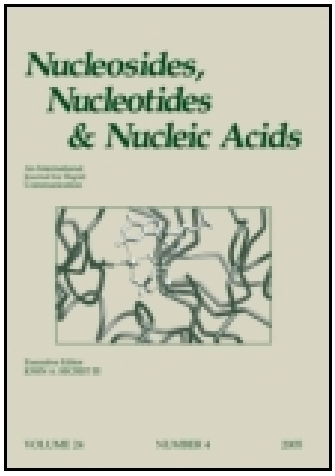

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Synthesis and Anti-HSV-1 Activity of 6 Substituted Pyrazolo[3,4-d]Pyridazin-7-one Nucleosides

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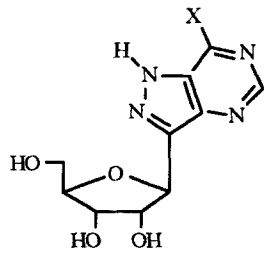
ABSTRACT: a new series of 6-substituted N^1- and N^2 -β-D-ribofuranosyl-pyrazolo[3,4-d]pyridazin-7(6H)-one derivatives (**8a-g, 9b-d** and **9f-g**) has been prepared in the aim to explore the structure-activity relationships and improve the antiviral activity of a series of N^1- and N^2 -β-D-ribofuranosyl-pyrazolo[4,3-d]pyrimidin-7(6H)-one derivatives previously reported by us. The studied compounds were preliminary tested for their *in vitro* antiviral activity against HSV-1. Among them **8f** and **9f** were found 2 fold more active than those belonging to the parent series.

Formycin A and B (**1**), two C-nucleoside antibiotics possessing the pyrazolo[4,3-d]pyrimidine ring system, rised considerable interest due to their broad spectrum of biological properties[1,2]. Synthetic efforts in this area have led both to sugar and base modified analogues[3,4] of **1.** Among the latter is a series of N^1-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7-one derivatives (**2**), whose synthesis has been previously reported from this laboratory[5]. The significant anti-herpes activity shown by some of these compounds together with the interesting biological properties of 2-azapurine analogues of natural nucleosides[6], have led us to design and synthesize new 5-aza-analogues of **2**, namely N^5- and N^6-β-D-ribofuranosylpyrazolo[4,3-d]1,2,3-triazin-4-ones (**3**)[7]. Continuing our studies, motivated by the isosteric modification of the pyrimidine ring of compounds **2**, we have investigated the structure-activity relationships (SAR) of compounds **2** and **3**. In this report, a new series of 6-substituted N^1- and N^2-β-D-ribofuranosylpyrazolo[3,4-d]pyridazin-7-one nucleosides were prepared and evaluated for their anti HSV-1 activity.

Chemistry

The synthesis of 1H-pyrazolo[3,4-d]pyridazin-7(6H)ones started from the 3-carboxyethyl pyrazole-4-carboxaldehyde **4**[8], which was treated with hydrazine or substituted hydrazines in



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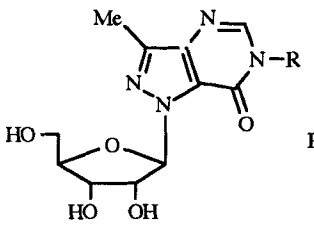

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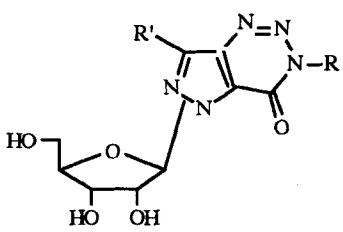


3 (N^5 and N^6 isomers)

Formycin A, X=NH_2 and B, X=OH

ethanol at reflux temperature. The following ring annulation with acetic acid gave the pyrazolo[3,4-d]pyridazin-7(6H)ones **5a-g**, with an improved yield when compared to the previously reported procedure[9] (SCHEME 1).

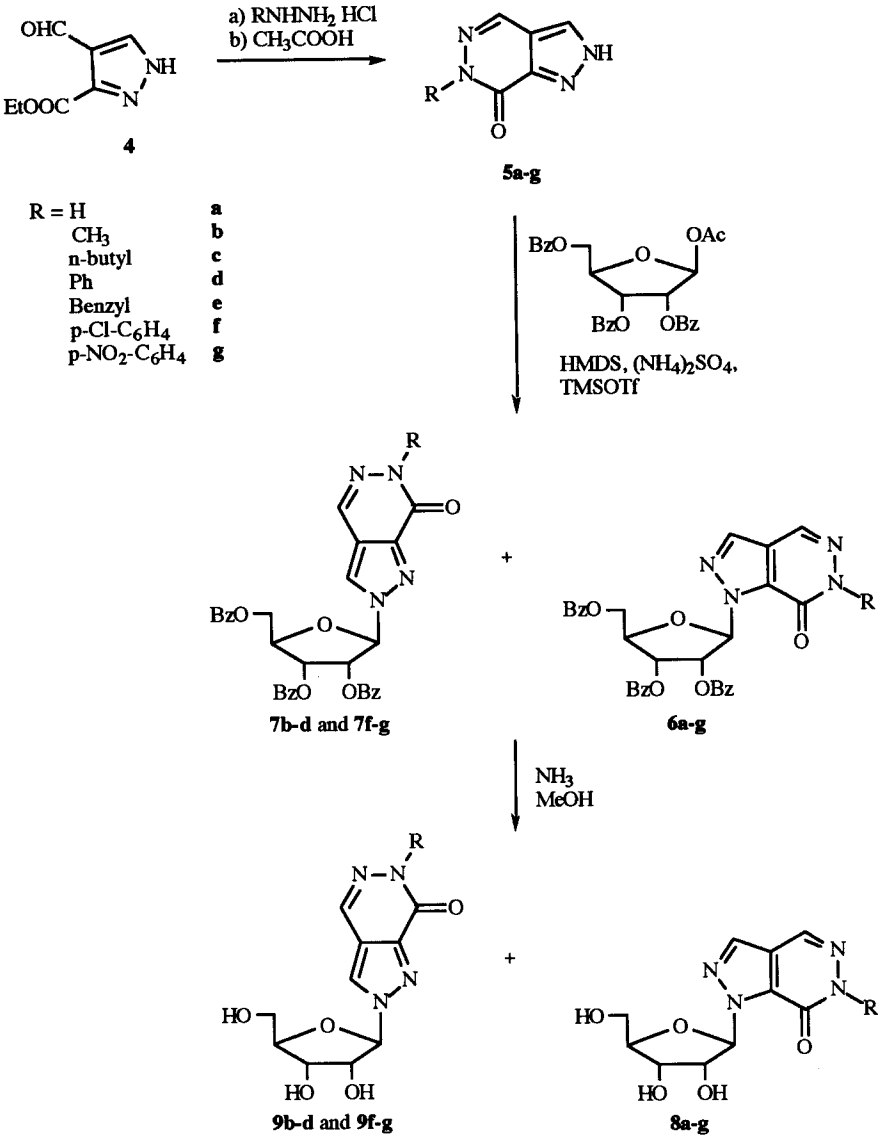
Compounds **5a-g** were glycosylated following the procedure previously reported by our group[7]: 6-substituted N^1- and N^2-β-D-ribofuranosylpyrazolo[3,4-d]pyridazin-7(6H)ones (**6a-g,7b-d**) and **7f-g** were obtained in good yields with only traces of the corresponding α-anomers. In the case of compounds **5a** and **5e**, glycosylation occurred only at N^1-atom giving **6a** and **6e**, respectively. After deprotection with methanolic ammonia, compounds **8a-g, 9b-d** and **9f-g** were obtained with an overall yield of 36-53% .

The regio and stereochemistry of glycosylation reactions were determined by NMR analysis in comparison with our previous data.[5,7] Thus, the N^1-β-anomeric protons appeared as a doublet centered at δ 6.5-7.1, with a pronounced downfield shift due to the close proximity of the anisotropic C_7 carbonyl group: the same effect has not been observed in the N^2 isomers **9b-d** and **9f-g** (δ 5.9-6.1). As expected, a downfield shift (about 0.24 ppm) was observed in the case of the corresponding α-anomers, whereas H1'-H2' coupling constants usually ranged about 5-7 Hz for α-anomers and 2-4 Hz for β ones[10,11,12].

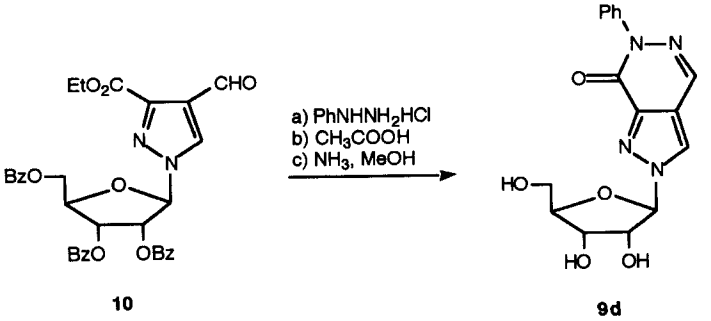
Moreover, in order to confirm the regiochemistry of the above prepared compounds, the 6-phenyl N^2-β-D-ribofuranosylpyrazolo[3,4-d]pyridazin-7(6H)one derivative **9d** was prepared by a different synthetic approach, involving cyclization of the suitable formyl-pyrazole-carboxylate nucleoside **10** with phenylhydrazine. Thus, ethyl 1-(β-D-2',3',5'-tri-O-benzoyl-ribofuranosyl)-4-formyl-pyrazole-3-carboxylate (**10**)[13] was converted into the corresponding 6-phenyl N^2-β-D-ribofuranosylpyrazolo[3,4-d]pyridazin-7(6H)one (**9d**) by reaction with phenylhydrazine hydrochloride and then acetic acid, as described above, with concomitant partial removal of the benzoyl protective groups. To complete the deprotection, the residue was treated with methanolic ammonia, following the general procedure described for derivatives **8a-g, 9b-d** and **9f-g** (SCHEME 2).

Antiviral Studies

The synthesized compounds were tested against herpes symplex type 1 (HSV-1). Preliminary *in vitro* assays indicated that these compounds were considerably less active than acyclovir



SCHEME 1



SCHEME 2

TABLE 1. Anti HSV-1 activity of the title compounds

Compound	ED50, μM
8f	25
9f	50
Acyclovir	0.1

ED_{50}: effective dose 50 was the compound concentration required to reduce by 50% the number of plaques. Plaque number in untreated cultures was 130. Compounds **8a-g**,**9b-d** and **9g** displayed an ED50 >100

(TABLE 1). However, in comparison with data obtained in previous studies for some corresponding pyrazolo[4,3-d]pyrimidin-7-one derivatives[5], in the case of compounds **8f** and **9f**, bearing a p-chloro phenyl substituent at position 6, a slight improvement in the activity (2-fold) was observed. Substitution of the halogen atom for a nitro group (compounds **8g** and **9g**) or an hydrogen atom abolished the activity.

In conclusion, in order to investigate the structure-activity relationships of ribofuranosylpyrazolo[4,3-d]pyrimidin-7-ones (**2**) and ribofuranosylpyrazolo[4,3-d]1,2,3-triazin-4-ones (**3**) we have synthesized a new series of isosteric analogs, namely the 6-substituted ribofuranosylpyrazolo[3,4-d]pyridazin-7(6H)ones **8a-g, 9b-d** and **9f-g**. In view of the preliminary biological results reported here it can be inferred that the substitution of the pyrimidine ring for the pyridazine ring in compounds **2**[5] has only marginal effects on the anti-HSV activity, whereas it substantially improved the activity of the corresponding triazine derivatives **3**.[7]

Experimental Section

All reactions were carried out under Argon atmosphere, unless otherwise stated. Standard syringe techniques were applied for tranferring dry solvents. Reaction courses were monitored on silica gel precoated F254 Merck plates with detection under 254 nm UV lamp and/or by spraying with 10% H_2SO_4/MeOH or 5% $KMnO_4/H_2O$ and heating. Nuclear magnetic resonance (^{1}H-NMR) spectra were determined for solution in $CDCl_3$-DMSO-*d6* on a Bruker AC-200 spectrometer and peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard. Melting points were determined on a Buchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded on a Jasco 510 spectrometer. Analytical HPLC were performed on a Waters 600E instrument on Rainin C_{18} (Dynamax 12μ) and Knauer silica gel (Eurospher 100, 5μ) columns. Column chromatographies were performed with Merck 60-200 mesh silica gel. Ambient temperature was 22-25°C. All drying operations were performed over anhydrous magnesium sulphate. Microanalysis, unless indicated, were in agreement with calculated values within ± 0.4 %.

General Procedure for the synthesis of 6-Aryl/alkyl 1H-Pyrazolo[3,4-d]Pyridazin 7 (6H) one. Derivatives (5a-g). To a solution of 3-carboxyethyl pyrazole-4-carboxaldehyde (3 mmol) in ethanol (20ml) was added in one portion the appropriate substituted-hydrazine hydrochloride (3.6 mmol). The resulting reaction mixture was heated at reflux condition for 5 h under an argon atmosphere. When TLC analysis (EtOAc/hexane) indicated that all of the starting material had disappeared, the reaction was cooled and concentrated under reduced pressure to give the corresponding hydrazone. This latter was dissolved in acetic acid (10 ml) and heated at reflux condition for 18 h. The mixture was then evaporated and the residue washed with 1M $NaHCO_3$ and water to give the 6-Aryl/alkyl 1H-Pyrazolo[3,4-d]Pyridazin 7 (6H) ones which were recrystallized from THF/hexane.

Pyrazolo[3,4-d]pyridazin-7(6H)one (5a): yield 69%; mp 270°C; ^{1}H-NMR(d6-DMSO): 8.16 (s, 1H), 8.35 (s, 1H), 12.7 (s, 1H), 14.6 (bs, 1H).

6-Methyl pyrazolo[3,4-d]pyridazin-7(6H)one (5b): yield 66%; mp 153-155°C; ^{1}H-NMR (d6-DMSO): 3.71 (s, 3H), 8.23 (s, 1H), 8.36 (s, 1H), 14.4 (bs, 1H).

6-Butyl pyrazolo[3,4-d]pyridazin-7(6H)one (5c): yield 69%; mp 171°C; ^{1}H-NMR (d6-DMSO): 0.88 (t, J=4.6 Hz, 3H), 1.29 (m, 2H), 1.68 (m, 2H), 4.12 (t, J=7.2 Hz, 2H), 8.22 (s, 1H), 8.38 (s, 1H), 14.6 (bs, 1H).

6-Phenyl pyrazolo[3,4-d]pyridazin-7(6H)one (5d): yield 63%; mp 250-255°C; ^{1}H-NMR (CDCl3): 7.44 (m, 5H), 8.29 (s, 1H), 8.47 (s, 1H), 14.4 (bs, 1H).

6-Benzyl pyrazolo[3,4-d]pyridazin-7(6H)one (5e): yield 65%; mp 211°C; ^{1}H-NMR (CDCl3): 5.52 (s, 2H), 7.32 (m, 5H), 8.07 (s, 1H), 8.31 (s, 1H), 14.1 (bs, 1H).

6-(p-Chloro)phenyl pyrazolo[3,4-d]pyridazin-7(6H)one (5f): yield 63%; mp 174°C; ^{1}H-NMR (d6-DMSO): 7.54 (d, J=9.2 Hz, 2H), 7.63 (d, J=8.2 Hz, 2H), 8.34 (s, 1H), 8.54 (s, 1H), 14.8 (bs, 1H).

6-(p-Nitro)phenyl pyrazolo[3,4-d]pyridazin-7(6H)one (5g): yield 58%; mp 192°C; ^{1}H-NMR (d6-DMSO): 2,5 (s, 3H), 7.30 (d, J=8.2 Hz, 2H), 7.43 (d, J=8.2 Hz, 2H), 8.31 (s, 1H), 8.50 (s, 1H), 14.8 (bs, 1H).

General Procedure for the preparation of 6-Aryl/alkyl 1H-Pyrazolo[3,4-d]Pyridazin 7 (6H) one glycosides 6a-g and 7b-g.

Method A. 1-*O* -Acetyl-2,3-5-tri-*O*-benzoyl-β-D-ribofuranose (0.76 g, 1.5 mmol), hexamethydisilazane (0.24 ml, 1.1 mmol) and a catalytic amount of ammonium sulfate were added, under positive argon pressure, to a suspension of the appropriate heterocycle (**5a-g**, 1 mmol) in 10 ml of freshly distilled dry acetonitrile. The mixture was heated at reflux conditions until complete dissolution was obtained. Then, trimethylsilyl chloride (0.15 ml, 1.2 mmol) and trifluoromethanesulfonic acid (0.21 ml, 2.4 ml) were added. After 3 h the reaction was cooled to room temperature, diluted with dichloromethane (10 ml) and extracted with a saturated aqueous $NaHCO_3$ solution (5 ml). The aqueous phase was then extracted with dichloromethane (2 x 10 ml), and the combined organic phases were washed with saturated NaCl/H_2O solution, dried (Na_2SO_4) and evaporated to dryness. The residue was eluted on a silica gel short column

(EtOAc/hexane as eluent) to be quickly purified from the lower moving unreacted heterocycles and to separe eventual N2-isomers. Evaporation of the collected fractions gave the benzoylated product which was used in the next step without any further purification.

General Procedure for Deprotection of Derivatives 6a-g and 7b-g. The protected compounds were dissolved at 4°C in 50 ml of methanolic ammonia (saturated at -15°C) and let to stirred at 4°C for 24 h. After this time, TLC (hexane/EtOAc, 7/3) indicated complete reaction and the mixture was evaporated to dryness. The residues were triturated with ether (3 x 50 ml) to give white microcrystalline products which were recrystallized from methanol/Et_2O.

N1-β-D-Ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (8a): yield 32%; mp 212°C; UV (MeOH): λ_{max} (ε) 276 (8.11), λ_{min} 241 (4.65). ^{1}H-NMR (d6-DMSO): 3.42 (m, 1H), 3.54 (m, 1H), 3.92 (m, 1H), 4.24 (m, 1H), 4.53 (m, 1H), 4.68 (t, J= 5.2 Hz, 1H), 5.13 (d, J=5.6 Hz, 1H), 5.41 (d, J=5.6 Hz, 1H), 6.70 (d, J= 3.8 Hz, 1H), 8.27 (s, 1H), 8.38 (s, 1H), 12.9 (s, 1H). Anal Calcd for $C_{10}H_{12}N_4O_5$: C, 44.76; H, 4.51; N, 20.89. Found C, 44.31; H, 4.16; N, 20.57.

6-Methyl-N1-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (8b): yield 32%; mp 231°C; UV (MeOH): λ_{max} (ε.) 267 (6.71), λ_{min} 242 (3.33). ^{1}H-NMR (d6-DMSO): 3.38 (m, 1H), 3.55 (m, 1H), 3.73 (s, 3H), 3.90 (m, 1H), 4.24 (m, 1H), 4.51 (m, 1H), 4.72 (t, J= 5.2 Hz, 1H), 5.22 (d, J=5.2 Hz, 1H), 5.46 (d, J=5.2 Hz, 1H), 6.74 (d, J=3.8 Hz, 1H), 8.27 (s, 1H), 8.40 (s, 1H). Anal Calcd for $C_{11}H_{14}N_4O_5$: C, 46.79; H, 5.00; N, 19.86. Found: C, 46.61; H, 4.68; N, 19.55.

6-Methyl-N2-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (9b): yield 32%; mp 264°C; UV (MeOH): λ_{max} (ε) 208 (7.64), λ_{min} 233 (1.24). ^{1}H-NMR (d6-DMSO): 3.43 (m, 1H), 3.55 (m, 1H), 3.57 (s, 3H), 4.01 (m, 1H), 4.14 (m, 1H), 4.34 (m, 1H), 5.03 (t, J= 5.2 Hz, 1H), 5.23 (d, J=5.4 Hz, 1H), 5.64 (d, J=5.4 Hz, 1H), 5.96 (d, J= 3.4 Hz, 1H), 8.33 (s, 1H), 8.77 (s, 1H). Anal Calcd for $C_{11}H_{14}N_4O_5$: C, 46.79; H, 5.00; N, 19.86. Found: C, 46.66; H, 4.72; N, 19.65.

6-*n* Butyl-N1-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (8c): yield 32%; mp 151°C; UV (MeOH): λ_{max} (ε) 266 (7.69), λ_{min} 240 (3.42). ^{1}H-NMR ($CDCl_3$): 1.0 (t, J=7.2 Hz, 3H), 1.45 (m, 2H), 1.90 (m, 2H), 3.73 (m, 1H), 3.79 (m, 1H), 3.93 (m, 1H), 4.02 (m, 1H), 4.18 (m, 2H), 4.24 (m, 1H), 4.30 (t, J= 5.2 Hz, 1H), 4.67 (d, J=5.4 Hz, 1H), 4.79 (d, J=5.4 Hz, 1H), 7.05 (d, J= 3.8 Hz, 1H), 8.02 (s, 1H), 8.26 (s, 1H). Anal Calcd for $C_{14}H_{20}N_4O_5$: C, 51.83; H, 6.22; N, 17.28. Found: C, 51.68; H, 5.89; N, 17.01.

As representative example, the data for the α-anomeric proton are reported **8c** (α: δ 7.34, J = 5.0 Hz).

6-*n* Butyl-N2-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (9c): yield 32%; mp 154-156°C; UV (MeOH): λ_{max} (ε) 268 (8.44) 209 (26.4), λ_{min} 242 (3.68). ^{1}H-NMR ($CDCl_3$): 0.92 (t, J=7.2 Hz, 3H), 1.33 (m, 2H), 1.76 (m, 2H), 3.73 (m, 1H), 3.79 (m, 1H), 3.93 (m, 1H), 4.02 (m, 1H), 4.18 (m, 2H), 4.24 (m, 1H), 4.30 (t, J= 5.2 Hz, 1H), 4.62 (d, J=5.4 Hz, 1H), 4.73 (d, J=5.4 Hz, 1H), 5.96 (d, J= 3.4 Hz, 1H), 7.99 (s, 1H), 8.2 (s, 1H).

Anal Calcd for $C_{14}H_{20}N_4O_5$: C, 51.83; H, 6.22; N, 17.28. Found: C, 51.71; H, 6.04; N, 17.09.

As representative example, the data for the α-anomeric proton are reported **9c** (α: δ 6.23, J = 5.2 Hz).

6-Phenyl-N1-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (8d): yield 32%; mp 196°C; UV (MeOH): λ_{max} (ε) 288 (6.47) 267 (6.58), λ_{min} 278 (6.42) 251 (5.7). ^{1}H-NMR (d6-DMSO): 3.42 (m, 1H), 3.58 (m, 1H), 3.92 (m, 1H), 4.26 (m, 1H), 4.55 (m, 1H), 4.75 (t, J= 5.8 Hz, 1H), 5.21 (d, J=5.6 Hz, 1H), 5.50 (d, J=5.6 Hz, 1H), 6.74 (d, J= 3.6 Hz, 1H), 7.50 (m, 5H), 8.35 (s, 1H), 8.56 (s, 1H). Anal Calcd for $C_{16}H_{16}N_4O_5$: C, 55.80; H, 4.69; N, 16.28. Found: C, 55.5; H, 4.37; N, 16.02.

6-Phenyl-N2-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (9d): yield 32%; mp 255°C; UV (MeOH): λ_{max} (ε) 210 (26.10), λ_{min} 255 (8.4) ^{1}H-NMR (d6-DMSO): 3.59 (m, 1H), 3.72 (m, 1H), 4.01 (m, 1H), 4.14 (m, 1H), 4.39 (m, 1H), 5.07 (t, J= 5.4 Hz, 1H), 5.27 (d, J=4.4 Hz, 1H), 5.71 (d, J=5.4 Hz, 1H), 6.0 (d, J= 3.4 Hz, 1H), 7.41 (m, 5H), 8.50 (s, 1H), 8.85 (s, 1H). Anal Calcd for $C_{16}H_{16}N_4O_5$: C, 55.80; H, 4.69; N, 16.28. Found: C, 55.46; H, 4.31; N, 15.89.

6-Benzyl-N1-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (8e): yield 76%; mp 153-155°C; UV (MeOH): λ_{max} (ε) 265 (7.5), λ_{min} 241 (3.87). ^{1}H-NMR (d6-DMSO): 3.39 (m, 1H), 3.53 (m, 1H), 3.89 (m, 1H), 4.24 (m, 1H), 4.53 (m, 1H), 4.71 (t, J= 6.6 Hz, 1H), 5.17 (d, J=5.6 Hz, 1H), 5.35 (s, 2H), 5.46 (d, J=5.6 Hz, 1H), 6.74 (d, J= 3.8 Hz, 1H), 7.30 (m, 5H), 8.29 (s, 1H), 8.46 (s, 1H). Anal Calcd for $C_{17}H_{18}N_4O_5$: C, 56.96; H, 5.07; N, 15.64. Found: C, 56.58; H, 4.77; N, 15.32.

6-(p-Chloro)phenyl-N1-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (8f): yield 34%; mp 183-185°C; UV (MeOH): λ_{max} (ε) 213 (25.3) 264 (6.95), λ_{min} 241 (3.16) ^{1}H-NMR (d6-DMSO): 3.41 (m, 1H), 3.55 (m, 1H), 3.92 (m, 1H), 4.26 (m, 1H), 4.52 (m, 1H), 4.74 (t, J= 6.6 Hz, 1H), 5.30 (d, J=5.6 Hz, 1H), 5.56 (d, J=5.6 Hz, 1H), 6.72 (d, J= 3.4 Hz, 1H), 7.6 (m, 4H), 8.35 (s, 1H), 8.57 (s, 1H). Anal Calcd for $C_{16}H_{15}N_4O_5Cl$: C, 50.78; H, 4; N, 14.82; Cl, 9.25. Found: C, 50.42; H, 3.61; N, 14.62; Cl, 9.01.

6-(p-Chloro)phenyl-N2-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (9f): yield 35%; mp 225-229°C; UV (MeOH): λ_{max} (ε) 212 (23), λ_{min} 228 (16.8) ^{1}H-NMR (d6-DMSO): 3.34 (m, 1H), 3.69 (m, 1H), 4.0 (m, 1H), 4.17 (m, 1H), 4.37 (m, 1H), 5.07 (m, 1H), 5.30 (m, 1H), 5.72 (m, 1H), 6.0 (d, J= 3.2 Hz, 1H), 7.57 (m, 4H), 8.51 (s, 1H), 8.85 (s, 1H). Anal Calcd for $C_{16}H_{15}N_4O_5Cl$: C, 50.78; H, 4; N, 14.82; Cl, 9.25. Found: C, 50.38; H, 3.55; N, 14.54; Cl, 8.95.

6-(p-Nitro)phenyl-N1-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (8g): yield 34%; mp 185°C; UV (MeOH): λ_{max} (ε) 315 (13.7) 205 (28.5), λ_{min} 289 (10.3) ^{1}H-NMR (d6-DMSO): 3.44 (m, 1H), 3.55 (m, 1H), 3.92 (m, 1H), 4.26 (m, 1H), 4.55 (m, 1H), 4.74 (t, J= 6.6 Hz, 1H), 5.22 (d, J=5.8 Hz, 1H), 5.50 (d, J=5.8 Hz, 1H), 6.72 (d, J= 3.6 Hz, 1H), 7.92 (d, J= 9 Hz, 2H), 8.36 (d, J=9 Hz, 2H), 8.40 (s, 1H), 8.65 (s, 1H). Anal Calcd for $C_{16}H_{15}N_5O_7$: C, 49.25; H, 3.89; N, 17.99. Found: C, 48.91; H, 3.52; N, 17.69.

6-(p-Nitro)phenyl-N2-β-D-ribofuranosyl pyrazolo[3,4-d]pyridazin-7(6H)one (9g): yield 35%; mp 221-222°C; UV (MeOH): λ_{max} (ε) 281 (8.83) 208 (15.1), λ_{min} 255 (6.27) ^{1}H-NMR (d6-DMSO): 3.59 (m, 1H), 3.63 (m, 1H), 4.02 (m, 1H), 4.16 (m, 1H), 4.37 (m, 1H), 5.08 (t, J= 6.6 Hz, 1H), 5.31 (d, J=5.8 Hz, 1H), 5.82 (d, J=5.8 Hz, 1H), 6.08 (d, J= 3.4 Hz, 1H), 7.91 (d, J=7 Hz, 2H), 8.37 (d, J=7 Hz, 2H), 8.60 (s, 1H), 8.88 (s, 1H). Anal Calcd for $C_{16}H_{15}N_5O_7$: C, 49.25; H, 3.89; N, 17.99. Found: C, 48.96; H, 3.59; N, 17.74.

Method B. In the case of 6-phenyl N^2-β-D-ribofuranosylpyrazolo[3,4-d]pyridazin-7(6H)one (**9d**) an alternative procedure has been developed in order to confirm the regiochemistry of the glycosylation reaction. To a solution of 613 mg (1 mmol) of 1-(β-D-2',3',5'-tri-O-benzoyl-ribofuranosyl)-4-formyl-pyrazole-3-carboxylate (**10**) in anhydrous ethanol (5 ml) was added phenylhydrazine hydrochloride 174 mg (1.2 mmol) and the resulting mixture was heated at reflux condition overnight. The reaction mixture was then cooled and concentrated in vacuo to give the corresponding hydrazone which was dissolved in glacial acetic acid (3 ml) and heated at reflux condition for 4 h (TLC hexane/EtOAc, 7/3). The mixture was then evaporated to dryness and the residue dissolved in EtOAc washed with 1M $NaHCO_3$ (2 x 10 ml) and water (1 x 10 ml). The organic phase was dried (Na_2SO_4) and evaporated to dryness. Since TLC analysis (hexane/EtOAc, 7/3) indicated incomplete deprotection, the residue was poured overnight in methanolic ammonia, following the general procedure for the deprotection of derivatives **8a-g, 9b-d** and **9f-g**. After purification,the product was obtained as a white solid (247 mg, 72% yield). Its analytical data were consistent with those reported for compound **9d**, obtained by route A.

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