THE N.M.R. SPECTROSCOPY OF DERIVATIVES OF 6-AMINO-6-DEOXY-D-GLUCOSE-*6*-^{15}N*. ^{13}C FOURIER-TRANSFORM AND INTERNUCLEAR, DOUBLE- AND TRIPLE-RESONANCE STUDIES**

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ABSTRACT

A series of ^{15}N-labeled 6-amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose derivatives and their ^{14}N analogs has been studied by continuous-wave proton, ^{13}C, and ^{19}F magnetic resonance spectroscopy, and by ^{13}C Fourier-transform techniques. Characteristic chemical shifts and the magnitudes of ^{1}H–^{1}H, ^{1}H–^{15}N, ^{1}H–^{19}F, ^{13}C–^{15}N, and ^{15}N–^{19}F coupling-constants are reported and discussed. The ^{1}H–^{15}N and ^{13}C–^{15}N coupling-constants over one bond have been used to assess the hybridization of the ^{15}N atom in three of the derivatives. Heteronuclear, internuclear, double-resonance (indor[1]) and triple-resonance ("intripler") experiments have been performed in which ^{15}NH proton transitions were monitored while the ^{15}N or ^{19}F frequencies were swept or maintained on resonance. Replicas of the computed, theoretical ^{15}N spectrum were recorded indirectly, and are discussed in relation to nuclear Overhauser effects.

INTRODUCTION

In the course of a program for the synthesis and structural and conformational characterization of isotopically labeled carbohydrate reference materials of biomedical interest, the synthesis and mass spectrometry of derivatives of 6-amino-6-deoxy-D-glucose labeled in high enrichment with ^{15}N have been reported[2,3]. One purpose of these studies is to attempt to define the applicability of measurements of ^{15}N magnetic resonance parameters to the structural and conformational analysis of amino sugars and their derivatives. For the direct recording of nitrogen n.m.r. spectra, both the ^{14}N and ^{15}N isotopes have the disadvantage that, at constant magnetic field, their

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*Dedicated to Dr. Nelson K. Richtmyer in honor of his 70th birthday.

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n.m.r. sensitivity is only one-thousandth that of protons[4]. However, the ^{14}N nucleus, although highly abundant normally, has the additional very marked disadvantage of an electric quadrupole moment associated with the nuclear spin of 1. The spin couplings of the ^{14}N nucleus are partially removed by quadrupolar relaxation[5], thus making their measurement difficult, and broadening the ^{14}N resonance and that of any other magnetic nucleus situated one or two bonds distant from the ^{14}N nucleus. The ^{15}N nucleus (spin 1/2) does not possess a quadrupole moment, but its natural abundance is[4] only 0.37%. At the present time, it appears that ^{15}N n.m.r. spectra can be obtained from complex molecules, by continuous-wave methods, only if these molecules are labeled with ^{15}N in high enrichment. However, this situation will undoubtedly change when methods[6] for Fourier-transform, ^{15}N n.m.r. spectroscopy become more highly developed. Some promise in this regard is shown by the recently described, driven-equilibrium, Fourier-transform ("deft"), spectroscopic method[7] (in which the nuclei are forced to relax artificially by the application of a second, 90° pulse that drives the refocused, macroscopic, magnetization vector back to the direction of the magnetic field), and the spin-echo, Fourier-transform ("seft") method[8] (in which the spin echoes produced by multiple, 180° pulses are accumulated coherently). Such multiple-pulse, Fourier-transform methods may be of particular value for nuclei having long relaxation times.

In this initial work on the n.m.r. parameters of ^{15}N-labeled amino sugars, the sensitivity problem inherent in direct observation of ^{15}N spectra has been avoided by studying the ^{15}N interactions present in continuous-wave, single-, double-, or triple-resonance spectra of the more sensitive nuclei, namely, protons, ^{13}C, and ^{19}F, and in ^{13}C Fourier-transform spectra obtained in the presence of noise-modulated, proton decoupling.

The key intermediate in the synthesis of the derivatives of 6-amino-6-deoxy-D-glucose-*6*-^{15}N is 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose-*6*-^{15}N (**1**-^{15}N) which was prepared[2,3] by reaction of 1,2:3,5-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose with potassium phthalimide-^{15}N. The *N*,*N*-phthaloyl derivative **1**-^{15}N was transformed successively into 6-amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose-*6*-^{15}N (**2**-^{15}N), and its *N*-acetyl (**3**-^{15}N) and *N*-trifluoracetyl (**4**-^{15}N) derivatives[3]. Quite naturally, the n.m.r. spectra of **1**-^{15}N–**4**-^{15}N have been studied in parallel with those of the normal ^{14}N derivatives (**1**–**4**), as an aid to the identification of chemical shifts and coupling constants.

RESULTS AND DISCUSSION

Initially, p.m.r. spectra were obtained of solutions of compound **1** in a variety of solvents, including acetone-d_6, benzene-d_6, benzene-d_6–pyridine-d_5, chloroform-d, and pyridine-d_5. At 100 MHz, all of these spectra were complex and not amenable to a simple analysis. For example, the spectrum of the solution in acetone-d_6 showed H-5, H-6, and H-6′ as a complex ABC sub-system that was sufficiently strongly coupled for the H-4 quartet to be broadened by virtual-coupling effects[9,10]. On recourse to a 220-MHz spectrometer, the spectrum of **1** in benzene showed H-1 as a doublet at low field, H-2 and H-3 as doublets at higher field, H-4 as a separate quartet, H-5 as a quartet overlapping the H-3 doublet, and H-6 and H-6′ as overlapping quartets at highest field. The spectrum of **1**-^{15}N at 220 MHz (see Fig. 1a) was similar, except that each line of the AB part of the ABX sub-system comprised of H-6, H-6′, and H-5, respectively, was further split by coupling of H-6 and H-6′ with the ^{15}N nucleus (1.0 and 1.1 Hz, respectively). Coupling of this nucleus with H-5 was expected, but was not resolved. At 100 MHz and 220 MHz, the phthalimido protons of **1** and **1**-^{15}N showed as similar, symmetrical, AA′XX′ systems at low field, and, in the spectrum of **1**-^{15}N, no coupling was obvious between these protons and the ^{15}N nucleus. However, in acetone-d_6 at 100 MHz, the phthalimido protons resonated as an A_4 system (that is, as a sharp singlet), but, in chloroform-*d*, as a complex AA′BB′ pattern. Changes of solvent therefore gave a complete range of chemical-shift differences between the two types of proton of the phthalimido group.

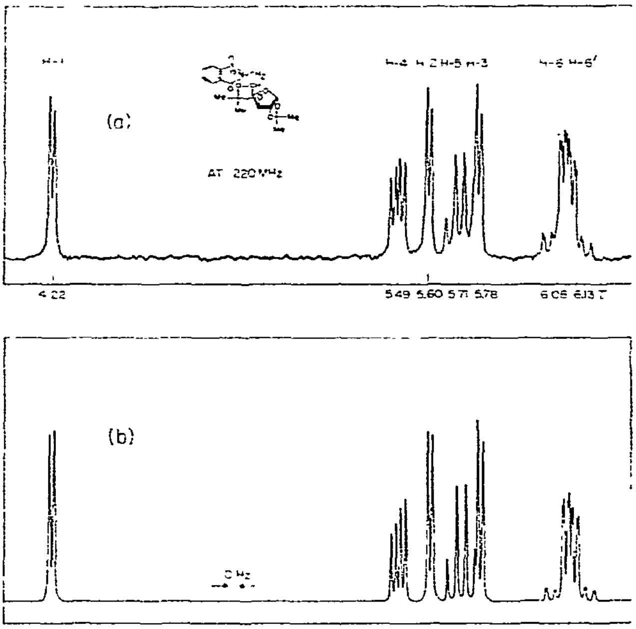

Fig. 1. Partial p.m.r. spectra of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose-6-^{15}N (**1**-^{15}N): (*a*) in benzene-d_6 solution at 220 MHz, (*b*) computed, theoretical spectrum from iterative analysis.

The sugar-chain proton regions of the 220-MHz spectra of **1** and **1**-^{15}N were analyzed approximately by first-order methods, and the parameters thus obtained were refined by iterative analysis, using a modified LAOCN3 program[11]. Compound **1** was treated as a seven-spin system, but, due to program limitations, **1**-^{15}N was reasonably assumed to be a composite of a six-spin sub-system comprised of (*a*) H-3–H-6′ and ^{15}N, and (*b*) a two-spin sub-system (H-1 and H-2), since H-2 was not observably coupled to H-3. The theoretical spectrum from the iterative analysis of **1**-^{15}N is shown in Fig. 1b, and its iterated chemical shifts and coupling constants in Tables I* and II*, respectively, together with those of **1**, and first-order parameters for the remaining derivatives. At 100 MHz, the spectrum of **1** in benzene showed H-6 and H-6′ as only three strong lines forming part of a deceptively simple, ABX sub-system.

The proton-decoupled, ^{13}C n.m.r. spectra of **1** and **1**-^{15}N at 25.2 MHz were next recorded, in order to obtain the characteristic, ^{13}C chemical-shifts and ^{13}C–^{15}N coupling-constants. ^{13}C spectra of solutions in benzene were accumulated by continuous-wave methods in 1024 channels of a time-averaging computer, whereas spectra of solutions in chloroform-*d* were obtained by Fourier-transform methods[6] in which 4096 time-averaged points in the time-domain spectrum were transformed into 2048 points in the frequency domain. For the same total accumulation time (~800 sec) and approximately the same quantity of compound, use of the Fourier-transform technique with a somewhat larger sample-tube afforded a signal:noise ratio approximately six times that obtained by the continuous-wave method. The ^{13}C spectra of **1** and **1**-^{15}N (see Fig. 2) show the carbon resonances of the methyl groups as singlets at high field (δ_C ~24–27 p.p.m. from internal tetramethylsilane), two of

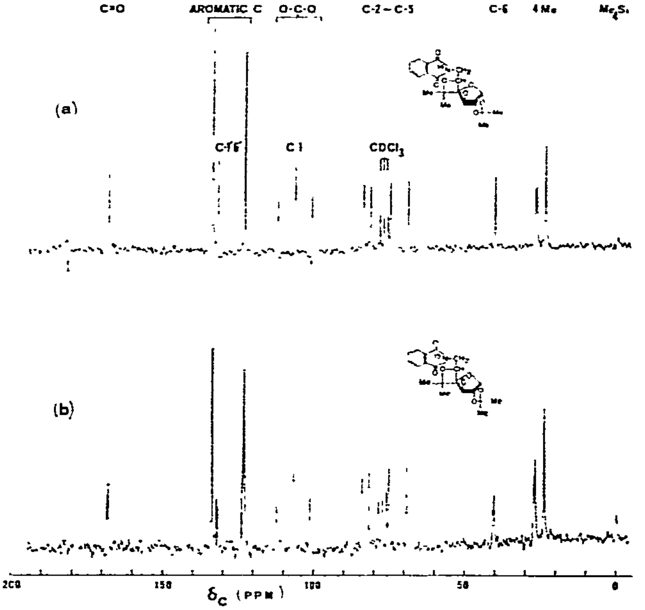

Fig. 2. Fourier-transform, ^{13}C n.m.r. spectra of solutions in chloroform-*d* at 25.2 MHz with proton decoupling: (*a*) 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose (**1**), (*b*) 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose -*6*-^{15}N (**1**-^{15}N)

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*Data are reported for the solvent system that allowed the most facile analysis of the spectra.

which were not resolved. Compound **1** shows ^{13}C-6 as a singlet at ~41 p.p.m. but **1**-^{15}*N* shows it as a doublet indicating $J_{^{13}C\text{-}6,^{15}N}$ 9.9 Hz. At lower field, both compounds display four singlets in the region of 69–84 p.p.m. consistent with carbon nuclei bound to one oxygen atom, namely, ^{13}C-2, ^{13}C-3, ^{13}C-4, and ^{13}C-5. At still lower field (101–112 p.p.m.), there appear three singlets due to ^{13}C nuclei bound to two oxygen atoms[12], that is, ^{13}C-1 and the 2-carbon nuclei of the isopropylidene groups. Further Fourier-transform spectra for which a larger pulse-width was used indicated that the singlet at δ_C 107 p.p.m. could be assigned to ^{13}C-1, as, in these spectra, the relative intensities of the singlets at 101 and 112 p.p.m. had diminished, and they were therefore assigned to the quaternary 2-carbon nuclei of the isopropylidene groups. The use of a larger pulse-width in a pulse experiment is akin to increasing the r.f. power in a continuous-wave experiment. However, the relaxation times of the ^{13}C nuclei that are bonded to protons are shortened by direct dipole–dipole interaction, and hence, on application of a longer pulse, the quaternary ^{13}C nuclei tend to be saturated first, because of their relatively longer relaxation-times. The aromatic, ^{13}C nuclei of **1** appear as three two-carbon singlets at 123, 132, and 134 p.p.m. The signal at 132 p.p.m. is very much less intense than those at 123 and 134 p.p.m. and may, therefore, be assigned to the 1- and 6-carbon nuclei* of the aromatic ring, which, because they are not directly bonded to protons, are not expected to experience a significant enhancement of signal intensity due to the nuclear Overhauser effect. For compound **1**-^{15}*N*, the signal at δ_C 132 p.p.m. is a sharp doublet (see Fig. 2b) due to the presence of the coupling constants $J_{^{13}C\text{-}1',^{15}N} = J_{^{13}C\text{-}6',^{15}N} = 11.4$ Hz, over two bonds. The carbonyl ^{13}C nuclei of **1** and **1**-^{15}*N* appear at lowest field (δ_C 168.5 p.p.m.) as a singlet and a doublet, respectively, that indicate the value $J_{^{13}C=O,^{15}N}$ 12.9 Hz. Again, these signals are of lower intensity because of the lack of an Overhauser enhancement.

The ^{13}C chemical shifts of **1** and **1**-^{15}*N* in chloroform-*d* are very similar and, referred to the same reference-compound (external carbon disulfide), are very similar to those measured for solutions in benzene. No evidence for a nitrogen isotope-effect was discerned.

The magnitudes of the ^{13}C–^{15}N coupling-constants over one bond are surprisingly small, but are comparable with values in the range <3–17.5 Hz that have been measured for simple aliphatic and aromatic nitrogen compounds by direct observation of ^{15}N n.m.r. spectra[13]. Many of these values have been correlated approximately[13] by the empirical relationship

$$S_C S_N = 80\, J_{^{13}C\text{-}^{15}N} \qquad (1)$$

where S_C and S_N are the percentages of *s*-character in the carbon and nitrogen orbitals that form the intervening σ-bond. This was done on the assumption that directly bonded ^{13}C–^{15}N couplings are dominated by the Fermi contact-interaction term, like those[14] of directly bonded ^{13}C-H and[13] also ^{15}N–H. This assumption

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*Labeled for convenience, in this paper, as C-1′ and C-6′.

TABLE I

CHEMICAL SHIFTS[a] (P.P.M.) OF ^{14}N- AND ^{15}N-LABELED 6-AMINO-6-DEOXY-1,2:3,5-DI-*O*-ISOPROPYLIDENE-α-D-GLUCOFURANOSE DERIVATIVES

Derivative	*Solvent*	*Me*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	[b]*H-6*	[b]*H-6'*	*Others*
1[c]	C_6D_6	8.94 8.88 8.84 8.58	4.23	5.60	5.79	5.49	5.72	6.09	6.14	^{13}CH$_3$, 169.5 (×2), 166.7, 166.2; ^{13}C-6, 153.5; ^{13}C-2–^{13}C-5, 124.2, 118.2, 111.8, 109.3; O^{13}CO, 92.7, 86.8; ^{13}C-1, 81.6; ^{13}C(Ar), 70.6 (×2), 61.1 (×2), 60.2 (×2); H(Ar), 3.17, 2.59
1-^{15}*N*[c]	C_6D_6	8.96 8.91 8.86 8.61	4.22	5.60	5.78	5.49	5.71	6.08	6.13	^{13}CH$_3$, 169.5 (×2), 166.7, 166.1; ^{13}C-6, 152.8; ^{13}C-2–^{13}C-5, 124.0, 118.0, 111.6, 109.1; O^{13}CO, 92.5, 86.6; ^{13}C-1, 81.4; H(Ar), 3.20, 2.61
2-d_2[d,e]	$CDCl_3$–D_2O[f]	8.69 8.65 (×2) 8.52	4.00	5.41	5.80	5.72	6.48	7.02	7.22	NH$_2$, ~8.5[g] (CDCl$_3$) or = 7.56[g] [(CD$_3$)$_2$SO]
2-^{15}*N*[d]	$CDCl_3$	8.69 8.65 (×2) 8.52	4.00	5.41	5.81	5.72	6.48	7.01	7.20	NH$_2$, ~8.5[g]
3[d]	C_5D_5N	8.70 (×3) 8.53	3.88	5.32	5.69	5.54	6.10	6.27	6.50	Ac, 7.98; NH, 1.51
3-^{15}*N*[d]	C_5D_5N	8.71 (×3) 8.53	3.87	5.32	5.68	5.53	6.09	6.23	6.50	Ac, 7.98; NH, 1.51
4-*d*[h]	C_5D_5N–D_2O (9:1 v/v)	8.64 (×3) 8.44	3.87	5.29	5.66	5.50	5.92	6.12	6.32	NH, −0.83[i]; CF$_3$, 74.9[j]
4-^{15}*N*-*d*[h]	C_5D_5N–D_2O (9:1 v/v)	8.63 (×3) 8.44	3.86	5.28	5.64	5.48	5.91	6.11	6.30	NH, −0.87[i]; CF$_3$, 74.8[j]

[a] First-order values (τ) for protons, unless indicated otherwise. [b] H-6 is to low field of H-6'. [c] Proton shifts were obtained by iterative (H-1–H-6') or first-order [Me and aromatic (Ar) protons] analysis of spectra recorded at 220 MHz and 5.17 T. The ^{13}C shifts tabulated were measured by continuous-wave methods for solutions in benzene at 25.2 MHz and 2.35 T, and are given in p.p.m. upfield from external carbon disulfide. An alternative set of ^{13}C shifts was obtained for solutions in chloroform-*d* by Fourier-transform methods at 25.2 MHz and 2.35 T, and are given here in p.p.m. downfield from internal tetramethylsilane, in the format: assignment, shift of **1** [shift of **1**-^{15}*N*]; CH$_3$, 24.1 [24.2] (×2), 27.0 [27.0], 27.4 [27.4]; C-6, 40.7 [40.7]; C-2–C-5, 69.5 [69.5], 75.4 [75.4], 81.8 [81.9], 84.2 [84.2]; OCO, 101.3 [101.4], 112.5 [112.5]; C-1, 106.8 [106.9]; C-1',6'(Ar), 132.4 [132.3]; C-2'–C-5' (Ar), 123.5 [123.5] (×2) and 134.2 [134.3](×2); C=O, 168.5 [168.5](×2). [d] Measured at 100 MHz and 2.35 T. [e] *N*-Deuterated. [f] Overlayer. [g] Nondeuterated derivative, measured at 60 MHz and 1.41 T. [h] Measured at 90 MHz and 2.11 T. [i] Nondeuterated derivative in pyridine-d_5. [j] ^{19}F shifts were measured on solutions in 9:1:1 (v/v) pyridine-d_5–deuterium oxide–trichlorofluoromethane at 84.7 MHz and 2.11 T, and are given in p.p.m. upfield from the internal trichlorofluoromethane.

TABLE II

COUPLING CONSTANTS (Hz) OF ^{14}N- AND ^{15}N-LABELED 6-AMINO-6-DEOXY-1,2:3,5-DI-*O*-ISOPROPYLIDENE-α-D-GLUCOFURANOSE DERIVATIVES

Derivative	*Solvent*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$^aJ_{5,6}$	$^aJ_{5,6'}$	$J_{6,6'}$	$^bJ_{6(')\text{,NH}}$	^{15}N *coupling-constants*^{c}
1^d	C_6D_6	3.2	<0.9	3.7	7.5	7.7	7.2	13.5	—	—
$1\text{-}^{15}N^d$	C_6D_6	3.5	<0.9	3.9	7.4	7.3	7.2	13.5	—	$^2J_{6,^{15}N}$ 1.0; $^2J_{6',^{15}N}$ 1.1; $^3J_{5,^{15}N}$ <0.9 $^1J_{^{13}C\text{-}6,^{15}N}$ 9.9^e; $^1J_{^{13}C=O,^{15}N}$ 12.9^e; $^2J_{^{13}C\text{-}1',^{15}N} = {}^2J_{^{13}C\text{-}6',^{15}N}$ 11.4^e
$2\text{-}d_2{}^{f,g}$	$CDCl_3$–D_2O^h	3.7	<0.4	3.9	6.6	4.1	7.9	13.5	—	—
$2\text{-}^{15}N^g$	$CDCl_3$	3.6	<0.4	3.8	6.7	4.0	7.5	13.6	—	$^3J_{5,^{15}N}$ 1.5; $^2J_{6,^{15}N} = {}^2J_{6',^{15}N}$ $\sim$0.5
$3\text{-}d^{f,g}$	C_5D_5N–D_2O (5:2 v/v)	3.6	0.3	3.7	6.4	4.7	7.5	13.9	—	—
$3\text{-}^{15}N^g$	C_5D_5N	3.7	<0.4	3.7	6.4	$\sim$4.4	$\sim$8,3	$\sim$14.4	5.7	$^3J_{5,^{15}N}$ 1.5; $^2J_{6',^{15}N}$ $\sim$0.5 $^1J_{^{15}N\text{-}H}$ 91.3; $^3J_{^{15}N,Me}$ 1.2
$3\text{-}^{15}N\text{-}d^{f,g}$	C_5D_5N–D_2O (5:2 v/v)	3.7	<0.4	3.6	6.5	4.3	7.6	13.9	—	$^3J_{5,^{15}N}$ 1.7; $^2J_{6,^{15}N}$ $\sim$0.7 $^2J_{6',^{15}N}$ $\sim$0.6; $^3J_{^{15}N,Me}$ 1.3
$4\text{-}d^{f,i}$	C_5D_5N–D_2O (9:1 v/v)	3.7	<0.4	3.7	6.6	4.5	7.8	13.3	—	—
$4\text{-}^{15}N^{i,j}$	C_5D_5N	3.7	<0.4	3.7	6.6	—	—	—	5.7	$^3J_{5,^{15}N}$ 1.4; $^1J_{^{15}N\text{-}H}$ 92.3
$4\text{-}^{15}N\text{-}d^{f,i}$	C_5D_5N–D_2O (9:1 v/v)	3.7	<0.4	3.7	6.6	$\sim$5	7.6	13.2	—	$^3J_{5,^{15}N}$ 1.3; $^2J_{6,^{15}N}$ $\sim$0.8 $^2J_{6',^{15}N}$ 0.6

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[a] H-6 resonates at lower field than H-6′. [b] Coupling constants of H-6 and H-6′ with NH. [c] Superscripts before *J* indicate the number of bonds separating the coupled nuclei. [d] Values from iterative analysis of spectra recorded at 220 MHz and 5.17 T. These values differed by up to 0.8 Hz from those obtained by first-order analysis. [e] Measured from ^{13}C spectra recorded at 25.2 MHz and 2.35 T. [f] *N*-Deuterated. [g] Measured at 100 MHz and 2.35 T. [h] Overlayer. [i] Measured at 90 MHz and 2.11 T. [j] $J_{F,NH}$ $\sim$0.7 Hz, and $^3J_{F,^{15}N} < 0.6$ Hz, for a solution in chloroform-*d*.

appears to be valid if (*a*) the electronegativity of the substituents does not vary over a large range, and (*b*) neither atom has valence s-electrons of low energy[15a].

If, for **1**-^{15}N, it is assumed (reasonably) that ^{13}C-6 is fully sp^3-hybridized, the *s*-character of the ^{13}C orbital directed towards ^{15}N is 25%, and a calculation using the observed value of $J_{^{13}C\text{-}6,^{15}N}$ in equation (*1*) gives the value of S_N as 31.7%. The electronic structure of the phthalimido group of **1**-^{15}N could be considered as a resonance hybrid of the nonpolar form **5** and the two polarized forms **6** and **7**, that are of equal energy. In all of these forms, each carbonyl carbon atom is sp^2-hybridized, and therefore, the s-character of each of their σ-bonds is 33.3%. Substitution of this value of S_C in equation (*1*), together with the value of $J_{^{13}C=O,^{15}N}$, gives $S_N = 31.0\%$, a proportion that agrees quite well with the value calculated from $J_{^{13}C\text{-}6,^{15}N}$. If the fractional contribution of canonical form **5** is x, and that of **6** and of **7** is y, then, by use of the mean value S_N 31.35,

$$25x + 2 \times 33.\dot{3}y = 31.35, \text{ and } x + 2y = 1,$$

from which, $x = 0.238$ and $y = 0.381$. Thus, the contributions of **5**. **6**, and **7** are weighted in the ratios 1:1.6:1.6, and the polarized, canonical forms **6** and **7** make a greater contribution than the nonpolar form **5**, in which the ^{15}N atom, including its lone pair of electrons, is sp^3-hybridized. This type of calculation has, however, less utility than appears at first sight, as the results obtained are very sensitive to slight changes in the coupling constants.

The signals of the amino protons of **2** and **2**-^{15}N in chloroform-*d* solution were found to lie underneath the methyl-group resonance, and it was necessary to confirm the assignment of the NH signals by measuring (at 60 MHz) the loss of integrated intensity from the methyl region on *N*-deuteration of the amines. At 60 MHz, the H-6 and H-6′ signals of these compounds were diffuse, but a full analysis was possible at 100 MHz. At the latter frequency, **2**-d_2 showed doublets for H-1, H-2, and H-3, a quartet for H-4, a sextet for H-5, and overlapping quartets (the AB part of an ABX sub-system) for H-6 and H-6′. The 100-MHz spectrum of **2**-^{15}N in chloroform-*d* showed an incompletely resolved dodecet for H-5, and, from the outer lines of this multiplet, the value $J_{5,^{15}N}$ 1.5 Hz was readily measured. Small, extra splittings in the H-6 and H-6′ quartets were attributed to small couplings (~0.5 Hz) of these protons with the ^{15}N nucleus, and, indeed, the simplicity of the H-6 and H-6′ signals, and the lack of splitting of the NH_2 signal by strong coupling with ^{15}N, indicated that the NH_2 protons were decoupled by chemical exchange. This appeared to be true even for solutions in methyl sulfoxide-d_6, from which the NH_2 resonance was recorded as a resolved, broad singlet.

The *N*-acetyl derivatives **3** and **3**-^{15}N were studied as their solutions in pyridine-d_5, or in a mixture of pyridine-d_5 and deuterium oxide, as the use of these solvents gave better spectral separation of H-3 from H-4, and of H-5 from H-6 and H-6′, than did chloroform-*d*. Solutions of **3** in pyridine-d_5 gave spectra at 100-MHz in which the NH resonance appeared at low field as a broad triplet, because of equal coupling (~5 Hz) with H-6 and H-6′, and broadening by the ^{14}N quadrupole. Under these conditions, 3-^{15}*N* showed an NH resonance composed of two sharp triplets, separated by 91.3 Hz which was assigned as the value of $J_{^{15}\mathrm{N-H}}$ (for spectra of **3** and **3**-^{15}*N*, see Ref. 2). The observation of these NH couplings demonstrates that, even in a basic solvent such as pyridine, the NH proton is undergoing exchange at a rate of less than 5 Hz. That this proton does exchange slowly was shown by adding deuterium oxide to the pyridine-d_5 solution; the triplets at low field were then replaced by a singlet at much higher field (HOD). These results may be contrasted with those from the amines, for which NH_2 proton-exchange is evidently autocatalytic. Compounds **3** and **3**-^{15}*N* showed a singlet and a doublet, respectively, for the acetyl methyl group, thus giving the value $J_{^{15}\mathrm{N,Me}}$ 1.2 Hz.

After *N*-deuteration of these compounds, the rest of their multiplet patterns (that is, for the protons attached to the sugar chain) were similar to those of the amines **2** and **2**-^{15}*N*, and analysis of the H-5, H-6, and H-6′ multiplets gave values $J_{5,^{15}\mathrm{N}}$ 1.5–1.7, and $J_{6,^{15}\mathrm{N}}$ and $J_{6',^{15}\mathrm{N}}$ 0.5–0.7 Hz.

The p.m.r. spectrum of **4** in chloroform-*d* at 90 MHz shows the NH signal as a broad hump having no obvious fine-structure; however, the NH resonance of **4**-^{15}*N* in chloroform-*d* is composed of two complex sub-multiplets separated by $J_{^{15}\mathrm{N-H}}$ 92.5 Hz (see Fig. 3). Moreover, the NH resonance of **4**-^{15}*N* in pyridine-d_5 was found to consist of the two widely spaced triplets that were expected on the basis of a first-order interpretation. Introduction of the fluorine atoms into the acetyl group (as in **4**-^{15}*N*) evidently caused extra deshielding (~2.4 p.p.m.) of the NH proton, as compared with the NH resonance of **3**-^{15}*N* in pyridine-d_5 solution. The complexity of the NH spectrum of **4**-^{15}*N* in chloroform-*d* is due to second-order effects produced by strong coupling of H-5, H-6, and H-6′, the chemical shifts of which are more similar to each other for this solution than for that in pyridine-d_5. This conclusion was confirmed by computation of a series of theoretical spectra for a seven-spin system composed of ^{15}N, ^{15}NH, and H-3–H-6′. It was found that second-order splittings were introduced into the theoretical, NH triplets only when H-5, H-6, and H-6′ were assigned similar chemical-shifts, for example, 346, 339, and 336 Hz, respectively. If one of these protons was assigned a shift more remote from those of the other two, the second-order effects were not evident in the theoretical spectrum. These effects are not often clearly observed in the NH resonances of acylamido sugars (^{14}N), and, if poorly resolved, could readily be mistaken for broadening by the ^{14}N quadrupole.

For **4**-^{15}*N*, one other complicating factor was discovered, namely, the presence of a long-range coupling (~0.7 Hz) between the fluorine nuclei and the NH. Thus, when **4**-^{15}*N* in chloroform-*d* was irradiated strongly in the fluorine region at 84.678105 MHz, the complex NH resonances became sharper (see Fig. 3). The long-range coupling could also be detected by means of an indor experiment in which the observing frequency (f_1) was held constant at the frequency of one of the strong, central lines of the NH sub-multiplets while the double-resonance frequency (f_2) swept through the fluorine region. A perturbation of the monitored NH line was detected as f_2 passed through 84.678105 MHz. Additional heteronuclear, indor experiments, and rare[15b] examples of heteronuclear, intripler experiments, were performed in

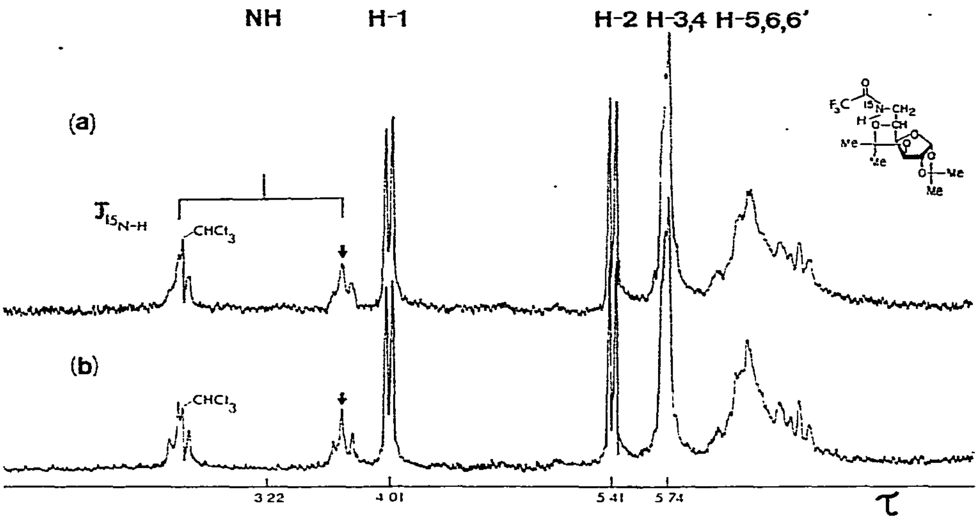

Fig. 3. Partial p.m.r. spectra of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-(trifluoroacetamido)-α-D-glucofuranose-6-^{15}N (4-^{15}N) in chloroform-*d* at 90 MHz: (*a*) single-resonance spectrum, (*b*) with irradiation at the fluorine frequency. The arrows in (*a*) and (*b*) indicate the NH peaks monitored in the indor and intripler experiments, respectively.

which one of the strong NH-proton peaks was monitored by f_1 while a perturbing frequency was swept through the ^{15}N resonance at ~9.12 MHz, either with simultaneous irradiation at the fluorine-resonance frequency (intripler experiments) or without this irradiation (indor). The responses observed in these experiments were studied in conjunction with the lists of progressively and regressively connected transitions that were obtained when the trial, theoretical spectra were computed for 4-^{15}N by using the modified LAOCN3 program[11]. For example, the output of the program indicated that the strong, central peak of the upfield NH submultiplet contains transitions that are regressively connected to the group of ^{15}N transitions at lower frequency, but are progressively connected to the group of ^{15}N transitions at higher frequency. These two groups of transitions are separated by a spacing of ~90 Hz due to $J_{^{15}N-H}$. An investigation[16] of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose-d_{12} by homonuclear, proton–proton, indor techniques has shown that it is possible to distinguish progressively and regressively connected transitions by observation of the positive and negative responses in the indor spectra, and to correlate these with the output of the magnetic-equivalence, factoring program[17] UEAITR. The crossover from positive and negative responses to purely negative responses, as the power of f_2 was increased, was also demonstrated[16]. This crossover is due to swamping-out of the general Overhauser effect caused by transfers of spin population between energy levels, by splitting of the energy levels (and, hence, of the monitored line) resulting from mixing of spin states (spin-tickling).

In the present work, for most of the sweep rates of f_2 and the power levels of f_1 and f_2 investigated, *positive* responses of the NH peak were observed (see Fig. 4b) as f_2 was swept through the resonance frequencies of *each* group of ^{15}N transitions. Thus, an exact *positive* replica of the computed, theoretical spectrum of the ^{15}N nucleus (see Fig. 4a) was obtained, that is, a doublet having a spacing of ~90 Hz.

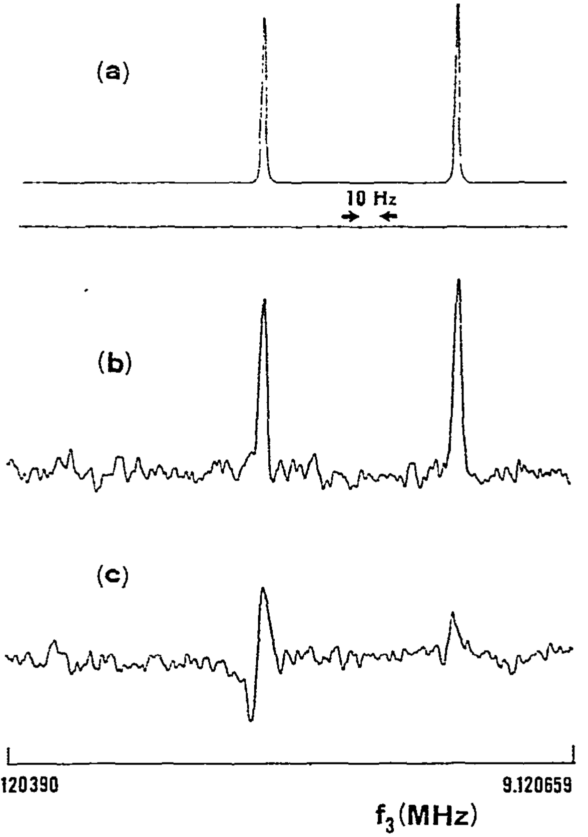

Fig. 4. ^{15}N-related n.m.r. spectra of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-(trifluoroacetamido)-α-D-glucofuranose-*6*-^{15}*N* (4-^{15}*N*) in chloroform-*d*: (*a*) computed, theoretical, ^{15}N n.m..r. spectrum*; (*b*) ^{15}N n.m.r. spectrum obtained indirectly from heteronuclear, intripler experiment in which a ^{19}F-decoupled, NH-proton peak was monitored by f_1 at ~90 MHz while the ^{15}N frequency (f_3) was swept; (*c*) the same, with f_1 at lower power.

These results are unusual, and are not in agreement with the negative and positive responses expected from considerations of connectivity as the ^{15}N frequency was swept from low to high values, or with the negative peaks expected from spin-tickling effects. In this instance, it appears that, for most of the conditions employed, general Overhauser and spin-tickling effects are dominated by a positive, intramolecular, nuclear Overhauser effect[18,19] due to magnetic dipole–dipole interaction between

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*If smaller sweep- and line-widths were assigned in the plotting routine, the doublet contained additional fine structure.

the ^{15}N and proton nuclei, which is known to be important for nuclei that are directly bonded[20] or in close spatial proximity[20,21].

The advantage of the intripler experiment was that positive responses of 12% greater amplitude, on average, were obtained in the presence of ^{19}F decoupling than in its absence, due, apparently (see Fig. 3b), to the fact that the decoupling gave a sharper NH peak to be monitored by f_L. These comparisons were conducted merely by offsetting the ^{19}F frequency from the resonance point by 100 or 200 Hz, instead of by switching this frequency off, as the latter procedure was found to cause undesirable changes of signal level in the transmitting-coil circuits. Maximum positive responses were obtained in both the indor and intripler experiments by using a comparatively high power (~0.5 W attenuated by 20 dB) for f_1 in combination with a high sweep-rate (6 Hz. sec^{-1}) for the ^{15}N frequency (f_2 or f_3, respectively). By using greater attenuation (30 dB) for f_1, it was possible to obtain spectra that contained two negative responses (spin-tickling only), or, more commonly, a negative response (*a*), followed by an immediate, positive response (*b*) at the lower ^{15}N-perturbation frequency, together with a positive response at the higher frequency (see Fig. 4c). This situation (and others like it) appears to correspond to superposition of spin-tickling effects on the dipole–dipole, Overhauser effect, the negative response *a* being observed just prior to the positive response *b*, because of a lower f_2 power-requirement for spin-tickling than for the dipole–dipole, Overhauser effect.

No particular dependence of the responses on the time between sweeps was noted. In some molecular environments, the ^{15}N nucleus is expected to have rather long relaxation-times[22], but, for indor experiments on 4-^{15}*N*, the continuous irradiation of some of the NH transitions by a strong f_1 frequency may be expected to provide a mechanism for dipolar relaxation of the ^{15}N nucleus, *i.e.*, the nuclear Overhauser effect in reverse.

For compound **4** in chloroform-*d*, the combined effects of the second-order splittings, long-range coupling, and broadening by the ^{14}N quadrupole produced an NH resonance that was so formless that none of the couplings of this proton could be measured. This fact emphasizes the utility of ^{15}N-labeled amino sugar derivatives in measurements of the coupling constants of NH protons.

The exact analysis of the complex p.m.r. spectra of solutions of **3**, **3**-^{15}*N*, **4**, and **4**-^{15}*N* in chloroform-*d* was deemed unnecessary, as, after simplification of the H-6 and H-6′ regions of their spectra in pyridine-d_5 solution by *N*-deuteration, these spectra were amenable to a first-order analysis. The analysis of **4**-^{15}*N*-*d* yielded the values $J_{5,^{15}N}$ 1.3, $J_{6,^{15}N}$ ~0.8, and $J_{6',^{15}N}$ 0.6 Hz, and from the spectrum of **4**-^{15}*N* in pyridine solution was obtained $J_{^{15}N-H}$ 92.3 Hz.

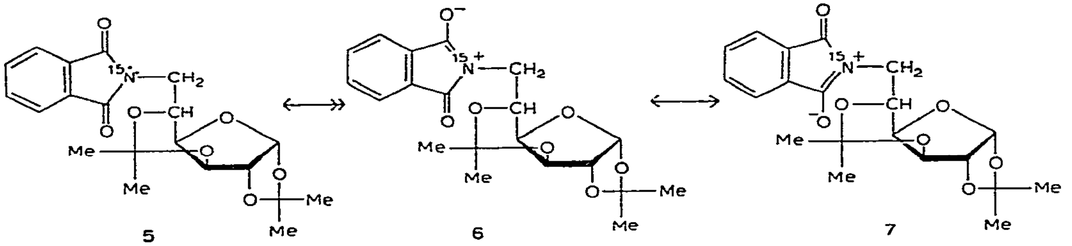
The ^{19}F spectra of compounds **4**, **4**-*d*, **4**-^{15}*N*, and **4**-^{15}*N*-*d* at 84.7 MHz all showed for the trifluoromethyl group quite narrow resonances that indicated $J_{F,^{15}N}$ <0.6 Hz, exact measurement of which was complicated by the long-range coupling between the fluorine nuclei and the NH, or ND.

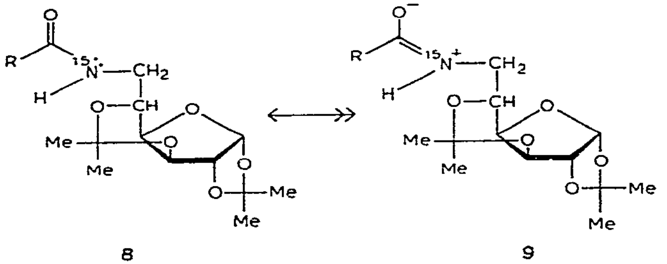
^{15}N–proton couplings through one bond. — Observed values of ^{15}N–proton coupling-constants over one bond have been correlated[13] with the s-character (S_N)

of the nitrogen-bond orbital directed towards the proton by means of the empirical expression

$$S_N = 0.43\,J_{^{15}N\text{-}H} - 6. \qquad (2)$$

This equation, which is based on the assumption of a dominating Fermi-contact interaction[23], sets an upper limit of $\sim$130 Hz (sp hybridization, $S_N = 50\%$) for couplings of a ^{15}N nucleus bonded to a proton and to at least one other atom. This limit has recently been observed[24] for *N*-protonated nitriles ($J_{^{15}N\text{-}H}$ $\sim$135 Hz). Many investigations[13,23-36] have shown, without exception, that one-bond couplings of ^{15}N and protons are considerably smaller than the analogous ^{13}C–H couplings. If equation (*2*) is applied to the range of values $J_{^{15}N\text{-}H}$ 91.3–92.5 Hz observed for the amides **3**-^{15}*N* and **4**-^{15}*N*, the values S_N 33.3–33.8% are obtained. These suggest that the nitrogen atom is fully *sp*^{2}-hybridized, and that the polarized, canonical form of the amide group (as in **9**) is much more important than the nonpolar form **8**, which has *sp*^{3}-hybridization of the nitrogen atom. The values of $J_{^{15}N\text{-}H}$ observed in the present study are comparable with those (93–94.5 Hz) reported for sugar osazones and formazans[34-36].


5 **6** **7**


8 **9**

R = Me or CF$_3$

^{15}*N Couplings through two or three bonds.* — From the data given in Table II, it may be seen that the proton–^{15}N coupling-constants over three intervening bonds are usually two to three times[26] those over two bonds, the *N*,*N*-phthaloyl derivative **1**-^{15}*N* being an exception, since the values observed for $J_{6,^{15}N}$ and $J_{6',^{15}N}$ were larger than that of $J_{5,^{15}N}$. However, these values are similar to the coupling (1.4 Hz) observed[13] between the methyl protons and the ^{15}N nucleus of *N*-methyl-^{13}*C*-phthalimide-

^{15}N. Two-bond, proton–^{15}N coupling-constants as large as 14.3–19.0 Hz have been observed for formamide[25] and its *N*-alkyl derivatives[29,37], but, for most simple derivatives of amines, imines, amides, thioamides, and isothiocyanates, values in the range of 0–8.4 Hz have been reported[13,23,26,28,30,31,37,38]. Larger values tend to be observed, if, as in the formamide derivatives, ^{15}N=CH is present[27,31,39–41] as a standard feature. The large value (11.4 Hz) of $J_{^{13}\mathrm{C}\text{-}1',^{15}\mathrm{N}}$ ($J_{^{13}\mathrm{C}\text{-}6',^{15}\mathrm{N}}$) observed for the structural feature ^{15}N=C–^{13}C in **1**-^{15}N may be compared here.

For the amides **3**-^{15}N and **4**-^{15}N, the presence of a considerable amount of π-character in the bond connecting the ^{15}N and carbonyl carbon atoms (as in **9**) did not enchance the magnitude of the three-bond coupling-constant between the ^{15}N nucleus and the acetyl methyl protons of **3**-^{15}N, as this coupling (1.2–1.3 Hz) is of the same order as $J_{5,^{15}\mathrm{N}}$ (<0.9–1.7 Hz), for which there are no intervening π-bonds. Additionally, for **4**-^{15}N, the value $J_{\mathrm{F},^{15}\mathrm{N}}$ <0.6 Hz is remarkably small in view of the fact that fluorine couplings are often substantial[42a,43]. This situation resembles that of pentafluoroethyl derivatives for which $^3J_{\mathrm{F,F}}$ is generally <1 Hz, and for which it has been supposed[42b] that the "through bond" contribution to this coupling constant is attenuated by the presence of a large number of electronegative substituents, that is, fluorine atoms. In **4**-^{15}N, the electronegative, carbonyl oxygen atom is present as a substituent on one of the carbon atoms intervening between the ^{15}N and ^{19}F nuclei.

Overall, it appears that, except for couplings over one bond, the ^{15}N coupling-constants of amino-^{15}N sugars are small, and that, for their utilization in structural and conformational analysis, good spectral resolution is essential. An obvious structural application is the differentiation of the proton resonances of *N*-acetyl groups from those of *O*-acetyl groups.

Proton–proton coupling. — The coupling constants of compounds **1**–**4** and their ^{15}N-labeled analogs are quite similar to those reported for other 1,2:3,5-di-*O*-alkylidene- (Ref. 19) and -arylidene- (Ref. 44) α-D-glucofuranose derivatives, except that, for **1**–**4** and **1**-^{15}N–**4**-^{15}N, the values of $J_{4,5}$ observed are much larger (see Table II). On the basis of small values observed for $J_{4,5}$, 1,2:3,5-di-*O*-benzylidene and 1,2-*O*-isopropylidene-3,5-*O*-(methoxymethylidene)-α-D-glucofuranose derivatives were assigned conformations in which the *m*-dioxane ring adopts a chair shape bearing an axially oriented H_2C-6 group. Were **1**–**4** and **1**-^{15}N–**4**-^{15}N to adopt the same conformation, there would be a strong 1,3-*syn*-diaxial interaction[19] between the H_2C-6 group and one of the 3,5-isopropylidene methyl groups. This interaction undoubtedly modifies the chair shape so that larger values of $J_{4,5}$ are observed. This effect is especially marked (see Table II) for **1** and **1**-^{15}N; these contain the (bulky) phthalimido substituent which has, in fact, been used previously as a conformational locking-group[45].

Investigations of these conformations are in progress, together with those of similar non-nitrogenous derivatives.

EXPERIMENTAL

General. — Derivatives **1–4** and their ^{15}N-labeled analogs were prepared as described previously[2,3]. P.m.r. spectra were recorded on commercial instruments* at 60, 90, 100, or 220 MHz, either in the field-sweep mode (60 and 220 MHz) with tetramethylsilane (Me_4Si) as the internal standard (τ 10.00 p.p.m.), or in the frequency-sweep mode (90 and 100 MHz) with internal, field-frequency (f_0) stabilization on the signal of Me_4Si, or on that of an isopropylidene methyl group.

^{19}F spectra were recorded in the frequency-sweep mode at 84.7 MHz, with internal, field-frequency stabilization on the signal of trichlorofluoromethane. The proton and fluorine spectra were obtained for solutions of 90–320 mg of solute in 0.35–0.55 ml of solvent.

Continuous-wave, ^{13}C magnetic resonance spectra were recorded in the frequency-sweep mode at 25.2 MHz and ~55°, with noise-modulated, broad-band irradiation of protons at 100 MHz, and internal, field-frequency stabilization on the ^{13}C signal of proton-decoupled benzene[12]. Solutions of 366–371 mg of solute in 1.0 ml of benzene were used in 8-mm (o.d.) sample-tubes. The ^{13}C spectral region was divided into four zones, each with a sweep width of 50 p.p.m., and 16–189 scans of each zone were accumulated separately in 1024 channels of a conventional, signal-averaging computer.

Fourier-transform, ^{13}C n.m.r. spectra were obtained by exciting the samples (at ~35°) with 400–2000 pulses at 25.2 MHz, using a pulse width of 40 or 75 μsec and a pulse interval of 0.4 or 1.6 sec. Solutions of 300–487 mg of solute in 2.5 ml of chloroform-*d* were employed in 12-mm (o.d.) sample-tubes, together with noise-modulated irradiation of protons at 100 MHz with a band-width of 4 kHz and internal, heteronuclear, field-frequency stabilization on the deuteron signal of the solvent at ~15.4 MHz.

Irradiation of the trifluoromethyl group of **4**-^{15}N was accomplished by connecting the proton stabilization and observing frequencies (f_0 and f_1) and the ^{19}F irradiation-frequency (f_2) to a single pair of transmitter coils tuned to f_2 (~84.7 MHz), increased power (~10 dB) being used for f_0 and f_1 to compensate for the loss at 90 MHz. The f_2 power used was ~0.5 W at 84.678105 MHz. However, for indor sweeps of f_2 from 84.678163 to 84.677978 MHz, this power was attenuated by 30–40 dB.

For indor sweeps of the ^{15}N frequency (f_2, from 9.120390 to 9.120659 MHz), one of the NH-proton peaks was monitored by a constant f_1, while f_2 was supplied at a power of ~0.5 W attenuated by 0–5 dB to a second pair of transmitter coils tuned approximately to 9.1 MHz by means of a plug-in, matching network. For the foregoing experiments, f_0, f_1, and f_2 were obtained from the internal, quartz oscillators of the spectrometer.

Intripler experiments were performed by monitoring one of the NH-proton

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*The choice of an instrument for a particular type of experiment was dictated solely by its availability, and not by its scientific merit.

peaks with a constant f_1, while the fluorine nuclei were irradiated with $f_2 = 84.678105$ MHz and the ^{15}N frequency (f_3) from the internal, quartz oscillator was swept in the region of 9.12 MHz as before. The maximum output (~2.5 mW) of a commercial, frequency synthesizer was used for f_2, which was matched to the ^{19}F frequency obtained previously from the internal, quartz oscillator by means of a high-frequency counter. The synthesizer was synchronized with the internal oscillators of the spectrometer by means of a 1-MHz output from it. In these experiments, f_0, f_L, and f_2 were connected to the first pair of transmitter coils (tuned to f_2), and f_3 was connected to the second pair of coils.

The large spacing in the ^{15}N spectrum was measured approximately, and indirectly, by counting the difference between the two stationary, ^{15}N frequencies that gave maximum responses of the monitored NH transitions under triple-resonance conditions.

Spectral analysis. — Trial, theoretical, p.m.r. spectra were computed for compounds **1** and **1**-^{15}*N* by use of a modified[19] LAOCN3 program[11]. Initial chemical-shifts were obtained from weighted means of the line positions and first-order spacings, respectively, in the 220-MHz proton spectra. Compound **1** was treated as a seven-spin system consisting of H-1–H-6′, and, owing to the limitations of the program, **1**-^{15}*N* was analyzed as a composite of a six-spin subsystem consisting of ^{15}N and H-3–H-6′, and a two-spin subsystem comprised of H-1 and H-2. This was possible because, as for many other compounds[19,44] of this type, H-2 and H-3 are only very weakly coupled.

The trial spectra of **1** and **1**-^{15}*N* were displayed as digital, incremental plots, and these resembled their observed spectra quite well, except that the relative chemical-shifts of H-3 and H-5, and of H-6 and H-6′, were incorrect.

The parameters of **1** were refined by an iterative analysis in which (*a*) 66 theoretical transitions were assigned to 21 observed peaks, and (*b*) variation was permitted of all of the chemical shifts and of the coupling constants ($J_{1,2}$, $J_{2,3}$, $J_{3,4}$, $J_{4,5}$, $J_{5,6}$, $J_{5,6'}$, and $J_{6,6'}$. A good fit of observed and theoretical spectra was obtained after one iteration, by using Gaussian and Lorentzian, half-line widths of 1.1 Hz for the plot of the theoretical spectrum.

In the iterative analysis of the six-spin system of **1**-^{15}*N*, 60 theoretical transitions were assigned to 26 observed peaks, and the coupling constants $J_{3,4}$, $J_{4,5}$, $J_{5,6}$, $J_{5,6'}$, $J_{6,6'}$, $J_{6,^{15}N}$, and $J_{6',^{15}N}$ were allowed to vary together with all of the chemical shifts, except the arbitrarily assigned value for the ^{15}N nucleus. A good fit of the theoretical to the experimental spectrum (see Fig. 1) was obtained after four iterations, by using Gaussian and Lorentzian, half-line widths of 0.9 Hz.

The p.m.r. spectra of the remaining derivatives were analyzed by first-order methods, *N*-deuteration being employed routinely to confirm the assignments of the NH signals and to simplify the analysis by diminishing the complexity of the H-6 and H-6′ regions of the spectra. A limited number of proton–proton, decoupling experiments were performed at 90 and 100 MHz to confirm the assignments of methine-proton signals.

A series of trial, proton spectra were computed for 4-^{15}N by using the modified LAOCN3 program and various values for the chemical shifts of H-5, H-6, and H-6′. However, due to program limitations, the CF_3 group was excluded. The following coupling-constants were used: $J_{^{15}N-H}$ -92.5, $J_{5,^{15}N}$ -1.3, $J_{6,^{15}N}$ 0.8, $J_{6',^{15}N}$ 0.6, $J_{6,NH}$ and $J_{6',NH}$ 5.7, $J_{3,4}$ 3.7, $J_{4,5}$ 6. 6, $J_{5,6}$ 4.5, $J_{5,6'}$ 7.6, and $J_{6,6'}$ -13.2 Hz. For the purposes of trial computations, the signs of the ^{15}N coupling-constants[15] and of $J_{6,6'}$ were assumed without direct proof.

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