Design and Synthesis of a New Fluorescent Tricyclic Nucleoside, 3-β-D-Ribofuranosylpyrazolo[3,2-*i*]purine

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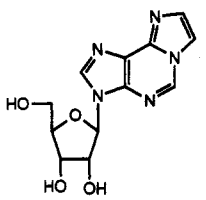
The novel nucleoside, 3-β-D-ribofuranosylpyrazolo[3,2-*i*]purine, has been prepared in seven steps from a fully protected 6-chloropurine derivative including a one-step reaction for the preparation of an ethyl 3-β-D-ribofuranosylpyrazolo[3,2-*i*]purine derivative from 6-enamino purine and hydrazine. The mechanism for the preparation of 9-ethyl-substituted pyrazolo[3,2-*i*]purines was elucidated. First, the hydrazino moiety of 6-enamino purine attacks at the C-6 carbon of the purine ring to give a spiro intermediate; this is followed by ring opening and cyclization. The new tricyclic nucleoside exhibited stronger fluorescence than that of 1,N^6-ethenoadenosine. Also, the compound and the 9-bromo-substituted pyrazolo[3,2-*i*]purine nucleoside showed cytotoxic activities against human leukemia CCRF-HSB-2 cells in culture.

Introduction

1,N^6-Ethenoadenosine derivatives are interesting compounds for the studies of mutagenesis[2] and enzymology.[3] It is known that 1,N^6-etheno-substituted nucleotides are formed as metabolites from the reaction of DNA or RNA with vinyl chloride, chloroacetaldehyde, and chloroethylene oxide. Barbin et al. reported that 1,N^6-etheno-substituted DNAs were found to be misincorporated *in vitro*, and the base-pairing was proposed for the 1,N^6-ethenoadenosine-guanosine pair.[4] The etheno-substituted polynucleotides are also resistant to nuclease action,[5] and 1,N^6-ethenoadenosine (1)[6] and its derivatives[7] have not exhibited cytotoxicity. Until now, the synthesis and the biological properties of a 9-deaza tricyclic nucleoside as an analogue of 1 have yet to be studied. Also, it is not known whether the carbon at the position 9 has an important role for cytotoxicity. Herein, we describe the synthesis of 3-β-D-ribofuranosylpyrazolo[3,2-*i*]purine (2), its fluorescence, as well as its cytotoxic activity.[8]



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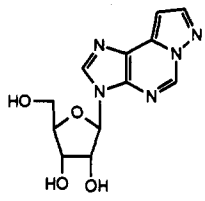

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Results and Discussion

A fully protected 6-chloropurine derivative **3** was prepared in 91% yield from 6-chloro-9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)purine[9] and 3,4-dihydropyran. Reaction of **3** with ethyl cyanoacetate using sodium hydride in DMF gave **4** in 86% yield (Scheme 1). Hydrogenation of **4** over 5% Pd–C in a benzene-DMF solvent system afforded the enamino ester **5** in 71% yield, and the over-reduced product **6** was obtained in 28% yield. The benzene–DMF solvent was most useful for catalytic hydrogenation of the 6-(cyanomethylene)purine derivatives.[10] With other solvents, the enamino ester **5** was obtained in lower yields. In the ^{1}H NMR spectrum, it was found that compound **5** existed in an enamino ester tautomeric equilibrium.[11] The ratio (*E*/*Z*) was 36:64 in acetone at room temperature.

Reaction of enamino ester **5** with hydrazine sulfate in pyridine–ethanol at 100 °C gave **7** and pyrazolone derivative[12] **8** in 87 and 9% yield, respectively. Compound **8** was converted to the methoxypyrazole derivative **9** by diazomethane. After removal of the protecting groups of **7** with trifluoroacetic acid, **10** was obtained in 84% yield. The structures of **7** and **10** were confirmed by ^{1}H and ^{13}C NMR and by chemical degradation. Thus, the ^{1}H NMR

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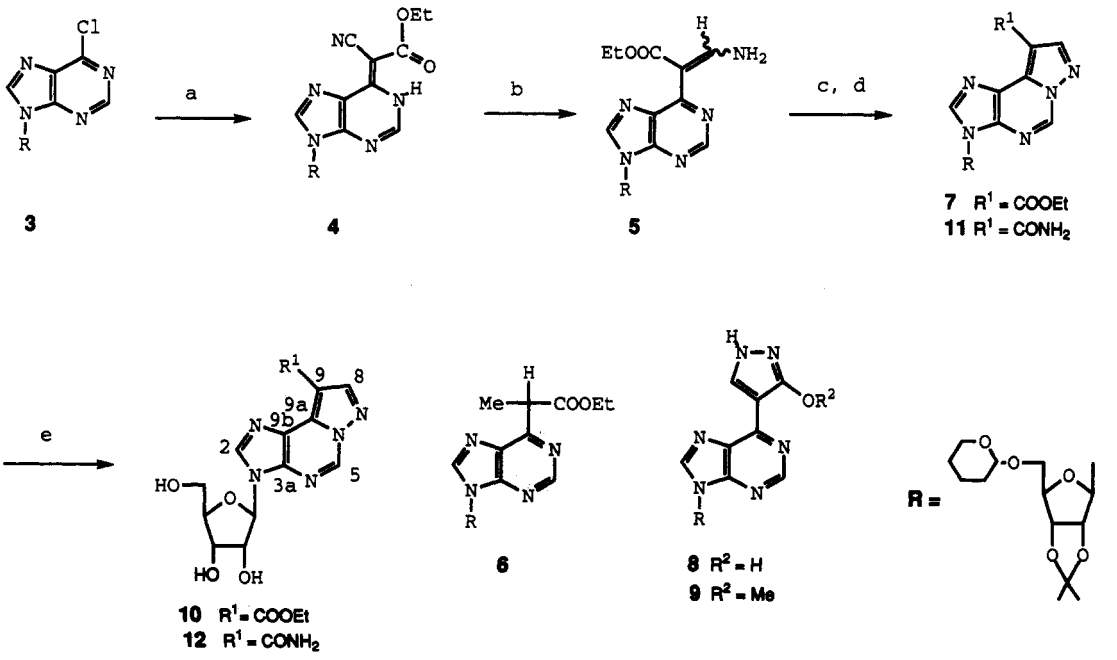
Scheme 1



[a] (a) $CH_2(CN)COOMe$, NaH; (b) H_2, 5% Pd–C; (c) NH_2NH_2; (d) saturated NH_3, MeOH; (e) CF_3COOH–H_2O.

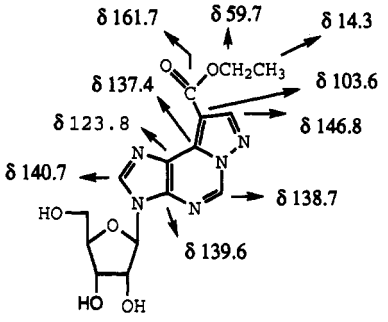


Figure 1. ^{13}C NMR assignment for the aglycon moiety of **10** in DMSO-d_6.

Table 1. ^{13}C–H Coupling Constant (Hz) for the Aglycon Moiety of 10

carbon	C_2–H	C_5–H	C_8–H
C_2	^1J = 214.8		
C_{3a}	*a*	*a*	
C_5		^1J = 216.7	
C_8			^1J = 189.8
C_9			^2J = 9.1
C_{9a}		^3J = 4.4	^3J = 4.4
C_{9b}	^3J = 12.8		

[a] Data not clear.

spectrum of **10** showed three aromatic protons at δ 8.57 (H-5), 8.71 (H-2) and 9.57 (H-8).[13] In the ^{13}C NMR spectrum, the carbon of the pyrazole ring[14] fused to the pyrimidine ring appeared at δ 103 (C-9), 137.4 (C-9a) and 146.8 (C-8) (Figure 1, Table 1). It is known that tricyclic compounds such as 1,N^6-ethenoadenosine and pyrimido[2,1-*i*]purine derivatives undergo ring-opening upon alkaline treatment.[15] As expected, treatment of **7** with NaOH gave the pyrazolylimidazole **13**, and heating of **13** with triethyl orthoformate gave **7** (Scheme 2).

On the basis of the ^1H NMR, ^{13}C NMR spectra and chemical degradation, compounds **7** and **10** were determined to be 9-(ethoxycarbonyl)pyrazolo[3,2-*i*]purine derivative. Reaction of **5** with hydrazine monohydrochloride gave the pyrazolo[3,2-*i*]purine derivative **7** and the pyrazolone derivative **8** in 87 and 10% yield, respectively. With hydrazine monohydrate, compounds **7** and **8** were obtained as a ca. 1:1 mixture. The ester of **7** was converted to the amide **11** with ammonia, the protecting groups of which were removed with trifluoroacetic acid to give **12**.

The reaction mechanism for the formation of **7** and **8** was elucidated (Scheme 3). Reaction of **5** with hydrazine in ethanol at 50 °C for 10 min gave the unstable hydrazino derivative **14** in 25% yield, which was heated in ethanol for 2.5 h to give **7** and **8** in 53 and 25% yield, respectively. Because of the instability of **14** at room temperature, the structure was confirmed by ^1H NMR spectroscopy after conversion to the acetonide **15**. Heating of **5** with dimethylhydrazine in ethanol for 6 h afforded **16** in 80% yield. When **5** was heated with methylhydrazine in ethanol at 50 °C for 20 h, compound **17** was obtained in 54% yield. The position of the methyl group of the pyrazole ring was determined by NOE. Thus, irradiation of the signal for the methyl group led to an 8% enhancement of the methine signal assigned to C-10. On the basis of these results, it seems that first addition elimination of hydrazine to the enamino moiety takes place to give **14**, whose hydrazino moiety attacks at the C-6 carbon to give a spiro intermediate [path a] (Scheme 4). The linkage between C-6 and N-1 is then cleaved to give the pyrazole derivative, which cyclizes to **7** with elimination of ammonia. Alternatively, pyrazolone derivative **8** is obtained by the cyclization of the hydrazino group to the ester of **14** [path b].

Reaction of amide **11** with 4 equiv of bromine in potasium phosphate buffer (pH 6.9) at 0–5 °C gave **18** in 91% yield, the protecting groups of which were removed with acid to

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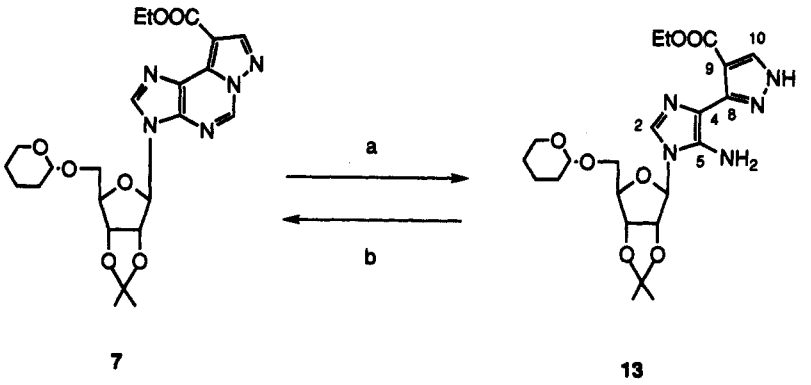
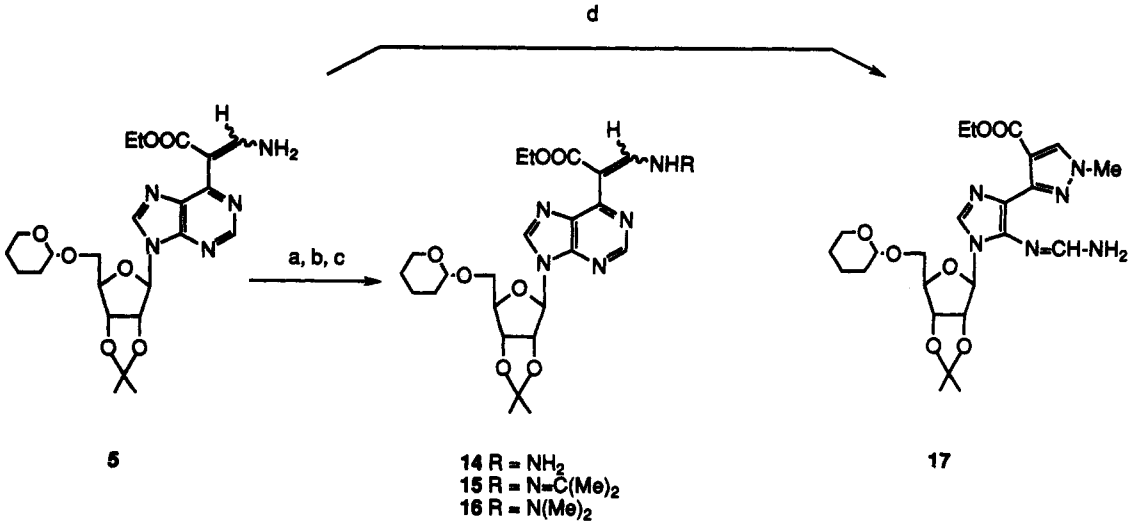
Scheme 2



7 13

[a] (a) NaOH, H_2O; (b) $HC(OEt)_3$, 135 °C.

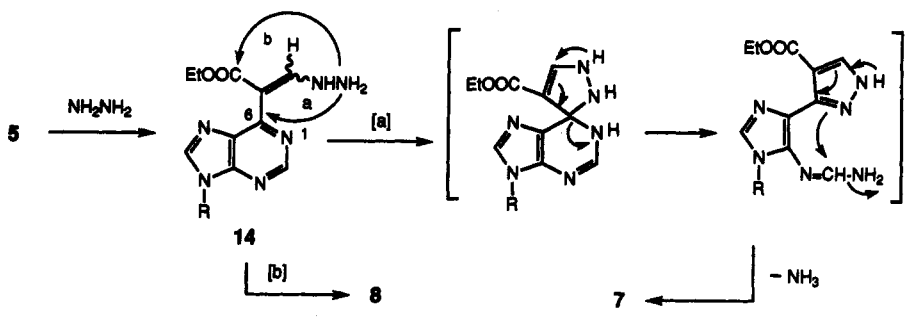
Scheme 3



5 14 R = NH_2, 15 R = $N{=}C(Me)_2$, 16 R = $N(Me)_2$ 17

[a] (a) $NH_2NH_2{\cdot}H_2O$; (b) CH_3COCH_3; (c) $H_2NN(Me)_2$; (d) H_2NNHMe.

Scheme 4



give 9-bromo-3-β-D-ribofuranosylpyrazolo[3,2-*i*]purine (**19**) (Scheme 5). The structures of **18** and **19** were verified by spectroscopy and elemental analyses. It is known that nitrodebromination occurs in bromo-substituted pyrazole derivatives by nitration as an ipso nitration.[16] Therefore, the bromo compound **18** could possibly be obtained from the amide **11** by an ipso bromination. Attempts at direct amination of the amide of **11** using the Hoffmann rearrangement were unsuccessful. Hydrogenolysis of **18** over 5% Pd–C, followed by removal of the protecting groups of **20** gave the highly fluorescent 3-β-D-ribofuranosylpyrazolo[3,2-*i*]purine (**2**). Also, bromination of **20** afforded **18** in 97% yield.

Fluorescence intensity data for **2**, **10**, **12**, and **19** are shown in Table 2. The quantum yield of **2** was stronger than those of the other tested compounds, and the 9-substituted amide **12** produced a similar quantum yield to that of $1,N^6$-ethenoadenosine. The cytotoxic activities of these compounds against human leukemia CCRF-HSB-2 cells[7] in culture are shown in Table 3. Compound **2** and the 9-bromo-substituted compound **19** exhibited an ID_{50} of 0.21 to 0.26 μg/mL against mouse leukemia L5178Y cells.[8b] However, aginst human cells compound **2** exhibited weak cytotoxic activity, while **19** displayed activity similar

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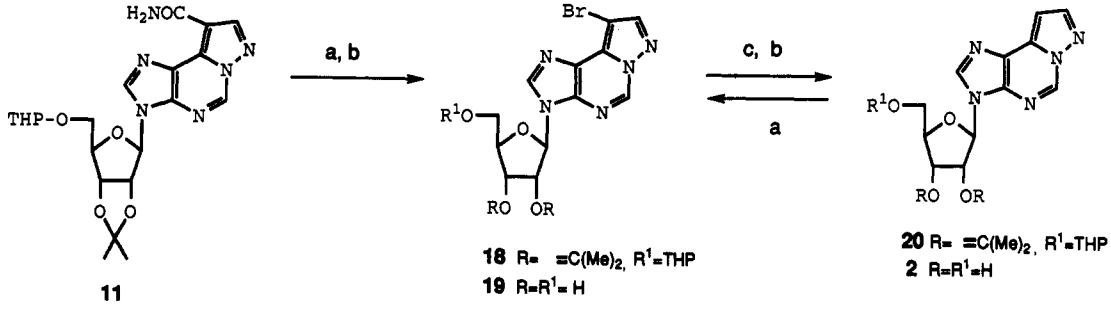
Scheme 5



[a] (a) Br_2, AcOEt–potassium phosphate buffer (pH 6.9); (b) CF_3COOH–H_2O; (c) H_2, 5% Pd–C.

Table 2. Fluorescence Data of 9-Substituted Pyrazolo[3,2-*i*]purine Derivatives[a]

compd	excitation max, nm	emission max, nm	quantum yield
2	233	406	0.68
10	233	384	0.40
12	236	392	0.57
19	233	424	0.42
1	300	412	0.57[b]

[a] Quantum yield calculated relative to quinine sulfate in 0.025 M phosphate buffer pH 7.0 at 21 °C. [b] Literature,[3a] 0.56.

Table 3. Cytotoxic Activities of 2, 10, 12, and 19 against CCRF-HSB-2 Cells

compd	ID_{50} (μg/mL)
2	5.31
10	>100
12	>100
19	0.92
1	>100

to that against mouse leukemia L5178Y cells. On the other hand, 1,N^6-ethenoadenosine (1), 9-ethyl- and amide-substituted compounds did not show cytotoxicity.

In conclusion, the desired compound 2 has been prepared from 6-enamino ester purine 5 by a new one-step reaction for the preparation of a pyrazolo[3,2-*i*]purine derivative including a novel bromodeamidation reaction (ipso bromination). It has been found that the fluorescence intensity of 2 was higher than that of 1. Interestingly, compound 2 exhibited different cytotoxic activities against human leukemia and mouse leukemia, while 9-bromo-substituted pyrazolo[3,2-*i*]purine 19 showed strong cytotoxic activities. Therefore, the position 9 of pyrazolo[3,2-*i*]purine ribonucleosides may be important for the observed cytotoxic activity.

Experimental Section

All melting points are uncorrected. IR spectra were taken on a JASCO A-102 spectrophotometer. UV spectra were measured on a Hitachi EPS-3T spectrophotometer. Fluorescence spectra were obtained on a Hitachi F-4000 spectrofluorometer. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-FX 100 spectrometer, using tetramethylsilanes as an internal standard. Mass spectra were measured with a JEOL JMS-D300 spectrometer.

6-Chloro-9-[2,3-*O*-isopropylidene-5-*O*-(2-tetra-hydropyranyl)-β-D-ribofuranosyl]purine (3). A solution of 6-chloro-9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)purine (9.801 g, 0.03 mol), 3,4-dihydropyran (5.312 g, 0.06 mol) and pyridinium *p*-toluenesulfonate (3.00 g, 0.006 mol) in CH_2Cl_2 (120 mL) was stirred for 15 h at room temperature. The solution was diluted with CH_2Cl_2 (200 mL). The combined organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was recrystallized from CH_2Cl_2–hexane to give 3 (11.25 g, 91%) as colorless needles: mp 118–119 °C; UV (MeOH) λ max (ε) 246 (sh, 6640), 362 (8800) nm; ^1H NMR ($CDCl_3$) δ 1.36 (s, 3H, Me), 1.40–1.50 (m, 6 H, pyran), 1.56 (s, 3 H, Me), 3.40–3.83 (m, 4 H, pyran, H-5′), 4.49–4.52 (m, 2 H, H-4′, pyran), 5.02 (dd, *J* = 6, 2 Hz, 1 H, H-3′), 5.48 (dd, *J* = 6 Hz, 1 H, H-2′), 6.30 (dd, *J* = 6, 2 Hz, 1 H, H-1′), 8.78 (s, 1 H), 8.82 (s, 1 H). Anal. Calcd for $C_{18}H_{23}ClN_4O_5$: C, 52.62; H, 5.64: Cl, 8.63; N, 13.63. Found: C, 52.61; H, 5.62; Cl, 8.49; N, 13.59.

Ethyl α-Cyano-9-[2,3-*O*-isopropylidene-5-*O*-(2-tetrahydropyranyl)-β-D-ribofuranosyl]purine-6-acetate (4). To a cold (0–5 °C), stirred suspension of 60% NaH (2.40 g, 0.06 mol) in dry DMF (100 mL) was added ethyl cyanoacetate (6.787 g, 0.06 mol) over a period of 20 min under argon and the solution was stirred for 10 min at the same temperature. After this time, a solution of 3 (8.216 g, 0.02 mol) in dry DMF (50 mL) was added to the above solution and stirring was continued at 85 °C overnight. After cooling, the solvent was evaporated *in vacuo*, and the residue was diluted with H_2O (100 mL) and adjusted to pH 5 with 1% aqueous HCl. The resulting precipitate was collected by filtration and dissolved in AcOEt (300 mL). The solution was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (50 g) with AcOEt as eluent, and the eluate was evaporated *in vacuo* to give 4 (8.414 g, 86%) as a colorless solid: IR (KBr) 3100 (NH), 2200 (CN), 1655 (CO) cm^{-1}; UV (MeOH) λ max (ε) 328 (29500, sh), 339 (34000) nm; ^1H NMR (DMSO-d_6) δ 1.28 (t, *J* = 7 Hz, 3 H, Me), 1.35 (s, 3 H, Me), 1.40–1.50 (m, 6 H, pyran), 1.54 (s, 3 H, Me), 3.10–3.87 (m, 4 H, H-5′, pyran), 4.23 (q, *J* = 7 Hz, 2 H, CH_2), 4.35–4.52 (m, 2 H, H-4′, pyran), 4.97 (dd, *J* = 7, 2 Hz, 1 H, H-3′), 5.36 (dd, *J* = 7, 2 Hz, 1 H, H-2′), 6.18 (br s, 1 H, H-1′), 8.46 (s, 0.5 H), 8.51 (s, 1.5 H), 13.59 (br s, 1 H, NH); MS *m/z* 487 (M^+), 403, 231 (B + 1). Anal. Calcd for $C_{23}H_{29}N_5O_7$: C, 56.66; H, 6.00; N, 14.37. Found: C, 56.87; H, 6.01; N, 14.37.

Catalytic Hydrogenation of 4. A solution of 4 (2.437 g, 5 mmol) in DMF–benzene (1:1 v/v, 120 mL) was hydrogenated over 5% Pd–C (1.7 g) at room temperature and 4 atm of hydrogen for 48 h. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*. The residue was purified by column chromatography on Florisil (100 g) with 5% EtOH in CH_2Cl_2 as eluent and the eluate was evaporated *in vacuo* to give 6 (0.690 g, 28%) as a colorless solid: IR (neat) 2930, 1735 (CO), 1590 cm^{-1}; UV (MeOH) λ max (ε) 246 (7080), 262 (8750) nm; ^1H NMR (DMSO-d_6) δ 1.09 (t, *J* = 7 Hz, 3 H, Me), 1.36 (s, 3 H, Me), 1.37–1.52 (m, 6 H, pyran), 1.52 (d, *J* = 7 Hz, 3 H, Me), 1.56 (s, 3 H, Me), 3.22–3.78 (m, 4 H, H-5′, pyran), 4,07 (q, *J* = 7 Hz, 2 H, CH_2), 4.39–4.53 (m, 3 H, CH, H-4′, pyran), 5.02 (dd, *J* = 6, 2 Hz, 1 H, H-3′), 5.49 (m, 1 H, H-2′), 6.29 (br s, 1 H, H-1′), 8.67 (s, 0.5 H), 8.72 (s, 0.5 H), 8.89 (s, 1 H); MS *m/z* 476 (M^+), 460, 220 (B + 1). Anal. Calcd for $C_{23}H_{32}N_4O_7$: C, 57.97; H, 6.77; N, 11.76. Found: C, 57.81; H, 6.73; N, 11.58.

Further elution with 50% EtOH in CH_2Cl_2 gave ethyl α-(aminomethylene)-9-[2,3-*O*-isopropylidene-5-(2-tetrahydropyranyl)-β-D-ribofuranosyl]purine-6-acetate (5) (1.750 g, 71%) as a colorless solid: IR (KBr) 3400 (NH), 3310 (NH), 1670 (CO), 1570 cm^{-1}; UV (MeOH) λ max (ε) 236 (11300), 263 (11500), 320 (17000) nm; MS *m/z* 489 (M^+), 473; ^1H NMR (CD_3COCD_3) δ 1.16, 1.18 (2t, *J* = 7 Hz, 3 H, Me), 1.38 (s, 3 H, Me), 1.42–1.55

(m, 6 H, pyran), 1.59 (s, 3 H, Me), 3.30–4.00 (m, 4 H, H-5′, pyran), 4,16, 4.18 (2q, J = 7 Hz, 2 H, CH_2), 4.41–4.49 (m, 1 H, H-4′), 4.52 (br s, 1 H, pyran), 5.13 (dd, J = 6, 2 Hz, 1 H, H-3′), 5.52 (dd, J = 6, 2 Hz, 1 H, H-2′), 6.29 (d, J = 2 Hz, 1 H, H-1′), 7.25 (br s, 0.64 H, NH), 7.89 (t, J = 12 Hz, 0.36 H, C=CH, E), 8.12–8.32 (m, 1 H, NH, C=CH, Z), 8.38 (s, 0.64 H, H-8, Z), 8.44 (s, 0.36 H, H-8, E), 8.67 (s, 0.64 H, H-2, Z), 8.74 (s, 0.36 H, H-2, E); MS m/z 489 (M^+), 473, 233 (B + 1). Anal. Calcd for $C_{23}H_{31}N_5O_7$: C, 56.43; H, 6.38; N, 14.31. Found: C, 56.34; H, 6.41; N, 14.20.

Preparation of Ethyl 3-[2,3-*O*-Isopropylidene-5-*O*-(2-tetrahydropyranyl)-β-D-ribofuranosyl]pyrazolo[3,2-*f*]purine-9-carboxylate (7). Method A. A solution of 5 (2.447 g, 5 mmol) and hydrazine sulfate (1.952 g, 15 mmol) in EtOH-pyridine (1:1 v/v, 80 mL) was heated at 100 °C with stirring for 5 h. After cooling, the resulting precipitate was filtered off, and the filtrate was evaporated *in vacuo*. The residue was partitioned between AcOEt (100 mL) and H_2O (20 mL). The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (200 g) with 1% MeOH in $CHCl_3$ as eluent, and the eluate was evaporated *in vacuo* to give 7 (2.117 g, 87%) as a colorless foam: IR (KBr) 1700 (CO), 1625 cm^{-1}; UV (MeOH) λ max (ε) 230 (19000), 287 (15000) nm; ^1H NMR (DMSO-d_6) δ 1.37 (s, and t, 6 H, 2 × Me), 1.42–1.54 (m, 6 H, pyran), 1.58 (s, 3 H, Me), 3.31–3.89 (m, 4 H, H-5′ and pyran), 4.34 (q, J = 7 Hz, 2 H, CH_2), 4.42–4.54 (m, 2 H, H-4′, pyran), 5.04 (dd, J = 6, 2 Hz, 1 H, H-3′), 5.44 (br d, J = 6 Hz, 1 H, H-2′), 6.34 (br s, 1 H, H-1′), 8.58 (s, 1.5 H, H-8 and H-2), 8.63 (s, 0.5 H, H-2), 9.58 (s, 1 H, H-5); MS m/z 487 (M^+), 231 (B + 1). Anal. Calcd for $C_{23}H_{29}N_5O_7$: C, 56.66; H, 6.00; N, 14.37. Found: C, 56.59; H, 6.14; N, 14.15.

Further elution with the same solvent gave 9-[2,3-*O*-isopropylidene-5-*O*-(2-tetrahydropyranyl)-β-D-ribofuranosyl]-6-(3-hydroxypyrazol-4-yl)purine (8) (0.157 g, 7%) as a colorless solid: $FeCl_3$ test, green color; IR (KBr) 3250 (NH and OH), 2940, 1650 (CO), 1610 cm^{-1}; UV (MeOH) λ max (ε) 268 (6100), 319 (sh, 11900), 341 (17800) nm; ^1H NMR (DMSO-d_6) δ 1.36 (s, 3 H, Me), 1.32–1.48 (m, 6 H, pyran), 1.56 (s, 3 H, Me), 3.26–3.86 (m, 4 H, H-5′, pyran), 4.42–4.50 (m, 2 H, H-4′, pyran), 5.01 (dd, J = 6, 2 Hz, 1 H, H-3′), 5.50 (dd, J = 6, 2 Hz, 1 H, H-2′), 6.29 (br s, 1 H, H-1′), 8.25 (br s, 1 H, pyrazole H), 8.74, 8.78 (2s, 2 H, H-2 and H-8); MS m/z 458 (M^+), 202 (B + 1). Anal. Calcd for $C_{21}H_{26}N_6O_6$: C, 55.01; H, 5.72; N, 18.33. Found: C, 55.31; H, 5.97; N, 18.05.

Method B. A solution of 5 (0.489 g, 0.001 mol) and hydrazine monohydrochloride (0.205 g, 0.003 mol) in EtOH–pyridine (1:1 v/v, 16 mL) was heated at 100 °C with stirring for 3 h. The workup was carried out according to the above method A. Compound 7 was obtained (0.425 g, 87%) and 8 was obtained (0.044 g, 10%). These products were identical (TLC and ^1H NMR) with those of authentic samples prepared by method A.

Method C. A solution of 5 (2.447 g, 5 mmol) and hydrazine monohydrate (0.152 g, 0.003 mmol) in EtOH (4 mL) was heated at 100 °C with stirring for 3 h. The workup was carried out according to the above method A. Compound 7 was obtained (0.203 g, 41%) and 8 was obtained (0.139 g, 30%). These products were identical (TLC and ^1H NMR) with those of authentic samples prepared by above reactions.

Reaction of 8 with Diazomethane. A solution of 8 (0.114 g, 0.25 mmol) and diazomethane (prepared from *N*-methyl-*N*-nitrosourea and 50% aqueous NaOH) in ether solution (5 mL) was allowed to stand for 12 h at room temperature. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g) with 12% CH_3COCH_3 in $CHCl_3$ as eluent and the eluate was evaporated *in vacuo* to give 9 (95 mg, 81%) as a colorless solid: IR (KBr) 3200 (NH), 2940, 1590 cm^{-1}; UV (MeOH) λ max (ε) 230 (sh 7100), 300 (17600), 310 (sh 15700) nm; ^1H NMR (DMSO-d_6) δ 1.35 (s, 3 H, Me), 1.35–1.45 (m, 6 H, pyran), 1.55 (s, 3H, Me), 3.44-3.76 (m, 4 H, H-4′, pyran), 3.94 (s, 3 H, OMe), 4.30–4.51 (m, 2 H, H-4′, pyran), 5.03 (dd, J = 6, 2 Hz, 1 H, H-3′), 5.52 (dd, J = 6, 2 Hz, 1 H, H-2′), 6.28 (d, J = 2 Hz, 1 H, H-1′), 8.60 (s, 0.5 H), 8.64 (s, 0.5 H), 8.80 (s, 2 H), 12.57 (s, 1 H, NH); MS m/z 472 (M^+), 387. Anal. Calcd for $C_{22}H_{28}N_6O_6$: C, 55.92; H, 5.97; N, 17.79. Found: C, 55.87; H, 5.96; N, 18.09.

Ethyl 3-β-D-Ribofuranosylpyrazolo[3,2-*f*]purine-9-carboxylate (10). A solution of 7 (0.487 g, 1 mmol) in CF_3COOH–H_2O (9:1 v/v, 12 mL) was stirred for 3 h at 0 °C. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (60 g) with $CHCl_3$–CH_3COCH_3–EtOH (12:7:1, v/v) as eluent, and the eluate was evaporated *in vacuo*. The residue was recrystallized from EtOH–hexane to give 10 (0.308 g, 84%) as colorless needles: mp 182–184 °C; UV (MeOH) λ max (ε) 230 (14800), 287 (14800) nm; ^1H NMR ($CDCl_3$) δ 1.38 (d, J = 7 Hz, 3 H, Me), 3.67 (q, J = 5 Hz, 2 H, H-5′), 3.94-4.06 (m, 1 H, H-4′), 4.13–4.24 (m, 1 H, H-3′), 4.35 (q, J = 7 Hz, 2 H, CH_2), 4.48-4.65 (m, 1 H, H-2′), 5.09 (t, J = 5 Hz, 1 H, OH), 5.25 (d, J = 5 Hz, 1 H, OH), 5.57 (d, J = 6 Hz, 1 H, OH), 6.10 (d, J = 5 Hz, 1 H, H-1′), 8.57 (s, 1 H, H-8), 8.71 (s, 1 H, H-2), 9.57 (s, 1 H, H-5); MS m/z 363 (M^+), 231 (B + 1). Anal. Calcd for $C_{15}H_{17}N_5O_6$: C, 49.58; H, 4.72; N, 19.28. Found: C, 49.43; H, 4.74; N, 19.28.

Reaction of 7 with NaOH. A solution of 7 (0.232 g, 0.5 mmol) and NaOH (0.120 g, 3 mmol) in H_2O (3 mL) was stirred for 20 h at room temperature. The solution was neutralized with 1% aqueous HCl and extracted with AcOEt (2 × 50 mL). The combined organic layers were washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (60 g) with 3% EtOH in $CHCl_3$ as eluent, and the eluate was evaporated *in vacuo*. The residue was recrystallized from CH_3COCH_3–hexane to give 5-amino-1-[2,3-*O*-isopropylidene-5-(2-tetrahydropyranyl)-β-D-ribofuranosyl]-4-[4-(ethoxycarbonyl)pyrazol-3-yl]imidazole (13)-(74 mg, 32%) as colorless needles: mp 132–134 °C dec; IR (KBr) 3140(NH), 2940, 1690 (CO) cm^{-1}; UV (MeOH) λ max (ε) 218 (19500), 293 (6920) nm; ^1H NMR (DMSO-d_6) δ 1.27 (t, J = 7 Hz, 3H, Me), 1.34 (s, 3H, Me), 1.44–1.55 (m, 6 H, pyran), 1.55 (s, 3 H, Me), 3.42–3.80 (m, 4 H, H-5′, pyran), 4.11–4.32 (m, 3 H, CH_2, H-4′), 4.60 (br s, 1 H, pyran), 4.89–4.93 (m, 1 H, H-3′), 5.14–5.22 (m, 3 H, NH_2, H-2′), 5.87 (d, J = 2 Hz, 1 H, H-1′), 7.60 (br s, 1 H, H-2), 7.84 (br s, 1 H, H-10), 13.06 (br s, 1 H, NH); ^{13}C-NMR ($CDCl_3$) (aglycon moiety) δ 14.3 (Me), 60.1 (CH_2), 106.4 (C-9), 111.5 (C-4), 130.3 (C-2), 136.4 (C-5), 139.7 (C-8), 142.2 (C-10), 165.4 (CO). MS m/z 477 (M^+), 393. Anal. Calcd for $C_{22}H_{31}N_5O_7$: C, 55.33; H, 6.54; N, 14.67. Found: C, 55.58; H, 6.60; N, 14.80.

Cyclization of 13 with Ethyl Orthoformate. A solution of 13 (64 mg, 0.13 mmol) in ethyl orthoformate (3 mL) was heated with stirring at 135 °C for 4 h. After cooling, the solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (20 g) with 5% EtOH in $CHCl_3$ as eluent, and the eluate was evaporated *in vacuo* to give 7 (52 mg, 80%) as a foam. The compound was identical (TLC and ^1H NMR) with those of an authentic sample prepared from compound 5 and hydrazines.

3-[2,3-*O*-Isopropylidene-5-*O*-(2-tetrahydropyranyl)-β-D-ribofuranosyl]pyrazolo[3,2-*f*]purine-9-carboxamide (11). A solution of 7 (4.875 g, 0.01 mol) in methanolic ammonia (250 mL) was heated at 75 °C for 4 days in a sealed tube. The solvent was evaporated *in vacuo*. The residue was precipitated from CH_3COCH_3–hexane to give 11 (3.988 g, 87%) as a colorless foam: IR (KBr) 2950, 1660, 1645 cm^{-1}; UV (MeOH) λ max (ε) 228 (sh 21200), 232 (21700), 287 (14800) nm; ^1H NMR (DMSO-d_6) δ 1.37 (s, 3 H, Me), 1.37–1.51 (m, 6 H, Pyran), 1.58 (s, 3 H, Me), 3.48–3.90 (m, 4 H, H-5′, pyran), 4.53-4.54 (m, 2 H, H-4′, pyran), 5.03 (dd, J = 6, 2 Hz, 1 H, H-3′), 5.48 (dd, J = 6, 2 Hz, 1 H, H-2′), 6.36 (br s, 1 H, H-1′), 7.62 (br s, 1 H, NH), 8.52 (s, 1 H), 8.67 (s, 0.5 H), 8.71 (s, 0.5 H), 8.78 (br s, 1 H, NH), 9.58 (s, 1 H); MS m/z 458 (M^+), 443, 202 (B + 1). Anal. Calcd for $C_{21}H_{26}N_6O_6$: C, 55.01; H, 5.72; N, 18.33. Found: C, 54.98; H, 5.75; N, 18.17.

3-β-D-Ribofuranosylpyrazolo[3,2-*f*]purine-9-carboxamide (12). A solution of 11 (2.292 g, 5 mmol) in CF_3COOH–H_2O (9:1 v/v, 75 mL) was stirred for 30 min at room temperature. The solvent was evaporated *in vacuo*. The residue was recrystallized from CH_3COCH_3–hexane to give 12 (1.292 g, 80%) as colorless needles: mp 206–208 °C dec; IR (KBr) 1645, 1605 cm^{-1}; UV (MeOH) λ max (ε) 228 (sh 19600), 232 (20500), 287 (14500) nm; ^1H NMR (DMSO-d_6) δ 3.61–3.75 (m, 2 H, H-5′), 3.97–4.08 (m, 1 H, H-4′), 4.23 (dd, J = 9, 5 Hz, 1 H, H-3′), 4.59 (dd, J = 10, 5 Hz, 1 H, H-2′), 5.08 (t, 1 H, J = 5 Hz, 1 H, OH), 5.26 (d, J = 5 Hz, 1 H, OH), 5.60 (d, J = 6 Hz, 1 H, OH), 6.10 (d, J = 5 Hz, 1 H, H-1′), 7.61 (br s, 1 H, NH), 8.51 (s, 1 H, H-8), 8.82 (s, 2 H, H-2, NH), 9.56 (s, 1 H, H-5); MS m/z 202 (B + 1), 186. Anal. Calcd

for $C_{13}H_{14}N_6O_5$: C, 46.70; H, 4.22; N, 25.14. Found: C, 46.62; H, 4.13; N, 25.30.

Reaction of 5 with Hydrazine Monohydrate. A solution of 5 (61 mg, 0.12 mmol) with hydrazine monohydrate (7 μL, 0.15 mmol) in EtOH (0.5 mL) was stirring at 50–55 °C for 10 min. The solvent was evaporated *in vacuo*, and the residue was purified by preparative TLC using 5% MeOH in $CHCl_3$ as developing solvent. Starting compound 5 was recovered in 9 mg (15%). Compound 7 was obtained in 4 mg (7%) as a solid. The pyrazole derivative 8 was obtained in 9 mg (16%) as a colorless solid. These compounds 7 and 8 were identical with those of authentic samples prepared from 5 and hydrazine sulfate. Compound 14 was obtained in 17 mg (25%) as a colorless amorphous solid. UV (MeOH) λ 235 (max), 250 (min), 270 (max), 296 (min), 332 (max) nm; ^{1}H NMR (CD_3CN) δ 1.19 (t, 3 H, J = 7 Hz, Me), 1.38 (s, 3 H, Me), 1.42–1.52 (m, 6 H, pyran), 1.59 (s, 3 H, Me), 2.34 (br s, 2H, NH_2), 3.30–4.01 (m, 4H, pyran, H-5′), 4.16 (q, J = 7 Hz, 2 H, CH_2), 4.43–4.51 (m, 2 H, H-4′, pyran), 5.00 (dd, J = 6, 2 Hz, 1 H, H-3′), 5.38 (dd, J = 6, 2 Hz, 1 H, H-2′), 6.18 (d, J = 2 Hz, 1 H, H-1′), 8.02 (br s, 1 H, C=CH), 8.22, 8.27 (2s, 1 H), 8.67 (s, 1H); FABMS m/z 505 (MH^+), 249 (B + 1). Further, the compound was confirmed as the acetonide derivative. Thus, compound 14 (36 mg, 0.073 mmol) was heated with acetone (5 mL) at 50 °C for 20 min to give a solid, which was recrystallized from CH_3COCH_3–H_2O to give 15 (39 mg, 97%) as slightly yellow needles: mp 103–104 °C; IR (KBr) 2950, 1710 (CO), 1620 cm^{-1}. UV (MeOH) λ max (ε) 240 (11500), 283 (12300), 340 (28200) nm; ^{1}H NMR (CD_3COCD_3) δ 1.22 (t, J = 7 Hz, 3 H, Me), 1.38 (s, 3 H, Me), 1.41–1.53 (m, 6 H, pyran), 1.59 (s, 3 H, Me), 2.02 (s, 3 H, Me), 2.05 (s, 3 H, Me), 3.43–4.12 (m, 4 H, pyran, H-5′), 4.23 (d, J = 7 Hz, 2 H, CH_2), 4.34–4.57 (m, 2 H, H-4′, pyran), 5.10 (dd, J = 6, 2 Hz, 1H, H-3′), 5.50 (dd, J = 6, 2 Hz, 1 H, H-2′), 6.30 (d, J = 2 Hz, 1 H, H-1′), 8.12 (d, J = 11 Hz, 0.5 H, C=CH), 8.43 (s, 0.5 H, H-8), 8.45 (d, J = 11 Hz, 0.5 H, C=CH), 8.50 (s, 0.5 H, H-8), 8.73 (s, 0.5H, H-2), 8.84 (s, 0.5 H, H-2), 11.23 (br s, 0.5 H, NH), 12.51 (br s, 0.5 H, NH); MS m/z 544 (M^+), 288 (B + 1). Anal. Calcd for $C_{26}H_{36}N_6O_7$: C, 57.34; H, 6.66; N, 15.43. Found: C, 57.50; H, 6.73; N, 15.38.

Heating of 14. A solution of 14 (18 mg, 0.0035 mmol) in EtOH (0.5 mL) was refluxed for 2.5 h. After cooling, the solvent was evaporated *in vacuo*. The residue was purified by preparative TLC using 5% MeOH in $CHCl_3$ as developing solvent. Compound 7 was obtained in 9 mg (53%) and compound 8 was obtained in 4 mg (25%). These products were identical (TLC and ^{1}H NMR) with those of authentic samples prepared from compound 5 and hydrazines.

Reaction of 5 with Dimethylhydrazine. A solution of 5 (61 mg, 0.12 mmol) and dimethylhydrazine (22 mg, 0.37 mmol) in EtOH (0.5 mL) was refluxed with stirring for 6 h. After cooling, the solvent was evaporated *in vacuo*, and the residue was purified by column chromatography on silica gel using 4% MeOH in CH_2Cl_2 as eluent, and the eluate was evaporated *in vacuo* to give 16 (52 mg, 80%) as a colorless solid: UV (MeOH) λ 240 (max), 251 (min), 269 (max), 295 (min), 329 (max) nm; ^{1}H NMR (CD_3COCD_3) δ 1.19 (t, J = 7 Hz, 3 H, Me), 1.39 (s, 3 H, Me), 1.43–1.55 (m, 6 H, pyran), 1.59 (s, 3 H, Me), 2.69 (s, 6 H, $N(Me)_2$), 3.32–3.91 (m, 4 H, H-5′, pyran), 4.18 (q, J = 7 Hz, 2 H, CH_2), 4.40–4.57 (m, 2 H, H-4′, pyran), 5.13 (dd, J = 6, 2 Hz, 1 H, H-3′), 5.52 (dd, J = 6, 2 Hz, 1 H, H-2′), 6.29 (d, J = 2 Hz, 1 H, H-1′), 8.15 (br s, 1H, C=CH), 8.39 (s, 0.69 H, H-8), 8.45 (s, 0.31 H, H-8), 8.69 (s, 1 H, H-2), 9.00 (br s, 1 H, NH); MS m/z 532 (M^+), 276 (B + 1); high-resolution MS m/z 532.262 (M^+, calcd $C_{25}H_{36}N_6O_7$, 532.264).

Reaction of 5 with Methylhydrazine. A solution of 5 (61 mg, 0.12 mmol) and methylhydrazine (11.5 mg, 0.25 mmol) in EtOH (3 mL) was stirred at 50 °C for 2 days in a sealed tube. The solvent was evaporated *in vacuo*, and the residue was purified by preparative TLC using 5% MeOH in $CHCl_3$ as developing solvent. Starting material 5 was recovered in 33 mg (54%). Compound 17 was obtained in 7 mg (11%) as a colorless solid: IR ($CHCl_3$) 3420 (NH), 1704 (CO) cm^{-1}; UV (MeOH) λ 220 (max), 254 (min), 263 (max) nm; ^{1}H NMR (CD_3COCD_3) δ 1.19 (t, J = 7 Hz, 3 H, Me), 1.35 (s, 3 H, Me), 1.50–1.53 (m, 6 H, pyran), 1.55 (s, 3 H, Me), 3.44–3.48 (m, 1 H, pyran), 3.60–3.70 (m, 1 H, H-5′), 3.74 (s, 3 H, NMe), 3.78–3.96 (m, 2 H, H-5′, pyran), 4.11 (q, J = 7 Hz, 2 H, CH_2), 4.25–4.29 (m, 1 H, H-4′), 4.63–4.66 (m, 1 H, pyran), 4.98, 5.03 (2dd, J = 6, 3 Hz, 1 H, H-3′), 5.17 (dd, J = 6, 3 Hz, 1 H, H-2′), 5.97, 5.98 (2d, J = 3 Hz, 1 H, H-1′), 6.29–6.48 (m, 2 H, NH_2), 7.47 (t, J = 8 Hz, 1 H, N=CH), 7.65, 7.68 (2s, 1 H, H-2), 7.79 (s, 1 H, H-10); ^{13}C NMR ($CDCl_3$) (aglycon moiety) δ 14.5 (Me), 37.6 (NMe), 59.8 (CH_2), 112.8 (C-4), 113.0 (C-9), 131.3, 131.7 (C-2), 139.1 (C-5), 139.5 (C-8), 140.2, 140.8 (C-10), 152.1, 152.2 (C=N), 162.5 (CO); MS m/z 518 (M^+), 262 (B + 1); high-resolution MS m/z 518.2457 (M^+, calcd $C_{24}H_{34}N_6O_7$, 518.2487).

Deamidation of 11 by Bromine. To a cold (0–5 °C) stirred solution of 11 (0.458 g, 1 mmol), $NaHCO_3$ (0.571 g, 6.8 mmol) and 0.025 M potassium phosphate buffer (pH 6.9) (30 mL) in AcOEt (40 mL) was added dropwise a solution of bromine (0.239 g, 4 mmol) in AcOEt (5 mL) over a period of 10 min, and the solution was stirred for 30 min at the same temperature. Saturated aqueous $NaHSO_3$ was added to the solution, and the excess bromine was quenched. The solution was adjusted to pH 7 with saturated aqueous $NaHCO_3$. The solution was extracted with AcOEt (2 × 20 mL), and the combined organic layers was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g) using 4% CH_3COCH_3 in $CHCl_3$ as eluent and the eluate was evaporated *in vacuo* to give 18 (0.449 g, 91%) as a colorless foam: UV (MeOH) λ max (ε) 231 (20200), 276 (5750, sh), 285 (6850), 313 (4980) nm; ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 3 H, Me), 1.41–1.53 (m, 6 H, pyran), 1.57 (s, 3 H, Me), 3.31–3.88 (m, 4 H, H-5′, pyran), 4.40–4.59 (m, 2 H, H-4′, pyran), 5.02 (dd, J = 6, 2 Hz, 1 H, H-3′), 5.43 (dd, J = 6, 2 Hz, 1 H, H-2′), 6.30 (d, J = 2 Hz, 1 H, H-1′), 8.35 (s, 1 H, H-8), 8.50 (s, 0.73 H, H-2), 8.54 (s, 0.27 H, H-2), 9.45 (s, 1 H, H-5); MS m/z 495 (M^+), 493 (M^+), 237 (B + 1), 239 (B + 1). Anal. Calcd for $C_{20}H_{24}N_5O_5Br$: C, 48.59; H, 4.89; N, 14.17; Br, 16.16. Found: C, 48.84; H, 4.91; N, 13.87; Br, 16.27.

9-Bromo-3-(β-D-ribofuranosyl)pyrazolo[3,2-*f*]purine (19). A solution of 18 (0.494 g, 1 mmol) in CF_3COOH–H_2O (9:1 v/v, 10 mL) was stirred for 15 min at room temperature. The solvent was evaporated *in vacuo* to give a solid, which was recrystallized from CH_3COCH_3–hexane to give 19 (0.277 g, 75%) as colorless needles: mp 195–197 °C dec; UV (MeOH) λ max (ε) 231 (20600), 276 (6500 sh), 285 (7360), 313 (5300) nm; ^{1}H NMR (DMSO-d_6–D_2O) δ 4.01 (dd, J = 7 , 5 Hz, 1 H, H-4′), 4.20 (t, J = 5 Hz, 1 H, H-3′), 4.56 (t, J = 5 Hz, 1 H, H-2′), 6.05 (d, J = 5 Hz, 1 H, H-1′), 8.32 (s, 1 H, H-8), 8.61 (s, 1 H, H-2), 9.48 (s, 1 H, H-5); MS m/z 369 (M^+), 371 (M^+), 237 (B + 1), 239 (B + 1). Anal. Calcd for $C_{12}H_{12}N_5O_4Br$: C, 38.98; H, 3.27; N, 18.92; Br, 21.59. Found: C, 39.42; H, 3.33; N, 18.79; Br, 21.22.

Hydrogenolysis of 18. A solution of 18 (0.494 g, 1 mmol) and Et_3N (0.285 g, 2 mmol) in EtOH–benzene (1:1 v/v, 20 mL) was hydrogenated over 5% Pd–C (0.250 g) for 3 h at room temperature. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*. The residue was passed through a short silica gel (30 g) column using 4% CH_3COCH_3–$CHCl_3$ as eluent, and the eluate was evaporated *in vacuo* to give 20 (0.395 g, 95%) as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 3H, Me), 1.40–1.51 (m, 6H, pyran), 1.57 (s, 3 H, Me), 3.52–3.88 (m, 4 H, H-5′, pyran), 4.39–4.53 (m, 2 H, H-4′, pyran), 5.03 (dd, J = 6 , 2 Hz, 1 H, H-3′), 5.43 (dd, J = 6 , 2 Hz, 1 H, H-2′), 6.56 (d, J = 2 Hz, 1 H, H-1′), 6.86 (dd, J = 2, 1 Hz, 1 H, H-9), 8.22 (d, J = 2 Hz, 1 H, H-8), 8.44, 8.49 (2s, 1 H, H-2), 9.43 (d, J = 1 Hz, 1 H, H-5); MS m/z 415 (M^+), 159 (B + 1). Anal. Calcd for $C_{20}H_{25}N_5O_5$: C, 57.82; H, 6.07; N, 16.86. Found: C, 58.01; H, 6.21; N, 16.53.

Bromination of 20. To a cold (0–5 °C), stirred solution of 20 (0.207 g, 0.5 mmol) and 0.025 M potassium phosphate buffer (pH 6.9, 10 mL) in AcOEt (10 mL) was added dropwise a solution of bromine (0.048 g, 0.015 mol) in AcOEt (10 mL). After being stirred for 5 min, a solution of $NaHCO_3$ (0.065 g, 0.75 mmol) in H_2O (1.5 mL) was added to the solution, followed by addition of a solution of bromine (0.079 g, 0.5 mmol) in AcOEt (1.5 mL). The mixture was stirred for 10 min, and the solution was diluted with AcOEt (10 mL), and the excess bromine was quenched by saturated aqueous $NaHSO_3$. The organic layer was washed with saturated $NaHCO_3$, H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was passed through a short column on silica gel (20 g), 4% CH_3COCH_3 in $CHCl_3$ as eluent, and the eluate was evaporated *in vacuo* to give 18 (0.240 g, 97%) as a colorless foam: The compound was identical (TLC, and ^{1}H NMR) with those of an authentic sample prepared from compound 11 and bromine.

3-β-D-Ribofuranosylpyrazolo[3,2-*i*]purine (2). A solution of **20** (0.415 g, 1 mmol) in CF_3COOH–H_2O (9:1, v/v, 10 mL) was stirred for 15 min at room temperature. The solvent was evaporated *in vacuo* to give a solid, which was recrystallized from MeOH–hexane to give **2** (0.210 g, 72%) as colorless needles: mp 211–213 °C dec; UV (MeOH) λ max (ε) 230 (26100), 258 (3700, sh), 268 (5200), 279 (6210), 307 (5970) nm; ^{1}H NMR (DMSO-d_6) δ 3.57–3.73 (m, 2 H, H-5′), 3.94–4.05 (m, 1 H, H-4′), 4.19 (dd, *J* = 11, 5 Hz, 1 H, H-3′), 4.58 (dd, *J* = 11, 5 Hz, 1 H, H-2′), 3.04 (t, *J* = 6 Hz, 1 H, OH), 5.21 (d, *J* = 5 Hz, 1 H, OH), 5.51 (d, *J* = 5 Hz, 1 H, OH), 6.04 (d, *J* = 5 Hz, 1 H, H-1′), 6.85 (dd, *J* = 2, 1 Hz, 1 H, H-9), 8.23 (d, *J* = 2 Hz, 1 H, H-8), 8.57 (s, 1 H, H-2), 9.42 (d, *J* = 1 Hz, 1 H, H-5); MS *m/z* 291 (M^+), 159 (B + 1). Anal. Calcd for $C_{12}H_{13}N_5O_4$: C, 49.48; H, 4.50; N, 24.05. Found: C, 49.62, H, 4.48; N, 24.34.

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