Determination of the Anomeric Configuration of 2'-Deoxy-D-ribonucleosides by ^{1}H NMR and by Crystallographic Studies of a Novel 2'-Deoxy *C*-Nucleoside

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2'-Deoxy-D-ribonucleoside analogs of biologically active 2-β-D-ribofuranosylthiazole-4-carboxamide were synthesized and the structure of the β anomer was determined by X-ray crystallography and ^{1}H nmr. 2'-Methylene protons of α- and β-deoxyribonucleosides were observed to exhibit characteristic patterns in the ^{1}H nmr which was used to distinguish between the two anomers. The method could be used to determine the anomeric configuration of both *N*- and *C*-2'-deoxyribonucleosides.

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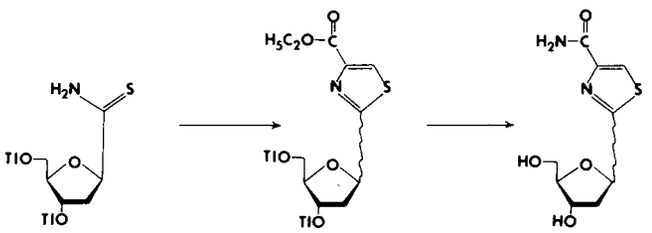
The ^{1}H nmr spectra of various 2'-Deoxy-D-ribonucleosides have been studied in our laboratories and it was observed that methylene protons (H2', H2") next to the anomeric center in α-2'-deoxy-D-ribonucleosides display more chemical shift non-equivalence than those in the corresponding β-2'-deoxy-D-ribonucleosides. Consequently the H2', H2" resonance "band width" observed for α-2'-deoxynucleosides is generally larger than that for β-2'-deoxynucleosides. This criterion has for the first time been used to readily distinguish between the α and β configurations of deoxynucleosides, and has been found especially useful for the anomeric pairs which deviate from the "triplet-quartet-peak width" (TQPW) rule (1-3). The β-configuration determined for 2-(2'-deoxy-D-*erythro*-pentofuranosyl)thiazole-4-carboxamide by ^{1}H nmr was further confirmed by X-ray crystallography.

Recently 2-β-D-ribofuranosylthiazole-4-carboxamide synthesized in our laboratory was found to exhibit potent antitumor activity (4). Subsequently the synthesis of the corresponding 2'-deoxynucleoside, 2-(2-deoxy-β-D-*erythro*-pentofuranosyl)thiazole-4-carboxamide (**4**) was investigated. Treatment of 2,5-anhydro-3-deoxy-4,6-di-*O*-*p*-toluoyl-D-*ribo*-hexonothiamide (4) (**1**) with ethyl bromopyruvate provided ethyl 2-(2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl)thiazole-4-carboxylates as a mixture of two anomers which were separated by silica gel column chromatography, and for convenience the anomers with fast and slow tlc mobility were denoted β (**2**, syrup, 52%) and α (**3**, syrup, 23%), respectively. Treatment of the β and α anomer with methanolic ammonia provided 2-(2-deoxy-β-D-*erythro*-pentofuranosyl)thiazole-4-carboxamide (**4**, crystals (ethanol) yield 60%, mp 144-145°) and 2-(2-deoxy-α-D-*erythro*-pentofuranosyl)-

Scheme 1



1 β - 2 α - 3 β - 4 α - 5

Table 1

2'-Deoxy-D-ribonucleoside Anomer	Type	H-2', H-2" Absorption Spaced Between δ (ppm)	Band Width δ (ppm)
2'-Deoxy-5-azacytidine (17)	(α) Set of Separate Multiplets (SM)	2.00-3.12	1.12
	(β) Clustered Multiplet (CM)	2.36-2.67	0.31
4-Amino-1-(2-deoxy-D-ribofuranosyl)imidazole-5-carboxamide (17)	(α) SM	1.94-2.95	1.01
	(β) CM	2.20-2.63	0.43
2'-Deoxy-5-methyluridine (18)	(α) SM	2.06-3.16	1.10
	(β) CM (triplet)	2.37-2.58	0.21
2'-Deoxy-5-fluorouridine (18)	(α) SM	2.45-3.45	1.00
	(β) CM (triplet)	2.73-2.93	0.20
2-(2-Deoxy-D-ribofuranosylthiazole)-4-carboxamide	(α) SM	1.74-2.86	1.12
	(β) CM	1.89-2.52	0.63

thiazole-4-carboxamide (**5**, syrup, yield 50%), respectively (5) (Scheme 1).

The β configuration of **4** was confirmed by X-ray crystallography. The molecule crystallizes in space group $P2_1$ with cell dimensions a = 7.921(2), b = 6.752(2), c = 10.550(3) Å, β = 102.53(2) Å, and Z = 2. Diffraction data were obtained on a Syntex $P2_1$ diffractometer using CuKα radiation (λ = 1.5418 Å) with a graphite monochromator. The structure was determined by location of the sulfur atom in the Patterson map and the other atoms including all hydrogens in successive Fourier syntheses. It was refined with a block diagonal least square procedure to yield a final discrepancy R factor of 0.032 for all 1122 data with $2\theta < 140°$.

The results of the determination show that the configuration at C1′ is β, (Figure 1) the conformation of the deoxyribose sugar ring is C2′ endo and the glycosidic torsion angle (O1′-C1′-C2-S) is 40.2°. The interbond H2′,H2″ angle is 105° and the torsion angles H1′-C1′-C2′-H2″ and H1′-C1′-C2′-H2′ are 39° and 155° respectively. The data correlated closely with the ^{1}H nmr of **4** as described below.

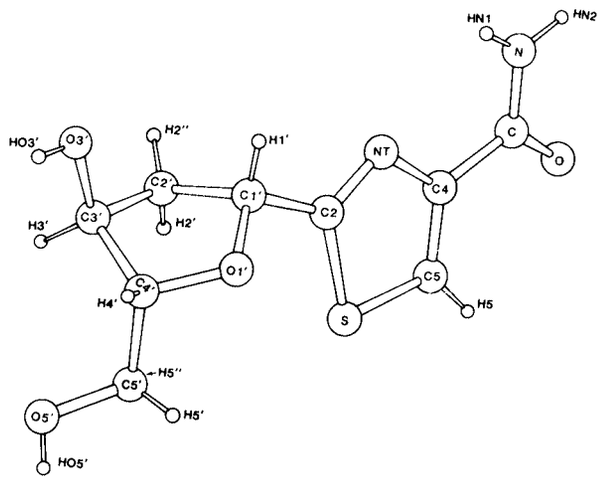


Figure 1

The formation of both the α and β anomers during the synthesis of 2′-deoxy-ribonucleosides by the direct glycosylation of an aglycone or by elaboration of the latter in a suitably functionalized sugar is not uncommon. However,

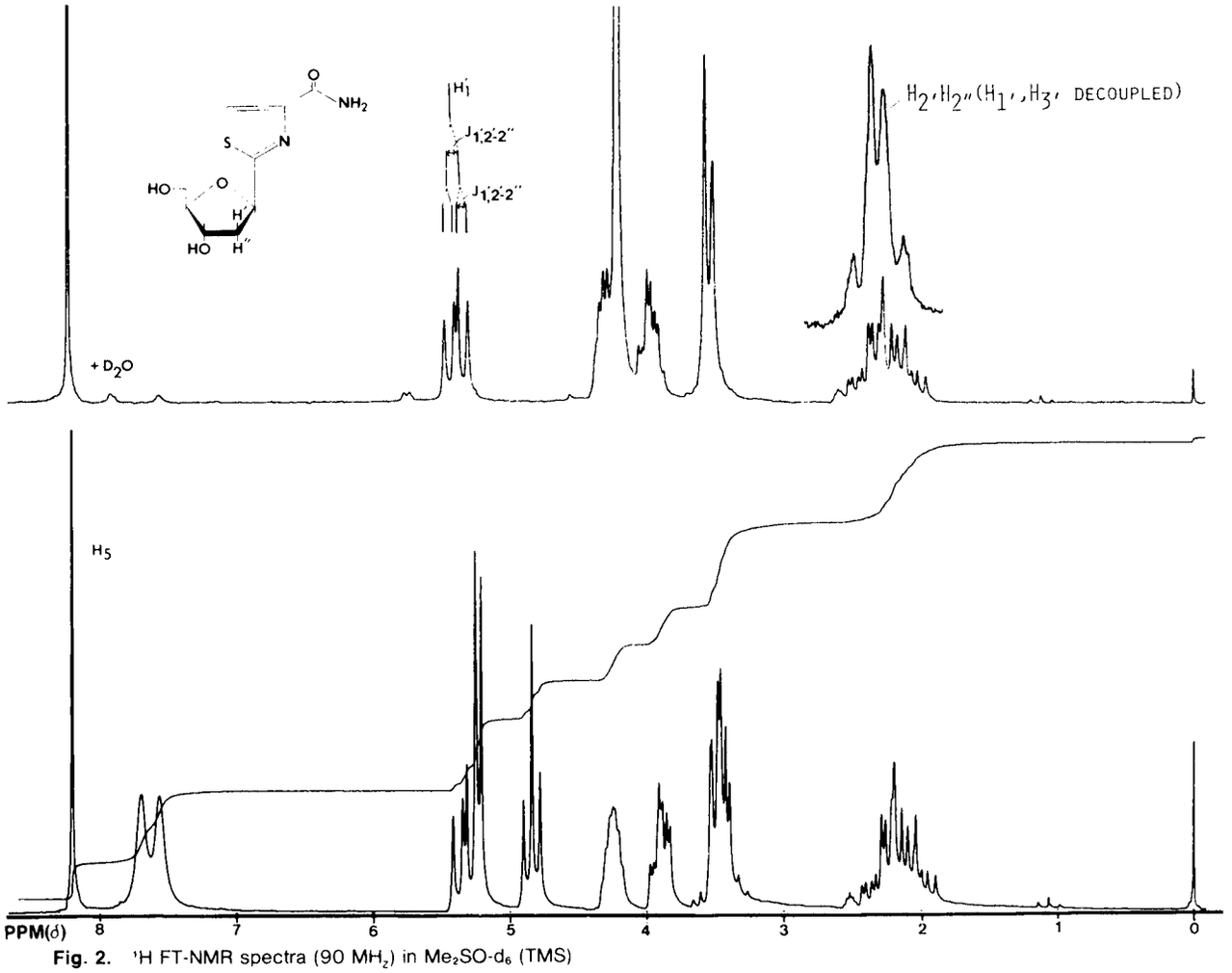


Fig. 2. ^{1}H FT-NMR spectra (90 MH_z) in Me_2SO-d_6 (TMS)

the only direct method (2) available to date to readily distinguish between the α and β anomers in solution is that by ^{1}H nmr in which a "pseudo-triplet" with a peak width of 13.7 $\pm$0.5 Hz and a quartet with a peak width of 10.4 $\pm$0.4 Hz for the anomeric proton is indicative of β and α configurations, respectively. Based on the pioneering work by Karplus (6) and Jardetzky (1) the "triplet-peak width" rule, however, assumes that in the β-deoxynucleosides there is very little (1-3 Hz) or no difference (7) in the chemical shifts of H2' and H2". The deviations from this triplet-peak width rule become apparent when the difference in the chemical shift increases. Compound β-**4** (Figure 2) exhibited a quartet centered at δ 5.30 $\pm$0.02 $J_{1'-2',2''}$ = 9 Hz and 6.2 Hz, peak width 15.2 Hz). This pattern, although a departure from the "triplet-peak width" rule, was very similar to that for α-**5** (Figure 3) which also exhibited a quartet centered at δ 5.3 ($J_{1'-2',2''}$ = 8 Hz and 5.2 Hz, peak width 13.2 Hz). Similar deviations from the "triplet-peak width" rule among the β deoxynucleosides have previously been reported for 2'-deoxy-5-(trifluoromethyl)uridine (8) and certain 8-substituted derivatives of 2'-deoxyadenosine (9) which also show a quartet with a peak width of 15.2 Hz for anomeric protons. Indeed, the situation in the case of **4** and **5** was more intriguing. The splitting pattern of the quartet for H1' of β-**4** was almost identical to that observed for the quartet for H1' of α-**5**. Also among the α deoxynucleosides a peak width of 13.2 Hz observed for the quartet for H1' of α-**5** was unusal and to the best of our knowledge has never been reported before. On the basis of the data available for H1' a fair assignment of the anomeric configuration of **4** and **5** by "TQPW" rule was not possible. Consequently, we focused our attention toward the methylene protons (H2', H2") next to the anomeric center. Certain changes in the splitting pattern of H2', H2" would be expected with conformational changes attributable to the difference in the steric interactions between the heterocycle and the sugar of a nucleoside. The steric interactions would be even more pronounced with the configurational changes (10) ($\alpha \leftrightarrow \beta$) in which case the difference in the splitting pattern of H2', H2" should become more apparent.

In the ^{1}H-nmr of β-**4** the signal for H2',H2" appeared as a complex multiplet clustered at 2.20 and spaced between 1.89 to 2.52 ppm. This multiplet, after spin-spin decoupling of H1' and H3', was roughly resolved into an overlapping pair of doublets with $J_{2'-2''} \cong$ 20 Hz which is within the range of the theoretical value (11) (19.7 Hz) for an interbond H2' H2" angle of 105° but the difference in the chemical shifts ($\Delta\delta \cong$ 12.6) was larger than normally observed (1,7) for β-2'-deoxynucleosides. Due to small $\Delta\delta/J$ ($\sim$0.6) the coupling of H2', H2" with H1' and H3' would be of an ABMX system with a second order splitting pattern (7,12) and the spectrum of H2', H2" would be like that of a "complex multiplet" as indeed observed in the case of β-**4** (Figure 2). When $\Delta\delta$ for H2', H2" is very small the coupling of H2', H2" with H1' and H3' would be like that of an AA'MX system with an approximate first order splitting pattern and the spectrum of H2', H2" would be like that of a simple multiplet (or a triplet) as normally observed for β-2'-deoxynucleosides (13). In the ^{1}H-nmr of α-**5** the signal for H2', H2" appeared as a set of three separate multiplets spread between δ 1.74 and 2.86. A multiplet accounting for one proton was centered at δ 2.62 and the other two multiplets accounting for the other one proton were spaced by 20 Hz ($J_{2'-2''}$) and centered at 2.0 (Figure 3). (Some unaccounted secondary splitting observed is due to the 3'-OH group.) A relatively large $\Delta\delta$2'-2" (56 Hz) observed for α-**5** as compared to that for β-**4** would be expected due to the spacing of H2' (*trans* to the thiazole ring) at an "equatorial" orientation of the puckered furanose ring and the deshielding region of the thiazole ring. Consequently, H2' resonates at lower field (14) as compared to H2" (*trans* to H1') which is shielded by the *cis* 3'-OH group and the *cis* thiazole ring (15,16). Inspection of the Corey-Pauling-Koltun model of α-**5** shows that in a "locked" glycosidic conformation H2" is located directly below the shielding plane of the thiazole ring. It also appears that due to the relatively large "S" atom the rotation around the glycosidic bond is restricted and a conformation close to that in the locked glycosidic position may be predominant even when in solution and on the nmr time scale. The shielding behavior of H2" on one hand and that deshielding of H2' on the other in α deoxynucleosides is responsible for extended absorption "band width" of H2', H2" as compared to that for the corresponding β anomers. Visual examination of the Dreiding model of α-**5** shows that both H1' and H3' have very small dihedral angles ($\sim$5°) with respect to the *cis* oriented H2' whereas those of $\sim$130° with respect to the *trans* oriented H2". According to the relationship of the coupling constants with the dihedral angles (1,13), H2' which would be expected to strongly couple with H1' and H3' (observed $J_{2'-1',3'}$ = 8 Hz) appears as a multiplet whereas H2" which is weakly coupled with H1' and H3' (observed $J_{2'-1',3'}$ = 5.2 Hz) appears as a pair of multiplets. Due to the relatively large $\Delta\delta$ (56 Hz) and $\Delta\delta/J$ (2.8) for H2', H2", the coupling of these protons with H1' and H3' in case of α-**5** would be similar to that of an AMXY system with and approximate first order splitting pattern as seen in Figure 3.

We have examined the ^{1}H nmr of several anomeric pairs of 2'-deoxy-*N*-nucleosides (Table 1) and observed in each case that the absorption of H2', H2" for α anomers extends to both higher and lower fields, giving a larger absorption "band width" and $\Delta\delta$ as compared to the corresponding β anomers. Consequently, the signal for H2',

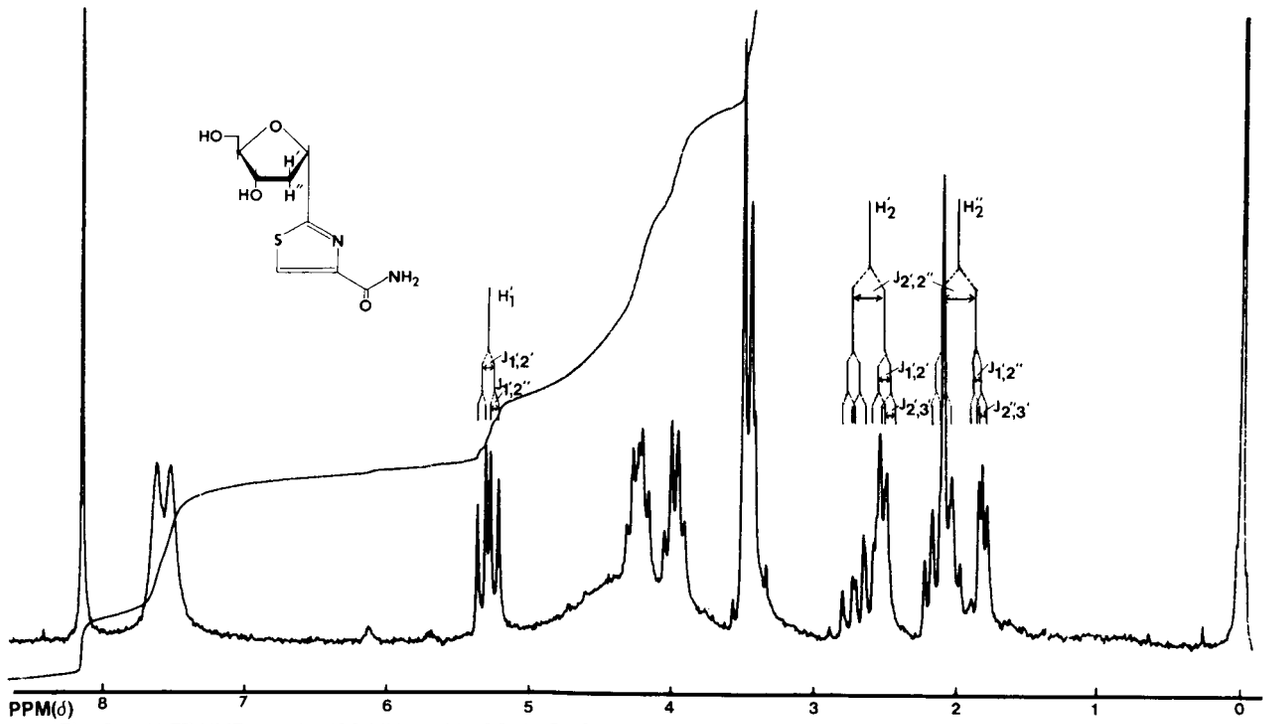


Fig. 3. ^{1}H FT-NMR spectra (90 MH_z) in Me_2SO-d_6 (TMS)

H2'' for α anomers appears as a set of three separate multiplets (or peaks) as compared to a clustered multiplet (or triplet) observed for the same protons in the corresponding β anomers. The values for some anomeric pairs are given in Table 1 (and correlate well with β and α assignments of **4** and **5** respectively). These characteristic ^{1}H nmr properties of methylene protons should prove helpful in distinguishing between the α and β anomers of 2'-deoxynucleosides especially when the "TQPW" rule fails to differentiate the two anomers.

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