^{13}C NMR Studies on Trifluoroacetyl Derivatives of 2-Acetamido-2-deoxyhexoses

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The effect of trifluoroacetylation on the ^{13}C chemical shifts of 2-acetamido-2-deoxyhexoses was examined. Studies of the 2-acetamido derivatives of glucose, galactose and mannose established that no regular trend in the ^{13}C shifts occurred on trifluoroacetylation. This was in marked contrast to the results obtained for the ^{1}H chemical shifts.

INTRODUCTION

Acetylation of hydroxy groups leads to a significant downfield shift of the proton attached to the carbon bearing the hydroxy substituent. This effect has been used to advantage in NMR studies of hydroxylic compounds, and is probably best exemplified by investigations on sugars.[1,2] While acetylation requires product purification prior to NMR analysis, trifluoroacetylation can often be readily accomplished by dissolving the compound in trifluoroacetic anhydride [$(CF_3CO)_2O$] or $(CF_3CO)_2O$–trifluoroacetic acid (TFA) mixtures. These solvent systems are transparent in the ^{1}H and ^{13}C NMR regions of interest, and the spectra can, therefore, be recorded without specific isolation of the products. Large ^{1}H downfield shifts are observed on trifluoroacetylation of 2-acetamido-2-deoxyhexoses, generally yielding highly resolved spectra amenable to detailed analysis.[3] This paper shows the influence of trifluoroacetylation on the ^{13}C chemical shifts of these systems. The results established that no regular shift patterns are discernible for the ring carbons in the 2-acetamido derivatives of glucose, galactose and mannose.

EXPERIMENTAL

NAG (2-acetamido-2-deoxy-D-glucose), NAM (2-acetamido-2-deoxy-D-mannose), NAGal (2-acetamido-2-deoxy-D-galactose) and trifluoroacetic anhydride were obtained from Sigma Chemical Co. and were used without further purification.

The tetratrifluoroacetate of NAG (2-acetamido-2-deoxyglucopyranose 1,3,4,6-tetratrifluoroacetate) (**1**) was prepared by allowing a solution of 200 mg of NAG in 1.5 ml of $(CF_3CO)_2O$ to stand at room temperature for approximately 8 h. A similar procedure was adopted in the preparation of the tetratrifluoroacetate of NAGal (2-acetamido-2-deoxygalactopyranose 1,3,4,6-tetratrifluoroacetate) (**2**) and that of NAM (2-acetamido-2-deoxymannopyranose 1,3,4,6-tetratrifluoroacetate) (**3**). The structures of **1–3** are shown in Fig. 1.

^{13}C NMR spectra were recorded on a Bruker WH-270 Fourier transform NMR spectrometer at an operating frequency of 67.89 MHz. Concentrations of 200 mg of the sample in 1.5 ml of $(CF_3CO)_2O$ were used and contained 30% (v/v) $CDCl_3$ to provide the internal deuterium lock. The sweep width was 17 421 Hz with an acquisition time of 0.475 s. The data after Fourier transformation were stored in 8K memory locations, yielding a digital resolution of 2.1 Hz per point. Chemical shifts are expressed as δ (ppm) downfield from internal TMS for the solutions in $(CF_3CO)_2O$. The spectrum of NAG in D_2O was measured with respect to internal DSS, and the observed δ values are expressed with respect to TMS using a correction factor of -1.89 ppm.

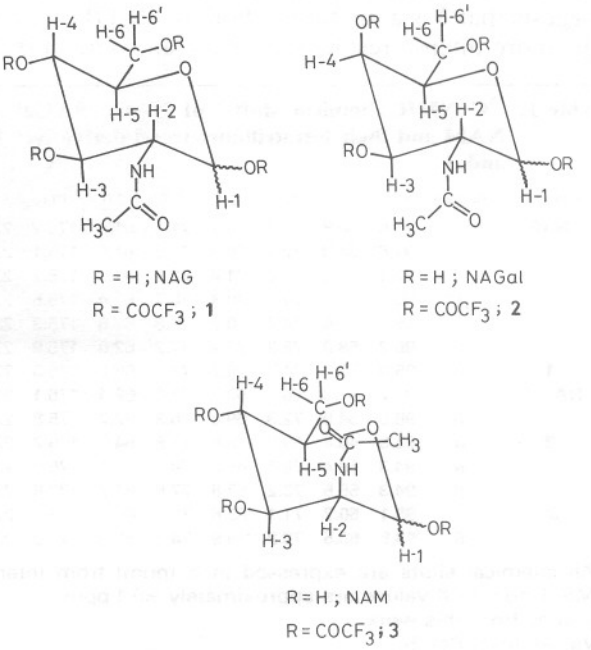

Figure 1. Tetratrifluoroacetyl derivatives of NAG, NAGal and NAM, **1**, **2** and **3**, respectively.

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RESULTS AND DISCUSSION

The chemical shifts of the ^{13}C resonances observed for NAG in D_2O are summarized in Table 1. Resonances corresponding to both α- and β-anomers are easily detected. Earlier studies[4,5] established that the α- and β-anomers exist in the ratio 68:32 in D_2O. The assignment of the carbon resonances of NAG, taken from Perkin *et al.*[5], is given in Table 1. The signals at δ 71.5 and 72.3 have been assigned to C-5 and C-3, respectively, while the C-6 resonance can be assigned unambiguously by its appearance as a triplet in the off-resonance decoupled spectrum. The C-2 peak is easily distinguished by its high-field position (~δ 55–58) in 2-acetamido sugars compared with free sugars[6] (δ 70–76).

Eight resonances are observed in the ^{13}C NMR spectrum of **1**, corresponding to eight non-equivalent carbon atoms in the molecule, and the absence of any additional peaks suggests that **1** exists only in one anomeric form. This is assigned to the α-anomer, on the basis of the J(H-1, H-2) value of 3.9 Hz observed in the ^1H NMR spectra reported elsewhere.[3] The assignment of the carbon resonances is summarized in Table 1.

Figure 2(a) shows the partial proton decoupled 67.89 MHz ^{13}C NMR spectrum of **2**. Seven intense resonances are observed in the high-field region, corresponding to the CH_3, CH_2 and CH groups in the molecule. The lines of low intensity marked with asterisks may belong to another anomeric form. The major species is assigned to the α-anomer on the basis of the low J(H-1, H-2) value of *ca* 3.5 Hz observed in the ^1H NMR spectrum of **2**, reported elsewhere.[3] The assignment of the carbon resonances is summarized in Table 1.

Figure 2(b) shows the partial ^{13}C NMR spectrum of **3**. Fourteen resonances are observed, corresponding to the CH_3, CH_2 and CH carbons of the molecule. This suggests that **3** is a mixture of both α- and β-anomers. The more intense resonances of the anomeric mixture

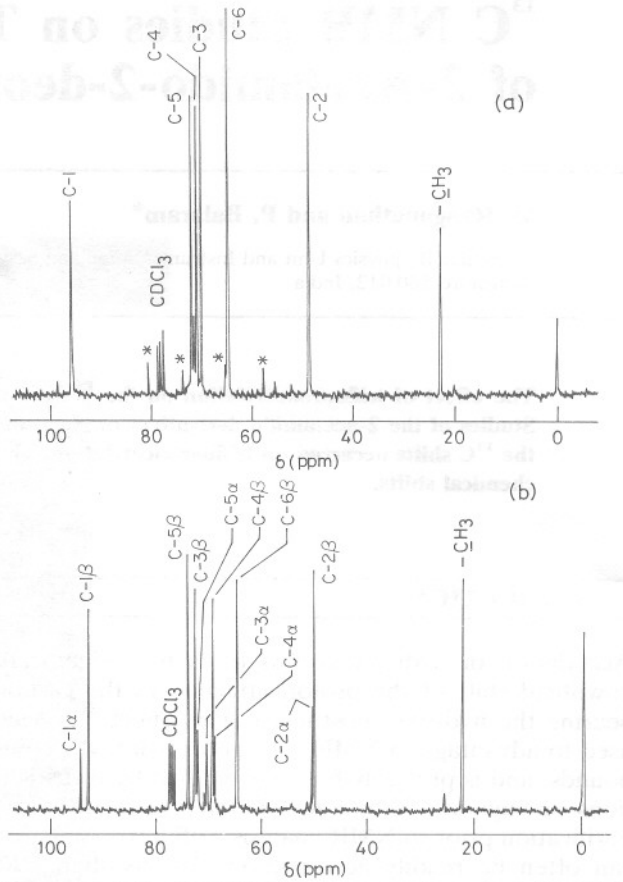

Figure 2. (a) Partial 67.89 MHz proton decoupled ^{13}C NMR spectrum of the tetratrifluoroacetyl derivative of NAGal (**2**). (b) 67.89 MHz proton decoupled ^{13}C NMR spectrum of the tetratrifluoroacetyl derivative of NAM (**3**).

are assigned to the β-form, on the basis of the higher field position of the more intense H-1 resonance in the ^1H NMR spectrum (reported elsewhere[3]). The anomeric composition obtained from the ^{13}C NMR spectrum is 74%β and 26%α. The assignment of the carbon resonances is summarized in Table 1.

Figures 3 and 4 show the ^{13}C and ^1H chemical shifts, respectively, of derivatives **1, 2** and **3**. The C-1, C-2 and C-6 resonances of **1, 2** and **3** are easily assigned by comparison with their parent compounds, viz. NAG, NAGal and NAM. The CH_3 and CO resonances are also easily assigned, and are not shown in Fig. 3. The unambiguous assignment of the C-3, C-4 and C-5 resonances is not straightforward. A tentative assignment is made in Fig. 3 and Table 1 based on probable steric effects on the ^{13}C chemical shifts.[7–9] The C-2 resonance (Fig. 3) shows a fairly large upfield shift, ranging from 2.5 to 3.8 ppm when the parent hexoses are trifluoroacetylated. The C-1 and C-6 carbons move downfield by *ca* 3.5 ppm on trifluoroacetylation in the case of NAG and NAGal. However, in the case of the NAM derivative **3**, C-1 shows a small downfield shift in the α-anomer and a slight upfield shift in the β-anomer. The large downfield shift of C-1 in **1** and **2** is probably a consequence of the electron-withdrawing ability of the $COCF_3$ substituent. The lack of such an effect in the NAM derivative **3** suggests that steric influences on the chemical shifts may be dominant. In all three compounds C-6 shows a large downfield shift on trifluoro-

Table 1. ^{13}C NMR chemical shifts[a] of NAG, NAGal and NAM and their tetratrifluoroacetyl derivatives 1, 2 and 3

Compound	Anomer	C-1	C-2	C-3	C-4	C-5	C-6	CO	CH_3
NAG	α[b]	91.6	54.8	72.3	70.7	71.5	61.4	175.2	22.7
	[c]	91.6	54.9	72.4	70.9	71.5	61.5	175.1	22.8
	[d]	92.1	55.3	72.0	71.4	72.8	61.9	175.7	23.3
	β[b]	95.7	57.5	74.7	70.4	76.7	61.4	175.5	22.9
	[c]	95.7	57.6	74.7	70.7	76.8	61.6	175.3	23.1
	[d]	96.2	58.0	75.2	71.2	77.2	62.0	175.9	23.5
1	α	95.0	52.3	74.7	70.7	71.6	65.2	176.3	22.6
NAGal[d]	α	92.2	51.4	68.6	69.7	71.6	62.4	176.1	23.2
	β	96.5	54.9	72.3	69.0	76.3	62.2	175.8	23.4
2	α	95.5	48.4	69.8	70.8	71.8	64.7	176.2	22.6
NAM[d]	α	94.3	54.4	70.1	68.0	78.2	61.7	175.9	23.2
	β	94.3	55.5	73.2	67.8	77.5	61.7	176.8	23.2
3[e]	α	95.1	50.7	71.1	69.6	72.9	65.3	176.6	22.6
	β	93.5	50.5	73.2	69.9	74.6	65.3	176.9	22.6

[a] All chemical shifts are expressed in δ (ppm) from internal TMS. Errors in δ values are approximately ±0.1 ppm.
[b] Values from this work.
[c] Values from Ref. 5.
[d] Values from Ref. 4.
[e] β-Anomer is more intense.

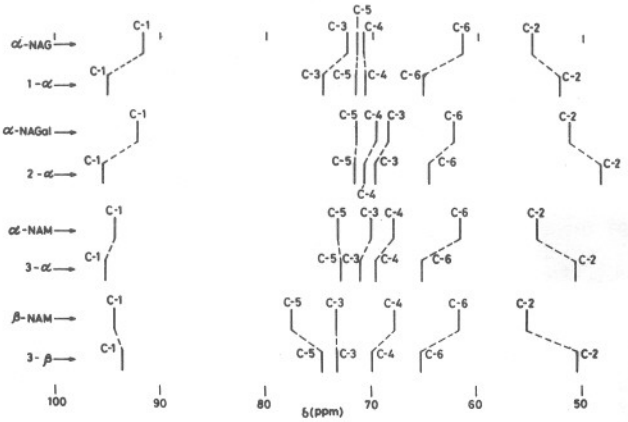

Figure 3. Schematic representation of the ^{13}C chemical shifts of the tetratrifluoroacetyl derivatives **1, 2** and **3.**

acetylation. While the assignments of C-3, C-4 and C-5 are not completely definitive, it should be noted that one of these ring carbons shows a downfield shift on trifluoroacetylation whereas the other two are not significantly shifted downfield. In the case of the NAGal derivative, small downfield shifts are observed for all three resonances C-3, C-4 and C-5. In the α-anomer of the NAM derivative **3**, downfield shifts are seen for two resonances, whereas a very small upfield shift is observed for the third.

In the case of the β-anomer, an unusually large upfield shift of the C-5 resonance is observed on trifluoroacetylation. The C-3 resonance appears to be unaffected by trifluoroacetylation, while C-4 shows the expected low-field shift.

The above results clearly demonstrate distinctly different influences of trifluoroacetylation on ^{1}H and ^{13}C NMR chemical shifts of 2-acetamido-2-deoxyhexoses. Whereas electronic influences dominate ^{1}H chemical shifts, resulting in uniform downfield shifts on trifluoroacetylation of the hydroxy groups, no such regular trend is noted for the ^{13}C resonances. This is unusual, since attachment of a highly electron-withdrawing $COCF_3$ substituent would have been expected to deplete the electron density at the carbon nucleus, leading to downfield shifts. The absence of such an effect suggests that more complex stereochemical factors may determine the ^{13}C chemical shifts. It therefore appears that acetylation, or trifluoroacetylation, may prove less useful in ^{13}C analysis of complex oligosaccharides than in ^{1}H NMR analyses.

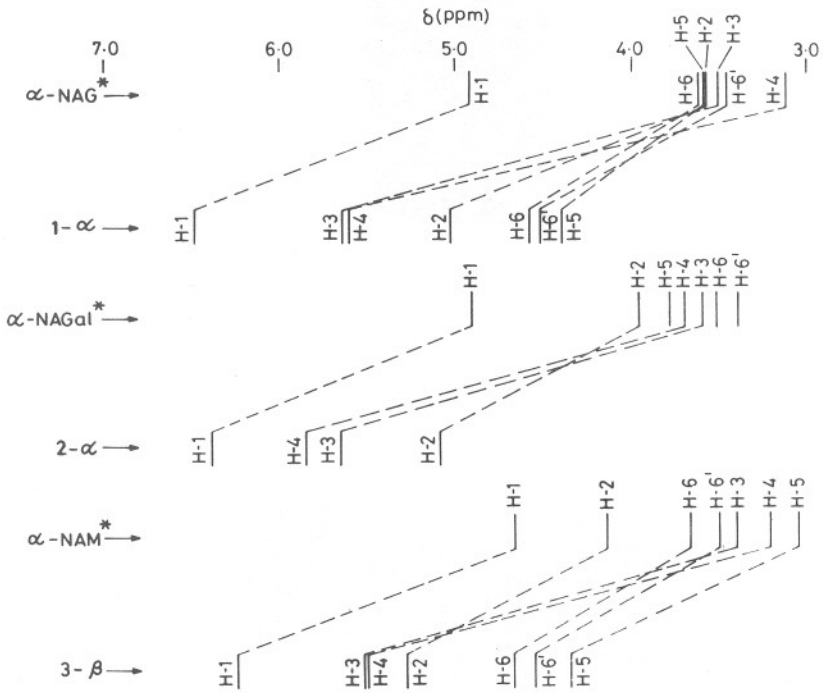

Figure 4. Schematic representation of the ^{1}H chemical shifts of the tetratrifluoroacetyl derivatives **1, 2** and **3.** * Values taken from Ref. 10.

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