5 h at 20 °C. The solution was reduced to one-third of the volume, filtered to remove *m*-chlorobenzoic acid, and evaporated and the residue was purified by TLC (dichloromethane/5% methanol) to yield 21 mg (62%) of epoxide **68**: mp 50–51 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.52 (t, *J* = 7.5 Hz, 3 H, 9-H), 1.07–1.39 (m, 8 H, 3-, 4-, 5-, 7-H), 2.30 (m, 1 H, 1-H), 2.44 (dt, *J* = 3.5, 10, 10 Hz, 1 H, 7-H), 2.55 (dt, *J* = 4.5, 10, 10 Hz, 1 H, 6-H), 2.60 (m, 1 H, 1-H), 2.67 (m, 1 H, 2-H), 4.66 (br s, 2 H, ArOH), 6.78 (2 d, *J* = 8.5 Hz, 4 H, ArH), 7.01 (2 d, *J* = 8.5 Hz, 4 H, ArH); MS (250 °C) *m/z* 326 (M^+), 297, 241, 227, 212, 191; exact mass calcd for $C_{21}H_{26}O_3$ 326.1882, found 326.1875.

***erythro*-5,6-Bis(4-hydroxyphenyl)-1-octyl 2-Hydroxyethyl Ether (69).** A solution of 244 mg (0.45 mmol) of benzyl ether **60** in 100 mL of cyclohexane was hydrogenated for 4 h in the presence of ca. 50 mg of Pd/C. The catalyst was filtered off, the filtrate was evaporated, and the residue was crystallized from diethyl ether to yield 162 mg (100%) of bisphenol **69**: mp 114–115 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 0.51 (t, *J* = 7.5 Hz, 3 H, 8-H), 0.97 (m, 2 H, 3-H), 1.21–1.40 (m, 6 H, 2-, 4-, 7-H), 2.46 (dt, *J* = 3.5, 10, 10 Hz, 1 H, 6-H), 2.61 (m, 1 H, 5-H), 3.61 (dt, 2 H, 1-H), 3.32 (m, 2 H, $ROCH_2CH_2OH$), 3.51 (m, 2 H, CH_2OH), 6.81 (2 d, *J* = 8.5 Hz, 4 H, ArH), 7.05 (2 d, *J* = 8.5 Hz, 4 H, ArH), 8.08 (s, 2 H, ArOH); MS (150 °C) *m/z* 358 (35, M^+), 241 (16), 223 (61), 161 (100), 147 (8), 135 (87). Anal. $C_{22}H_{30}O_4$) C, H.

***erythro*-5,6-Bis(4-hydroxyphenyl)octyl Diethylene Glycol Monoether (70).** Benzyl ether **61** (254 mg, 0.44 mmol) was hydrogenated as described for **69** to afford 158 mg (90%) of oily bisphenol **70**: ^{1}H NMR (300 MHz, acetone-d_6) δ 0.51 (t, 3 H, 8-H), 0.97 (m, 2 H, 3-H), 1.21–1.42 (m, 6 H, 2-, 4-, 7-H), 2.47 (dt, *J* = 3.5, 10, 10 Hz, 1 H, 6-H), 2.60 (dt, *J* = 5.0, 10, 10 Hz, 1 H, 5-H), 3.18 (dt, 2 H, 1-H), 3.38 (m, 2 H, OCH_2R), 3.47 (m, 4 H, $OCH_2CH_2OCH_2CH_2OH$), 3.58 (m, 2 H, CH_2OH), 6.79 (2 d, *J* = 8.5 Hz, 4 H, ArH), 7.06 (2 d, *J* = 8.5 Hz, 4 H, ArH, 8.07 (s, 2 H, ArOH); MS (190 °C) *m/z* 402 (5, M^+), 345 (8), 267 (17), 207 (8), 161 (100); exact mass calcd for $C_{24}H_{34}O_5$ 402.2406, found 402.2385.

***erythro*-5,6-Bis(4-hydroxyphenyl)-1-triethylene Glycol Monooctyl Ether (71).** Benzyl ether **62** (268 mg, 0.43 mmol) was hydrogenated as described for **69** to yield 173 mg (91%) of oily bisphenol **71**: ^{1}H NMR (300 MHz, acetone-d_6) δ 0.51 (t, *J* = 7.5 Hz, 3 H, 8-H), 0.98 (m, 2 H, 3-H), 1.20–1.42 (m, 6 H, 2-, 4-, 7-H), 2.47 (dt, *J* = 3.5, 10, 10 Hz, 1 H, 6-H), 2.60 (dt, *J* = 5, 10, 10 Hz, 1 H, 5-H), 3.18 (dt, 2 H, 1-H), 3.36–3.63 (m, 12 H, $R(OCH_2CH_2)_3OH$), 6.81 (2 d, *J* = 8.5 Hz, 4 H, ArH), 7.07 (2 d, *J* = 8.5 Hz, 4 H, ArH), 8.08 (s, 2 H, ArOH); MS (270 °C) *m/z* 447 (MH^+), 329, 311, 161, 151, 135; exact mass calcd for $C_{26}H_{39}O_6$ 447.27464, found 447.27411.

Registry No. (±)-*erythro*-**1**, 120578-46-3; (±)-*threo*-**1**, 120579-13-7; (±)-**2**, 120578-47-4; (±)-**3**, 120608-65-3; (±)-**4**, 120578-48-5; (±)-**5**, 120578-49-6; (±)-**6**, 120608-66-4; (±)-**8**, 120578-50-9; (±)-**9**, 120578-51-0; (±)-**10**, 120578-52-1; (±)-*threo*-**10**, 120579-15-9; (±)-**11**, 120578-53-2; (±)-**12**, 120578-54-3; (±)-**13**, 120578-55-4; (±)-**14**, 120578-56-5; (±)-**15**, 120578-57-6; (±)-**16**, 120578-58-7; (±)-**17**, 120578-59-8; (±)-**18**, 120578-60-1; (±)-**19**, 120578-61-2; (±)-**20**, 120578-62-3; (±)-**21**, 120578-63-4; (±)-**22**, 120578-64-5; (±)-**23**, 120578-65-6; (±)-**24**, 120578-66-7; (±)-**25**, 120578-67-8; (±)-**26**, 120578-68-9; (±)-**27**, 120578-69-0; (±)-**28**, 120578-70-3; (±)-**29**, 120578-71-4; (±)-**30**, 120578-72-5; (±)-**31**, 120578-73-6; (±)-**32**, 120578-74-7; (±)-**33**, 120578-75-8; (±)-**34**, 120578-76-9; (±)-**35**, 120578-77-0; (±)-**36**, 120579-12-6; (±)-**36**·HCl, 120578-78-1; (±)-**37**, 120579-11-5; (±)-**37**·HCl, 120578-79-2; (±)-**38**, 120578-80-5; **40**, 60784-40-9; (±)-**41**, 120578-81-6; **42**, 127-88-8; (±)-**43**, 120578-82-7; (±)-**44**, 120578-83-8; (±)-**45**, 120578-84-9; (±)-**46**, 120578-85-0; (±)-**47**, 120578-86-1; (±)-**48**, 120578-87-2; (±)-**49**, 120578-88-3; (±)-**50**, 120578-89-4; (±)-**51**, 120578-90-7; (±)-**52**, 120578-91-8; (±)-**53**, 120578-92-9; (±)-**54**, 120578-93-0; (±)-**55**, 120578-94-1; (±)-**56**, 120578-95-2; (±)-**57**, 120578-96-3; (±)-**58**, 120578-97-4; (±)-**59**, 120578-98-5; (±)-**60**, 120578-99-6; (±)-**61**, 120579-00-2; (±)-**62**, 120579-01-3; (±)-**63**, 120579-02-4; (±)-**64**, 120579-03-5; (±)-**65**, 120579-04-6; (±)-**66**, 120579-05-7; (±)-**67**, 120579-06-8; **68**, 120579-07-9; (±)-**69**, 120579-08-0; (±)-**70**, 120579-09-1; (±)-**71**, 120579-10-4; 4-$MeOC_6H_4C(Et)$=$C(n$-$C_4H_9)C_6H_4OMe_4$, 120579-14-8; $H_2N(CH_2)_2CO_2Et$·HCl, 4244-84-2; $(C_6H_5)_3P^+Me\ Br^-$, 1779-49-3; diethanolamine, 111-42-2.

Synthesis and Biological Evaluation of Certain 3-β-D-Ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazines Related to Formycin Prepared via Ring Closure of Pyridazine Precursors

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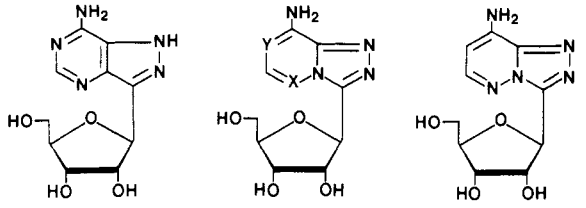
All three amino-substituted 3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazines (**5**, **19**, and **20**) structurally related to formycin A were prepared and tested for their antitumor and antiviral activity in cell culture. Dehydrative coupling of 4-amino-5-chloro-3-hydrazinopyridazine (**7**) with 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonic acid (**6**) in the presence of DCC and subsequent thermal ring closure of the reaction product (**8**) provided 8-amino-7-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (**9**). Dehalogenation of **9**, followed by debenzoylation, gave the formycin congener 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**5**). Similar condensation of 5-amino-4-chloro-3-hydrazinopyridazine (**13**) with **6** and dehalogenation of the cyclized product (**16**), followed by debenzoylation, gave the isomeric 7-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**19**). DCC-mediated coupling of **6** with 6-chloro-3-hydrazinopyridazine (**12**), followed by ammonolysis of the cyclized product (**21**) with liquid NH_3, provided a convenient route to 6-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**20**). The structural assignment of **5** was made by single-crystal X-ray diffraction analysis. Compounds **5**, **19**, **20**, and certain deprotected nucleoside intermediates were evaluated against L1210, WI-L2, and CCRF-CEM tumor cell lines, as well as against DNA and RNA viruses in culture. These compounds did not exhibit any significant antitumor or antiviral activity in vitro.

Since the isolation[1,2] and structural elucidation[3,4] of the naturally occurring C-nucleoside antibiotic formycin A (7-amino-3-β-D-ribofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine, **1**), several reports have appeared in the literature describing its diverse biological properties.[5-7] Formycin

(1) Hori, M.; Ito, E.; Takita, T.; Umezawa, H. *J. Antibiot., Ser. A* **1964**, *17*, 96.
(2) Koyama, G.; Umezawa, H. *J. Antibiot., Ser. A* **1965**, *18*, 175.
(3) Robins, R. K.; Townsend, L. B.; Cassidy, F. C.; Gerster, J. F.; Lewis, A. F.; Miller, R. L. *J. Heterocycl. Chem.* **1966**, *3*, 110.
(4) Koyama, G.; Maeda, K.; Umezawa, H.; Iitake, Y. *Tetrahedron Lett.* **1966**, 597.
(5) Suhadolnik, R. J. *Nucleoside Antibiotics*; Wiley-Interscience: New York 1970, pp 354–389.

0022-2623/89/1832-1547$01.50/0



1
Formycin A

2, X = Y = CH
3, X = CH; Y = N
4, X = Y = N

5

A, a cytotoxic isostere of adenosine, is readily deaminated by the catabolic enzyme adenosine deaminase (ADA) to the less-active inosine analogue formycin B (3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(1*H*,6*H*)-one).[8] However, interest in the potential of formycin A as an antitumor agent has been rekindled since, in combination with an ADA inhibitor, formycin A is effective in prolonging the life of mice infected with L1210 leukemia.[9] Moderate antiviral activity with formycin A has also been observed in cell culture.[10-13] By virtue of its C-glycosidic linkage, formycin A is stable to the action of purine nucleoside phosphorylase[14,15] and this resistance to glycosidic cleavage provides a distinct advantage of this group of nucleosides in clinical trials.

In an effort to obtain metabolically more stable C-nucleoside analogues, we[16] and others[17-19] have recently reported the synthesis of several bridgehead aza/deaza congeners (**2**–**4**) of formycin A. Although the deaza analogue 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*a*]-pyridine[19] (**2**) was reported to be less effective in its cytotoxic properties than 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*a*]pyrazine[18] (**3**), the aza analogue 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine[16] (**4**) exhibited inhibitory effects against L1210, WI-L2, and CCRF-CEM cell lines with ID_{50} values ranging from 5.0 to 7.3 μM. Compound **3** was also found to be a very poor substrate for calf intestinal mucosa type I adenosine deaminase (EC 3.5.4.4).[18] In continuation of this work, we have now prepared 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**5**) and its 6- and 7-amino isomers as well (**20** and **19**, respectively).

Results and Discussion

Chemistry. As reasoned in our previous publication in this series,[16] the successful synthesis of the bridgehead-nitrogen C-nucleosides could be approached by the ring annulation of the glycosyl derivative of an appropriately functionalized hydrazino heterocycle. The synthesis of such a substituted 3-hydrazinopyridazine, 4-amino-5-chloro-3-hydrazinopyridazine (**7**), was accomplished as reported.[20] A mild dehydrative coupling of **7** with 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonic acid (**6**)[21] in the presence of 1,3-dicyclohexylcarbodiimide (DCC) in dichloromethane at room temperature gave the intermediate **8** (Scheme I), which on further heating in ethylene glycol at 150 °C ring closed to furnish 8-amino-7-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]-pyridazine (**9**) in 66% yield. Debenzoylation of **9** with $MeOH/NH_3$ at room temperature afforded 8-amino-7-chloro-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**10**) in 70% yield. The fact that the chloro group is still intact and not nucleophilically displaced by an amino group was established by the elemental analysis. Moreover, catalytic (Pd/C) hydrogenation of **9** gave a compound characterized as 8-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (**11**). The appearance of two sharp doublets (for C6 and C7 protons), rather than a singlet (as in the case of **9**), in the ^1H NMR spectrum of **11** clearly established that the halogen at the C-7 position had been displaced by hydrogen. Treatment of **11** with $MeOH/NH_3$ at room temperature for 16 h readily gave crystalline 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**5**) in 75% yield. The structure of **5** was assigned by single-crystal X-ray diffraction analysis.

For the synthesis of the isomeric 7-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**19**), we again elected to employ 5-amino-4-chloro-3-hydrazinopyridazine (**13**) as our starting material. Compound **13** was prepared in a multistep synthesis, as reported by Kuraishi and Castle,[20] and condensed with **6** by using essentially the identical reaction conditions as were used for the preparation of **9**, to yield 7-amino-8-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (**16**) via the intermediate **15**. Treatment of **16** with $MeOH/NH_3$ at room temperature furnished 7-amino-8-chloro-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**17**) in good yield. Presence of the chloro group in **17** was established by elemental analysis. Catalytic hydrogenation of **16** with Pd/C in the presence of K_2CO_3 furnished a 52% yield of 7-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (**18**), which on subsequent debenzoylation with $MeOH/NH_3$ afforded the isomeric C-nucleoside **19** in 76% yield.

Although the synthesis of the third target C-nucleoside 6-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**20**) was reported recently by Legraverend and co-workers[22] and involved the condensation of 6-chloro-3-hydrazinopyridazine[23] (**12**) with benzyl (5-*O*-benzoyl-D-ribofuranosyl)thioformimidate hydrochloride,[24] the yield

(6) Townsend, L. B. In *Handbook of Biochemistry and Molecular Biology*, Nucleic Acids, 3rd ed.; Fasman, D. G., Ed.; CRC Press: Cleveland, OH, 1975; Vol. 1, pp 271–401.
(7) Suhadolnik, R. J. *Nucleosides as Biological Probes*; Wiley-Interscience: New York, 1979, pp 169–183.
(8) Ishizuka, M.; Sawa, T.; Koyama, G.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 1.
(9) Hidaka, T.; Katayama, K.; Yamashita, K.; Yamashita, T.; Watanabe, K.; Shimasaki, M.; Ohno, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 303.
(10) Ishida, N.; Homma, M.; Kumagai, Y.; Schimizu, Y.; Matsumoto, S.; Isawa, A. *J. Antibiot.* **1967**, *20A*, 49.
(11) Giziewicz, J.; De Clercq, E.; Luczak, M.; Shugar, D. *Biochem. Pharmacol.* **1975**, *24*, 1813.
(12) Schneller, S. W.; Thompson, R. D.; Cory, J. G.; Olsson, R. A.; De Clercq, E.; Kim, I-K.; Chiang, P. K. *J. Med. Chem.* **1984**, *27*, 924.
(13) Sarih, L.; Agoutin, B.; Lecoq, O.; Weill, D.; Jullien, P.; Heyman, T. *Virology* **1985**, *145*, 171.
(14) Sheen, M. R.; Kim, B. K.; Parks, R. E., Jr. *Mol. Pharmacol.* **1968**, *4*, 293.
(15) Parks, R. E., Jr.; Stoeckler, J. D.; Cambor, C.; Savarese, T. M.; Crabtree, G. W.; Chu, S-H. In *Molecular Actions and Targets for Cancer Chemotherapeutic Agents*; Sartorelli, A., Lazo, J. S., Bertino, J. R., Eds.; Academic Press: New York, 1981; pp 229–252.
(16) Ramasamy, K.; Ugarkar, B. G.; McKernan, P. A.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.* **1986**, *29*, 2231.
(17) Huynh-Dinh, T.; Sarfati, R. S.; Gouyette, C.; Igolen, J. *J. Org. Chem.* **1979**, *44*, 1028.
(18) Same as ref 12.
(19) Kobe, J.; Brdar, B.; Soric, J. *Nucleosides Nucleotides* **1986**, *5*, 135.
(20) Kuraishi, T.; Castle, R. N. *J. Heterocycl. Chem.* **1964**, *1*, 42.
(21) Bobek, M.; Farkas, J. *Collect. Czech. Chem. Commun.* **1969**, *34*, 247.
(22) Legraverend, M.; Bisagni, E.; Lhoste, J-M. *J. Heterocycl. Chem.* **1981**, *18*, 893.
(23) Schönbeck, R.; Kloimstein, E. K. *Monatsh. Chem.* **1968**, *99*, 15.

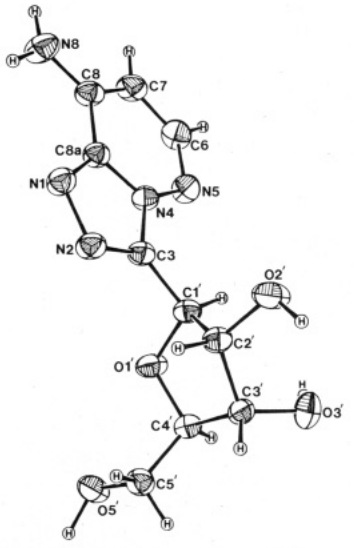


Figure 1. ORTEPII drawing of compound **5**.

of the desired **20**, after separation of the α- and β-anomers and further transformation of the functional groups, was only 5.6%. Therefore, in an effort to obtain **20** in better yield, we treated compound **12** with **6** in anhydrous dichloromethane in the presence of DCC at room temperature. A 42% yield of the desired, anomerically pure 6-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (**21**) was obtained after ring closure of the intermediate **14** in ethylene glycol at 150 °C. When compound **21** was heated at 80 °C with liquid NH_3 in a sealed steel reaction vessel, an 85% yield of **20** was obtained after silica gel column chromatography and crystallization from a mixture of EtOH/EtOAc. Compound **20** was found to be identical with the nucleoside prepared by the reported procedure.[22] Treatment of **21** with either sodium methoxide in methanol or methanolic ammonia furnished a good yield of 6-methoxy-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (**22**), which was isolated as anhydrous crystals of mp 187–188 °C. A melting point of 96 °C was reported by Legraverend and co-workers[21] for the monohydrated form of **22**. It is of particular interest that, under the methanolysis conditions, solvolytic displacement of the C-6 halogen of **21** occurred to give the 6-methoxy derivative **22**. Treatment of **22** with 1 N NaOH at 80 °C for 5 h furnished 3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazin-6(5*H*)-one (**23**) in 46% yield.

Single-Crystal X-ray Diffraction Analysis of Compound 5. Colorless, transparent crystals of **5** grew from aqueous solution as long, flat prisms. The compound crystallizes in the orthorhombic space group $P2_12_12_1$ [cell parameters: a = 6.7976 (5) Å, b = 7.4170 (8) Å, and c = 22.1104 (17) Å] with one molecule per asymmetric unit. The structure was solved by direct methods [SHELXS86][25] and refined by full-matrix least-squares [SHELX76].[26] The final R was 0.0273 for 2217 observed reflections ($F \geq 4\sigma_F$).

The molecular conformation of **5** is illustrated [ORTEPII][27] in Figure 1. The bonding geometry of the triazolopyridazine base is similar to that of the unsubstituted heterocycle[28] with the exception of C8 bonds distorted by the conjugation of the attached amino group. Comparison of the structure of **5** with formycin A[29] and its 5′-monophosphate (FMP)[30] reveals that the glycosidic bond of 1.491 (2) Å in the former is slightly shorter than that found in the latter compounds. The glycosidic torsion angle (χ = O4′–C1′–C3–N4) is −112.56 (13)°, corresponding to an anti orientation whereas formycin A (χ = −73.83°) is high anti and FMP is syn (χ = −6.43°). The ribose moiety has a pseudorotation angle (P) of 141.2° and an amplitude of pucker (τ_m) of 42.4°.[31] The conformation is C1′ exo–C2′ endo ($_1T^2$). These characteristics are similar to those of formycin (P = 148.3°; C2′ endo) but radically different from FMP (P = 20.3°; C3′ endo) and AMP (P = 12.2°, C3, endo).[32] The side chain is gauche-trans which is also consistent with formycin A. All hydroxyl groups are hydrogen-bond donors; the amino group donates only one of its protons. N1, N2, O2′, and O5′ act as hydrogen-bond acceptors. The D···A distances are N8···O2′, 2.871 (2) Å; O2′···O5′, 2.714 (2) Å; O3′···N1, 2.929 (2) Å; and O5′···N2, 2.863 (1) Å. The range of D–H···A angles is 159–168°. Details of the structure determination and results will be published elsewhere.[33]

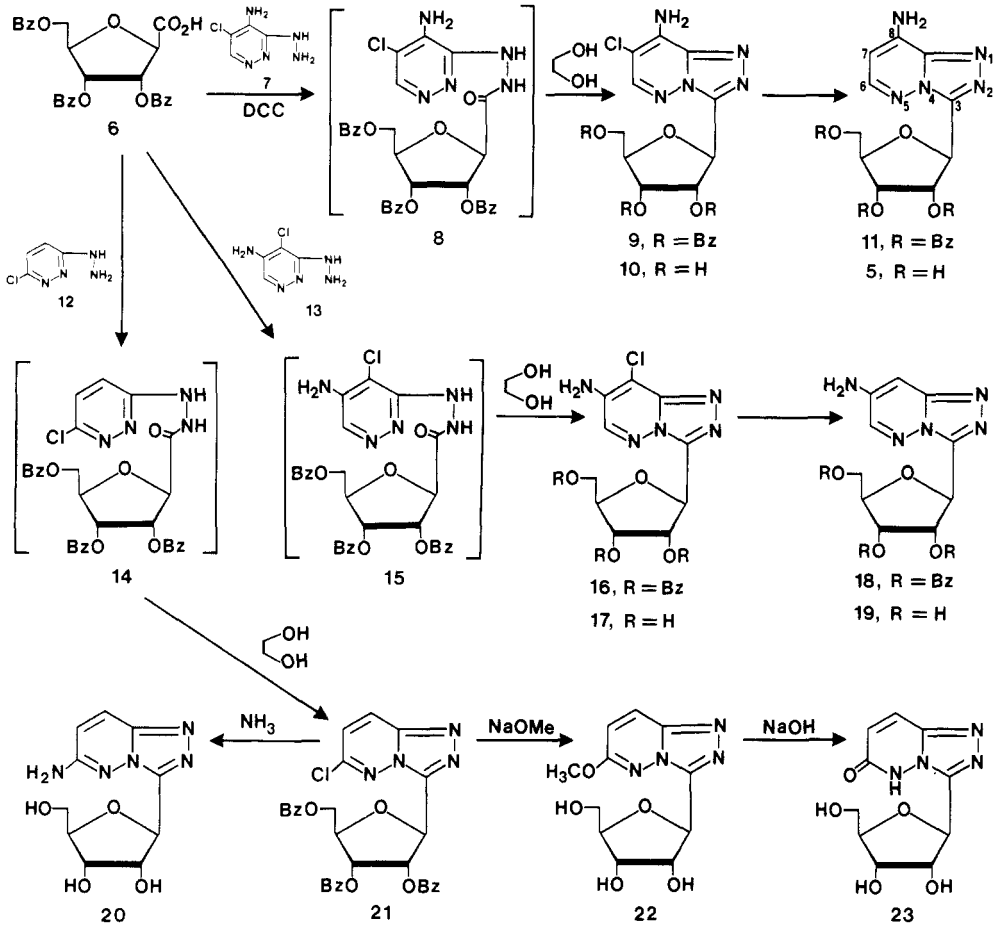
Biological Evaluations. Compounds **5**, **10**, **17**, **19**, **20**, **22**, and **23** were evaluated in vitro for their ability to inhibit the growth of L1210 murine lymphocytic leukemia, WI-L2 human B-lymphoblastic leukemia, and CCRF-CEM human T-lymphoblastic leukemia cell lines using the methodology as reported from our laboratory.[16] The highest concentration of compounds tested was 100 μM. These compounds were also evaluated in vitro for their antiviral effects against several unrelated DNA and RNA viruses, which include herpes simplex virus type 2 (MS), adenovirus type 2 (Adenoid 6), parainfluenza virus type 3 (C243), rhinovirus type 1-A (2060), influenza A virus (Chile), semliki forest virus (original), and visna virus (1513). Evaluation of the virus-induced cytopathic effect and calculation of the virus rating were performed by the method described by Sidwell and Huffman,[34] except that concentrations of compounds tested were from 1 to 1,000 μM instead of from 1 to 1,000 μg/mL. None of the compounds tested exhibited any significant antitumor or antiviral effects in these cell lines.

Experimental Section

General Procedures. Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (TLC) was conducted on plates of silica gel 60 F-254 (EM Reagents). Silica gel (E. Merck; 230–400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components in TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30 °C. Infrared (IR) spectra were recorded with a Perkin-Elmer 1420 spectrophotometer and ultraviolet (UV) spectra were recorded on a Beckman DU-50 spectrophotometer. Nuclear

(24) Huynh-Dinh, T.; Kolb, A.; Gouyette, C.; Igolen *J. Heterocycl. Chem.* **1975**, *12*, 111.
(25) Sheldrick, G. M. SHELXS86; Program for crystal structure determination; University of Göttingen: Gottingen, Federal Republic of Germany, 1986.
(26) Sheldrick, G. M. SHELX76; Program for crystal structure determination; University of Cambridge: Cambridge, England, 1976.
(27) Johnson, C. K. ORTEPII; A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations (3rd Revision); Oak Ridge National Laboratory ORNL-5138, March, 1976.
(28) Golic, L.; Leban, I.; Stanovnik, B.; Tisler, M. *Acta Crystallogr.* **1978**, *B34*, 1136.
(29) Prusiner, P.; Brennan, T.; Sundaralingam, M. *Biochemistry* **1973**, *12*, 1196.
(30) Giranda, V. L.; Berman, H. M.; Schramm, V. L. *Biochemistry* **1988**, *27*, 5813.
(31) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.
(32) Kraut, J.; Jensen, L. H. *Acta Crystallogr.* **1963**, *16*, 79.
(33) Larson, S. B.; Kang, Y.; Revankar, G. R.; Robins, R. K. *Acta Crystallogr., Sect. C.* In press.
(34) Sidwell, R. W.; Huffman, J. H. *Appl. Microbiol.* **1971**, *22*, 797.

Scheme I



magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The signals are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The presence of solvent as indicated by elemental analysis was verified by ^{1}H NMR spectroscopy.

8-Amino-7-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (9). To a stirred suspension of 4-amino-5-chloro-3-hydrazinopyridazine[20] (7, 2.0 g, 12.5 mmol) and 3,4,6-tri-O-benzoyl-2,5-anhydro-D-allonic acid[21] (6, 5.88 g, 12 mmol) in dry dichloromethane (400 mL) was added 1,3-dicyclohexylcarbodiimide (DCC, 2.47 g, 12 mmol). The reaction mixture was stirred at room temperature for 18 h under anhydrous conditions and then filtered. The filtrate was evaporated to dryness and the residue was purified on a flash silica gel column (2.5 × 30 cm) using EtOAc as the eluent. The homogeneous fractions were pooled and evaporated to dryness, and the residual intermediate 8 (5.8 g) was used without characterization for the ring-closure reaction.

The above intermediate (8) was dissolved in ethylene glycol (10 mL) and heated at 150 °C (bath temperature) for 2 h. After cooling to room temperature, the reaction mixture was partitioned between EtOAc (75 mL) and water (75 mL). The organic layer was separated and the aqueous phase was further extracted with EtOAc (2 × 50 mL). The combined EtOAc extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified on a silica gel column (2 × 20 cm), using EtOAc as the solvent to yield 4.9 g (66%) of 9 as colorless foam: IR (KBr) ν 710 (C–Cl), 1720 (C=O), 3320–3420 (NH_2) cm^{-1}; UV λ_{max} (MeOH) 230 (ϵ 40900), 272 (8600), 308 nm (8900); partial ^{1}H NMR (Me_2SO-d_6) δ 5.86 (d, 1 H, J = 4.0 Hz, $C_{1'}H$), 7.42–7.92 (m, 17 H, $3C_6H_5$ and NH_2), and 8.19 (s, 1 H, C_6H). Anal. ($C_{31}H_{24}ClN_5O_7$) C, H, N, Cl.

8-Amino-7-chloro-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (10). A solution of 9 (2.0 g, 3.25 mmol) in MeOH/NH_3 (150 mL, saturated at 0 °C) was stirred at room temperature for 18 h in a pressure bottle. The solvent was evaporated to dryness and the residue was purified on a flash silica gel column (2.5 × 25 cm) using EtOAc/EtOH (6:1, v/v) as the eluent. The homogeneous fractions were pooled and evaporated to dryness, and the residue was crystallized from aqueous EtOH to yield 0.69 g (70%) of **10**: mp 203–205 °C; IR (KBr) ν 760 (C–Cl), 3160–3450 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1, 7, and 11) 235 (ϵ 10200), 261 (sh) (7300), 268 (7300), 306 nm (10600); ^{1}H NMR (Me_2SO-d_6) δ 3.47 (m, 2 H, $C_{5'}CH_2$), 3.88 (m, 1 H, $C_{4'}H$), 4.10 (m, 1 H, $C_{3'}H$), 4.63 (m, 1 H, $C_{2'}H$), 4.75 (t, 1 H, $C_{5'}OH$), 5.26 and 5.70 (2d, 2 H, $C_{2',3'}OH$), 5.16 (d, 1 H, J = 4.3 Hz, $C_{1'}H$), 7.91 (br s, 2 H, NH_2), and 8.32 (s, 1 H, C_6H). Anal. ($C_{10}H_{12}ClN_5O_4$) C, H, N, Cl.

8-Amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (11). A mixture of 9 (2.0 g, 3.25 mmol), K_2CO_3 (0.45 g, 3.3 mmol), and Pd/C (1.4 g, 5%) in aqueous EtOH (90%, 150 mL) was hydrogenated at 30 psi for 24 h at room temperature. The reaction mixture was filtered through a Celite pad and washed with EtOH (3 × 25 mL). The combined filtrates were evaporated to dryness. The residue was purified on a flash silica gel column (2 × 20 cm) using EtOAc as the eluent. The homogeneous fractions were pooled and evaporated to dryness to yield 0.99 g (52%) of 11 as a white amorphous solid: mp 187 °C (sintered at 83 °C); IR (KBr) ν 1720 (C=O), 3350–3430 (NH_2) cm^{-1}; UV λ_{max} (MeOH) 231 (ϵ 44600), 273 (8000), 300 nm (11400); partial ^{1}H NMR (Me_2SO-d_6) δ 5.86 (d, 1 H, J = 4.5 Hz, $C_{1'}H$), 6.14 (d, 1 H, J = 5.0 Hz, C_7H), 7.40–7.94 (m, 17 H, $3C_6H_5$ and NH_2), and 8.03 (d, 1 H, J = 5.0 Hz, C_6H). Anal. ($C_{31}H_{25}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

8-Amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (5). A solution of 11 (2.0 g, 3.45 mmol) in MeOH/NH_3 (100 mL, saturated at 0 °C) was stirred at room temperature for 16 h and worked up as described for **10** to yield 0.69 g (75%) of crystalline (from aqueous EtOH) title compound: mp 230 °C; IR (KBr) ν 3220–3430 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 235 (ϵ 8700), 269 (5300), 301 nm (12200); UV λ_{max} (pH 7) 235 (ϵ 11200), 259 (4800), 267.5 (5000), 301 nm (12800); UV λ_{max} (pH 11) 235 (ϵ 11600), 258 (5100), 267 (5300), 301 nm (13000); ^{1}H NMR (Me_2SO-d_6) δ 3.50 (m, 2 H, $C_{5'}CH_2$), 3.90 (m, 1 H, $C_{4'}H$), 4.12 (m,

1 H, $C_{3'}H$), 4.67 (m, 1 H, $C_{2'}H$), 4.82 (t, 1 H, $C_{5'}OH$), 5.07 and 5.25 (2d, 2 H, $C_{2',3'}OH$), 5.19 (d, 1 H, J = 4.3 Hz, $C_{1'}H$), 6.13 (d, 1 H, C_7H), 7.55 (br s, 2 H, NH_2), and 8.08 (d, 1 H, J = 4.0 Hz, C_6H). Anal. ($C_{10}H_{13}N_5O_4$) C, H, N.

7-Amino-8-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (16). To a stirred suspension of 5-amino-4-chloro-3-hydrazinopyridazine[20] (**13**, 4.78 g, 30 mmol) and **6** (14.7 g, 30 mmol) in dry dichloromethane (800 mL) was added DCC (6.2 g, 30 mmol). The mixture was stirred at room temperature for 16 h with the exclusion of moisture and then filtered. The filtrate was evaporated to dryness and the residue was purified on a flash silica gel column (5 × 20 cm) using EtOAc as the eluent. The homogeneous fractions were pooled and evaporated to dryness, and the residual intermediate **15** (8.2 g) was used without characterization for the ring-closure reaction.

The above intermediate (**15**) was dissolved in ethylene glycol (25 mL) and heated at 145–150 °C for 2 h. The reaction mixture was worked up as described for **9** to yield 7.4 g (40%) of **16** as an analytically pure foam: IR (KBr) ν 710 (C–Cl), 1720 (C=O), 3320–3420 (NH_2) cm^{-1}; UV λ_{max} (MeOH) 230 (ϵ 49 000), 263 (14 900), 339 nm (4100); partial ^{1}H NMR (Me_2SO-d_6) δ 5.81 (d, 1 H, J = 4.3 Hz, $C_{1'}H$), 6.86 (br s, 2 H, NH_2), 7.41–7.93 (m, 15 H, $3C_6H_5$), and 8.25 (s, 1 H, C_6H). Anal. ($C_{31}H_{24}ClN_5O_7$) C, H, N, Cl.

7-Amino-8-chloro-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (17). Debenzoylation of **16** (1.0 g, 1.62 mmol) with MeOH/NH_3 (80 mL, saturated at 0 °C) at room temperature for 16 h and subsequent workup of the reaction mixture, as described for **10**, gave the title compound. Recrystallization of the product from a mixture of EtOH/EtOAc gave 0.29 g (59%) of crystalline **17**: mp 295 °C dec; IR (KBr) ν 745 (C–Cl), 3210–3460 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 212 (ϵ 16 800), 268 (12 600), 321 nm (6600); UV λ_{max} (pH 7) 212 (ϵ 19 900), 236 (sh) (13 300), 333 nm (4000); UV λ_{max} (pH 11) 238 (sh) (ϵ 13 200), 261 (8200), 333 nm (4000); ^{1}H NMR (Me_2SO-d_6) δ 3.40 (m, 2 H, $C_{5'}CH_2$), 3.86 (m, 1 H, $C_{4'}H$), 4.08 (m, 1 H, $C_{3'}H$), 4.59 (m, 1 H, $C_{2'}H$), 4.72 (t, 1 H, $C_{5'}OH$), 5.05 and 5.25 (2d, 2 H, $C_{2',3'}OH$), 5.08 (d, 1 H, J = 4.3 Hz, $C_{1'}H$), 6.79 (br s, 2 H, NH_2), and 8.28 (s, 1 H, C_6H). Anal. ($C_{10}H_{12}ClN_5O_4$) C, H, N, Cl.

7-Amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (18). A mixture of **16** (1.0 g, 1.62 mmol), K_2CO_3 (0.23 g, 1.62 mmol), and Pd/C (0.7 g, 5%) in aqueous EtOH (90%, 80 mL) was hydrogenated at 30 psi at room temperature for 24 h and worked up as described for **11** to yield 0.49 g (52%) of **18** as an analytically pure amorphous solid: mp 106–110 °C; IR (KBr) ν 1720, 1800 (C=O), 3520–3600 (NH_2) cm^{-1}; UV λ_{max} (MeOH) 231 (ϵ 34 000), 264 nm (8400); partial ^{1}H NMR (Me_2SO-d_6) δ 5.79 (d, 1 H, J = 4.3 Hz, $C_{1'}H$), 6.51 (br s, 1 H, NH_2), 6.76 (d, 1 H, J = 3.5 Hz, C_8H), 7.42–7.96 (m, 15 H, $3C_6H_5$), and 8.19 (d, 1 H, J = 3.5 Hz, C_6H). Anal. ($C_{31}H_{25}N_5O_7$) C, H, N.

7-Amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (19). In a similar manner, as described for the preparation of **10**, treatment of **18** (0.75 g, 1.29 mmol) with MeOH/NH_3 (100 mL) at room temperature for 18 h provided 0.26 g (76%) of **19**, which was crystallized from MeOH: mp 233–234 °C; IR (KBr) ν 3230–3450 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 224 (ϵ 17 100), 264 (15 400), 319 nm (7400); UV λ_{max} (pH 7) 210 (ϵ 18 400), 234 (18 500), 262 (sh) (9400), 335 nm (3700); UV λ_{max} (pH 11) 234 (ϵ 19 000), 262 (sh) (10 100), 335 nm (3900); ^{1}H NMR (Me_2SO-d_6) δ 3.42 (m, 2 H, $C_{5'}CH_2$), 3.84 (m, 1 H, $C_{4'}H$), 4.06 (m, 1 H, $C_{3'}H$), 4.59 (m, 1 H, $C_{2'}H$), 4.74 (t, 1 H, $C_{5'}OH$), 5.02 and 5.22 (2d, 2 H, $C_{2',3'}OH$), 5.07 (d, 1 H, J = 4.3 Hz, $C_{1'}H$), 6.39 (br s, 2 H, NH_2), 6.70 (d, 1, J = 2.6 Hz, C_8H), and 8.20 (d, 1 H, J = 2.6 Hz, C_6H). Anal. ($C_{10}H_{13}N_5O_4$) C, H, N.

6-Chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*b*]pyridazine (21). To a mixture of 6-chloro-3-hydrazinopyridazine[23] (**12**, 4.33 g, 30 mmol) and **6** (14.7 g, 30 mmol) in anhydrous dichloromethane (500 mL) was added DCC (6.2 g, 30 mmol) and the solution was stirred at room temperature for 16 h. Additional DCC (6.2 g, 30 mmol) was added to the reaction mixture and it was stirred for 6 h. After filtration, the filtrate was evaporated to dryness. The residue was purified on a flash silica gel column (5 × 20 cm) using a hexanes/acetone 9:1 (v/v) → 4:1 (v/v) gradient. The homogeneous fractions were pooled and evaporated to dryness, and the residual intermediate **14** (8.5 g) was ring-closed without characterization.

The above intermediate (**14**) was dissolved in ethylene glycol (50 mL) and heated at 150 °C for 2 h. After cooling, the reaction mixture was worked up as described for **9** to yield, after crystallization from EtOH, 7.5 g (42%) of the title compound: mp 159–161 °C; IR (KBr) ν 710 (C–Cl), 1730 (C=O) cm^{-1}; UV λ_{max} (MeOH) 227 (ϵ 56 300), 268 (4500), 304 nm (2500); partial ^{1}H NMR (Me_2SO-d_6) δ 5.94 (d, 1 H, J = 4.3 Hz, $C_{1'}H$), 7.42–7.93 (m, 16 H, $3C_6H_5$ and C_7H), and 8.51 (d, 1 H, J = 6.0 Hz, C_8H). Anal. ($C_{31}H_{23}ClN_4O_7$) C, H, N, Cl.

6-Amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (20). Compound **21** (0.70 g, 1.1 mmol) and liquid NH_3 (50 mL) were placed in a sealed steel reaction vessel (125 mL) and heated at 80 °C for 16 h. After cooling, the NH_3 was allowed to evaporate at room temperature and the residue was subjected to a vacuum overnight to remove the last traces of NH_3. The residual solid was purified on a silica gel column (2 × 25 cm) using a EtOAc/EtOH 9:1 (v/v) → 4:1 (v/v) gradient. The homogeneous solid was crystallized from a mixture of EtOH and EtOAc as white needles to yield 0.27 g (85%) of **20**: mp 252–253 °C [lit.[22] mp >250 °C]; IR (KBr) ν 3200–3400 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 226 (ϵ 23 000), 267 (3100), 308 nm (1600); UV λ_{max} (pH 7 and 11) 226 (ϵ 24 500), 280 nm (4200); ^{1}H NMR (Me_2SO-d_6) δ 3.48 (m, 2 H, $C_{5'}CH_2$), 3.83 (m, 1 H, $C_{4'}H$), 4.08 (m, 1 H, $C_{3'}H$), 4.60 (m, 1 H, $C_{2'}H$), 4.80 (t, 1 H, $C_{5'}OH$), 5.01 and 5.21 (2d, 2 H, $C_{2',3'}OH$), 5.06 (d, 1 H, J = 6.3 Hz, $C_{1'}H$), 6.81 (d, 1 H, J = 8.0 Hz, C_7H), 6.86 (br s, 2 H, NH_2), and 7.95 (d, 1 H, J = 8.0 Hz, C_8H). Anal. ($C_{10}H_{13}N_5O_4$) C, H, N.

6-Methoxy-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazine (22). Method A. Compound **21** (0.60 g, 1 mmol) was dissolved in anhydrous MeOH (50 mL) containing sodium methoxide (0.27 g, 5 mmol) and the mixture was stirred at ambient temperature for 2 h. The pH of the solution was adjusted to ~4–5 with 20% HCl and the mixture was evaporated to dryness. The residue was purified on a silica gel column (1.5 × 20 cm) using EtOAc/EtOH (2:8, v/v) as the eluent. The homogeneous solid was crystallized from a mixture of EtOH and EtOAc as white needles to yield 0.23 g (80%) of **22**: mp 187–188 °C [lit.[22] mp 96 °C for the monohydrate]; IR (KBr) ν 3060–3320 (OH) cm^{-1}; UV λ_{max} (pH 1) 213 (ϵ 13 300), 262 nm (3300); UV λ_{max} (pH 7) 217 (ϵ 17 300), 272 nm (3200); UV λ_{max} (pH 11) 216 (ϵ 17 100), 268 nm (3200); ^{1}H NMR (Me_2SO-d_6) δ 3.51 (m, 2 H, $C_{5'}CH_2$), 3.89 (m, 1 H, $C_{4'}H$), 3.98 (s, 3 H, OCH_3), 4.16 (m, 1 H, $C_{3'}H$), 4.67 (m, 1 H, $C_{2'}H$), 4.72 (t, 1 H, $C_{5'}OH$), 5.10 and 5.26 (2d, 2 H, $C_{2',3'}OH$), 5.18 (d, 1 H, J = 5.5 Hz, $C_{1'}H$), 7.07 (d, 1 H, J = 8.0 Hz, C_7H), and 8.25 (d, 1 H, J = 8.0 Hz, C_8H). Anal. ($C_{11}H_{14}N_4O_5$) C, H, N.

Method B. A solution of compound **21** (1.0 g, 1.67 mmol) in MeOH/NH_3 (50 mL) was stirred in a pressure bottle at room temperature for 18 h. The solvent was evaporated and the residue was purified on a silica gel column (2 × 25 cm) using EtOAc/EtOH 9:1 (v/v) → 4:1 (v/v) gradient. The homogeneous solid that was obtained after evaporation of the solvent was crystallized from EtOH as white needles to yield 0.25 g (52%) of **22**; mp 187–188 °C. This product was found to be identical with **22** prepared by method A.

3-β-D-Ribofuranosyl-1,2,4-triazolo[4,3-*b*]pyridazin-6-(5*H*)-one (23). A solution of **22** (1.13 g, 4 mmol) in 1 N NaOH (50 mL) was heated at 80 °C for 5 h. After cooling, the pH of the reaction mixture was adjusted to ~4–5 with Dowex-50 (H^+ form) resin. The resin was removed by filtration and the filtrate was evaporated to dryness. The residue was crystallized from EtOH to yield 0.49 g (46%) of **23**: mp 215–217 °C; IR (KBr) ν 1680 (C=O), 3280–3400 (OH) cm^{-1}; UV λ_{max} (pH 1) 211 (ϵ 18 700), 262 nm (3700); UV λ_{max} (pH 7 and 11) 224 (ϵ 21 800), 274 nm (3900); ^{1}H NMR (Me_2SO-d_6) δ 3.48 (m, 2 H, $C_{5'}CH_2$), 3.40–4.0 (br s, 3 H, $C_{2',3',5'}OH$), 3.86 (m, 1 H, $C_{4'}H$), 4.09 (m, 1 H, $C_{3'}H$), 4.65 (m, 1 H, $C_{2'}H$), 5.11 (d, 1 H, J = 5.5 Hz, $C_{1'}H$), 7.04 (d, 1 H, J = 7.0 Hz, C_7H), 8.20 (d, 1 H, J = 7.0 Hz, C_8H), and 12.61 (br s, 1 H, N_5H). Anal. ($C_{10}H_{12}N_4O_5 \cdot {}^3/_4H_2O$) C, H, N.

Registry No. **5**, 120525-46-4; **6**, 23316-68-9; **7**, 53180-75-9; **8**, 120525-42-0; **9**, 120525-43-1; **10**, 120525-44-2; **11**, 120525-45-3; **12**, 17284-97-8; **13**, 61071-29-2; **14**, 120525-52-2; **15**, 120525-47-5; **16**, 120525-48-6; **17**, 120525-49-7; **18**, 120525-50-0; **19**, 120525-51-1; **20**, 80504-69-4; **21**, 120525-53-3; **22**, 80504-68-3; **23**, 120525-54-4.