The Use of Carbon-13 Magnetic Resonance Chemical Shifts and Long-range $^{13}C-^{1}H$ Coupling Constants for assigning the Site of Glycosylation on Nitrogen Heterocycles [1]

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A series of *v*-triazolo[4,5-*b*]pyridine nucleosides, which are structurally related to the biologically active 8-aza-purines, has been prepared. The use of natural abundance carbon-13 n.m.r. in establishing the site of ribosylation of the nucleoside 5-amino-3-(β-D-ribofuranosyl)-*v*-triazolo[4,5-*b*]pyridin-7-one (6) is reported. The potential diagnostic utility of long-range (two- and three-bond) $^{13}C-^{1}H$ coupling constants as an aid in the unequivocal assignment of the site of ribosylation and other signals in the ^{13}C n.m.r. spectrum are described.

In synthetic nucleoside chemistry, a successful synthesis of the requisite nucleoside often represents a small fraction of the effort spent on the overall project when compared to that devoted toward establishing the site of glycosylation. The time involved in the latter is usually proportional to the number of possible sites of glycosylation on the nitrogen heterocycle used in the reaction. The methods of choice for determining the specific site of glycosylation involve either (1) the chemical conversion of the newly synthesized nucleoside into a known, fully characterized derivative or (2) comparison of the u.v. spectra of the nucleoside with those of all possible *N*-methylated derivatives of the heterocycle, *i.e.*, derivatives synthesized by unambiguous routes. Recent investigations in our laboratories which led to the synthesis of certain imidazo[4,5-*b*]pyridine nucleosides [2] related to guanosine, utilized method (1) to determine the site of ribosylation. To our surprise, when this approach was applied to a similar series of *v*-triazolo[4,5-*b*]pyridine nucleosides [3] it failed. The problem was further complicated in that the appropriate model methyl derivatives [method (2)] of our ring system and target nucleoside had not been synthesized, and their unambiguous preparation was highly improbable. To solve our dilemma we used an alternate spectroscopic technique, carbon-13 n.m.r.

Previous investigations [4,5] have demonstrated the value of ^{13}C n.m.r. in determining the site of *N*-substitution on nitrogen heterocycles. This spectral method relies on changes in carbon chemical shifts of those carbon atoms α and β [see (A) and (B)] to the site of *N*-substitution. Like any empirical technique, minor limitations arise [6] and certain precautions [7,8] should be followed prior to using any set of ^{13}C parameters as an unequivocal assignment of structure. Having developed the application of α- and β-carbon chemical shifts as a means of establishing sites of *N*-substitution, we explored the possibility of improving the technique. We now report an improvement which utilizes a combination of the α- and β-carbon chemical shift data with certain long-range two- and three-bond $^{13}C-^{1}H$ spin–spin couplings.

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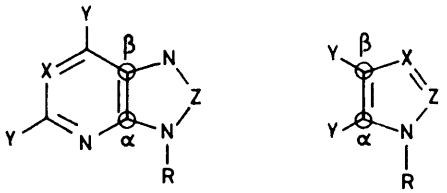
EXPERIMENTAL

^{13}C N.m.r. spectra were obtained on a Varian XL-100-15 spectrometer operating in the Fourier transform mode. All compounds used in this investigation were prepared and characterized by literature procedures.[2,3] These compounds (20—100 mg) were dissolved in [$^{2}H_{6}$]dimethyl sulphoxide (0.3 ml) containing dioxan (2.5 μl) as an internal reference. Noise-decoupled ^{1}H spectra were obtained at ambient probe temperature (37 °C) for self-consistent chemical shift data, while coupled spectra were generally obtained at 80 °C for purposes of greater resolution and sensitivity.

Noise-decoupled ^{1}H spectra were recorded over a 5 KHz spectral width with an 8K word data table and a digital line broadening of *ca.* 1 Hz. An IF crystal filter



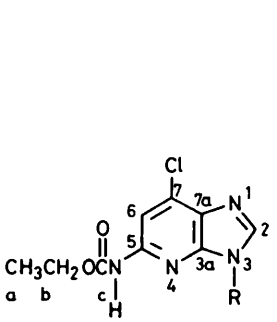
General representations of heterocyclic systems which lend themselves to the n.m.r. spectroscopic method. The signal for the carbon atoms α to the site of substitution (R = alkyl or glycosyl) shift upfield and the signal for the carbon atoms β to the site of substitution shift downfield relative to the signals of the unsubstituted heterocycle (R = H). X and Z can be either carbon or nitrogen.

was used to increase sensitivity and to reduce aliasing and fold-over artifacts. The coupled spectra were obtained over a narrower spectral width (typically 1500 Hz with 0.2 Hz digital line broadening) than the decoupled spectra to increase resolution for more accurate measurements of the spin–spin coupling constants. Sensitivity was significantly increased by gating the proton decoupler *on* during delay times and *off* during the acquisition of the free induction decays to obtain nuclear Overhauser enhancement of the signals.[9] A Krohn–Hite model 3202 audio filter was also used to further increase sensitivity and reduce aliasing effects from the ribosyl (upfield) portion of the coupled spectra.

RESULTS AND DISCUSSION

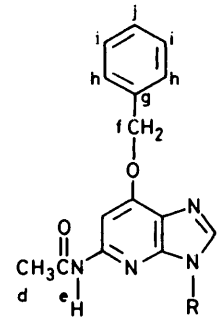
Carbon-13 Chemical Shifts.—Shifts for the imidazo-[4,5-*b*]pyridine and *v*-triazolo[4,5-*b*]pyridine heterocycles and nucleosides are summarized in Table 1. Initially,

these assignments were made on the basis of one-bond $^{13}C-^{1}H$ coupling constants, comparison with data obtained from other heterocyclic systems,[4c,8,10] and chemical intuition. To simplify the presentation of (3a—c), and (4a and b) the carbon resonances for the methyl (C_a), methylene (C_b), and carbonyl (C_e) carbons of the 5-carbamate group were assigned to the signals at δ 15.4, 61.9, and 154.7 p.p.m., respectively. These data



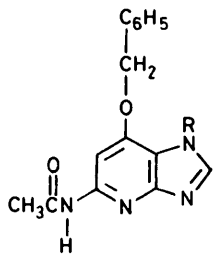
(1)

a; R = H
b; R = β-D-ribofuranosyl

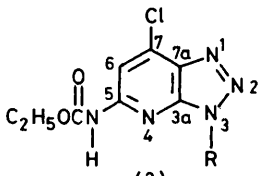


(2)

a; R = H
b; R = β-D-ribofuranosyl

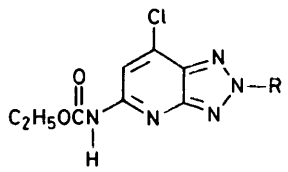


(2c) R = β-D-ribofuranosyl

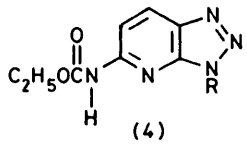


(3)

a; R = H
b; R = β-D-ribofuranosyl

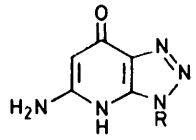


(3c) R = β-D-ribofuranosyl



(4)

a; R = H
b; R = β-D-ribofuranosyl



(5)

a; R = H
b; R = β-D-ribofuranosyl

these assignments, we have divided the data into several categories.

A. *5-Carbamate, 5-acetamido, and 7-benzyloxy substituents.* In the noise-decoupled spectra of (1a and b), were in good agreement with those reported for ethyl methylcarbamate.[11] The methyl (C_d) and carbonyl (C_e) carbon lines of the 5-acetamido group in (2a—c) were attributed to the signals at δ 25.1 and 170.3 p.p.m.,

TABLE 1

Carbon-13 chemical shifts for certain imidazo[4,5-*b*]-pyridines and *v*-triazolo[4,5-*b*]pyridines

	Aglycone						Ribose				
Compound	C(2)	C(3a)	C(5)	C(6)	C(7)	C(7a)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
(1a)	144.2	149.1	149.4	108.4	133.8	128.2					
(1b)	144.1	146.6	149.7	109.1	135.6	130.1	88.5	74.7	71.5	86.5	62.5
(2a)	141.6	(153)	150.3	95.1	156.7	118.2					
(2b)	141.1	147.9	150.6	95.3	158.3	123.1	87.5	74.8	71.7	86.2	62.5
(2c)	143.8	156.3	150.6	94.6	154.0	113.9	90.7	75.8	70.7	85.9	62.0
(3a)		152.9	151.8	110.0	134.4	131.6					
(3b)		146.3	154.6	111.6	138.8	133.0	90.4	74.1	71.7	87.0	62.9
(3c)		157.3	152.4	115.3	129.2	121.7	92.2	74.6	71.6	87.1	62.4
(4a)		149.3	153.3	112.4	128.8	131.3					
(4b)		145.4	154.1	112.2	131.2	134.9	90.7	74.1	71.9	87.0	63.4
(5a)		149.3	163.6	92.2	159.3	126.1					
(5b)		148.9	163.2	92.2	158.2	126.7	90.1	74.0	72.1	87.0	63.4

[a] Chemical shifts are in p.p.m. from Me_4Si. Values were measured relative to internal dioxan and converted to the Me_4Si scale using $\delta(Me_4Si) = \delta(\text{dioxan}) + 67.4 - 17.5 \times 10^{-4}\,T(°C)$. Concentration: 100 mg in 0.3 ml [$^2H_6$]DMSO. *T ca.* 37 °C.

respectively, and these assignments correspond to those reported for acetanilide.[11] Similarly, the carbon resonances of the benzyloxy substituent of (2a—c) [depicted in structure (2a) as C_{f-j}] were in agreement with those published for benzyl alcohol. The three closely spaced

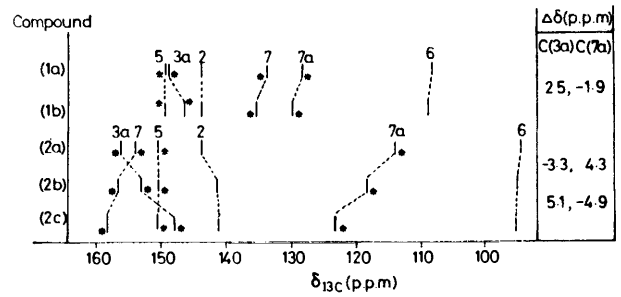


FIGURE 1 ^{13}C Chemical shift correlation diagram (with respect to Me_4Si) for certain imidazo[4,5-*b*]pyridines. The asterisks refer to the quaternary carbon lines

lines centred at δ 129.0 p.p.m. were assigned to $C_{h,j}$, while the signal at δ 137.4 p.p.m. [136.9 p.p.m. for (2c)] was attributed to C_g. The methylene (C_f) resonance was observed at δ 71.7 p.p.m.

B. β-D-*Ribofuranosyl moiety*. The carbon-13 chemical shifts for the β-D-ribofuranosyl moiety are listed in Table 1. The values show little deviation with those

TABLE 2

Fine splitting patterns [a] and $^{13}C-^1H$ coupling constants [b] for certain imidazo[4,5-*b*]pyridines

Compound	C(2)	C(3a)	C(5)	C(6)	C(7)	C(7a)
	d	d	s	dd	d	dd
(1a)	$^1J_{H2}$ 209.6	$^3J_{H2}$ 5.0		$^1J_{H6}$ 171.9	$^2J_{H6}$ 4.2	$^3J_{H6}$ 6.0
				$^3J_{NH}$ 2.5		$^3J_{H2}$ 11.5
	dd	dd	s	dd	d	dd
(1b)	$^1J_{H2}$ 214.1	$^3J_{H2}$ 5.3		$^1J_{H6}$ 174.0	$^2J_{H6}$ 4.5	$^3J_{H6}$ 6.4
	$^3J_{H1'}$ 4.0	$^3J_{H1'}$ 3.0		$^3J_{NH}$ 2.5		$^3J_{H2}$ 11.6
	d	d	s	dd	d	dd
(2a)	$^1J_{H2}$ 208.0	n.a.		$^1J_{H6}$ 169.0	$^2J_{H6}$ 3.8	n.a.
				$^3J_{NH}$ 3.0		
	dd	dd	s	dd	d	dd
(2b)	$^1J_{H2}$ 212.2	$^3J_{H2}$ 4.6		$^1J_{H6}$ 168.9	$^2J_{H6}$ 3.3	$^3J_{H2}$ 11.4
	$^3J_{H1'}$ 4.2	$^3J_{H1}$ 2.1		$^3J_{NH}$ 3.0		$^3J_{H6}$ 5.4
	dd	d	s	dd	d	ddd
(2c)	$^1J_{H2}$ 212.0	$^3J_{H2}$ 12.4		$^1J_{H6}$ 169.0	$^2J_{H6}$ 3.2	pr
	$^3J_{H1'}$ 4.0			$^3J_{NH}$ 3.0		

[a] d = Doublet, s = singlet, dd = doublet of doublets, pr = poor resolution, na = not available, ddd = doublet of doublets of doublets (pseudo-octet). [b] In Hz ± 0.4.

published for a variety of *N*-nucleosides.[8] The resonance line sequence (from downfield to upfield) has been established [12] as: C(1′), C(4′), C(2′), C(3′), and C(5′).

C. *Ring carbons of the imidazo*[4,5-b]*pyridine and* v-*triazolo*[4,5-b]*pyridines*. The aromatic carbon chemical shift data for the imidazo[4,5-*b*]pyridine and *v*-triazolo[4,5-*b*]pyridine ring systems are compiled in Table 1 and illustrated in the correlation diagrams depicted in Figures 1 and 2. The carbon chemical shift assignments for these aromatic carbons were corroborated by their

TABLE 3

Fine splitting patterns [a] and $^{13}C-^1H$ coupling constants [b] for certain *v*-triazolo[4,5-*b*]pyridines

Compound	C(3a)	C(5)	C(6)	C(7)	C(7a)
(3a)	s	s	dd	d	d
			$^1J_{H6}$ 172.4	$^2J_{H6}$ 4.6	$^3J_{H6}$ 7.5
			$^3J_{NH}$ 3.0		
	d	s	dd	d	d
(3b)	$^3J_{H1'}$ 2.0		$^1J_{H6}$ 173.7	$^2J_{H6}$ 4.9	$^3J_{H6}$ 6.8
			$^3J_{NH}$ 3.0		
	s	s	dd	d	d
(3c)			$^1J_{H6}$ 174.8	$^2J_{H6}$ 4.4	$^3J_{H6}$ 7.8
			$^3J_{NH}$ 3.0		
	d	d	dd	d	d
(4a)	$^3J_{H7}$ 5.7	$^3J_{H7}$ 8.6	$^1J_{H6}$ 171.3	$^1J_{H7}$ 169.5	$^3J_{H6}$ 9.0
			$^3J_{NH}$ 3.0		
	dd	d	dd	d	d
(4b)	$^3J_{H7}$ 6.8	$^3J_{H7}$ 8.6	$^1J_{H6}$ 170.9	$^1J_{H7}$ 169.9	$^3J_{H6}$ 9.0
	$^3J_{H1'}$ 3.0		$^2J_{NH}$ 3.0		
	s	s	d	d	d
(5a)			$^1J_{H6}$ 162.0	$^2J_{H6}$ 2.8	$^3J_{H6}$, pr
	d	s	d	d	d
(5b)	$^3J_{H1'}$ 2.0		$^1J_{H6}$ 161.8	$^2J_{H6}$ 2.8	$^3J_{H6}$ 6.5

[a] d = Doublet, s = singlet, dd = doublet of doublets, pr = poor resolution. [b] In Hz ± 0.4.

one-, two-, and three-bond $^{13}C-^1H$ spin–spin couplings (see Tables 2 and 3) and selective proton-decoupling experiments.

Signals for the bridgehead carbons C(3a) and C(7a) could be distinguished by their reduced peak intensities which have been attributed to the saturation effect of

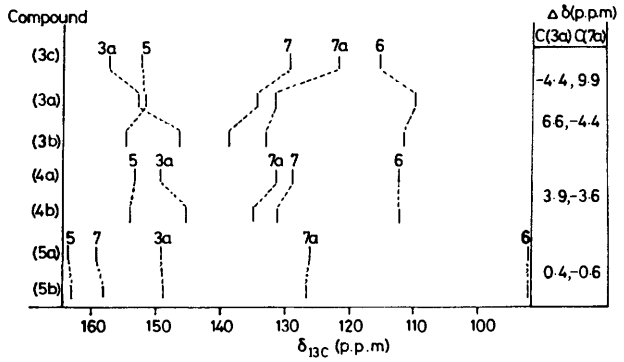


FIGURE 2 ^{13}C Chemical shift correlation diagram (with respect to Me_4Si) for certain *v*-triazolo[4,5-*b*]pyridines

the longer T_1 relaxation times of these quaternary carbon atoms.[13] The C(2) resonances of all imidazo[4,5-*b*]pyridines examined also exhibited this reduced peak intensity (possibly because of the line broadening

due to the quadrupolar relaxation effects of the adjacent ring nitrogens); however, this carbon could be distinguished from the bridgehead carbons by its large (*ca.* 210 Hz) one-bond $^{13}C-^{1}H$ coupling in the off-resonance spectrum. Of the two bridgehead carbons, C(3a) was expected to be the furthest downfield. This assignment was consistent with those made on similar heterocycles.[4c,8,10] It is worthwhile mentioning that the carbon resonances for C(3a) and C(7a) of 5-acetamido-7-benzyloxyimidazo[4,5-*b*]pyridine (2a) were not visible in the noise-decoupled spectrum operating under normal conditions (60° pulse at a 0.8 s repetition rate). Presumably, this was caused by the long T_1 relaxation times for these carbon atoms. By optimizing the conditions (10° pulse at a 5 s repetition) while still avoiding saturation, the C(7a) signal was adequately visualized; however, the C(3a) line still remained too broad and low in intensity to assign an exact chemical shift.

The carbon chemical shifts of the remaining carbon atoms C(5), C(7), and C(6) appeared in the order as written in all the spectra [from downfield to upfield (see Table 1)], and this resonance line sequence was consistent with chemical shift data reported for pyridine and certain substituted pyridines.[14-17]

The differences in carbon chemical shifts ($\Delta\delta$) between the parent heterocycle and their *N*-nucleosides are illustrated in Figures 1 and 2. These correlation diagrams clearly indicate the upfield shift of the α-carbon C(3a) and the downfield shift of the β-carbon C(7a) (relative to the parent heterocycle) for those nucleosides (1b)—(5b) which were designated as the N(3) isomers. The downfield shift of C(3a) and the upfield shift of C(7a) for 5-acetamido-7-benzyloxy-1-(β-D-ribofuranosyl)imidazo[4,5-*b*]pyridine (2c) confirmed the tentative assignment of this nucleoside as N(1). Ethyl 7-chloro-2-(β-D-ribofuranosyl)-*v*-triazolo[4,5-*b*]pyridin-5-ylcarbamate (3c) which had been originally assigned as an N(1) nucleoside according to the differences in carbon chemical shift data of the α- and β-carbons was later tentatively designated as the N(2) isomer based on the long-range two- and three-bond $^{13}C-^{1}H$ spin–spin coupling constant data.

D. $^{13}C-^{1}H$ *spin–spin coupling constants.* A recent attempt to determine the site of ribosylation of certain 4-chloro-*v*-triazolo[4,5-*c*]pyridine nucleosides [18] using the α,β-carbon chemical shift method was unsuccessful. The method failed in this particular case because the two bridgehead carbons of these nucleosides could not be differentiated due to the near-degeneracy of their carbon signals. However, ^{13}C n.m.r. technology has recently progressed to the point that small, long-range two- and three-bond $^{13}C-^{1}H$ spin–spin couplings (2—12 Hz) can be resolved using short accumulation times (9—10 h) and small sample sizes (50—100 mg). Long-range coupling constants observed for compounds (1)—(5) not only allowed an unambiguous assignment of the ^{13}C signals in the decoupled spectra, but also allowed an unequivocal assignment of the site of ribosylation for these nucleosides.

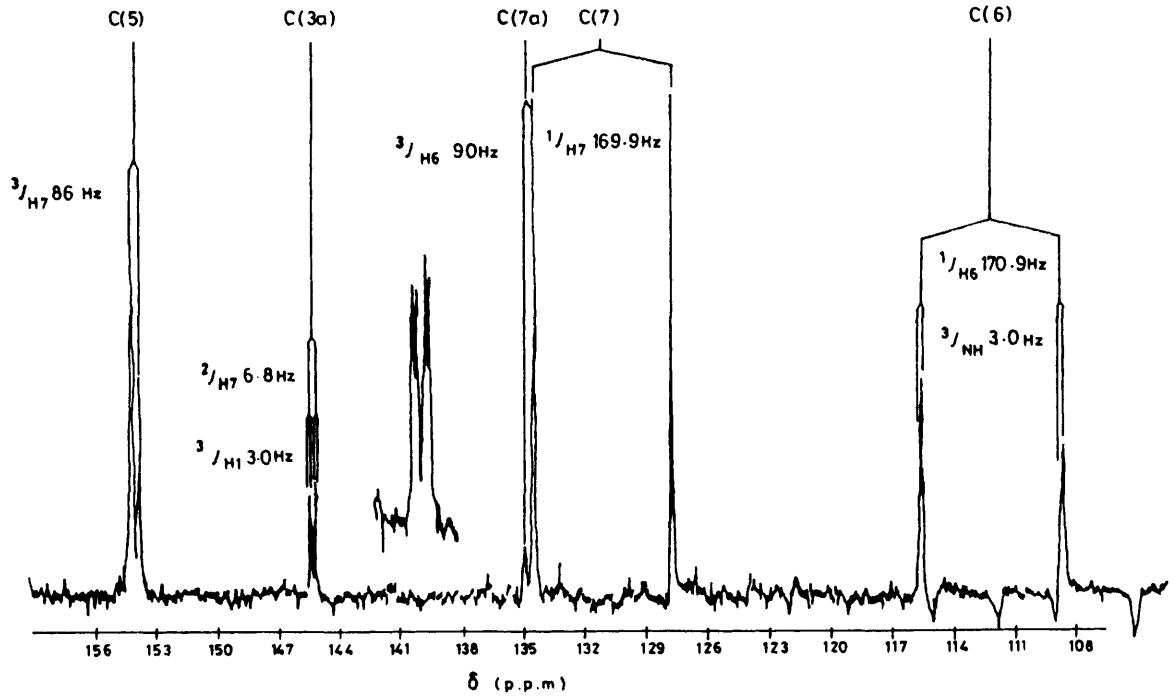


FIGURE 3 Coupled ^{13}C n.m.r. spectrum of ethyl 3-(β-D-ribofuranosyl)-*v*-triazolo[4,5-*b*]pyridin-5-ylcarbamate (4b)

The fine-splitting patterns along with the one- (^{1}J), two- (^{2}J), and three-bond (^{3}J) coupling constants data obtained in this study are tabulated in Tables 2 and 3 and illustrated in Figure 3. An examination of this data in the light of published observations on sub-

stituted pyridines,[14-17] allows the following general observations to be made concerning the long-range ^{13}C–^{1}H coupling constants for this series of compounds. First, as observed in the pyridine investigations,[14-17] the ^{2}J coupling constants are smaller than the ^{3}J coupling constants for the aromatic carbon–proton couplings, but the range of values observed in our bicyclic systems is slightly different. The ^{2}J values are observed between 0 and 4.9 Hz, while the through-carbon ^{3}J values are between 5.4 and 9.0 Hz. Secondly, substitution on an aromatic carbon appears to increase the ^{2}J coupling to that carbon, *e.g.*, compare the $J_{H6,C7}$ coupling (Table 3) constants determined for ethyl 7-chloro-3-(β-D-ribofuranosyl)-*v*-triazolo[4,5-*b*]pyridin-5-ylcarbamate (3b) with that found for ethyl 3-(β-D-ribofuranosyl)-*v*-triazolo[4,5-*b*]pyridin-5-ylcarbamate (4b). The magnitude of this effect seems to depend on the nature of the substituent. Thirdly, the difference in the $^{3}J_{H2,C3a}$ and $^{3}J_{H2,C7a}$ values for the nucleosides (1b), (2b), and (2c) indicate that coupling through a pyridine-type nitrogen increases the ^{3}J coupling compared with a coupling through carbon or through a substituted ring nitrogen. Fourthly, no ^{2}J coupling is observed between the 5-NH proton and C(5); however, a small ^{3}J (3.0 Hz) is observed with C(6). The observation of this coupling constant depends on the dryness of the sample. Fifthly, a ^{3}J coupling of 2.0—4.0 Hz is observed between the anomeric proton * H(1′) of the carbohydrate moiety and the aromatic carbon α to the site of glycosylation. This key $^{3}J_{H1',C\alpha}$ spin–spin coupling provides an unequivocal assignment of the site of *N*-glycosylation. With the exception of (2c) and (3c), all the nucleosides examined possessed this long-range coupling. The data indicates that these nucleosides are substituted at N(3) of their respective heterocyclic aglycones and supports the α- and β-carbon chemical shift data. Unfortunately, for the N(1) nucleoside (2c) the low intensity and broad linewidth of the C(7a) signal prevented the resolution of the expected pseudo-octet and we were not able to measure the ^{3}J couplings for this carbon. However, the linewidth (12 Hz) of the C(7a) multiplet as well as the splitting patterns for each of bridgehead carbons C(3a) and C(7a) in the off-resonance spectra of (2b and c) confirm our assignment of this nucleoside as the N(1) isomer.

The absence of any long-range coupling with the anomeric proton and bridgehead carbons in the off-resonance spectra of (3c) has influenced our decision to designate this nucleoside as the N(2) isomer. Although the carbon chemical shifts of the bridgehead carbons suggest ribosylation at N(1) we place more credence in the $J_{H1',C\alpha}$ coupling or lack of it in this case. Obviously, this negative data does not constitute an unequivocal proof of structure, but this evidence, coupled with the fact that the N(2) isomer is the major side-product in other ribosylations of fused triazole heterocyclic systems [18,19] and that anomalous α,β carbon shifts have been reported [6] leads us to believe that (3c) is ribosylated at N(2).

Conclusions.—The increasing availability of high resolution ^{13}C coupled spectra exhibiting long-range ^{13}C–^{1}H spin–spin couplings should greatly facilitate the resolution of difficult structural assignments of benzenoid and heteroaromatic compounds. Application of the three-bond coupling constant, $^{3}J_{H1',C\alpha}$, between the anomeric proton and the carbon atom α to the site of substitution as a means of determining sites of *N*-glycosylation of nucleosides is demonstrated in this study. However, it is not yet known whether a change in the configuration or conformation of the glycosyl moiety will greatly alter the magnitude of this small coupling constant. Recent work [20] from our laboratory concerning the ^{13}C n.m.r. spectra of methylated formycin derivatives revealed a three-bond coupling constant between the methyl protons and the carbon atoms α to the site of methylation. This implies that the stereochemistry of the exocyclic proton does not greatly affect that important $^{3}J_{H1',C\alpha}$ value. Further studies of a variety of *N*-alkyl- and *N*-glycosyl-substituted heterocycles are needed to define this phenomenon fully.

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* The imidazo[4,5-*b*]pyridine nucleosides (1b), (2b), and (2c) also exhibit a ^{3}J coupling between H(1′) and C(2).

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