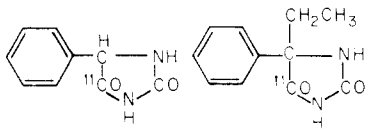
Table V. Relative Carbon-11 Activity in Tissues Following Administration of [^{11}C]Hydantoins[c]



Organ	[^{11}C]-5-Phenylhydantoin[a]	[^{11}C]-5-Ethyl-5-phenylhydantoin[b] (nirvanol)
Brain	0.73	1.25
Lungs	1.05	0.75
Heart (washed)	0.71	1.00
Temporal muscle	1.00	1.00
Liver	2.25	1.33
Bile	0.83	1.58
Spleen	0.81	1.25
Stomach	0.76	0.92
Pancreas	0.63	1.67
Kidneys	1.91	1.25
Blood	0.93	1.00
Mesenteric fat	0.25	0.75
Urine		0.67
CSF		2.67

[a] 80 min after intravenous administration. [b] 73 min after intravenous administration. [c] Expressed as cpm/g of tissue/cpm/g of temporal muscle.

a carboxyl moiety showed fairly uniform distribution of label in all cellular tissues of the body. Due to the large skeletal muscle mass in the body, ^{11}C label from hydantoins deposited in skeletal muscle represents the largest extravascular pool of label following completion of initial mixing. No evidence was noted of concentration of label in highly perfused lipid stores such as in omentum or mesentery. The relatively uniform distribution of activity in cellular tissues and slow excretion from the body support the thesis that the pharmacologic action of the hydantoins is related to physical effects on biomembranes rather than to specific chemical interactions with cell constituents.

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Synthesis of Tetrazole Ribonucleosides and Their Evaluation as Antiviral Agents

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Synthesis of 1-β-D-ribofuranosyltetrazole and two 5-substituted derivatives, i.e., the 5-carboxamide and 5-acetamide, is described. The stereochemical structure of the parent tetrazole ribonucleoside has been established by means of nuclear Overhauser effect and x-ray crystallography. By analogy to the parent compound, the two 5-substituted tetrazole nucleosides are also assigned the β configuration on the basis of the NMR coupling constant of the anomeric proton and the site of N-ribosylation is determined by ^{13}C NMR studies. Results are also presented on antiviral testing of these synthetic tetrazole nucleosides against influenza A2/Asian/J-305 virus infection in mice.

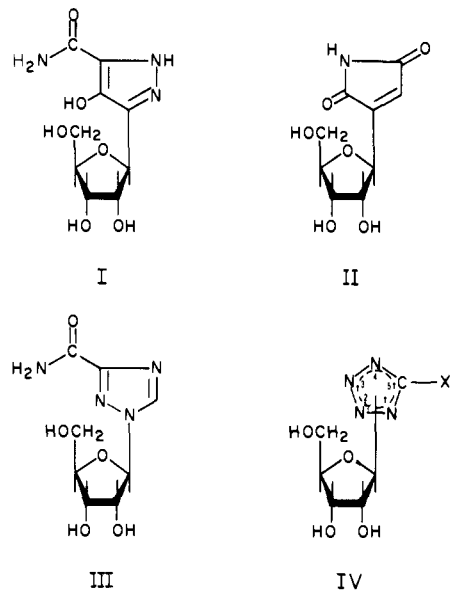
During the past several years quite a few ribofuranosyl nucleosides in which the base moiety is a five-membered heterocycle have been found to display chemotherapeutic activity. More significant among these are pyrazomycin (I),[1-3] showdomycin (II),[4] and Virazole (Ribavirin) (III)[5] (Chart I). Whereas the first two are *C*-nucleosides, Ribavirin is an example of an *N*-nucleoside. The broad-spectrum antiviral activity of Ribavirin has stimulated interest in the synthesis of ribonucleosides of tetrazole (IV) which is an isosteric ring equivalent of triazole. Although tetrazole derivatives have previously been implicated in certain biological activities[6-8] there is no report hitherto on the synthesis of tetrazole nucleosides or their biological effects.

The present paper describes the synthesis of 1-β-D-ribofuranosyltetrazole and some of its derivatives (IV). The biological screening results against influenza A2/Asian/J-305 virus infection in mice are presented for compounds XIII–XV. Since these are the first examples of tetrazole nucleosides, we have provided rigorous proof of their structural configuration by conducting intramolecular nuclear Overhauser and x-ray crystallographic studies on the parent tetrazole nucleoside (XIII) in addition to proton and ^{13}C NMR studies on all derivatives.

Results and Discussion

Synthesis of Tetrazole Ribonucleosides. The synthesis of tri-O-benzoylated tetrazole nucleosides described here was achieved either by coupling of tetrazole derivatives[9] and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide[10] in the presence of mercuric cyanide[11] and nitromethane or, alternatively, by the acid-catalyzed fusion[12] of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and a tetrazole. While the rest of the coupling reactions proceeded to give the expected nucleoside product, in the case of the attempted synthesis of X by the mercuric cyanide method, partial loss of the C-5 carboethoxy substituent was found to have occurred and the resultant coupled product was IX instead (Chart II). Consequently, this approach was abandoned and nucleoside XI was obtained by starting

Chart I



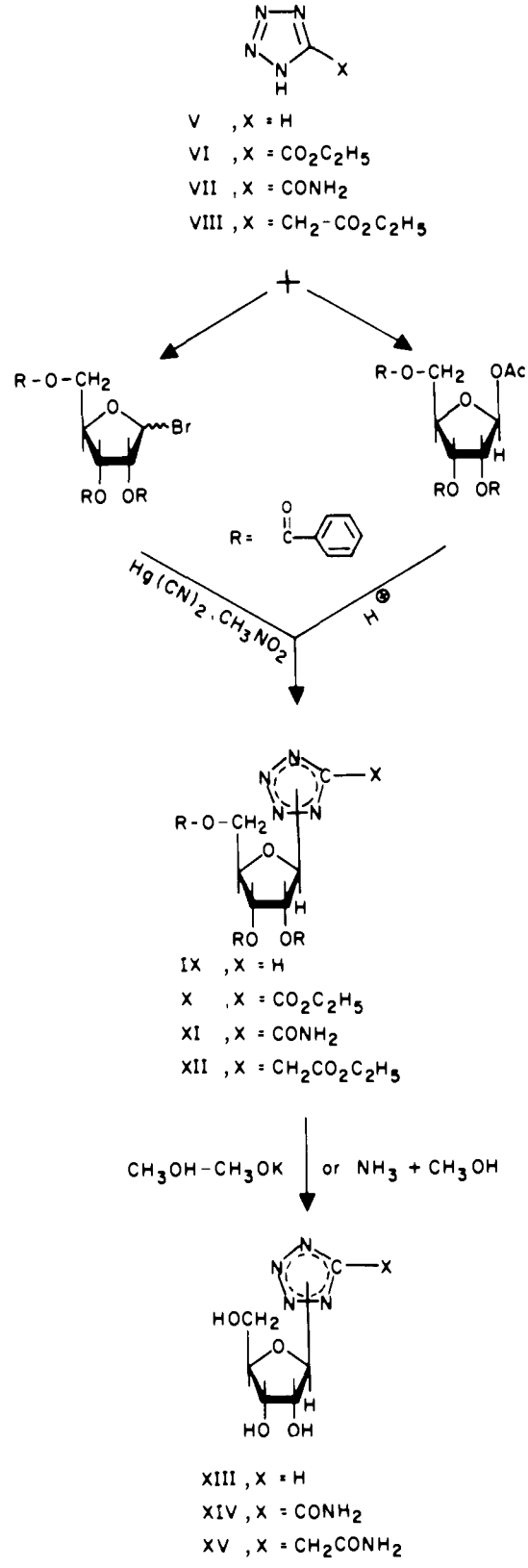
I II III IV

with tetrazole-5-carboxamide (VII). The deacylated tetrazole nucleosides were then obtained by alkaline hydrolysis (NH_3 or $NaOCH_3$ in CH_3OH) of the corresponding blocked compounds.

Structural Assignments of Tetrazole Nucleosides. General molecular structures for all new compounds were established on the basis of elemental analysis and ir, NMR, and mass spectrometry. In the case of tetrazole nucleosides, two questions of stereochemical interest had to be resolved for any complete structural assignment: first, whether tetrazole is linked with the sugar moiety at N_1 or N_2; and second, whether the tetrazole attachment on the sugar is in the α or β configuration. The answers to these two questions will in fact decide which of the four possible structural isomers (XVI, XVII, XVIII, or XIX) is the correct one (Chart III). In the case of pyrimidine and triazole nucleosides, the distinction between α and β configuration has generally been provided by the value of the NMR coupling constant between 1'C–H and 2'C–H ($J_{1'-2'}$). This criterion, however, when applied to tetrazole nucleosides, does not supply a clear choice. According to the literature[13] the $J_{1'-2'}$ coupling constant for furanoid derivatives mentioned above will be in the approximate region of 3.5–8.0 Hz for the α-D and 0.0–8.0 Hz for the β-D configuration. The observed value of this coupling constant in all examples of deblocked tetrazole nucleosides reported here is 4 Hz. This value, although indicative of the β configuration, is not sufficient for a definitive assignment.[13]

A series of nuclear Overhauser experiments[14] on the parent tetrazole ribonucleoside (XIII) was performed to decide between N_1 and N_2 attachment of the tetrazole moiety. Irradiation of 1'C–H produced an increase of 12.3% in the signal intensity of C_5-H. In agreement with the reported literature values[15] for the model compounds, uridine and thymidine, this would indicate N_1 attachment. Dreiding models show that the internuclear distance between protons on C-1' and C-5 in structure XVI ($N_1\beta$ attachment) is 3 Å and in structure XVII ($N_2\beta$ attachment) 5 Å. In general, a nuclear Overhauser effect is observed between protons separated by 3.5 Å or less. This result, therefore, strongly points to N_1 attachment of the tetrazole moiety. That the $N_1\beta$ assignment is indeed correct was finally confirmed by x-ray crystallographic studies on the parent tetrazole ribonucleoside XIII. The details about these studies are presented in the following section. The nuclear Overhauser and x-ray crystallographic studies were performed only on one nucleoside (i.e., XIII). Since the NMR spectra of the other derivatives of tetrazole ribonucleoside (i.e., compounds XIV and XV) reported here also show $J_{1'-2'}$ = 4 Hz and their ORD Cotton effect is negative as in the case of the parent tetrazole nucleoside, these also are assigned β configuration. In order to determine the site of N-ribosylation in compounds XIV and

Chart II



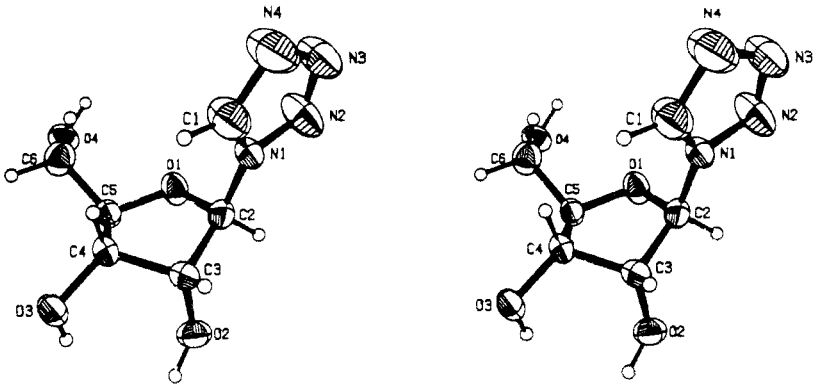
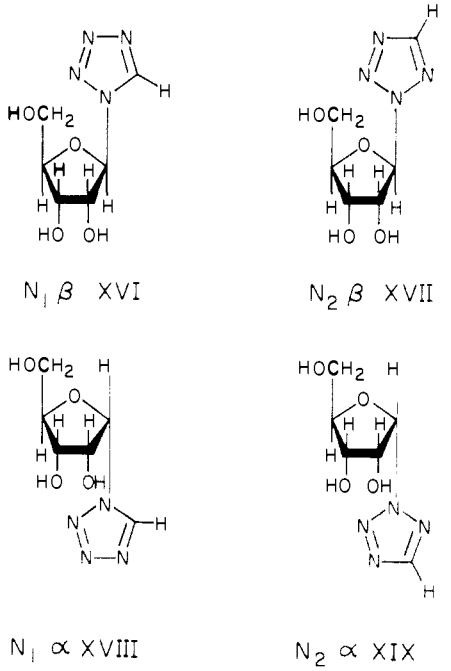


Figure 1. Stereodrawing of the tetrazole ribonucleoside, 1-β-D-ribofuranosyltetrazole (XIII), showing its conformation in the crystalline state.

Chart III



XV, the effect on the ^{13}C NMR chemical shift of carbon-5 upon ribosylation of the base was measured and compared with the corresponding effect in the parent tetrazole nucleoside XIII for which N1-ribosyl attachment was established by x-ray and nuclear Overhauser studies. The results listed in Table I show that there is only minor change in the ^{13}C chemical shift of C-5 of tetrazole VIII (X = CH_2CONH_2) and V on glycosylation. In contrast, tetrazole VII exhibited a significant downfield shift (8.62 ppm) on transformation to nucleoside XIV. These results suggest that glycosyl attachment for nucleoside XV is N1 and for nucleoside XIV, it is N2. It should, however, be noted here that ^{13}C results from compounds XIII and XV are at variance with the general behavior of heterocycles upon nitrogen substitution as described in the literature.[16,17] According to these studies the ^{13}C signal of an α-carbon undergoes a significant upfield shift upon nitrogen substitution (alkyl or glycoside). However, ^{13}C studies on N_1-methyl- and N_2-methyltetrazoles[18] have shown small (1.3 ppm) shift in the α-carbon signal and larger (9.8 ppm) downfield shift in the β-carbon resonance as compared to the unsubstituted tetrazole. The data on compounds XIII and XV in conjunction with N_1-methyltetrazole seem to point that in tetrazole the α-carbon undergoes none or small ^{13}C signal shift on nitrogen substitution.[19]

Table I. ^{13}C Chemical Shifts (ppm from Me_4Si) of Carbon-5 of the Tetrazole Anions and Their Protonated and Glycosylated Derivatives

Tetrazole base	Anion[a]	Protonated species	Glycosylated compd
V	143.0	143.38	143.21
VIII[b]	150.9	151.5	151.8
VII	151.3	151.8	160.42

[a] Anions of various tetrazole derivatives were formed by neutralization with LiOH in Me_2SO-d_6. [b] VIII, X = CH_2CONH_2.

Table II. Crystal Data for 1-β-D-Ribofuranosyltetrazole (XIII)

Formula	$C_6H_{10}N_4O_4$
Formula wt	202.17
Space group	$P2_1$
a	5.171 (2) Å
b	7.545 (3) Å
c	11.058 (3) Å
β	89.98 (3)°
Z	2
d_{calcd}	1.555 g cm^{-3}
μ (Cu Kα)	11.5 cm^{-1}

Table III. R Values for the Trial Models

Structure	Trial model	R_1	R_2
$N_1\beta$	C(1) as C	0.052	0.081
$N_1\beta$	N(2) as C, C(1) as N	0.063	0.093
$N_2\beta$	N(3) as C, C(1) as N	0.061	0.096
$N_2\beta$	N(4) as C, C(1) as N	0.060	0.091

X-Ray Crystallographic Results. The crystal data are summarized in Table II. The intensity data were collected on a Hilger-Watts diffractometer from a crystal which was approximately 0.05 × 0.15 × 0.30 mm in size. Of 844 accessible reflections with $\theta < 68.5°$, 741 had intensities significantly greater than background and these data were used for the structure analysis. The structure was solved by a multiple solution procedure.[20] All refinements were carried out by full-matrix least squares. For the final refinement (and the test refinements described below) anisotropic thermal parameters were used for all atoms except the hydrogens which had isotropic temperature factors. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy index is $R = 0.052$ for the 741 observed reflections.

In order to distinguish between N1 and N2 attachment of the tetrazole ring, it was necessary to refine four trial structures. Structure XVI (Table III) was the one indicated for tetrazole ribonucleoside. In each of the other three structures, one of the nitrogen atoms N(2), N(3), or

Table IV. Effect of 14 Intraperitoneal Treatments with Ribavirin and Tetrazole Ribonucleosides against Influenza A2/Asian/J-305 Virus Infection in Mice[a]

Compd	Dose, mg/kg ip × 14[b]	No. of mice surviving/no. tested at 21 days		p^c
		Treated	Control	
Ribavirin	20	5/10	0/9	0.022
XIII	100	2/8	0/9	>0.05
XIV	100	2/10	0/9	>0.05
XV	25	2/7	4/9	>0.05

[a] Weanling mice were infected intranasally with 3 LD_{50} of influenza A2/Asian/J-305 virus. [b] Treatment (0.5 ml intraperitoneal) was administered 4 hr before virus infection, 1 hr after infection, and then twice daily for the next 6 days for a total of 14 treatments. [c] Fisher exact test.

N(4) (Figure 1), was treated as a carbon atom and the tetrazole carbon atom was treated as a nitrogen atom. In each case, the hydrogen atom was placed on the atom assumed to be carbon. The unweighted and weighted R values, R_1 and R_2, for the four trial structures are given in Table III. The R values for structure XVI are significantly lower than those for any of the other structures, thus establishing the structure of the tetrazole ribonucleoside as XVI.

The conformation of the tetrazole ribonucleoside in the crystalline state can be seen in the stereodrawing (Figure 1). Note that in the crystal the proton H-5 is turned away from the proton H-1'. The H-5 to H-1' distance in this orientation is 3.8 Å. When the tetrazole ring is rotated 180° about the N-1 to C-1' bond (which can easily occur in solution) the H-5 to H-1' distance becomes 2.5 Å, thus permitting an intramolecular nuclear Overhauser effect to be observed in the NMR spectrum.

Biological Results. Procedure. Swiss albino mice (Royalhart) weighing 10–12 g were lightly anesthetized with ether and infected intranasally with three lethal doses (LD_{50}) of influenza A2/Asian/J-305 virus. Mice received intraperitoneal injections (0.5 ml) of the test substances 4 hr before virus infection, 1 hr after infection, and then twice per day for the next 6 days for a total of 14 treatments. Animals were observed for 21 days following infection and the number of survivors was recorded on the 21st day.

The results of antiviral testing are shown in Table IV. Ribavirin produced an effect ($p = 0.022$) at 20 mg/kg while XIII, XIV, and XV were inactive ($p > 0.05$) at the doses tested.

Experimental Section

General. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Varian XL-100 spectrometer with tetramethylsilane as internal reference. The values are given in parts per million downfield from Me_4Si. Infrared spectra were scanned either on Perkin-Elmer 621 or Beckman IR9. Thin-layer chromatographic analysis was performed on 0.25-mm silica gel plates (60F-254) purchased from Brinkman Instruments, Westbury, N.Y. In the case of tribenzoylated tetrazole nucleosides, solvent system A (chloroform–acetone, 13:1, v/v) was used in developing the analytical TLC plates. These compounds were visualized under a uv lamp. For the TLC development of debenzoylated tetrazole nucleosides, however, solvent system B (toluene–methanol, 1:1, v/v) was found useful. These materials were visualized by spraying the TLC plates with a solution of glacial acetic acid, sulfuric acid, and *p*-anisaldehyde (50:1:0.5, v/v) and then heating the plates at 110° for about 5–15 min. Components containing the ribonucleoside moiety gave yellowish-green or green color.

Synthesis of Tetrazole Derivatives. The parent 1*H*-tetrazole is commercially available from Aldrich Chemical Co. For synthesis of the 5-substituted tetrazole derivatives a literature method[9] was followed with some modification.

5-Ethoxycarbonyltetrazole (VI). A mixture of ethyl cyanoformate (99 g, 1 mol), sodium azide (130 g, 2 mol), and ammonium chloride (117 g, 2 mol) in 500 ml of dimethylformamide was heated with stirring at 95° for 17 hr. After cooling to room temperature, the solid material was removed by filtration. The filtrate and the DMF washings (3 × 50 ml) were combined and concentrated on a flash evaporator under vacuum to an oil. More (7.5 g) of a high-melting material (>300°) had precipitated as a solid during concentration. This was collected by filtration and the solid material was washed with dry acetonitrile. The combined filtrate and washings (200 ml) gave a further 0.85 g of the unknown high-melting material on keeping at 4° overnight. At this point the desired product crystallized from the acetonitrile solution. A first crop weighed 8.4 g; a second crop of another 8.7 g of crystals was obtained by allowing the solution to stand at 4° overnight. This combined material was found to be the ammonium salt of 5-ethoxycarbonyltetrazole: yield 10.7%; mp 180–181°. Anal. ($C_4H_9N_5O_2$) C, H, N.

This material was then converted to the acid form by passing its aqueous solution (4 g, 25.1 mmol in 30 ml of water) through a 2.0 × 30 cm column of Dowex AG 50W-X8 (H^+) resin. The material so obtained had mp 87.5–89° (lit.[21] 85–86°).

Tetrazole-5-carboxamide (VII). The hydrogen form of 5-ethoxycarbonyltetrazole (3.56 g, 25.1 mmol) was taken up in methanol (100 ml) and cooled in a dry ice–acetone bath, and ammonia gas was bubbled into this solution for 1 hr. The reaction vessel was stoppered securely and allowed to warm to room temperature where it was kept for 2.5 days. The reaction vessel was then cooled in a dry ice–acetone bath and carefully opened. On concentration, the ammonium salt of tetrazole-5-carboxamide was obtained. Before coupling with the ribonucleoside sugar it was always converted into the hydrogen form by the procedure described above. The desired material was recrystallized from ethanol to afford a yield of 62%, mp 241°. Anal. ($C_2H_3N_5O$) C, H, N.

Ethyl Tetrazole-5-acetate (VIII). A mixture of ethyl cyanoacetate (22.6 g, 0.2 mol), sodium azide (14.3 g, 0.22 mol), and ammonium chloride (11.7 g, 0.22 mol) in dimethylformamide (100 ml) was heated at 104° for 7 hr with mechanical stirring. After cooling it was concentrated to an oil which was coevaporated thrice with water. Finally, it was taken up in water (100 ml) and the pH of this solution brought to 1.8 by addition of hydrochloric acid. On cooling in an ice bath, a solid product was deposited. The solid was separated and washed with small portions of cold water. The total material obtained was 15.7 g (50.2% yield): mp 126–128° (lit.[9] 128–130°); NMR (Me_2SO-d_6) δ 1.19 (t, 3, J = 7 Hz, CH_3), 4.12 (q, 2, J = 7 Hz, CH_2CH_3), 4.12 (s, 2, CH_2CO), 11.3 (broad, 1, NH).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole (IX). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-ribose (25.2 g, 50 mmol) was converted to the 2,3,5-tri-*O*-benzoyl-D-ribosyl bromide as described in the literature.[10] The syrupy ribosyl bromide residue so obtained was dissolved in nitromethane and concentrated again to a syrup. It was then dissolved in nitromethane (100 ml) and was added dropwise to a heated (90°) and mechanically stirred suspension of 1-H tetrazole (3.5 g, 50 mmol), mercuric cyanide (12.62 g, 50 mmol), and white Drierite (20 g) in nitromethane (400 ml) over a period of 1 hr. The reaction mixture was then heated at reflux for an additional 3 hr after which it was cooled to room temperature and purged with a stream of nitrogen gas. The solid suspension was filtered off and the solid washed with nitromethane (3 × 50 ml). The filtrate was concentrated to a syrup, coevaporated with chloroform (3 × 100 ml), and finally dissolved in 200 ml of chloroform. Some of the salts precipitated at this stage and were removed by filtration. The chloroform layer was extracted with 30% potassium iodide solution (6 × 150 ml) to remove remaining mercuric salts. The chloroform solution was concentrated to a residue which was coevaporated with toluene. The desired product precipitated as a solid from toluene. The yield of this material (which was found to be about 95% pure by TLC, solvent A) was 9.2 g (35%). Recrystallization from ethanol furnished an analytically pure sample of colorless needles: mp 166–168°; NMR (Me_2SO-d_6) δ 4.79 (m, 2, 5'CH_2), 5.04 (m, 1,

4'C-H), 6.26 (m, 2, 3' and 2'C-H), 6.97 (d, 1, 1'C-H, $J_{1'-2'}$ = 2 Hz), 7.58 (m, 9, aromatic protons), 8.0 (m, aromatic protons), 9.82 (s, 1, 5C-H); ir (KBr) 3100, 1710, 1590, 1440, 1260, 1080, 700 cm^{-1}. Anal. ($C_{27}H_{22}N_4O_7$) C, H, N.

1-β-D-**Ribofuranosyltetrazole (XIII).** A sample of 1-(2,3,-5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole (8.05 g, 15.6 mmol) was dissolved in 40 ml of sodium methoxide–methanol solution (concentration of $NaOCH_3$, 2 mg/ml) and allowed to stand at room temperature for 3 hr. The reaction was quenched by the addition of AG 50W-X8 (H^+) resin (40 ml, 1.7 mequiv/ml of resin bed). The resin was filtered and washed with methanol. The filtrate and washings were combined and concentrated. In order to remove methyl benzoate from the mixture, the residue was dissolved in water and extracted with toluene (3 × 100 ml). The aqueous phase was concentrated and the tetrazole nucleoside recrystallized from ethyl acetate to give colorless needles: mp 127°; yield of the product 2.94 g (86%); NMR (Me_2SO-d_6) δ 3.62 (m, 2, 5'C-H_2), 4.06 (m, 1, 4'C-H), 4.21 (m, 1, 3'C-H), 4.49 (dd, 1, 2'C-H, $J_{2'-3'} = J_{1'-2'}$ = 4 Hz), 5.16 (broad, 3, 5',3',2'C-OH), 6.06 (d, 1, 1'C-H, $J_{1'-2'}$ = 4 Hz), 9.63 (s, 1, 5C-H); ir (KBr) 2950–3600 (very broad band), 1480, 1440, 1060, 1040, 960, 900, 820 cm^{-1}. Anal. ($C_6H_{10}N_4O_4$) C, H, N.

1-β-D-**Ribofuranosyltetrazole-5-acetamide (XV).** A 50-mmol preparation of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide was obtained from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose as described. This was then condensed with ethyltetrazole 5-acetate (7.8 g, 50 mmol) by the mercuric cyanide method. The residual syrup obtained after the work-up as described in the preparation of 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole was dissolved in toluene (100 ml) and one-half of this solution was loaded on silica gel 70–230 mesh column (3.6 × 60 cm). The column was eluted successively with toluene (1 l.), toluene–chloroform (75:25, v/v, 1 l.), toluene–chloroform (50:50, v/v, 1 l.), and finally with toluene–chloroform (25:75, 4 l.). The last eluent contained 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-ethoxycarbonyltetrazole (XII), as shown by NMR on the crude sample. Small amounts of contaminants were then removed by further purification of this sample by preparative silica TLC (2-mm plates, solvent A). Even this purified material failed to crystallize. Subsequently it was hydrolyzed in methanol saturated with ammonia at room temperature (3 days). The solvent was evaporated, the residue dissolved in water, and the aqueous phase extracted with ether. The aqueous phase which contained the desired material was concentrated and the residual material dissolved in ethanol for crystallization. After a slow crystallization extended over several days, colorless material, mp 95–97°, was obtained (yield 0.34 g, 2.6%): NMR (Me_2SO-d_6) δ 3.45 (m, 2, 5'C-H_2), 3.93 (s, 2, CH_2CO), 3.93 (m, 1, 4'C-H), 4.20 (m, 1, 3'C-H), 4.55 (m, 1, 2'C-H), 4.61 (m, 1, 5'C-OH), 5.17 (d, 1, OH), 5.55 (d, 1, OH), 5.83 (d, 1, 1'C-H, $J_{1'-2'}$ = 4 Hz), 7.24 and 7.73 (s, 2, NH_2). Anal. ($C_8H_{13}N_5 \cdot 0.5H_2O$) C, H, N.

1-(**2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole-5-carboxamide (XI).** A mixture of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose (10.08 g, 20 mmol) and tetrazole-5-carboxamide (2.26 g, 20 mmol) was melted in vacuo by heating in a 175° bath. To the molten mass was added bis(*p*-nitrophenyl) phosphate (40 mg) and heating at 175° in vacuo continued for 15 min. The mass was cooled to room temperature, dissolved in hot methanol, and allowed to stand overnight. The solid deposited was isolated by filtration to give 6.4 g (57%) of the crude desired product. An analytically pure sample of XI was obtained on recrystallization from methanol: mp 176–176.5°; NMR (Me_2SO-d_6) δ 4.65 (m, 2, 5'C-H_2), 5.06 (m, 1, 4'C-H), 6.24 and 6.38 (m, 1 each, 2' and 3'C-H), 7.21 (d, 1, 1'CH, $J_{1'-2'}$ = 2 Hz), 7.55 and 8.0 (m, 15, aromatic protons of benzoyl substituent), 8.14 and 8.43 (singlets, 2, NH_2); ir (KBr) 3375, 3200, 1740, 1680, 1600, 1450, 1280, 1100, 1000, 920, 710 cm^{-1}. Anal. ($C_{28}H_{23}N_5O_8$) C, H, N.

1-β-D-**Ribofuranosyltetrazole-5-carboxamide (XIV).** A sample of 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole-5-carboxamide (4.0 g, 7.17 mmol) was dissolved in 1800 ml of methanol. Ammonia gas was bubbled through the chilled (dry ice–acetone temperature) methanol solution for 1 hr. Subsequently the reaction vessel was securely stoppered and placed at 5° for 72 hr. The solvent was evaporated and the material applied on a 3.7 × 70 cm silica gel column which was previously equilibrated with a toluene–acetone (2:1, v/v) mixture. The column was eluted by varying compositions of toluene and acetone as follows (v/v): 2:1 (4 l.), 3:2 (1.5 l.), 2:3 (2 l.), 3:7 (4.5 l.), and 0:1 (1 l.). The desired product emerged in the last two fractions. These fractions were pooled and evaporated and the oil was dissolved in 95% ethanol. The material crystallized the following day. Two crops of pure material yielded 0.96 g (52%) of colorless crystals: mp 87–89°; NMR (Me_2SO-d_6) δ 3.55 (m, 2, 5'C-H_2), 4.05 (m, 1, 4'C-H), 4.30 (m, 1, 3'C-H), 4.60 (m, 1, 2'C-H), 4.75 (t, 1, 5'CH_2-OH), 5.3 and 5.75 (doublets, 1 each, 2' and 3'OH, J = 5.5 Hz), 6.25 (d, 1, 1'C-H, $J_{1'-2'}$ = 4 Hz), 8.00 and 8.33 (singlets, 1 each, $CONH_2$); ir (KBr) 3500–3000 (broad band), 1665, 1600, 1058, 1018, 850, 700, 650 cm^{-1}. Anal. ($C_7H_{11}N_5O_5$) C, H, N.

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References and Notes

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