Synthesis and Antiviral Evaluation of Some Novel [1,2,4]Triazolo[4,3-*b*][1,2,4]triazole Nucleoside Analogs

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Ribosylation of 3-amino-5*H*-[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**1**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose and stannic chloride resulted in the following protected nucleoside analogs: 3-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**4**), 3-amino-1-(2,3,5-tri-*O*-benzoyl-α-D-ribofuranosyl)[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**5**), 3-imino-2*H*-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**6**), and 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)amino-5*H*-[1,2,4]triazolo[4,3-*b*]-[1,2,4]triazole (**7**). Compounds **4-6** were deprotected to 3-amino-1-β-D-ribofuranosyl[1,2,4]triazolo[4,3-*b*][1,2,4]-triazole (**3**), 3-amino-1-α-D-ribofuranosyl[1,2,4]triazolo[4,5-*b*][1,2,4]triazole (**8**), and 3-imino-2*H*-2-β-D-ribofuranosyl[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**9**), while **7** could not be deprotected without decomposition. Compounds **1**, **4**, **6**, **7**, and **9** were screened and found to have no antiviral activity.

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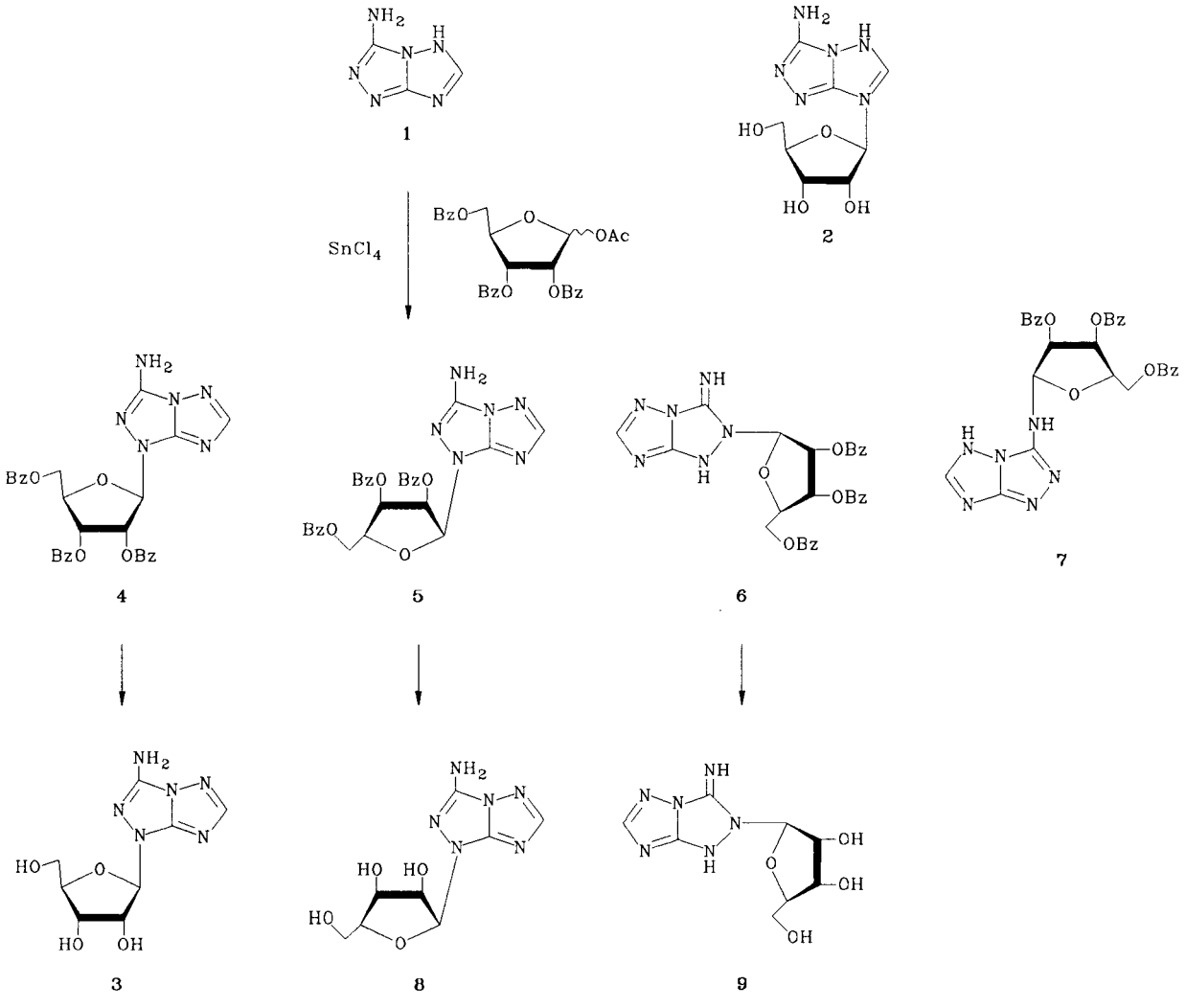
Based upon the antiviral activity found with certain triazolotriazoles [1], we prepared a number of compounds containing this ring system, including some novel nucleoside analogs of 3-amino-5*H*-[1,2,4]triazolo[4,3-*b*][1,2,4]-triazole (**1**) [2]. According to the literature, Robins *et al.* [3] had already attempted to synthesize adenosine analog **2** by treating triazole **1** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose using four catalytic systems: (1) the general trimethylsilyl-Lewis acid procedure; (2) direct glycosylation of the unsilylated triazolotriazole with TMS-triflate; (3) addition of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide with the triazolotriazole sodium salt at 50-55°; and (4) the high temperature glycosylation procedure with boron trifluoride etherate in nitromethane. His attempt using system 1 was unsuccessful, while the attempts with systems 2-4 resulted, after deprotection, in the isolation of only one structurally elucidated product, 3-amino-1-β-D-ribofuranosyl[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**3**). The product of system 3 was especially surprising, since the predicted site of sodium salt formation had been on the 7-nitrogen, on the ring without the exocyclic amino function. Ribosylation of this intermediate was therefore expected to give the tribenzoylated precursor to adenosine analog **2**. This result led them to further speculate that the expected isomer might form only as an intermediate that spontaneously rearranged to the isolated *N*-1 isomer **4**.

We decided to investigate this ribosylation using a different catalyst. Triazolotriazole **1** was treated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose and stannic chloride at 0° under argon. After stirring at room temperature for 24 hours, a mixture of four main products was isolated. In addition to **4**, we also obtained three other protected triazolotriazole nucleosides including: 3-amino-1-(2,3,5-tri-*O*-benzoyl-α-D-ribofuranosyl)[1,2,4]triazolo[4,3-*b*][1,2,4]-triazole (**5**), 3-imino-2*H*-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**6**), which was the major product isolated, and 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)amino-5*H*-[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**7**). Deprotection of compounds **4-6** gave the expected triazolotriazole nucleosides **3**, **8** (characterized only by nmr), and **9**, respectively. Similar attempts to deprotect **7** only resulted in its decomposition.

The structures for our compounds were determined by careful analysis of the nmr data. As previously mentioned, the X-ray crystal structure and the ^{1}H nmr spectrum of 3-amino-1-β-D-ribofuranosyl[1,2,4]triazolo[4,3-*b*][1,2,4]triazole had already been reported [3]. The ^{1}H nmr spectrum for our compound **3** was found to agree with the reported spectral data for 3-amino-1-β-D-ribofuranosyl[1,2,4]triazolo[4,3-*b*][1,2,4]triazole. The ^{13}C nmr spectrum of **3** and **8** and the corresponding precursors **4** and **5** contained ^{13}C spectral data for the heterocyclic portions that were nearly identical, indicating that the site of glycosylation was the same in both pairs and that the only differences between **3** and **4** and between **8** and **5** were their anomeric configurations. Since the δ for H-4′ of the α-anomers was greater than the δ for the H-4′ of the β-anomer, compounds **8** and **5** identified were as the α-anomers of **3** and **4**. For compound **7**, the ^{1}H nmr spectrum showed that H-1′ was coupled to both the NH of the base and to the H-2′. The anomeric configuration was determined by more closely scrutinizing the data for H-4′. The initial spectrum of **7** showed that this compound was actually a 1:3 mixture of α:β anomers, but that upon standing, this mixture became a 45:55 mixture of the α and β anomers. The anomeric configuration of **6** was determined to be β since the cou-



pling between H-1′ and H-2′ was small ($J_{1',2'}$ = 1.7 Hz), indicating that H-1′ and H-2′ have a *trans* relationship. Using the deprotected compound **9**, we then performed NOE experiments that further confirmed the β-anomeric configuration. When H-1′ was irradiated, there was a 1.3% enhancement of H-4′, and when H-4′ was irradiated, there was a 1.1% enhancement of H-1′; therefore H-1′ and H-4′ are on the same side of the ring. When H-1′ was irradiated, there was also a 2% enhancement of the NH's at 8.25 ppm, and when the NH's were irradiated, there was a 14.2% enhancement of H-1′, thus indicating that H-1′ was near the exocyclic NH group. There were no enhancements of any signals when H-6 was irradiated. These data supported the view that the riboside was attached to the ring containing the NH_2 group. The site of ribosyl attachment was further confirmed by long range C-H coupling. If the ribose was attached to N-5 or N-7, H-1′ would be coupled to C-6 and/or C-7a; however, no such coupling was found. Instead, H-1′ was shown by selective decoupling of H-1′ to be coupled to C-3 with a $^3J_{C\text{-}3,H\text{-}1'}$ = 1.7 Hz. This data combined with the NOE experiments confirmed the attachment of the ribose to N-2.

Biological Results.

Compounds **1**, **4**, **6**, **7**, and **9** were evaluated *in vitro* by the U.S. Army Medical Research Institute of Infectious Diseases for their antiviral activities against RNA viruses: Punta Toro (PT), Venezuelan equine encephalomyelitis (VEE), Yellow fever (YF), Japanese encephalitis (JE), Pichinde (PIC), Vesicular stomatitis (VSV), and Sandfly (SF). DNA viruses: Vaccinia (VV) and Adenotype 2 (AD2). The antiviral quantitative MTT [4-7] assay was used to determine the 50% inhibition (IC_{50}) of virus-induced cell death. The concentration of test compound that was cytotoxic to 50% of uninfected cells (TC_{50}) was also determined, as was the therapeutic index, TI_{50}, a ratio of these two values (TC_{50}/IC_{50}). No significant *in vitro* activity was observed for any of the compounds against these viruses.

EXPERIMENTAL

All solvents and materials were reagent grade and were either used as received or purified as required. The ^{1}H nmr and ^{13}C nmr spectra were run with a Nicolet NT-300 NB spectrometer operating at 300.635 and 75.60 MHz, respectively, with tetramethylsilane as an internal reference. Chemical shifts (δ) for multiplets were measured from the appropriate centers. The mass spectral data were obtained from a Varian MAT 311A mass spectrometer in fast atom bombardment (FAB) or electron-impact (EI) mode (direct probe temperature 20°), as indicated. Infrared data were obtained with a Nicolet 10-MX spectrometer. In most cases, only strong or medium peaks in the 1800-600 cm^{-1} range were reported. The uv absorption spectra were determined in the appropriate *p*H 1 (0.1 *N* hydrochloric acid), *p*H 7 buffer, and *p*H 13 (0.1 *N* sodium hydroxide) solutions with either a Cary 17 spectrometer or a Perkin Elmer Model Lambda 9 UV/VIS/NIR spectrophotometer. Melting point data was obtained with a Mel-Temp Capillary Melting Point apparatus, and all melting points were uncorrected. Elemental analysis data were obtained on a Perkin Elmer Model 240 Elemental Analyzer or from Atlantic Microlab of Atlanta, Georgia.

The NOE experiments were conducted in a non-degassed solution of DMSO-d_6. While non-spinning, eight fid's were acquired with the decoupler set at a desired frequency, and eight fid's were recorded with the decoupler off-resonance. The process was repeated until 400 fid's had been accumulated for each experiment. Subsequent subtraction of the two spectra gave the net enhancement.

2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl Triazolotriazoles **4-7**.

The 3-amino-5*H*-[1,2,4]triazolo[4,3-*b*][1,2,4]triazole [2] (**1**, 6.97 g, 56.2 mmoles) was suspended in a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (28.35 g, 56.2 mmoles) in anhydrous acetonitrile (915 ml). Stannic chloride (14.7 ml, 125.6 mmoles) was added slowly at 0° under argon atmosphere. The reaction mixture was stirred at room temperature for 24 hours. It was then concentrated to a small volume and saturated sodium bicarbonate was added until the vigorous evolution of carbon dioxide had ceased. The mixture was evaporated under reduced pressure and the residual gum was extracted several times with hot chloroform. The combined extracts were dried and concentrated. Thin-layer chromatographic analysis of this crude product (silica gel, chloroform-methanol, 9:1 v/v) showed four products with *Rf* values of 0.45, 0.81, and 0.9 along with unchanged and decomposed sugar derivatives (*Rf* 0.97). The mixture was applied to a column of silica gel and eluted with chloroform followed by 1%, 2%, and 3% methanol in chloroform. The three products were separated and further purified by repeating the silica gel column chromatography. A fourth product was isolated from the chromatographic purification of the fraction containing compound **4**, when the solvent system was changed to cyclohexane-ethyl acetate (1:1). Structures of these compounds were assigned on the basis of the following spectral data.

3-Amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)[1,2,4]triazolo-[4,3-*b*][1,2,4]triazole (**4**).

The above cited compound with *Rf* 0.9, yield 2.34 g (7.3%), mp 86-87°; ms: (FAB) m/z 569 (M + 1); uv: λ max 239 (30,500), shoulder at 280 (13,300) at *p*H 1; 240 (30,700), shoulder at 280 (13,300) at *p*H 7; 229 (27,700), shoulder at 275 (2200) at *p*H 13; ir (potassium bromide): 3425, 3335 (NH_2), 3175, 3065 (CH), 1727 (C=O), 1655 (C=N), 1600, 1570 (aromatic), 1452, 1317, 1269, 1175, 1160, 1121, 1096, 1070, 1025, 709 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 8.15-7.25 (m, 16, H-6 and benzoyl), 6.31 (d, 1, H-1′, $J_{1',2'}$ = 3.86 Hz), 6.28 (dd, 1, H-2′, $J_{2',3'}$ = 5.32 Hz), 6.15 (dd, 1, H-3′, $J_{3',4'}$ = 5.31 Hz), 4.85 (dd, 1, H-5′a, $J_{4',5'a}$ = 3.8 Hz, $J_{5'a,5'b}$ = 12.6 Hz), 4.76 (m, 1, H-4′), 4.68 (dd, 1, H-5′b, $J_{4',5'b}$ = 4.5 Hz); ^{13}C nmr (deuteriochloroform): δ 166.1, 165.2, 165.0 (C=O), 158.3 (C-6, $^1J_{CH}$ = 207.4 Hz), 156.0 (C-7a, $^3J_{C\text{-}7a,H\text{-}6}$ = 9.8 Hz, $^3J_{C\text{-}7a,H\text{-}1'}$ = 1.7 Hz), 140.9 (C-3), 133.6, 133.5, 133.0, 129.8, 129.77, 129.6, 128.8, 128.7, 128.4, 128.37, 128.3 (aromatic), 88.5 (C-1′, $^1J_{CH}$ = 168.7 Hz), 79.9 (C-4′), 73.7 (C-2′), 71.7 (C-3′), 63.8 (C-5′).

Anal. Calcd. for $C_{29}H_{24}N_6O_7 \cdot 0.33H_2O$: C, 60.62; H, 4.33; N, 14.63. Found: C, 60.81; H, 4.36; N, 14.27.

3-Amino-1-(2,3,5-tri-*O*-benzoyl-α-D-ribofuranosyl)[1,2,4]triazolo-[4,3-*b*][1,2,4]triazole (**5**).

The above cited compound with *Rf* 0.85, yield 238 mg, mp 83-86°; ms: (FAB) m/z 569 (M + 1); ir (potassium bromide): 3425, 3335 (NH_2), 3175, 3065 (CH), 1727 (C=O), 1653 (C=N), 1602, 1569 (aromatic), 1452, 1317, 1268, 1176, 1165, 1110, 1069, 1025, 708 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 8.07 (m, 2, *ortho* H's), [7.84 (m, *ortho* H's) and 7.83 (s, H-6)] (2), 7.56-7.21 (m, 9, *meta* and *para* H's), 6.45 (d, 1, H-1′, $J_{1',2'}$ = 5.5 Hz), 5.98 (t, 1, H-2′, $J_{2',3'}$ = 6.5 Hz), 5.37 (m, 1, H-4′), 4.91 (br s, 2, NH_2), 4.81 (dd, 1, H-5′a, $J_{4',5'a}$ = 3.3 Hz, $J_{5'a,5'b}$ = 12.3 Hz), 4.65 (dd, 1, H-5′b, $J_{4',5'b}$ = 3.8 Hz); ^{13}C nmr (deuteriochloroform): δ 166.1, 165.4 and 164.8 (C=O), 158.4 (C-6, $^1J_{CH}$ = 206.7 Hz), 155.9 (C-7a, $^3J_{C\text{-}7a,H\text{-}6}$ = 9.9 Hz, $^3J_{C\text{-}7a,H\text{-}1'}$ = 3.5 Hz), 139.9 (C-3), 133.4, 133.4, 133.3, 129.7, 129.7, 129.7, 129.4, 128.9, 128.5, 128.4, 128.3 (aromatic), 87.2 (C-1′, $^1J_{C,H}$ = 168.0 Hz), 81.5 (C-4′), 71.4 (C-2′), 71.0 (C-3′), 63.9 (C-5′).

Anal. Calcd. for $C_{29}H_{24}N_6O_7 \cdot 0.2C_6H_{12}$: C, 61.96; H, 4.55; N, 14.36. Found: C, 61.73; H, 4.55; N, 14.36.

3-Imino-2*H*-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**6**).

The above cited compound with *Rf* 0.45, yield 9.46 g (30%), mp 125-127°; ms: (FAB) m/z 569 (M + 1); uv: λ max 234 (39,400), shoulder at 280 (3200) at *p*H 1; 236 (33,000), shoulder at 276 (17,300) at *p*H 7; 225 (29,300), 276 (8600) at *p*H 13; ir (potassium bromide): 3500-2500 (broad NH, NH, CH), 1727 (C=O), 1666 (C=N), 1615, 1602 (aromatic), 1585, 1475, 1450, 1315, 1267, 1200, 1175, 1116, 1093, 1070, 1024 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 8.1-7.8 and 7.6-7.1 (m, 18, NH, NH, H-6, and benzoyl), 6.35 (d, 1, H-1′, $J_{1',2'}$ = 1.7 Hz), 6.25 (dd, 1, H-2′, $J_{2',3'}$ = 5.2 Hz), 6.19 (dd, 1, H-3′, $J_{3',4'}$ = 6.8 Hz), 4.90 (m, 1, H-4′, $J_{4',5'a}$ = 5.2 Hz, $J_{4',5'b}$ = 3.6 Hz), 4.79 (d, 1, H-5′b), 4.71 (dd, 1, H-5′a, $J_{5'a,5'b}$ = 12.1 Hz); ^{13}C nmr (deuteriochloroform): δ 166.3, 165.2, 165.1 (C=O), 160.3 (C-6, $^1J_{C,H}$ = 202.6 Hz), 160.1 (C-7a, $^3J_{C\text{-}7a,H\text{-}6}$ = 4.4 Hz), 138.3 (C-3), 133.7, 133.4, 133.0, 129.7, 129.64, 129.6, 129.2, 128.6, 128.43, 128.4, 128.39 (aromatic carbons), 88.8 (C-1′, $^1J_{C\text{-}1',H}$ = 167.8 Hz), 780.4 (C-4′), 75.3 (C-2′), 71.7 (C-3′), 63.9 (C-5′).

Anal. Calcd. for $C_{29}H_{24}N_6O_7 \cdot H_2O$: C, 59.34; H, 4.47; N, 14.33. Found: C, 59.12; H, 4.42; N, 14.50.

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)amino-5*H*-[1,2,4]triazolo-[4,3-*b*][1,2,4]triazole (**7**).

The above cited compound with *Rf* 0.81, yield 7.5 g (24%), mp

189-192°; ms: (FAB) m/z 569 (M + 1); uv: λ max 233 (19,500), shoulder at 278 (1350) at *p*H 1; 236 (18,800), shoulder at 280 (5400) at *p*H 7; 226 (14,000) at *p*H 13; ir (potassium bromide): 3200, 3070, 3010, 2975-2900 (NH and CH), 1734, 1725, 1716 (C=O), 1630, 1587 (broad, aromatic), 1465, 1450, 1315, 1284, 1267, 1180, 1155, 1126, 1114, 1093 cm^{-1}. Both the ^1H nmr and ^{13}C nmr spectra indicated a 1:3 mixture of α,β-isomers; ^1H nmr (DMSO-d_6): δ 12.52 (br s, 1, H-5), 8.64 (d, NHβ, $J_{NH,1'}$ = 9.6 Hz), 8.1-7.8, 7.8-7.3, and 8.02 (s, H-6β) (s, H-6α), 7.96 (m, 16, H-6 and benzoyl), 6.35 (dd, H-1'α, $J_{1',NH}$ = 10.4 Hz, $J_{1',2'}$ = 4.6 Hz), 5.91 (dd, H-1'β, $J_{1',2'}$ = 5.2 Hz), 5.88 (t, H-3'β, $J_{2',3'}$ = 5.8 Hz), 5.79 (t, H-2'β), 5.91-5.78 (m, H-2'α, H-3'α), 4.76 (m, H-4'α), 4.65 (m, H-4'β, H-5' of α and β anomers); ^{13}C nmr (DMSO-d_6): δ 165.4, 165.4, 164.7, 164.6 (C=O), 158.5 (C-6β, $^1J_{C-6,H}$ = 205.4 Hz), 158.2 (C-6α, $^1J_{C-6\alpha,H}$ = 205.1 Hz), 156.7 (C-8α, C-8β), 139.5 (C-3α), 139.1 (C-3β), 133.7, 133.6, 133.5, 133.4, 129.7, 129.3, 129.2, 129.1, 128.8, 128.7, 128.6, 128.4 (aromatic carbons), 85.6 (C-1'β), 83.3 (C-1'α), 77.7 (C-4'β), 76.5 (C-4'α), 73.5 (C-3'β), 71.7 (C-3'α), 64.1 (C-5'α and β).

Anal. Calcd. for $C_{29}H_{24}N_6O_7$: C, 61.26; H, 4.26; N, 14.79. Found: C, 61.42; H, 4.56; N, 14.78.

3-Amino-1-β-D-ribofuranosyl[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**3**).

Tribenzoyl derivative **4** (2.0 g, 3.5 mmoles) was stirred with sodium methoxide (0.2 g, 3.7 mmoles) in methanol (100 ml) under dry conditions for 5 hours at room temperature. The *p*H of the solution was brought to 7 by the careful addition of 6 *N* hydrochloric acid. The reaction mixture was concentrated under reduced pressure and charged on a silica gel column. Methyl benzoate was removed by eluting with chloroform. Elution with chloroform-methanol (4:1) yielded the pure product, 0.86 g (96%), mp 141-143° (lit [3] mp 144-145°); ms: (FAB) m/z 257 (M + 1); uv: λ max 240 (6400) at *p*H 1; 240 (7000) at *p*H 7; 242 (6800) at *p*H 13; ir (potassium bromide): 3600-2600 (broad, OH, NH_2, CH), 1657, 1626 (C=N), 1580, 1505, 1455, 1445, 1410, 1325, 1285, 1265, 1215, 1185, 1140, 1105, 1052 cm^{-1}; ^1H nmr (DMSO-d_6): δ 8.02 (s, 1, H-6), 6.85 (s, 2, NH_2), 5.48 (d, 1, H-1', $J_{1',2'}$ = 5.3 Hz), 5.43 (d, 1, 2'-OH, $J_{2',2'-OH}$ = 6 Hz), 5.15 (d, 1, 3'-OH, $J_{3',3'-OH}$ = 5.2 Hz), 4.82 (t, 1, 5'-OH, $J_{5',5'-OH}$ = 5.9 Hz), 4.57 (m, 1, H-2'), 4.09 (m, 1, H-3'), 3.85 (m, 1, H-4'), 3.58-3.33 (m, 2, H-5'); ^{13}C nmr (DMSO-d_6): δ 157.8 (C-6, $^1J_{CH}$ = 206.7 Hz), 155.2 (C-7a, $^3J_{C-7a,H-6}$ = 3.7 Hz, $^3J_{C-7a,H-1'}$ = 9.6 Hz), 141.2 (C-3), 90.5 (C-1'), 84.9 (C-4'), 72.0 (C-2'), 70.3 (C-3'), 62.0 (C-5').

Anal. Calcd. for $C_8H_{12}N_6O_4$: C, 37.50; H, 4.72; N, 32.81. Found: C, 37.24; H, 4.93; N, 32.47.

3-Amino-1-α-D-ribofuranosyl[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**8**).

This sample was prepared from **5** by the method used for compound **3**; ^1H nmr (DMSO-d_6): δ 8.02 (s, 1, H-6), 6.98 (br s, 2, NH_2), 5.83 (d, 1, H-1', $J_{1',2'}$ = 6.6 Hz), 5.60 (d, 1, 3'-OH, $J_{3',3'-OH}$ = 11.5 Hz), 5.24 (d, 1, 2'-OH, $J_{2',2'-OH}$ = 5.8 Hz), 4.83 (t, 1, 5'-OH, $J_{5',5'-OH}$ = 5.7 Hz), 4.42 (m, 1, H-2', $J_{2',3'}$ = 6.8 Hz), 4.07 (m, 1, H-4'), 3.93 (ddd, 1, H-3', $J_{3',4'}$ = 2.9 Hz), 3.44 (m, 2, H-5'); ^{13}C nmr (DMSO-d_6): δ 157.9 (C-6, $^1J_{CH}$ = 206.9 Hz), 155.7 (C-7a, $^3J_{C-7a,H-6}$ = 9.7 Hz, $^3J_{C-7a,H-1'}$ = 3.1 Hz), 140.7 (C-3), 87.5 (C-4'), 87.3 (C-1', $^1J_{CH}$ = 166.1 Hz), 70.1 (C-3'), 70.0 (C-2'), 61.6 (C-5').

3-Imino-2*H*-2-β-D-ribofuranosyl[1,2,4]triazolo[4,3-*b*][1,2,4]triazole (**9**).

Tribenzoyl ribofuranoside **6** (8.6 g) was deprotected by stirring with sodium methoxide (0.85 g) in methanol (200 ml) under dry conditions for 3 hours at room temperature. The *p*H of the solution was brought to 7 by the careful addition of 6 *N* hydrochloric acid. The reaction mixture was concentrated under reduced pressure and charged onto a silica gel column. Methyl benzoate was first removed by eluting with chloroform. Elution with chloroform-methanol (4:1) yielded the pure product, 2.81 g (73%), mp 160-162° dec; ms: (FAB) m/z 257 (M + 1); uv: λ max 248 (7200) at *p*H 1; 264 (8500) at *p*H 7; 277 (9100) at *p*H 13; ir (potassium bromide): 3650-2600 (broad, OH, NH, CH), 1678, 1613 (C=N, C=NH), 1480, 1445, 1415, 1350, 1208, 1120, 1045 cm^{-1}; ^1H nmr (DMSO-d_6): δ 8.25 (s, 1, NH), 7.88 (s, 1, H-6), 5.83 (d, 1, H-1', $J_{1',2'}$ = 3.9 Hz), 5.45 (d, 1, 2'-OH, $J_{2',2'-OH}$ = 5.3 Hz), 5.14 (d, 3'-OH, $J_{3',3'-OH}$ = 5.3 Hz), 4.92 (t, 1, 5'-OH, $J_{5',5'-OH}$ = 5.4 Hz), 4.53 (m, 1, H-2'), 4.19 (m, 1, H-3'), 3.92 (m, 1, H-4'), 3.64-3.42 (m, 2, H-5'); ^{13}C nmr (DMSO-d_6): δ 160.1 (C-6, $^1J_{CH}$ = 201.0 Hz), 159.9 (C-7a, $^3J_{C-7a,H-6}$ = 10.6 Hz, $^3J_{C-3,H-1'}$ = 1.7 Hz, $^4J_{C-3,H-6}$ = 1.7 Hz), 138.3 (C-3), 89.8 (C-1', $^1J_{CH}$ = 164.8 Hz), 85.3 (C-4'), 73.2 (C-2'), 70.6 (C-3'), 62.1 (C-5').

Anal. Calcd. for $C_8H_{12}N_6O_4 \cdot CH_3OH \cdot 0.2H_2O$: C, 37.00; H, 5.09; N, 31.20. Found: C, 37.01; H, 4.99; N, 31.24.

In Vitro Antiviral and Cytotoxicity Assays.

Compounds were evaluated for antiviral efficacy against the following viruses (viral strain): (a) Punta Toro, PT (Adames); (b) Venezuelan equine encephalomyelitis, VEE (Trinidad donkey); (c) Yellow fever, YF (Ashibi); Japanese encephalitis, JE (Nakayama); Pichinde, PIC; Vesicular stomatitis, VSV; Sandfly, SF (Sicilian); Vaccinia, VV (Lederle vaccine); and Adenotype 2, AD2. The *in vitro* antiviral and cytotoxic effects of a test compound were measured by observing inhibition of viral cytopathic effect [4-7] using an MTT assay. All assays were carried out in Vero cells except for the use of Hep2 and L929 cells for AD2 and VSV, respectively.

Basic measurements and definitions used throughout these studies include (a) 50% cellular toxic concentration, TC_{50}, the drug concentration (μg/ml) that reduces the cell number and their metabolic activity by 50% as compared to the viability of uninfected control cells in duplicate test wells in the MTT assay; (b) 50% viral inhibitory concentration, IC_{50}, the drug concentration (μg/ml) at which 50% reduction of viral cytopathic effect (CPE) is observed in triplicate test wells; the therapeutic (or antiviral) index, TI_{50}, a value proportional to the overall *in vitro* activity calculated as a ratio of TC_{50}/IC_{50}. It is a single drug concentration measurement of the relative anticellular and antiviral effectiveness of a compound during the same test and time period. All *in vitro* MTT assay results represent an average of two to six individual test results.

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