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pH Dependent C6 and C8 ^{13}C Chemical Shift Assignment in *N*-Acetyl Neuraminic Acid.

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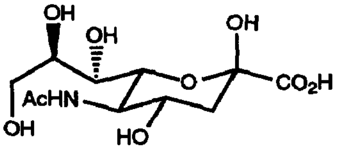
Abstract: Heteronuclear correlation NMR spectroscopy was employed in the unambigous carbon chemical shift assignment of *N*-acetyl neuraminic acid at pH 1.0 and 7.0. Our results show that at pH 1.0 the C6 resonance is slightly downfield from that of C8 and at pH 7.0 the C6 and C8 chemical shifts are transposed. These experiments firmly establish carbon chemical shift assignments in *N*-acetyl neuraminic acid for the first time.

We have initiated an NMR program targeting new methods for studying carbohydrates in solution as part of a larger goal of studying protein bound carbohydrates. We are especially interested in recognition processes involving sialoglycoconjugates and recently we reported ^{1}H and ^{13}C solution phase chemical shift anisotropy (CSA) data for *N*-acetyl neuraminic acid (NeuAc) **1**.[1] Interpretation of our results required complete carbon chemical shift assignment and we were surprised to find that this had not been firmly established in the literature.



N-acetyl neuraminic acid **1**

The first ^{13}C NMR studies on NeuAc were performed at 22.63 MHz in D_2O. The resonances corresponding to C6, C7, and C8 were poorly resolved at this low field and complete assignment was not possible.[2] Carbon chemical shift data was also reported at 25.16 MHz in D_2O and although better resolution allowed assignment of the C7 resonance (69.4 ppm), the C6 (71.3 ppm) and C8 (71.5 ppm) resonances were only tentatively assigned on the basis of chemical shift changes in acetylated derivatives.[3] It occurred to us that unambiguous chemical shift assignment could be achieved using heteronuclear correlation NMR spectroscopy (HETCOR). The results of those experiments on sialic acid and its sodium salt are reported herein.

Figure 1: 75.43 MHz HETCOR Spectrum of 0.45M Sialic Acid in D_2O, pH 1.

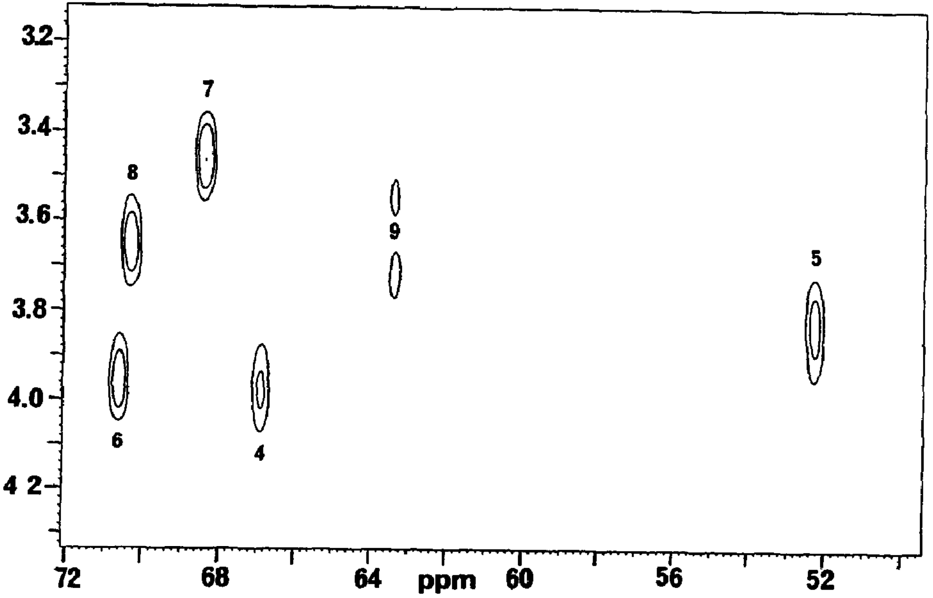
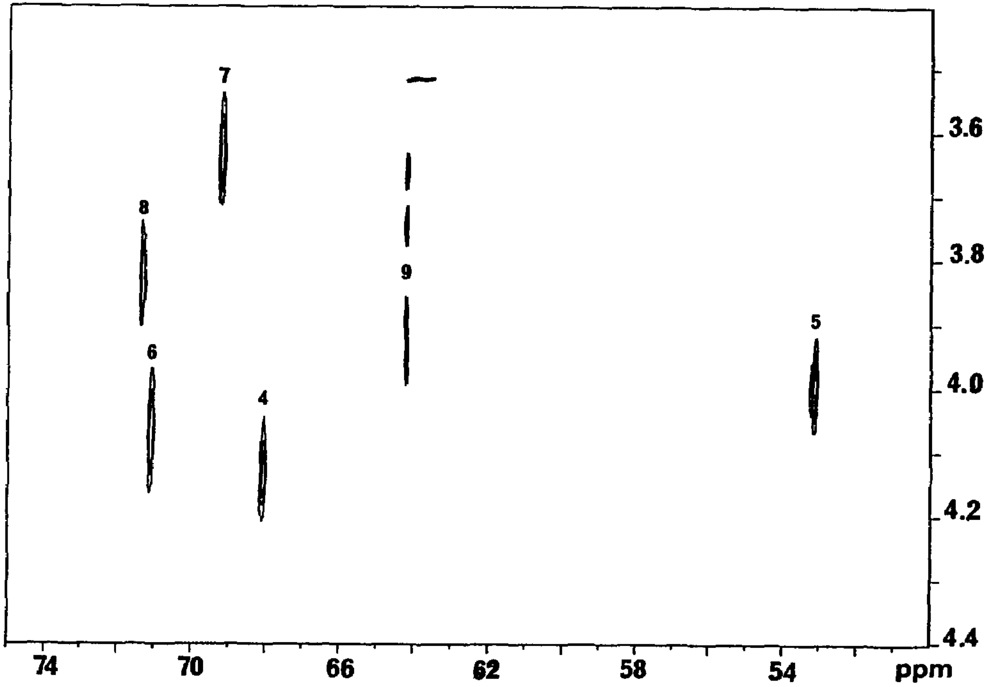


Figure 2: 62.5 MHz HETCOR Spectrum of 0.9M Sialic Acid Sodium Salt in D_2O, pH 7.



Proton chemical shift assignment of NeuAc was made by Brown et al. at 270 MHz in D_2O.[4] Our assignments were in agreement with theirs and the proton chemical shift values are listed in table 1.[5] Figure 1 shows the HETCOR of 0.45M NeuAc in D_2O at 75.43 MHz (pH 1.0).[6] It is clear from this experiment that the peak occurring at 71.19 ppm is coupled to the proton at 4.00 ppm which is H6. Similarly the 70.90 ppm resonance is coupled to H8. The resonances occurring at 68.98 and 67.41 ppm are well resolved and correspond to C7 and C4, respectively. C9 appears as a doublet at 63.92 ppm (coupled to H9 and H9'). The complete ^{13}C assignment for sialic acid is listed in table 2.

Table 1: Proton Chemical Shifts for *N*-acetyl neuraminic acid and *N*-acetyl neuraminic acid Sodium Salt

	$H3_{ax}$	$H3_{eq}$	H4	H5	H6	H7	H8	H9	H9'
NeuAc[a]	1.66	2.10	3.85	3.71	3.84	3.34	3.53	3.62	3.40
NeuAc[b] sodium salt	1.59	1.97	3.81	3.67	3.75	3.32	3.53	3.60	3.40

a. Chemical shifts are reported in ppm from an internal dioxane standard (δ=3.53ppm) for a 0.45M solution in D_2O at rt., pH 1. b. Chemical shifts are reported in ppm from an internal dioxane standard for a 0.9M solution in D_2O at rt., pH 7.

Since these assignments differed from earlier reports[3] we decided to do a HETCOR on the sodium salt of *N*-acetyl neuraminic acid which was prepared by titration of the acid with 1M NaOH. We also did a COSY experiment to confirm the proton chemical shift assignments which are listed in table 1. There were not large differences in the proton chemical shifts however H6 was shifted slightly upfield. The HETCOR spectrum shown in figure 2 clearly shows that the positions of C8 and C6 are transposed. The resonance peak at 71.33 ppm is coupled to H8 and the slightly upfield peak at 71.08 ppm is coupled to H6. The remaining assignments are listed in table 2.

Several ^{13}C NMR studies of NeuAc have appeared in the literature which cite tentative assignments placing C8 downfield from C6.[7] The results reported herein unambiguously confirm the earlier assignments at neutral pH. However, these pH dependent chemical shifts warrant caution in future studies involving NeuAc and care should be taken in reporting the pH of the solutions studied and in verifying the carbon chemical shifts.

Table 2: Carbon Chemical Shifts for *N*-Acetyl Neuraminic Acid and *N*-Acetyl Neuraminic Acid Sodium Salt

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C=O	CH_3
NeuAc[a]	173.79	95.97	39.55	67.41	52.83	71.19	68.98	70.70	63.92	175.63	22.86
NeuAc[b] Na salt	175.73	97.33	40.25	68.05	53.12	71.08	69.21	71.33	64.18	177.78	23.35

a. Chemical shifts are reported in ppm relative to dioxane internal standard (δ=67.4 ppm) for 0.45M solutions in D_2O at room temperature, pH 1. b. Chemical shifts are reported in ppm from an internal dioxane standard for a 0.9M solution in D_2O at rt., pH 7.

In conclusion, unambiguous carbon chemical shift assignments of sialic acid and its sodium salt were made possible using heteronuclear correlation NMR spectroscopy. At pH 1.0 the resonance occurring at 71.19 ppm is coupled to H6 and is therefore assigned to C6 whereas the slightly lower field resonance at 70.90 ppm is assigned to C8 due to coupling to H8. At neutral pH the positions of C6 and C8 are transposed with C8 occurring at 71.33 ppm and C6 at 71.08 ppm.

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References:

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1. Batta, Gy.; Gervay, J. *J. Am. Chem. Soc.* submitted for publication.

2. Eschenfelder, V.; Brossmer, R.; Friebolin, H. *Tetrahedron Lett.* **1975**, 3069-3072.

3. Bhattacharjee, A.K.; Jennings, H.J.; Kenny, C.P.; Martin, A.; Smith, I.C.P. *J. Biol. Chem.* **1975**, *250*, 1926-1932.

4. Brown, E.B.; Brey, W.S.Jr.; Weltner, W.Jr. *Biochim. Biophys. Acta* **1975**, *399*, 124-130.

5. The absolute values differ due to different reference points however the relative positions are identical.

6. We also perfomed a HETCOR on a 0.9M solution at pH 1 and no differences in the spectra were observed.

7. Czarniecki, M.F.; Thornton, E.R. *Biochem. Biophys. Res. Comm.* **1977**, *74*, 553-558. Czarniecki, M.F.; Thornton, E.R. *J. Am. Chem. Soc.* **1977**, *99*, 8273-8279. Jaques, L.W.; Brown, E.B.; Barrett, J.M.; Brey, W.S.; Weltner, W.Jr. *J. Biol. Chem.* **1977**, *252*, 4533-4538. Jaques, L.W.; Riesco, B.F.; Weltner, W.Jr. *Carb. Res.* **1980**, *83*, 21-32. Ogura, H.; Furuhata, K.; Saito, H.; Izumi, G.; Itoh, M.; Shitori, Y. *Chem. Lett.* **1984**, 1003-1006.

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