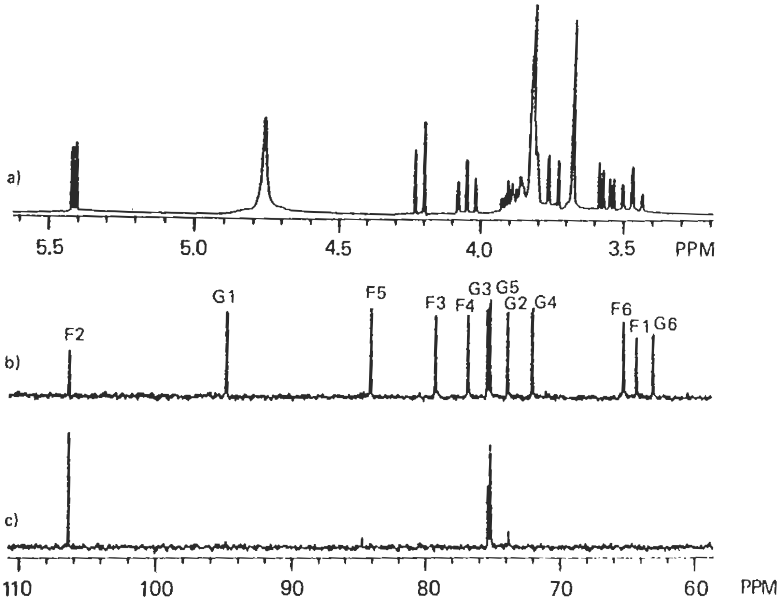
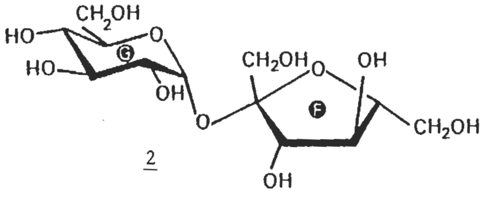


Figure 5. (a) Proton spectrum of sucrose recorded at 270 MHz, on a 0.6 M solution in a 5 mm sample tube. (b) ^{13}C spectrum obtained in 8 scans (c) Selective INEPT spectrum, pulsing proton G-1, obtained from 32 scans. All ^{13}C resonances that have a significant long-range coupling with proton G-1 appear in the selective INEPT spectrum.

of the long-range couplings, sensitivity will suffer, but will still be better than, for example, selective decoupling[30-32] or the two dimensional selective proton-flip experiment[29] or selective population transfer via long range couplings.[42]

As a first example, Fig. 5 shows the selective INEPT spectrum of a 0.6 M solution of sucrose (2) in ^2H_2O, selectively pulsing proton H-1 of the glucose ring, and yields glucose carbons G-3 and



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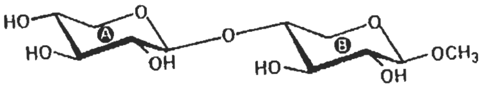
G-5 and fructose carbon F-2. The spectral assignments of sucrose were made originally by Pfeffer et al.[43] and later confirmed by a two-dimensional double quantum INADEQUATE experiment.[25] As expected, the two-bond coupling between H-1 and carbon G-2 is small (≈ 1Hz) (see references 44 and 45) and results in little polarization transfer in the selective INEPT experiment. The critical factor in applying the selective INEPT experiment for determination of the interglycosidic linkage position is the size of the three-bond coupling constant, $^3J_{HCOC}$. The magnitude of this coupling depends on the dihedral angle, in a Karplus-type relationship. The values of those three-bond couplings have been measured for sugars 1, 2 and 3, and are given in Table 3.



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All of the three-bond couplings across the glycosidic linkage lie between 3.5 and 5.5 Hz and are presumably typical values for this type of linkage. These couplings are sufficiently large to allow unambiguous use of the selective INEPT experiment. Figure 6 shows

TABLE 3. Magnitude of $^3J_{HCOC}$ Couplings in a Number of Oligosaccharides

Sugar	^1H	^{13}C	$^3J_{HCOC}$ (Hz)
1	A1	B4	4.6 ± 0.2
1	B4	A1	5.0 ± 0.1
1	C1	B2	3.5 ± 0.5
1	B2	C1	5.2 ± 0.1
1	B1	Me B1	5.1 ± 0.2
1	Me B1	B1	5.25 ± 0.1
1	Me C6	C6	7.0 ± 0.1
2	G1	F2	4.0 ± 0.1
3	A1	B4	4.5 ± 0.2
3	B4	A1	4.6 ± 0.2

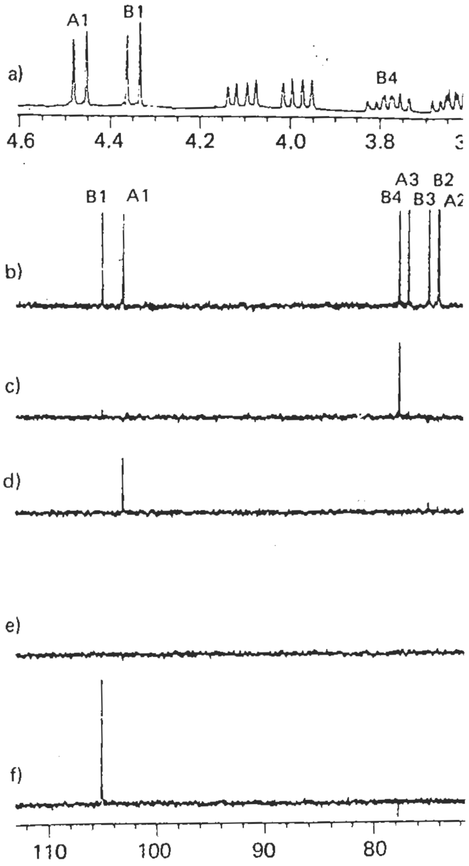


Figure 6. Spectra of a 0.3 M solution
in a 5 mm sample tube. (a) 270 MHz pro
spectrum obtained from 16 scans (c)-(f)
transferring from protons A1, B4, B1 an
respectively. All selective INEPT spec
In spectra (c)-(e), both delays, Δ_1 and
transfer from the methyl protons (g), d
Δ_2 to 24 ms.

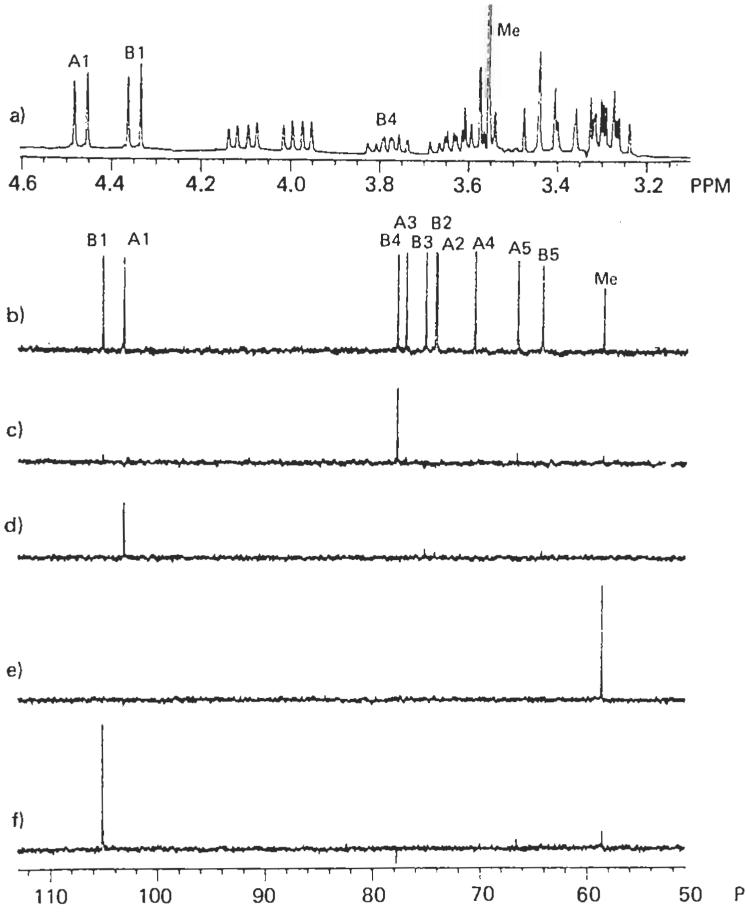


Figure 6. Spectra of a 0.3 M solution of disaccharide, 3, in ^2H_2O, in a 5 mm sample tube. (a) 270 MHz proton spectrum (b) 68 MHz ^{13}C spectrum obtained from 16 scans (c)-(f) Selective INEPT spectra, transferring from protons A1, B4, B1 and the methyl protons, respectively. All selective INEPT spectra result from 64 scans. In spectra (c)-(e), both delays, Δ_1 and Δ_2, were set to 50 ms. In transfer from the methyl protons (g), delay Δ_1 was set to 50 ms and Δ_2 to 24 ms.

the selective INEPT spectra for methyl β-xylobioside, 3. It is seen from Figure 6 that the interglycosidic linkage can be easily checked in both directions. Figure 6c shows transfer from proton A1 to carbon B4, and 6d shows transfer from proton B4 to carbon A1. Similarly, the methylated site can be found by transfer from proton B1 (Fig. 6e) or by transfer from the methyl protons (Fig. 6f). Transfer from overlapping, but not mutually coupled protons, is also possible but will generally show more ^{13}C resonances because magnetization is transferred from a number of different protons. (These ^{13}C resonance assignments are in agreement with those reported in the literature[46]). Figure 7 shows the selective INEPT spectra for the trisaccharide, I, that define the positions of interglycosidic linkages and that show the methylated sites. Figure 7g, which shows transfer from the methyl protons to carbon C6, also shows a low intensity resonance for carbon C4, due to transfer via $^2J_{CH}$ from proton C3, which is close in frequency to the methyl resonance selected. From the limited number of sugars investigated in this paper, one finds that the coupling across the interglycosidic linkage seems independent of the type of linkage (α and β). However, at least for the pyranose sugars, the couplings between the anomeric proton and carbons 3 and 5 of the same ring seem to be very small for the β configuration, but significant for the α configuration (the glucose unit in sucrose and ring C in 1).

Selective polarization transfer in a polymer

The use of the selective INEPT experiment is based on the presence of long-range heteronuclear scalar couplings. The question arises whether this technique is exclusively applicable to small molecules with a relatively long transverse relaxation time, T_2, or whether this method can also be applied to the study of macromolecules wherein long-range couplings are poorly or not at all resolved. It will be shown, that although the efficiency of the experiment decreases for short transverse relaxation times, a selective INEPT experiment is still feasible for T_2 values in excess of 100 msec. Experiments were performed on the Haemophilus influenzae type b capsular polysaccharide 4, a compound investigated in the past by

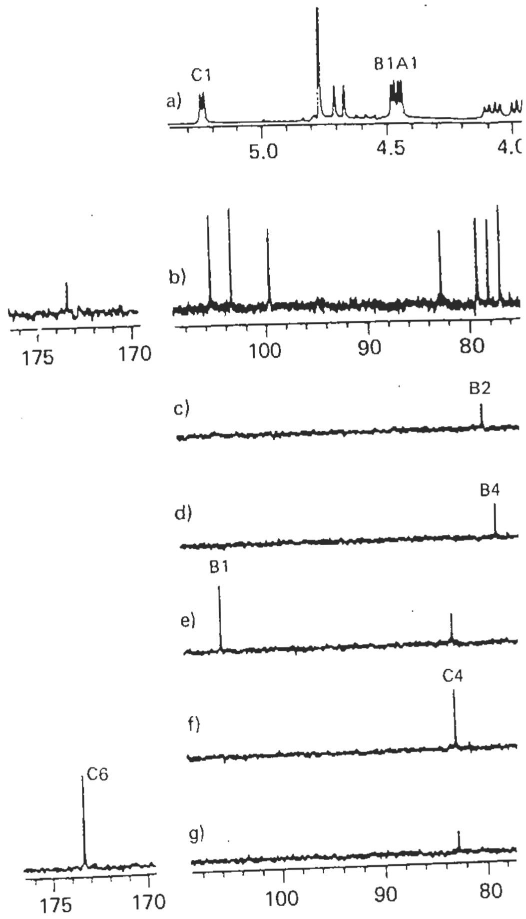


Figure 7. Spectra of a 0.15 M solution
^2H_2O, in a 5 mm sample tube. (a) 270 MH
MHz ^{13}C spectrum obtained from 64 scans.
spectra transferring from protons C1 (c)
and B1 (d), the methyl protons at 3.54 p
3.45 ppm (f) and the methyl protons at
INEPT spectra result from 256 scans.

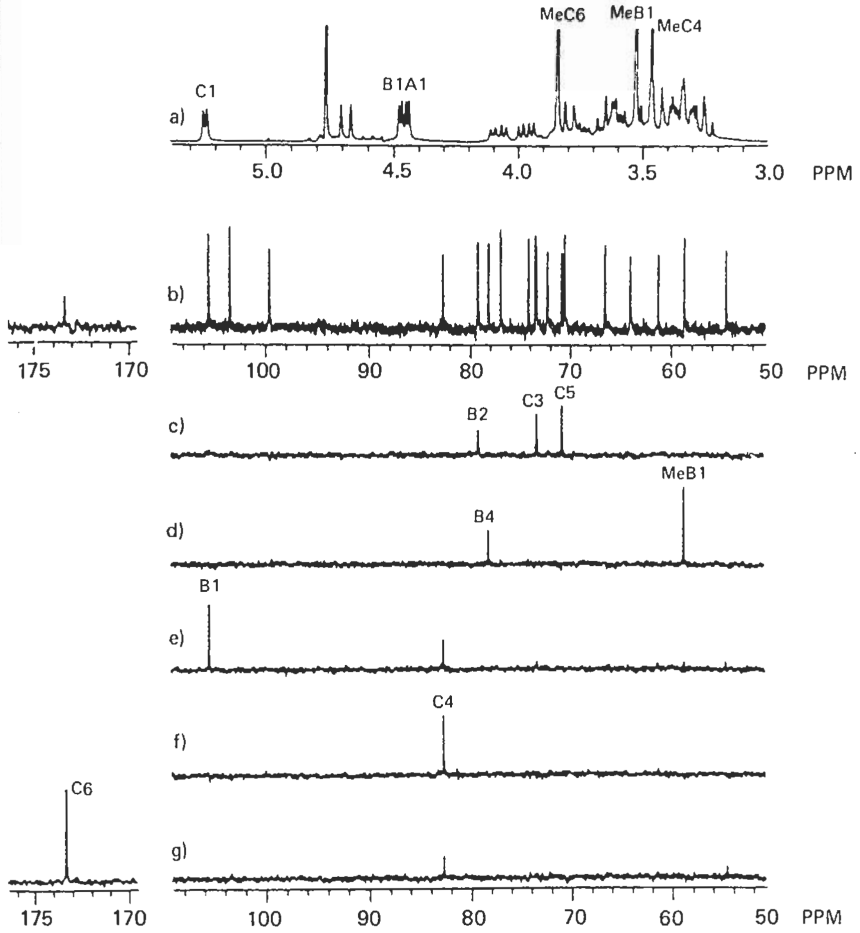


Figure 7. Spectra of a 0.15 M solution of the trisaccharide, 1, in ^2H_2O, in a 5 mm sample tube. (a) 270 MHz proton spectrum. (b) 68 MHz ^{13}C spectrum obtained from 64 scans. (c)-(g) Selective INEPT spectra transferring from protons C1 (c), overlapping protons A1 and B1 (d), the methyl protons at 3.54 ppm (e), the methyl protons at 3.45 ppm (f) and the methyl protons at 3.85 ppm (g). All selective INEPT spectra result from 256 scans.

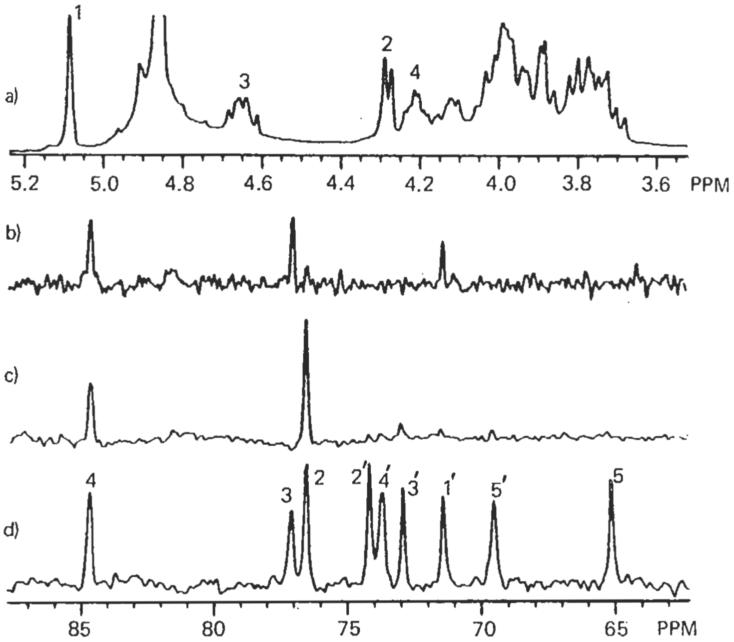
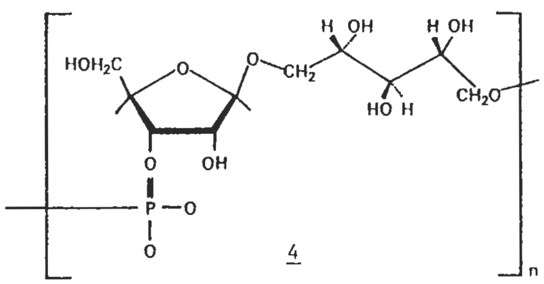


Figure 8. Spectra of a solution of 20 mg of polymer, 4, in 0.5 ml, ^2H_2O recorded on a NT-270 spectrometer. (a) ^1H spectrum. (b) ^{13}C spectrum resulting from 300 scans. (c) Selective INEPT spectrum, pulsing the anomeric proton H-1. The spectrum is the result of 3200 scans (1.2 hrs). (d) Decoupled selective population transfer spectrum, obtained from 300 scans, transferring from the down-field satellite of proton H-2. The smaller resonance is due to spurious transfer from the down-field satellite of proton H-4.

^{13}C NMR.[47] Figures 8a and b show the proton and ^{13}C spectra,



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recorded at 270 MHz. The proton transverse relaxation time of H-1 was determined using a selective T_2 experiment and found to be

160 ± 25 msec. ^{13}C T_2 values were measured
τ spin echo sequence with no proton decoupli
τ, and measured to be 80 ± 20 msec for the r
unit, and might possibly have been shortened
of the hydroxyl ^2H. Figure 8c shows the sel
obtained from pulsing proton H-1, and yields
(previously assigned[47]) and C-3. To show th
unassigned resonance is indeed C-3 and does
two-bond transfer to C-2, a ^1H-^{13}C correlat
H-2 was assigned using the COSY experiment.
selective population transfer (SPT) experim
low-field ^{13}C satellite of proton H-2, show
(high intensity) and C-4 (low intensity).
heteronuclear shift correlation is an extre
method in cases where only a small number
identified and where the proton spectrum i
allow a selective pulse for one of the ^{13}C
spectrum. The pulse sequence of this deco
sketched in Fig. 9. A number of 90° ^{13}C
selective 180° ^1H pulse saturates all ^{13}C
180° pulse, applied selectively to the ^{13}C
preselected proton, changes the intensity

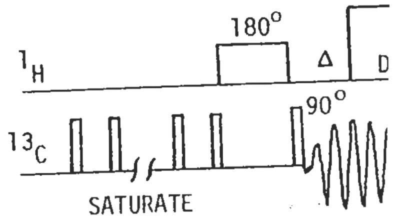

Figure 9. Pulse sequence of the deco
Saturation of the ^{13}C signal is obtai
number (>5) of 90° ^{13}C pulses spaced
radiofrequency field strength of the
the order of 20 Hz (pulse width 25 m
started immediately after the 90° ob
decoupling is switched on a time, Δ,
set to $1/(2J_{CH})$ for methine resonanc
methylene and methyl groups.

160 ± 25 msec. ^{13}C T_2 values were measured using a 90° – τ – 180° – τ spin echo sequence with no proton decoupling during the delays, τ, and measured to be 80 ± 20 msec for the ring carbons of the sugar unit, and might possibly have been shortened by slow exchange of the hydroxyl ^2H. Figure 8c shows the selective INEPT spectrum obtained from pulsing proton H-1, and yields resonances C-4 and C-1' (previously assigned[47]) and C-3. To show that the previously unassigned resonance is indeed C-3 and does not originate from a two-bond transfer to C-2, a ^1H-^{13}C correlation is necessary. First H-2 was assigned using the COSY experiment. Then the decoupled selective population transfer (SPT) experiment,[48] applied to the low-field ^{13}C satellite of proton H-2, shows resonances C-2 (Fig. 8d) (high intensity) and C-4 (low intensity). This one-dimensional heteronuclear shift correlation is an extremely sensitive and simple method in cases where only a small number of carbons have to be identified and where the proton spectrum is sufficiently resolved to allow a selective pulse for one of the ^{13}C satellites in the ^1H spectrum. The pulse sequence of this decoupled SPT experiment is sketched in Fig. 9. A number of 90° ^{13}C pulses prior to the selective 180° ^1H pulse saturates all ^{13}C magnetization. The proton 180° pulse, applied selectively to the ^{13}C satellite of one preselected proton, changes the intensity of the ^{13}C doublet

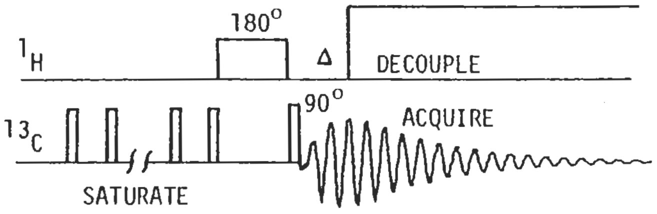

Figure 9. Pulse sequence of the decoupled SPT experiment. Saturation of the ^{13}C signal is obtained by the application of a number (>5) of 90° ^{13}C pulses spaced on the order of 15 msec. The radiofrequency field strength of the soft proton 180° pulse is on the order of 20 Hz (pulse width 25 ms). Data acquisition is started immediately after the 90° observe pulse, and broad-band decoupling is switched on a time, Δ, later. The duration of Δ is set to $1/(2J_{CH})$ for methine resonances and to $1/(4J_{CH})$ for methylene and methyl groups.

21. H. Kogler, O. W. Sorensen, G. Bodenhausen, and R. R. Ernst, J. Magn. Reson., 55, 157 (1983).
22. H. Kessler, M. Bernd, H. Kogler, J. Zarbock, O. W. Sorensen, G. Bodenhausen, and R. R. Ernst, J. Am. Chem. Soc., 105, 6944 (1983).
23. O. W. Sorensen and R. R. Ernst, J. Magn. Reson., 55, 338 (1983).
24. A. Bax, R. Freeman, and T. A. Frenkiel, J. Am. Chem. Soc., 103, 2102 (1981).
25. A. Bax, R. Freeman, T. A. Frienkiel, and M. H. Levitt, J. Magn. Reson., 43, 478 (1981).
26. T. H. Mareci and R. Freeman, J. Magn. Reson., 48, 158 (1982).
27. J. H. Prestegard, T. A. W. Koerner, P. C. Demou, and R. K. Yu, J. Am. Chem. Soc., 104, 4993 (1982).
28. G. Batta and A. Liptak, J. Am. Chem. Soc., 106, 248 (1984).
29. A. Bax and R. Freeman, J. Am. Chem. Soc., 104, 1099 (1982).
30. K. Bock and C. Pederson, J. Magn. Reson., 25, 227 (1977).
31. N. J. Koole and M. J. A. de Bie, J. Magn. Reson., 23, 9 (1976).
32. R. Freeman and H. D. Hill, J. Magn. Reson., 5, 278 (1971).
33. A. Bax, C. H. Niu, and D. Live, J. Am. Chem. Soc., 106, 1150 (1984).
34. A. Bax, J. Magn. Reson., 57, 314 (1984).
35. W. P. Aue, P. Bachmann, A. Wokaum, and R. R. Ernst, J. Magn. Reson., 29, 523 (1978).
36. M. H. Levitt, G. Bodenhausen, and R. R. Ernst, J. Magn. Reson., in press.
37. J. R. Garbow, D. P. Weitekamp, and A. Pines, Chem. Phys. Lett., 93, 504 (1982).
38. P. Kováč, J. Alfoldi, P. Kocis, E. Petrakova, and J. Hirsch, Cellulose Chem. Techn., 16, 261 (1982).
39. G. A. Morris and R. Freeman, J. Am. Chem. Soc., 101, 760 (1979).
40. G. A. Morris, J. Am. Chem. Soc., 102, 428 (1980).
41. D. P. Burum and R. R. Ernst, J. Magn. Reson., 39, 163 (1980).
42. H. J. Jakobsen and W. S. Brey, J. Am. Chem. Soc., 101, 774 (1979).
43. P. E. Pfeffer, K. M. Valentine, and F. W. Parris, J. Am. Chem. Soc., 101, 1265 (1979).
44. P. E. Hansen, Prog. Nucl. Magn. Reson. Spectrosc., 14, 175 (1981).
45. N. Cyr and C. S. Perlin, Can. J. Chem., 57, 2504 (1979).
46. P. Kováč, J. Hirsch, A. S. Shashkov, A. I. Usov, and S. Y. Yarotsky, Carbohydr. Res., 85, 177 (1980).

47. W. Egan in "Magnetic Resonance in Biology", J. New York, NY, 1980, Chapter 5.
48. A. Bax and S. K. Sarkar, J. Magn. Reson., in pre
49. K. G. R. Pachler and P. L. Wessels, J. Magn. Re

47. W. Egan in "Magnetic Resonance in Biology", J. S. Cohen, Ed., Wiley, New York, NY, 1980, Chapter 5.

48. A. Bax and S. K. Sarkar, J. Magn. Reson., in press.

49. K. G. R. Pachler and P. L. Wessels, J. Magn. Reson., 12, 337 (1973).