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Preliminary communication

Nitrogen-15 n.m.r. spectroscopy of amino sugars*

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In continuation of a program[1–3] for the synthesis and spectroscopic characterization of isotopically labeled and unlabeled amino-sugar derivatives of biomedical interest, the ^{15}N n.m.r. spectra of some of these derivatives have been observed directly at 9.1 MHz, by pulse–Fourier transform techniques. Derivatives of 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose-*6*-^{15}*N* (**1**) and the corresponding unlabeled compounds have been synthesized *via* reactions of either 1,2:3,4-di-*O*-isopropylidene-6-*O*-tolyl-*p*-sulfonyl-α-D-galactopyranose or its 6-deoxy-6-iodo derivative with potassium phthalimide-^{15}*N* (or ^{14}*N*) in hexamethylphosphoric triamide, followed by hydrazinolysis of the phthaloyl group.

The availability of 6-amino-6-deoxy-D-galactose-*6*-^{15}*N* and[1–3] 6-amino-6-deoxy-D-glucose-*6*-^{15}*N* derivatives having ^{15}N enrichments of 99% allowed (*a*) accurate optimization of the pulse power and pulse width prior to the more difficult observation of n.m.r. spectra of ^{15}N at natural abundance (0.36%), (*b*) the facile recording of ^{15}N spectra without decoupling of protons, and (*c*) rapid measurements of the nuclear Overhauser effect (n.o.e.) due to irradiation of protons.

In the absence of proton decoupling, the ^{15}N n.m.r. spectra of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose-*6*-^{15}*N* (**2**) and 6-deoxy-1,2:3,4-di-*O*-isopropylidene-6-phthalimido-α-D-galactopyranose-*6*-^{15}*N* (**3**) are poorly resolved, complex multiplets (see Fig. 1a) caused by small coupling constants (~1 Hz) of the ^{15}N nucleus with neighboring protons[1,3]. Irradiation of the protons of **2** and **3** at 90 MHz caused their ^{15}N resonances to appear as inverted singlets (see Fig. 1b) having linewidths of less than 0.1 Hz. The narrow bandwidth of the ^{15}N frequency in the proton-decoupled, free induction decay (f.i.d.) signal caused a severe sampling problem when large frequency ranges [for example, 5 kHz (548 p.p.m.)] were used to obtain the resonances of the amino-sugar derivative and chemical-shift reference (saturated $NH_4{}^{15}NO_3$ solution) simultaneously. Under these contions, the real and imaginary parts of the Fourier transform appeared to have a non-orthogonal phase relationship, and for many settings of the pulse frequency, phase correc-

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*Dedicated to Dr. Horace S. Isbell in honour of his 75th birthday.

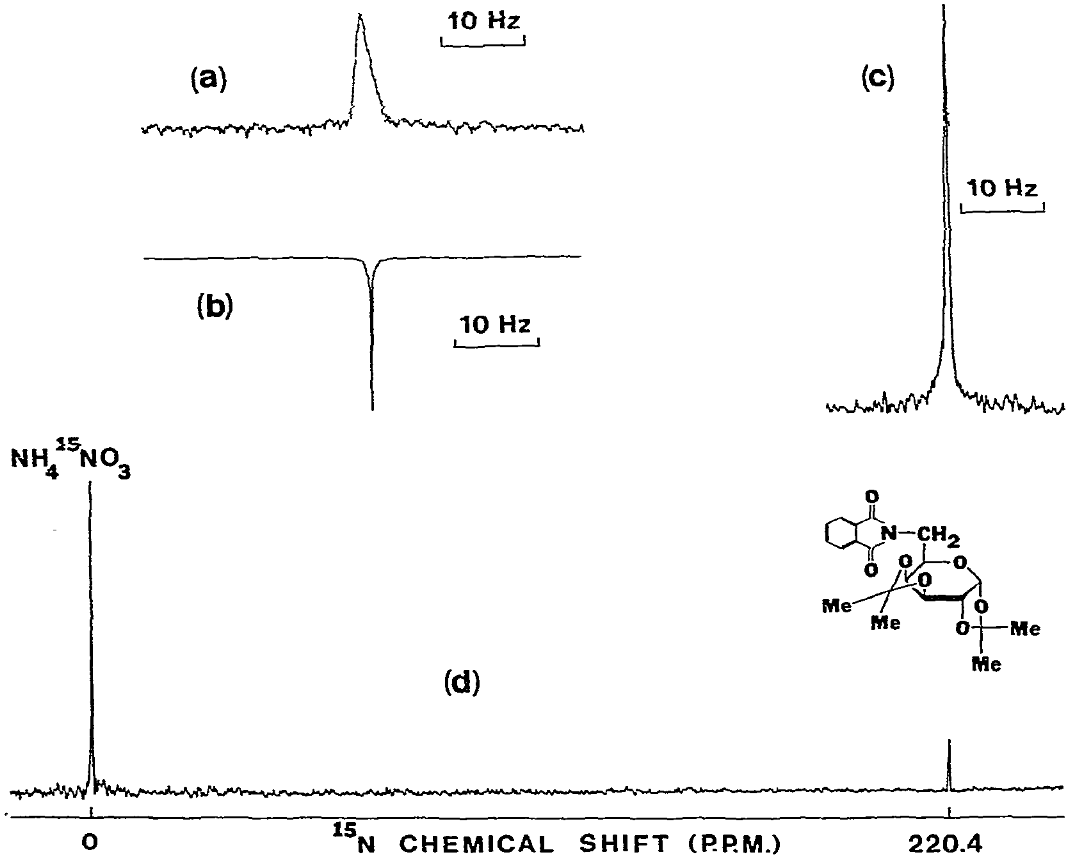

Fig. 1. ^{15}N n.m.r. spectra* of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-6-phthalimido-α-D-galactopyranose-6-^{15}N (3) in chloroform-*d*–hexafluorobenzene (9:1 v/v) at 9.12 MHz: (*a*) absorption-mode spectrum (128 scans) without proton decoupling, (*b*) absorption-mode spectrum (128 scans) with irradiation of protons at 90 MHz, (*c*) magnitude spectrum (one scan), with proton irradiation, and (*d*) magnitude spectrum (8,192 scans) of ^{15}N at natural abundance in 3-6-^{14}N, with irradiation of protons. *Recorded with internal, field-frequency stabilization on the ^{19}F signal of hexafluorobenzene at 84.6 MHz. The sign of the n.o.e. is lost in the computation of the magnitude spectra.

tion of these real or imaginary parts yielded an absorption mode (v) spectrum of greatly diminished or even zero intensity. In these instances, the majority of the signal intensity was present in the dispersion mode (u) spectrum. This problem was solved by broadening the resonances artificially, either by (*a*) exponential filtering of the f.i.d. signal[4], (*b*) taking the magnitude spectrum $(u^2 + v^2)^{1/2}$, or (*c*) sampling a smaller frequency range (for example, 125 Hz), while dispensing with the reference compound.

The recording of spectra of ^{15}N at natural abundance in the amino-sugar derivatives was aided by a substantial, negative n.o.e.[5,6], even for the *N*-phthaloyl derivatives 2 and 3, in which the ^{15}N nuclei are not directly bonded to protons. For example, digital integrations of the absorption-mode ^{15}N spectra of 3 indicate that irradiation of its protons enhances the intensity of the ^{15}N signal by a factor of 3.4 ±0.1 (± two standard deviations of the mean of five measurements, each consisting of 128 scans). Since the signal is inverted by the irradiation, this factor corresponds to a n.o.e. of −4.4 ±0.1. Derivative **3** (0.5 g; ^{15}N enrichment, 99%) yielded a ^{15}N signal:noise ratio of 36:1 when excited by a single pulse (see Fig. 1c). With ^{15}N at natural abundance in 0.75 g of **3**-*6*-^{14}*N*, 8,192 pulses (112 min) were required to give a ^{15}N signal:noise ratio of 13:1 (see Fig. 1d). The negative n.o.e. on the ^{15}N signal of **3** was removed (with reinversion of the signal[6], but no shift) by addition of the relaxation reagent chromium(III) acetylacetonate to a concentration of 0.02M.

The ^{15}N chemical shifts (computed in p.p.m. upfield from the signal of an external capillary of saturated $NH_4{}^{15}NO_3$ solution, ^{15}N 99 atom%) of the 6-amino-6-deoxy-D-*galacto* derivative **1** and 6-amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose-*6*-^{15}*N* were found to be 361.2* and 361.8 p.p.m., respectively, and those of the corresponding *N*-phthaloyl derivatives **3** and **2** were observed to be 220.3 and 220.8 p.p.m., respectively. Similar results were obtained for analogs having ^{15}N in natural abundance.

The results suggest that ^{15}N chemical shifts have major potential in structural analysis.

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