$CH_2Cl_2$. The uptake of 1 was followed by either measuring the UV spectra of the solutions or by analytical GC with benzophenone as an internal standard. The adsorption was followed until no further change in concentration was observed. Typically adsorption was complete in less than 24 h. The results are given in Figure 1.

**Desorption.** The composition of the adsorbed esters after irradiation was determined by treating the $CH_2Cl_2$ washed catalyst with methanol. The methanol solutions were concentrated and analyzed by GC with benzophenone as an internal standard.

**Photoisomerization with Solid Catalysts.** All reactions were carried out under a nitrogen atmosphere with conditions which maximized light falling on the catalyst and minimized irradiation of the solutions. (A) The outside limbs of the reactor shown in Figure 5 were packed with ground $SiO_2/Al_2O_3$ (10–20 mesh) or Nafion pellets. A $CH_2Cl_2$ solution of 1 was added to the central compartment of the reactor which was covered with black tape. The solution was stirred with a magnetic stirrer. The effect of the stirrer was to force the solution up through the external tubes and return it to the top of the central reservoir. The outside limbs were irradiated in the Rayonet reactor and the reactions followed by removing aliquots for GC analysis. (B) The ground $SiO_2/Al_2O_3$ (0.5 g) or Nafion pellets, solvent (30 mL), and substrate (30 mg) were placed in a stoppered test tube (100 mL) and stirred with a magnetic stirrer. The stirring rate was adjusted so that a suspension of the catalyst was maintained in the bottom 3–4 cm of the tube. The upper portion of the tube was covered with black tape to minimise light adsorption by the substrate in solution in the absence of the catalyst. The course of the reactions was followed by GC analysis with benzophenone as an internal standard.

**Registry No.** 1, 4192-77-2; 2, 4610-69-9; 3, 1896-62-4; 4, 937-53-1; 5, 1885-38-7; 6, 24840-05-9; Nafion, 39464-59-0.

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# $^{13}C$ and $^{1}H$ Chemical Shift Assignments and $^{1}H$–$^{19}F$ Spin–Spin Coupling Constants in Oligosaccharides and Fluorinated Oligosaccharides by Two-Dimensional $^{13}C$–$^{1}H$ Chemical Shift Correlation Spectroscopy with Proton Homonuclear Decoupling

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A version of the two-dimensional $^{13}C$–$^{1}H$ chemical shift correlation NMR spectroscopy which includes selective spin flip pulses has been used to resolve and assign $^{1}H$ and $^{13}C$ chemical shifts and to determine $^{1}H$–$^{19}F$ spin–spin couplings of a series of oligosaccharides and fluorinated oligosaccharides. The selective spin flip results in almost complete homonuclear decoupling in the $^{1}H$ dimension, leading to substantially better resolution and signal to noise ratio.

Two-dimensional (2-D) $^{13}C$–$^{1}H$ chemical shift correlation NMR spectroscopy has become in the last few years a powerful and indispensable technique in making complete proton and $^{13}C$ NMR assignments of complex molecules.[1-4] Recently a new pulse sequence, which selectively flips the protons not directly bonded to the $^{13}C$ nucleus observed, has been proposed.[5-9] This pulse sequence has been incorporated into the $^{13}C$–$^{1}H$ chemical shift correlation technique,[8,9] resulting in the elimination of most of the $^{1}H$–$^{1}H$ homonuclear spin–spin couplings in the $F_1$ dimension. This new $^{13}C$–$^{1}H$ chemical shift correlation technique has now been applied to the study of a steroid (progesterone)[10] and a fluorinated steroid (9α-fluorocortisol).[11] The advantages of this new technique, as observed from these studies, are manifold. The peaks along the $F_1$ dimension are sharper, facilitating more accurate measurement of the $^{1}H$ chemical shifts as well as increasing the signal to noise ratio. In the case of $CH_2$ with nonequivalent protons, the geminal coupling between the two protons attached to the same carbon is not eliminated by this pulse sequence. Furthermore, an additional advantage of this technique is that it facilitates convenient measurement of the magnitude and relative sign of the couplings between $^{1}H$ and additional heteronuclei (e.g., $^{19}F$, $^{31}P$),[9,11] which normally are almost impossible to measure in complex molecules even by elaborate double-resonance techniques. Therefore, this method is most suitable for the study of complex molecules with additional heteronuclei.

The work of Hall and Morris[3,12] have demonstrated that $^{13}C$–$^{1}H$ chemical shift correlation is the most useful and reliable means of assigning the $^{13}C$ and especially the $^{1}H$ spectra of oligosaccharides.

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Table I. $^{13}C$ and $^{1}H$ Chemical Shifts (in Hz) and $^{2}J_{HH}$ (in Hz) for Several Oligosaccharides[a]

| | δ($^{13}C$) | | | | | | δ($^{1}H$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| **1**, α[c] | 93.04 | 75.25 | 73.77 | 70.39 | 70.90 | 66.72 | 5.246 | 3.546 | 3.710 | 3.517 | 4.013 | 3.999, 3.713 (12.0)[b] |
| β[c] | 96.86 | 74.84 | 76.71 | 70.24 | 75.15 | 66.64 | 4.676 | 3.256 | 3.487 | 3.517 | 3.644 | 3.963, 3.765 (12.0) |
| | 99.03, 98.97[d] | 69.28 | 70.30 | 70.04 | 71.76 | 61.94 | 4.993, 4.983[d] | 3.827 | 3.891 | 4.000 | 3.977 | 3.749 |
| **2** | 88.95 | 69.33 | 69.33 | 76.10 | 71.98 | 61.55 | 6.249 | 5.011 | 5.446 | 3.813 | 3.696 | 4.406, 4.052 (12.5) |
| | 100.95 | 71.63 | 72.97 | 67.77 | 70.77 | 61.39 | 4.544 | 4.948 | 5.148 | 5.087 | 4.001 | 4.479, 4.123 (12.2) |
| **3** | 102.03 | 68.77 | 70.98 | 67.32 | 72.14 | 66.70 | 4.319 | 5.105 | 4.927 | 5.291 | 3.812 | 3.785, 3.701 (13.2) |
| | 100.63 | 68.58 | 70.93 | 67.49 | 71.89 | 66.44 | 4.463 | 5.089 | 4.894 | 5.290 | 3.803 | 3.753, 3.649 (11.5) |
| | 100.55 | 68.36 | 70.75 | 66.89 | 70.75 | 61.18 | 4.417 | 5.079 | 4.926 | 5.309 | 3.851 | 4.070 |
| **4**[e] | 88.85 | 69.38 | 69.32 | 76.20 | 71.94 | 61.50 | 6.222 | 4.981 | 5.409 | 3.798 | 3.661 | 4.350, 4.029 |
| | 100.78 | 71.73 | 72.58 | 76.11 | 72.66 | 62.21 | 4.496 | 4.812 | 5.101 | 3.773 | 3.619 | 4.401, 4.101 |
| | 100.55 | 71.85 | 72.58 | 76.11 | 72.75 | 62.15 | 4.463 | 4.841 | 5.079 | 3.773 | 3.586 | 4.396, 4.100 |
| | 100.78 | 71.63 | 72.85 | 67.72 | 70.94 | 61.38 | 4.496 | 4.889 | 5.110 | 5.055 | 3.982 | 4.463, 4.089 |

[a] Ordering of sugar rings starts with the reducing unit. [b] $^{2}J_{HH}$ in Hz in parentheses. [c] α and β form of the glucopyranose ring. [d] Corresponding to the α and β forms of the glucypyranose ring, respectively. [e] Some of the chemical shifts for carbons and protons with the corresponding positions in rings 2 and 3 may be interchangeable.

Fluorinated carbohydrates have been reported to have antitumor and many other biological activities.[13-16] They have been used as inhibitors in probing the active sites of hexokinase,[16] as well as in many other biochemical investigations (e.g., biosynthesis, monoclonal antibody–carbohydrate binding, lectin–carbohydrate affinities, etc.).[17-19] Besides $^{19}F$ NMR, $^{13}C$ and $^{1}H$ NMR are the most useful technique in probing their structures.

In the present investigation, a series of oligosaccharides and fluorinated oligosaccharides have been studied by this $^{1}H$–$^{13}C$ chemical shift correlation method. Assignments of the $^{1}H$ and $^{13}C$ spectra have been made unambiguously in most cases. The relative sign and magnitude of $^{1}H$–$^{19}F$ and $^{13}C$–$^{19}F$ couplings have also been measured.

## Experimental Section

The pulse sequence [A]

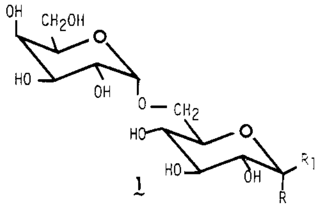
$$\begin{array}{lllll} {}^{1}\mathrm{H}: & \pi/2(\phi_1)-t_1/2-\pi/2(\phi_2)-\tau-\pi(\phi_1)-\tau-\pi/2(\phi_2)-t_1/2-\tau- & \pi(\phi_1)-\tau-\theta(\phi_3)-\tau\text{-dec.} & & \\ {}^{13}\mathrm{C}: & \qquad\qquad -\pi(\phi_3)- & -\pi/2(\phi_2)\quad -\pi(\phi_2)-\ \text{-acq.}(\pm) & & [A] \\ & |\qquad \text{evolution} \qquad | & \text{mixing} \qquad | & & \end{array}$$

has been used throughout this study. This pulse sequence combines the DEPT pulses[20] and the selective spin–flip pulses[5-9]

$$\begin{array}{lll} {}^{1}\mathrm{H}: & \ldots\pi/2(\phi_2)-\tau-\pi(\phi_1)-\tau-\pi/2(\phi_2)\ldots & \\ {}^{13}\mathrm{C}: & \qquad -\pi(\phi_3)- & [B] \end{array}$$

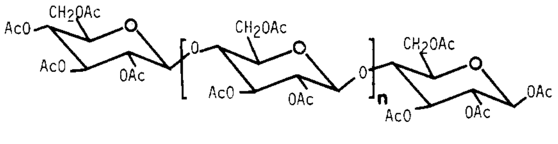
at the middle of the evolution time $t_1$ where $\tau$ is set equal to $1/(2^{1}J_{CH})$. It is identical with that used in ref 11 and has been discussed in detail there.[11] The variable proton pulse $\theta$ is usually set at $\pi/4$ to maximize the signal of the methylene carbons. The phase cycling scheme has been described previously.[11] Processing of the 2-D data sets also follows the procedure described in ref 10 and 11. Usually 128 × 2K or 128 × 4K data sets are generated by incrementing the evolution time $t_1$ in steps of 1.0–1.8 ms depending on the width of the proton spectral region of interest. In two of the more dilute samples, 64 data sets instead of 128 were obtained. Usually the data along the $t_1$ dimension are zero-filled to 4K or 8K points before Fourier transformation.

Chart I



1

| | R | R1 |
|---|---|---|
| α | OH | H |
| β | H | OH |



**2** n = 0

**4** n = 2

Melibiose (**1**) was purchased from Sigma Chemicals, cellobiose octaacetate (**2**) from Aldrich Chemicals. **3**,[21] **4**,[22] **5–9**,[23] and **10** and **11**[24] were synthesized according to published procedures.

Except for **1** and **8**, for which $D_2O$ was used as the solvent, all other samples were dissolved in $CDCl_3$. The concentration ranges from 0.07–0.7 M. A 5-mm $^{13}C$ probe in a Nicolet NT-300 spectrometer ($^{13}C$ frequency, 75.45 MHz; $^{1}H$ frequency, 300.05 MHz) was used. The measuring time ranges from 3 h to about 20 h for **11**, the concentration of which is 0.07 M.

Chemical shifts are referenced with respect to internal $Me_4Si$ for samples in organic solvents and internal TSP for aqueous samples (**1** and **8**). δ($^{13}C$) is usually determined to within 0.02 ppm, δ($^{1}H$) to 0.005 ppm, $^{2}J_{HH}$ to 1 Hz, and $J_{HF}$ to 0.5 Hz.

## Results and Discussion

**Chemical Shifts of Oligosaccharides.** The $^{13}C$–$^{1}H$ chemical shift correlation map of melibiose (**1**) (Table I) was obtained to compare with that obtained by Morris and Hall using the conventional heteronuclear chemical shift correlation technique.[12a] It is clear from the comparison

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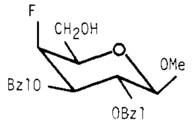
that the present results offer a better resolution and more precise determination of the proton chemical shifts. For example, in the present study it was possible to resolve easily the two $\delta(^{1}H)$ of proton 1 in the galactopyranosyl ring corresponding to the $\alpha$ and $\beta$ forms of the glucopyranose ring (0.01 ppm difference), while the work of Morris and Hall[12a] could only detect one chemical shift value for these two forms (uncertainty 0.05 ppm). Similarly, in the present study it was also possible to pinpoint the four chemical shifts (as well as the geminal couplings) of the two nonequivalent protons 6 of the $\alpha$ and $\beta$ glucopyranose ring. The previous study[12a] could only list two chemical shifts. This significant improvement obviously arises, not from the magnetic field difference (7.05 T vs. 6.35 T) but from the better resolution in the $F_1$ dimension due to the elimination of most of the homonuclear couplings.

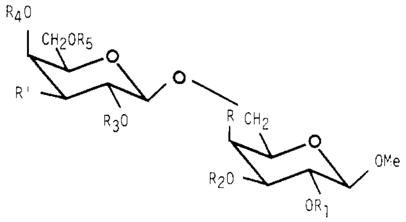
Three other oligosaccharides have also been studied and the data are presented in Table I. For cellotetraose tetradecaacetate (**4**), the overlapping of peaks both in the $F_1$ and $F_2$ dimensions made it difficult to make unambiguous assignments by itself even with the chemical shift correlation map. Therefore, cellobiose octaacetate (**2**) was studied as a model compound to aid the assignement of the spectra of **4**. By comparing the $^{13}C$–$^{1}H$ chemical shift correlation map and the $^{1}H$ 1-D spectrum of **2**, all the $^{1}H$ spin–spin coupling patterns and hence the connectivity of the protons were determined unequivocally. Therefore in **2** and in rings 1 and 4 of **4**, the $^{13}C$ and $^{1}H$ assignments are unambiguous. Some resonance assigned to corresponding carbons and protons in rings 2 and 3 of **4** may still be interchangeable. But in all these cases, the difference in chemical shifts between those interchangeable assignments is only of the order of 0.1 ppm for $^{13}C$ and 0.05 ppm for $^{1}H$. An assignment of the $^{13}C$ NMR spectra of **2** and **4** based on 1-D $^{13}C$ spectra alone has previously been made.[25] The present study shows that there are some descrepancies in that assignment, mainly in the identification of which ring the resonances should be assigned to. Considering the inadequate information offered by 1-D spectra, the discrepancies are relatively minor.

Likewise, the assignment for **3** is largely unambiguous. The $^{13}C$ and $^{1}H$ assignments makes it possible to infer from them the conformational properties of these oligosaccharides.[12,26] Although proton assignment for some disaccharides and even a few trisaccharides are known from continuous wave INDOR studies,[27] the INDOR method would prove to be extremely tedious or incapable of assigning more complicated oligosaccharides. A more recent and promising method[28] is by using a combination of 2-D $^{1}H$ techniques including 2D–$J$[29] and $^{1}H$ homonuclear chemical shift correlation (COSY)[30] spectroscopy. While this method is powerful in delineating assignments and sequencing of the oligosaccharides, the experimental and interpretation time required may be substantially greater except for samples with extremely limited quantity. Very recently, transient proton nuclear Overhauser effect (NOE) in the rotating frame has been applied to the assignment of a tetrasaccharide.[31] However the experiment was performed at 600 MHz (magnetic field, 14 T). Whether sufficiently good results can be obtained at lower field (e.g., 7–8 T) is yet to be determined. On the other hand, the $^{13}C$ assignment is generally less difficult. With the availability of the $^{1}H$–$^{13}C$ chemical shift correlation map any minor uncertainty in the assignment in both dimensions will usually be eliminated. Therefore $^{13}C$–$^{1}H$ chemical shift correlation method still remains as one of the most useful and convenient technique for spectral assignment in carbohydrates.

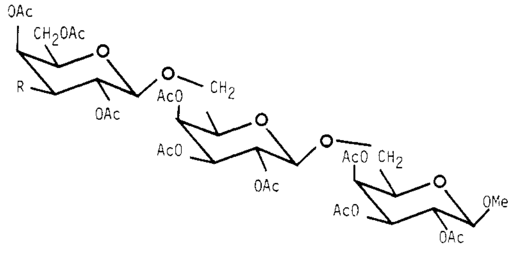
**Chart II**



**5**



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | R | R' |
|---|---|---|---|---|---|---|---|
| **6** | Bzl | Bzl | Ac | Ac | Ac | F | OAc |
| **7** | H | H | Ac | Ac | Ac | F | OAc |
| **8** | H | H | H | H | H | F | OH |
| **9** | Ac | Ac | Ac | Ac | Ac | F | OAc |
| **10** | Ac | Ac | Ac | Ac | Ac | OAc | F |



**3** R = OAc

**11** R = F

**$^{1}H$ and $^{13}C$ Chemical Shifts of Fluorinated Oligosaccharides.** $^{13}C$[32–34] and proton[34–39] chemical shift as-

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signments for a variety of fluorinated monosaccharides have been made in the past. Recently, proton COSY[30] has been used to make $^1$H assignments of a series of fluorinated monosaccharides.[40] However, no detailed investigation of the $^1$H spectra of fluorinated disaccharides or more complicated fluorinated oligosaccharides has been made. The present study represents the first such investigation. In addition to complete $^{13}$C and $^1$H assignments, the sign and magnitudes of the $^1$H–$^{19}$F couplings can be obtained (see next section). One monosaccharide (**5**), five disaccharides (**6–10**), and one trisaccharide (**11**) have been included in this study (Table II).

The $^1$H and $^{13}$C data derived from the correlation map showed that both of the chemical shifts of those nuclei on rings with the same structure and substituents are very consistent, i.e., usually within 0.1 ppm. Therefore, the substituent effect due to $^{19}$F can be easily deciphered. For example, when fluorine is in the axial position at carbon 4, $\delta(^1H)$ of the geminal proton shifts downfield by 0.5 ppm in acylated rings, and by more than 0.9 ppm for unacylated rings. In contrast, an equatorial fluorine at position 3 causes an upfield shift of ~0.3–0.4 ppm for the axial $H_3$. However, both axial and equatorial fluorines cause downfield shifts for vicinal protons by 0.1–0.2 ppm, the latter by a slightly larger magnitude. When the 1-D $^1$H spectrum of these fluorinated oligosaccharides are compared with the chemical shift correlation map, the complete $^1$H assignments, in many cases including most of the coupling patterns, can be made. These, and the conformational information derived, will be presented in more detail elsewhere.

Pulse sequence [A] eliminates all $^1$H–$^1$H homonuclear couplings, except in the case of methylene carbons with two nonequivalent protons.[9-11] In the latter situation, the geminal coupling between the protons remains, leading to four peaks in the $F_1$ dimension. Therefore, methylene carbons with equivalent and nonequivalent protons can be clearly distinguished by this technique. Several $C_6$ methylenes at the nonreducing end of the sugar chain contain nonequivalent protons (**2**, **4**, **5**, and **9**). The nonequivalence of the methylene protons is due to the unequal populations of the three staggered conformations along the $C_5$–$C_6$ bond. The asymmetry was caused by the nonsymmetrical environment at $C_5$ of the pyranose ring. The equivalence of $C_6$ protons in the other compounds was probably due to accidental equivalence. For the two compounds with benzylic substituents at positions 2 and 3 (**5** and **6**), the benzylic methylene carbon at about 75 ppm contains nonequivalent protons, while that at about 72 ppm contains equivalent protons (Table II). Since the $\delta(^{13}C)$ of benzylic methylene carbons at positions 3 and 4 were found to range from 71.4 to 73.0 ppm in several other closely related saccharides,[41] the peak at 75 ppm in **5** and **6** should then be assigned to the one at the position 2. The nonequivalency of the benzylic protons at position 2 in contrast to the equivalent benzylic protons at position 3 must reflect a more symmetrical environment for the OBzl group at position 3 than at position 2. Such information is useful in probing the difference in the steric factors and bonding at various positions of the sugar rings and is difficult to obtain from 1-D $^1$H spectrum of oligosaccharides.

**$^1$H–$^{19}$F Coupling Constants.** The $^1$H–$^{19}$F couplings constants have been measured for a variety of monosaccharides.[34-40] Double resonance technique has also been

Table II. $^{13}$C and $^1$H Chemical Shifts (in ppm), $J_{CF}$ and $^2J_{HH}$ (in Hz) of Some Fluorinated Oligosaccharides[a,b]

| | $\delta(^{13}C)$ | | | | | | | $\delta(^1H)$ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| compd | 1 | 2 | 3 | 4 | 5 | 6 | OMe | 1 | 2 | 3 | 4 | 5 | 6 | OMe |
| **5**[c,d] | 104.64 | 78.97 | 78.85 (17.7) | 86.04 (182.9) | 73.46 (17.9) | 60.93 (5.3) | 57.25 | 4.301 | 3.694 | 3.494 | 4.730 | 3.445 | 3.863, 3.735 (12.0) | 3.567 |
| **6**[c] | 104.53 | 78.81 | 78.67 (18.5) | 86.26 (183.4) | 72.72 (17.9) | 68.09 (4.8) | 57.07 | 4.273 | 3.670 | 3.474 | 4.726 | 3.562 | 4.028, 3.862 (12.7) | 3.569 |
| | 101.37 | 68.83 | 70.85 | 67.05 | 70.78 | 61.34 | | 4.593 | 5.204 | 5.010 | 5.323 | 3.920 | 4.145 | |
| **7** | 103.67 | 71.46 | 72.18 (18.2) | 88.73 (181.5) | 72.80 (17.9) | 67.87 (5.2) | 57.09 | 4.195 | 3.646 | 3.646 | 4.739 | 3.708 | 4.049, 3.838 (11.7) | 3.559 |
| | 101.26 | 68.79 | 70.80 | 67.09 | 70.80 | 61.30 | | 4.598 | 5.192 | 5.018 | 5.395 | 3.956 | 4.145 | |
| **8** | 103.35 | 70.67 | 71.32 (18.1) | 89.55 (178.2) | 72.33 (17.4) | 67.80 (4.6) | 57.40 | 4.366 | 3.493 | 3.724 | 4.819 | 3.981 | 4.065, 3.891 (12.9) | 3.535 |
| | 103.24 | 70.67 | 72.59 | 68.53 | 75.08 | 60.90 | | 4.409 | 3.487 | 3.601 | 3.873 | 3.645 | 3.721 | |
| **9** | 101.73 | 68.75 | 71.39 (17.6) | 86.16 (185.3) | 72.48 (18.0) | 67.40 (5.0) | 56.75 | 4.417 | 5.189 | 4.974 | 4.854 | 3.787 | 4.041, 3.868 (11.5) | 3.510 |
| | 101.31 | 68.71 | 70.86 | 67.13 | 70.86 | 61.40 | | 4.602 | 5.214 | 5.023 | 5.391 | 3.978 | 4.181, 4.118, (12.5) | |
| **10** | 102.06 | 68.76 | 70.92 | 67.54 | 72.19 | 66.93 | 57.05 | 4.335 | 5.126 | 4.954 | 5.314 | 3.840 | 3.795, 3.684 (10.4) | 3.483 |
| | 100.08 (10.3) | 69.54 (19.5) | 88.73 (195.6) | 66.79 (19.8) | 69.94 (6.6) | 61.24 | | 4.436 | 5.201 | 4.550 | 5.494 | 3.790 | 4.097 | |
| **11** | 102.12 | 68.83 | 71.04 | 67.63 | 72.34 | 66.88 | 57.03 | 4.380 | 5.178 | 4.993 | 5.364 | 3.872 | 3.788, 3.745 | 3.533 |
| | 100.74 | 68.68 | 70.97 | 67.47 | 72.36 | 66.61 | | 4.540 | 5.164 | 4.977 | 5.362 | 3.871 | 3.737, 3.693 | |
| | 100.06 (11.1) | 69.59 (19.3) | 88.71 (194.2) | 66.85 (16.5) | 70.11 (5.9) | 61.30 | | 4.449 | 5.231 | 4.603 | 5.553 | 3.828 | 4.154 | |

[a] Numbers in parentheses on the $^{13}$C side denote $J_{CF}$ in Hz, on the $^1$H side $^2J_{HH}$ in Hz. For sign of $J_{CF}$, see text.
[b] Ordering of sugar rings starts with the reducing unit.
[c] Chemical Shifts for benzylic methylenes: **5**, $\delta(^{13}C)$ 75.32, 72.46; $\delta(^1H)$ 4.822, 4.751 ($^2J_{HH}$ = 11.3), 4.734; **6**, $\delta(^{13}C)$ 75.25, 72.46; $\delta(^1H)$ 4.880, 4.742 ($^2J_{HH}$ = 12.0); 4.735.
[d] The assignment of the $^{13}$C spectrum of **5** in $D_2O$ has been made previously.[45] Minor variations in $\delta(^{13}C)$ due to solvent effects are found. In addition, the $^1$H–$^{13}$C chemical shift correlation and $^1$H–$^{19}$F coupling constants in the present study show that the earlier assignment of $C_3$ and $C_5$ should be interchanged.

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**Table III. The $^1H$–$^{19}F$ Coupling Constants (in Hz) for Some Fluorinated Oligosaccharides**

| | fluorine position[a] | $^{19}F$–$^1H_1$ | $^{19}F$–$^1H_2$ | $^{19}F$–$^1H_3$ | $^{19}F$–$^1H_4$ | $^{19}F$–$^1H_5$ | $^{19}F$–$^1H_6$[d] | $^{19}F$–$^1H_6$[d] |
|---|---|---|---|---|---|---|---|---|
| **5** | 4a | | | 31.5[b] | +50.4 | 27.7[b] | 1.3[b] | 1.4[b] |
| **6** | 4a | | | 31.0[b] | +49.6 | 27.0[b] | 1.1[b] | 1.8[b] |
| **7** | 4a | | | 27.7[b] | +49.3 | 26.9[b] | 1.4[b] | 1.9[b] |
| **8** | 4a | | | 32.6[b] | +49.7 | 28.7[b] | 1.2[b] | 1.8[b] |
| **9** | 4a | | | 30.3[b] | +50.0 | 26.9[b] | 1.5[c] | 1.2[c] |
| **10** | 3e | 1.3[c] | 12.7[b] | +47.5 | 5.1[b] | 2.8[c] | | |
| **11** | 3e | 1.5[b] | 12.0[b] | +47.6 | 6.3[b] | 1.0[b] | | |

[a] a and e denote axial and equatorial positions, respectively. The number indicates the carbon number of the sugar ring which bears the fluorine nucleus. [b] Coupling is of the same sign as the corresponding $^{13}C$–$^{19}F$ coupling. [c] Coupling is of opposite sign as the corresponding $^{13}C$–$^{19}F$ coupling. [d] Corresponding to the lower field and higher field proton of the nonequivalent methylene group, respectively.

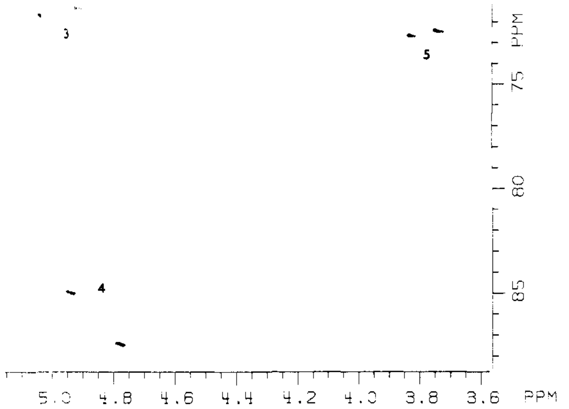

**Figure 1.** A portion of the $^{13}C$–$^1H$ chemical shift correlation map for $C_3$, $C_4$, and $C_5$ of **9**, where both the protons and carbons have observable coupling with $^{19}F$ at $C_4$. The orientation of the doublet of $C_4$ shows that $^2J_{H_4F}$ and the corresponding $^1J_{C_4F}$ are of opposite signs, while the orientation of the doublets of $C_3$ and $C_5$ indicate that $^3J_{H_3F}$ and $^3J_{H_5F}$ are of the same signs as those of the corresponding $^2J_{C_3F}$ and $^2J_{C_5F}$, respectively.

used, mainly by Hall and co-workers, to determine the sign of the $^1H$–$^{19}F$ coupling constants.[38,39] However, it would be rather difficult or even impossible to apply simple one-dimensional $^1H$ and $^{19}F$ NMR or double resonance method to fluorinated saccharides of greater complexity, since the $^1H$ spectrum usually cannot be assigned or resolved. Therefore, no work on even fluorinated disaccharides has been done.[42,43] In the present study, with the proton resonances in the $F_1$ dimension devoid of homonuclear coupling and free from overlapping with signals from other protons, $^1H$–$^{19}F$ coupling can be readily observed in an unambiguous fashion. From the two-dimensional chemical shift correlation map, the relative signs of the $J_{HF}$ and the corresponding $J_{CF}$ can be determined.[11] In Figure 1, the contour map of such correlation is shown for $C_3$, $C_4$, and $C_5$ of compound **9**. For $C_4$, the doublets due to coupling with $^{19}F$ in the $^{13}C$ and $^1H$ dimensions are shifted in opposite directions (i.e., the component of the doublet with the higher $^{13}C$ frequency has the lower $^1H$ frequency). The sign of $^2J_{H_4F}$ is, therefore, opposite to that of $^1J_{C_4F}$. Since $^1J_{CF}$ is always negative,[42,43] it follows that $^2J_{H_4F}$ is positive. On the other hand, for $C_3$ and $C_5$, the correlation map shows that the $^1H^{19}F$ and $^{13}C^{19}F$ doublets are shifted in the same direction (i.e., the component of the doublet with the higher $^{13}C$ frequency also is higher in $^1H$ frequency), indicating that the $^3J_{HF}$ and the corresponding $^2J_{CF}$ are of the same sign. Double resonance experiments in monosaccharides have shown that $^3J_{HF}$ are mostly positive.[38,39] Therefore the present study provides a correlation which establishes that $^2J_{CF}$ in fluorinated sugars are positive. While the sign of $J_{HF}$ in simple molecules can be determined by using double resonance techniques, there has been no study of the sign of $^2J_{CF}$ in fluorinated sugars nor in most other types of molecules except substituted fluorobenzenes and some very simple model compounds.[42,43] Thus the sign of $^nJ_{CF}$ for $n \gtrsim 2$ is essentially unknown. This technique would provide a simple and superior way of determining the sign of $^nJ_{CF}$. This applies, of course, to other heteronuclei of spin $^1/_2$, such as $^{31}P$, as well.

The magnitude and sign of $^1H$–$^{19}F$ coupling are summarized in Table III. There are clearly significant differences in $^1H$–$^{19}F$ couplings between $^{19}F$ in the axial position and $^{19}F$ at the equatorial position. For the former case, the $^3J_{HF}$ corresponding to $F_{ax}$–$H_{ax}$ is larger, ranging from 26.9 to 32.6 Hz (±0.5 Hz) in **5**–**9**. When $^{19}F$ is at the equatorial position, $^3J_{HF}$ are smaller. In compounds **10** and **11**, $^3J_{HF}(F_{eq}$–$H_{ax})$ was found to be about 12–13 Hz, while $^3J_{HF}(F_{eq}$–$H_{eq})$ ranges from 5 to 6 Hz. This is more or less consistent with those found in monosaccharides.[33-39] It is also consistent with the Karplus type of dependence on dihedral angles for $^3J_{HF}$.[44]

Equatorial $^{19}F$ has been reported to have relatively large coupling with protons four bonds or more away, especially with equatorial protons which form "planar M" configuration with the $^{19}F$.[38] In **10** and **11**, however, $^{19}F$ at 3 equatorial only can have $^4J_{HF}$ with axial protons 1 and 5. Both were observed to range in magnitude from 1.0 to 2.8 Hz. In previous works,[38] Foster et al. reported that $^4J_{HF}$(e,a) is negative in all compounds they studied. However, the present work revealed that (Table III), in **11**, all $^4J_{HF}$ are of the same sign as the corresponding $^3J_{CF}$, while in **10**, they are of opposite signs. This means either the $^4J_{HF}$ changes sign between **10** and **11** or the corresponding $^3J_{CF}$ do. For saccharides **5**–**9**, no $^4J_{HF}$ for $^{19}F$ at position 4 axial with ring proton 2 was observed. However, $^4J_{HF}$ with both protons at carbon 6 was clearly observed. Again the relative sign of these $^4J_{HF}$ and the corresponding $^3J_{CF}$ varies with compounds. Thus, in **5**–**8**, $^4J_{HF}$ and $^3J_{CF}$ are of the same sign, while in **9**, they are of opposite signs. The accuracy in measuring the $^4J_{HF}$ involving the nonequivalent methylene protons 6 suffers somewhat more than those of other protons. The uncertainty is likely to be close to 1 Hz. However, the determination of the relative sign is unmistakable in every case. Since the absolute sign of neither $^4J_{HF}$ nor $^3J_{CF}$ is known for certain in these compounds, the present result is not sufficient by itself to establish the absolute sign. However the information on the relative signs is valuable in providing a useful link between these parameters.

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The sign and magnitude of the $^1H$–$^{19}F$ couplings have been shown to be stereospecific, the present results can thus be used to shed some light on the conformations of these oligosaccharides. However a detailed discussion of the information offered by the $^1H$–$^{19}F$ couplings is beyond the scope of the present report and will be presented in a later communication.

## Conclusion

This investigation presents the first detailed study of the $^1H$ spectra of fluorinated and nonfluorinated oligosaccharides by a new $^{13}C$–$^1H$ chemical shift correlation technique which incorporates the selective spin–flip pulses resulting in homonuclear decoupling in the $^1H$ dimension. This study clearly demonstrates the improved resolution and signal to noise ratio achieved by this new version of the heteronuclear chemical shift correlation spectroscopy. We have demonstrated in this and previous studies[9-11] that this technique can be applied routinely to complex molecules with great advantage. Very high magnetic field ($B_0$ > 7 T) is not necessary for excellent resolution in the $^1H$ dimension. The provision of measuring both the relative sign and magnitude of $^1H$–X spin–spin coupling offered by this technique enables the measurement of these important parameters in complex molecules. Even in the case of simple molecules, it may be an attractive alternative to double resonance methods.

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**Registry No.** **1**α, 13299-20-2; **1**β, 13299-21-3; **2**, 20764-63-0; **3**, 92397-29-0; **4**, 52523-12-3; **5**, 51996-35-1; **6**, 92420-18-3; **7**, 92397-30-3; **8**, 92397-31-4; **9**, 92397-32-5; **10**, 89185-94-4; **11**, 89185-97-7.

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# Nonconventional Electrophilic Heteroaromatic Substitutions: Ring vs. Side-Chain Reactivity of 2,5-Dimethyl Five-Membered Ring Heterocycles toward Electron-Deficient Aromatics

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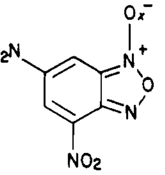
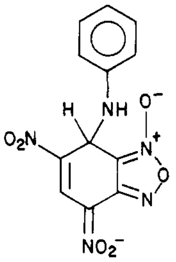
4,6-Dinitrobenzofuroxan (DNBF) and 4,6-dinitrobenzofurazan (DNBZ) behave as conventional electrophiles toward 2,5-dimethylpyrrole, 2,5-dimethylthiophene, and 2-methylbenzofuran, adding to the unsubstituted β-carbon to form the corresponding σ adducts in $Me_2SO$. In contrast reactions with 2,5-dimethylfuran result in electrophilic substitution of a methyl group as the only observed process. The mechanism of the latter reaction is discussed with reference to previously reported results. This leads to some reconsideration of current notions regarding the mechanism of electrophilic side-chain reactions of 2,5-dimethyl five-membered ring heterocycles. It is also concluded that DNBF and DNBZ have an electrophilic character of the same order as that of 2,4-dinitrobenzenediazonium cation.

## Introduction

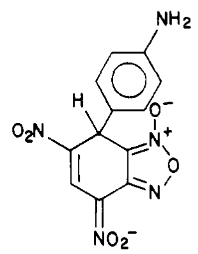
The possibility of using the extremely high electrophilic character of 4,6-dinitrobenzofuroxan (DNBF) to assess the reactivity of a number of very weak carbon nucleophiles has been recently emphasized.[1-3] This is best illustrated by the reaction of DNBF with aniline.[1,2] As expected, the nitrogen-bonded adduct 1 is formed immediately but subsequent rearrangement occurs leading to the carbon-bonded adduct 2 as the product of thermodynamic control.[4] This provides evidence that aniline can act, as do phenols,[5-7] as a carbon nucleophile in σ complex formation and related nucleophilic aromatic substitution reactions with nitroarenes.[8-10]



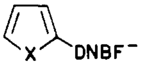
$x = 0$ DNBZ
$x = 1$ DNBF



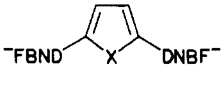
**1**



**2**

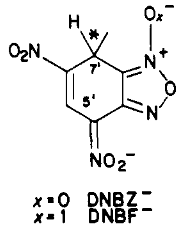


**3**



**4**

X = NH, S, O



$x = 0$ DNBZ⁻
$x = 1$ DNBF⁻

Also noteworthy are the reactions of DNBF with π-excessive heterocycles like furan, thiophene, and pyrrole,

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