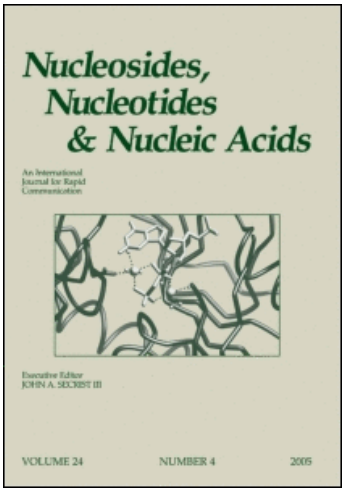

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SYNTHESIS OF PYRAZOLO[4,3-*c*]PYRIDINE C-RIBONUCLEOSIDES *VIA* AN EFFECTIVE TETRAZOLE TO PYRAZOLE TRANSFORMATION

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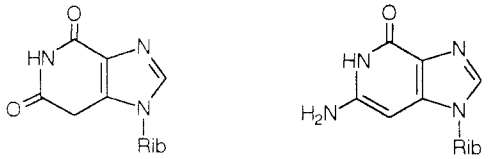
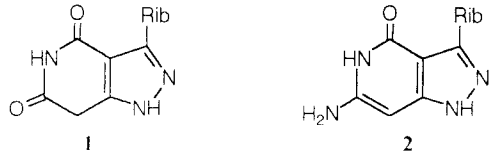
Abstract: Methyl 2-[4-methoxycarbonyl-5-(β-D-ribofuranosyl)-1H-pyrazolyl-3]-acetate (**7a**) obtained from ribofuranosyltetrazole **3** by conjugative addition to dimethyl 1,3-allenedicarboxylate or dimethyl 3-chloro-2-pentenedioate after electrocyclization of 2-propenyltetrazole **5** was used as a suitable intermediate to provide pyrazolo [4,3-*c*]pyridine C-ribonucleosides **1** and **2** related to 7-substituted 3-deazaxanthosine and -guanosine analogs.

Introduction: Recently, the expansion of the genetic alphabet as proposed by Benner *et al.*[1] began to deal with the strategy of design and synthesis of modified bases that can be incorporated by DNA and RNA polymerases. Several of the proposed nonnatural bases possess a carbon-carbon bond joining the ribose ring and incorporation of such functionalized bases in RNA should provide RNA molecules with the potential for an increase in catalytic power.[1] Furthermore, an old observation of the existence of triple helical DNA, as now documented for synthetic oligomers,[2] has shown the therapeutic significance of such triple helix forming oligonucleotides by blocking transcription of viral proteins.[3]

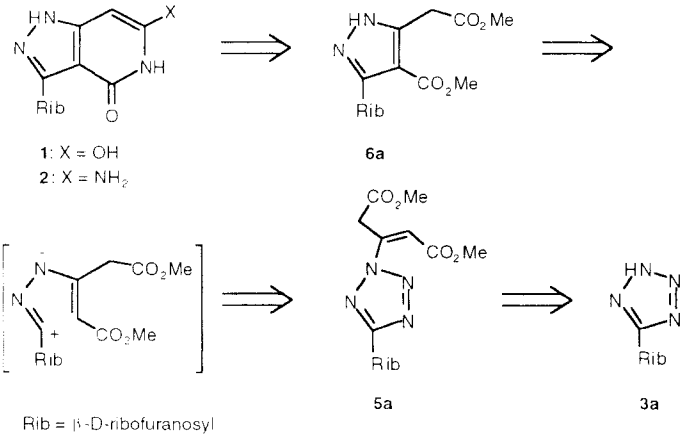
This paper reports on chemical synthesis of 3-(β-D-ribofuranosyl)-1H-pyrazolo[4,3-*c*]pyridine-4,6(5H,7H)-dione (**1**) and -6-amino-1H-pyrazolo[4,3-*c*]pyridine-4(5H)-one (**2**), related to 7-substituted 3-deazaxanthosine and -guanosine analogs, respectively. Both target compounds **1** and **2** are likely to be capable of forming three hydrogen bonds. In addition, **2** matches pyrazolopyridine analog **P1** [1-(2-deoxy-β-D-ribofuranosyl)-3-methyl-5-amino-1H-pyrazolo[4,3-*c*]pyrimidine-7(6H)-one], which can form a stable triade with Watson-Creek GC pair, as reported by Dervan *et al.*[4]





Rib = β-D-ribofuranosyl

Retrosynthetic analysis suggested that the synthesis of **1** and **2** might be realized by the cyclization of appropriate glycosyl pyrazole precursor **6a** and outlines the design *via* stereocontrolled rearrangement of tetrazolyl-2 acrylate **5a** prepared by regioselective conjugative addition of tetrazole **3a**.



Rib = β-D-ribofuranosyl

We reported the synthesis of such substituted pyrazole **6** at an earlier date.[5] Since that report the small-scale procedure was subjected to certain modifications on a semi-synthetic scale (0.05 mole).

Results and Discussion.

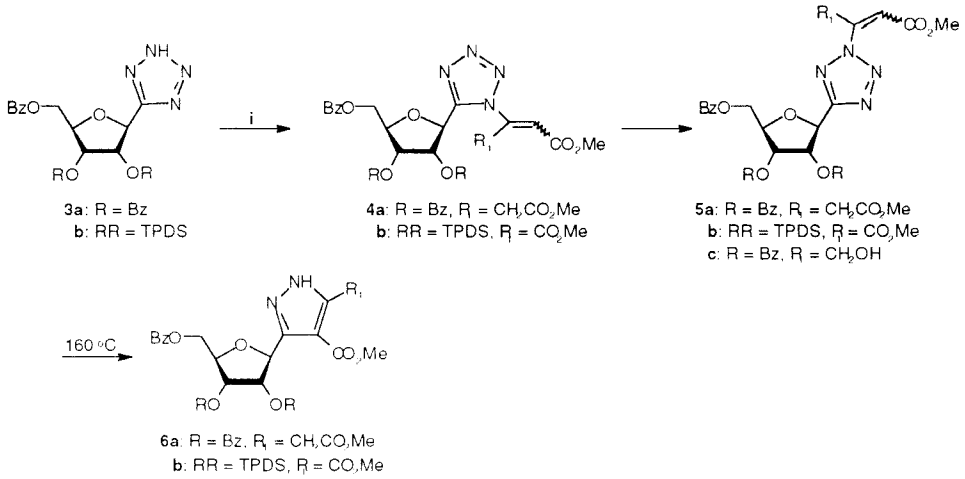
Preparation of 5-Ribofuranosylpyrazoles. Various attempts were made to effect conjugative addition of 5-substituted tetrazoles (Me, COOMe, Ph) to activated alkynes and alkenes, but largely without success.[6] Instead, lithiation of 2-alkyltetrazoles was

developed as a new synthetic route to 2-alkenyltetrazoles.[7] In their thorough studies Moody *et al.*[6] reported on thermal decomposition of the appropriate 2-alkenyltetrazoles as a general route to 3-phenyl-1H-pyrazoles, while no pyrazoles could be obtained from tetrazolyl-2 acrylates.[6] In contrast, we have communicated at the same time on thermolysis of 2-(1,3-dimethoxycarbonyl-1-propen-2-yl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole (**5a**) yielding the designed pyrazole **6a**.[5] **5a** was obtained from protected 5-(β-D-ribofuranosyl)-1H-tetrazole **3a** by conjugative addition to dimethyl 1,3-allenedicarboxylate (DMAL) or dimethyl 3-chloro-2-pentenedioate (DMCP) (**Scheme 1**). DMCP provided in DMF at 120°C a mixture of N-1 and N-2 alkenyltetrazoles **4a** and **5a** in an 1/6 ratio, whereas only N-2 propenyltetrazole **5a** was isolated with DMAL in benzene below 7°C. Since thermolysis affected **5a** only, the resulting glycosylpyrazole **6a** was easily separated from unchanged **4a** by filtration after treatment of the mixture of regioisomers **4a** and **5a** in hot xylene. Transformations of **5a** to **6a** were performed in quantitative yields. Schneller used DMAL for 1,3-dipolar cycloaddition to diazosugar thus obtaining a positional isomer to **6a**, *i.e.* methyl 2-[3-methoxycarbonyl-5-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-1H-pyrazolyl-4]-acetate.[8]

Additionally, dimethyl acetylenedicarboxylate (DMAD) and methyl 4-hydroxy-2-butynoate[9] (MHB) seemed to be reasonable α,β-unsaturated carbonyl compounds for use in the conjugative addition of 5-ribofuranosyl tetrazoles **3a-b**. DMAD afforded with **3b** in toluene at room temperature an equimolar mixture of N-1 and N-2 substituted terazoles **4b** and **5b** (which we were not able to separate by column chromatography), whereas only N-2 isomer **5c** was formed from **3a** with neat MHB[10] at 4°C (**Scheme 1**). Further treatment of the **4a**/**5b** mixture in hot xylene, followed by separation on silica gel, afforded unchanged **4b** and pyrazolyldicarboxylate **6b**. Farkas and Sorm prepared the 2',3',5'-tri-*O*-benzyl analog of **6b** *i.e.* dimethyl 2-[5-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-1H-pyrazol-3,4-diyl]di-carboxylate by a multi-step synthesis from allononitrile *via* the diazosugar with DMAD.[11]

Thermolysis of N-2 hydroxypropenyltetrazole **5c** did not provide the corresponding ribofuranosylpyrazole.

Cyclization of 5-Ribofuranosylpyrazoles 7. Deprotection of **6a** with methanolic sodium methylate at ambient temperature afforded methyl 2-[5-(β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]-acetate (**7a**). With **7a** in hand the synthesis of the 3-deazaxanthosine analog was realized in a straightforward manner. Diester **7a** was ammoniolyzed quantitatively at ambient temperature in two days to give monoamide **8a**, which was after treatment with sodium methylate in boiling methanol readily closed to pyrazolo[4,3-*c*]pyridine **1** (**Scheme 2**), similar to the cyclization of 3-deazaxanthine from 2-(4-methoxycarbonyl-1H-imidazolyl-3)-acetamide.[12] The position of the amide function



3a: R = Bz
b: RR = TPDS

4a: R = Bz, R_1 = CH_2CO_2Me
b: RR = TPDS, R_1 = CO_2Me

5a: R = Bz, R_1 = CH_2CO_2Me
b: RR = TPDS, R_1 = CO_2Me
c: R = Bz, R_1 = CH_2OH

6a: R = Bz, R_1 = CH_2CO_2Me
b: RR = TPDS, R_1 = CO_2Me

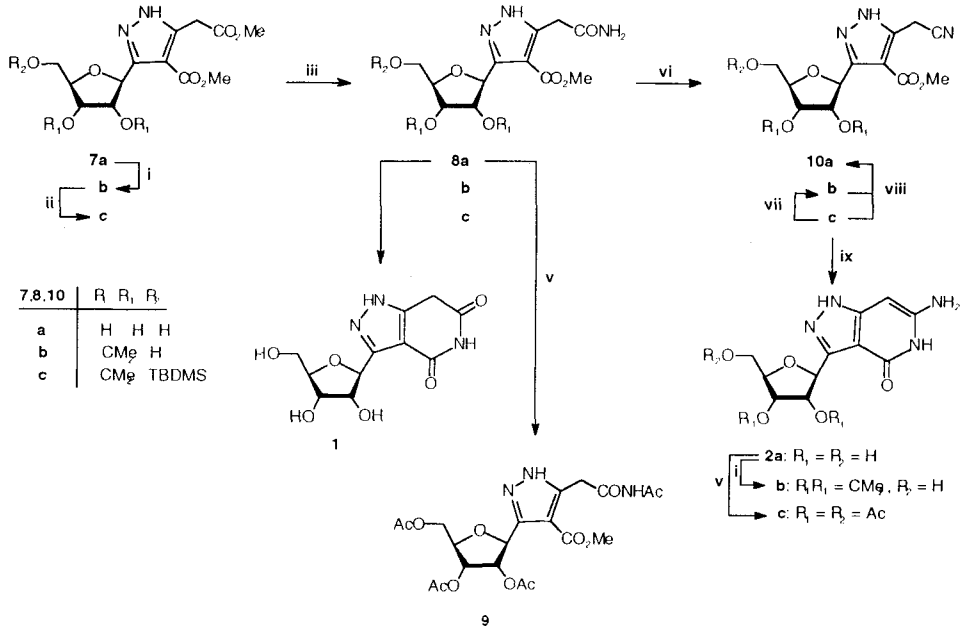
Reagents and conditions i	Ratio of isomers[a]	Yield[b]
3a + 1.1eq.DMAL / benzene / 7°C / 14h	**5a** = 100	62
3a + 1.0eq.DMCP / DMF / 120°C / 2h	**5a** : **5b** = 85 : 15	62
3b + 2.5eq.DMAD / toluene / rt / 3d	**5b** : 4b = 50 : 50	40
3a + 2.6 eq.MHB / 4°C / 5d	**5c** = 100	55

[a]Determined from ^1H spectra of crude reaction mixtures.
[b]Total yield after chromatographic purification.

SCHEME 1

in monoamide **8a** was expected due to the known greater reactivity of an aliphatic ester over aromatic one, and confirmed on the basis of IR spectra in comparison to literature data.[12] ^{13}C-NMR signals at 31.4, 161.7 and 170.6 ppm for C-7, C-4 and C-6 and two sharp CO bands at 1702 and 1682 cm^{-1} confirm the diketo form of **1**.

The synthesis of 3-deazaguanosine analog **2** was realized by the method of Robins *et al.* developed for the preparation of condensed aminopyridone systems *via* an acetonitrile intermediate.[13] To facilitate further transformations, monoamide **8a** was converted into a more organic soluble form. Acetylation with acetic anhydride and triethylamine in acetonitrile in the presence of a catalytic amount of DMAP[14] provided tetraacetyl derivative **9**, whereas isopropylidenation in acetone with iodine as a catalyst[15] afforded diisopropylidene derivative, characterized as $C_{18}H_{25}N_3O_7$ by HRMS.



SCHEME 2: *Reagents and conditions*: **i**, J_2, acetone, rt, 4-12h, 70-99%; **ii**, TBDMS-triflate, Et_3N, $(CH_2Cl)_2$, rt, 7h, 91%; **iii**, 27% NH_3/MeOH, rt, 1-2d, 100%; **iv**, 1. 1M MeONa/MeOH, Δ, 1h; 2. CH_3COOH, 61%; **v**, Ac_2O, DMAP, Et_3N, MeCN, rt, 2h, 35-58% (after FC); **vi**, TFAA, py, THF, 5 °C, 2h, then rt ovnt, 53-63%; **vii**, 27% NH_3/MeOH, 60 °C, 4h, 80%; **viii**, Dowex H+, MeOH, rt, ovnt, 95%; **ix**, liq.NH_3, 130-150 °C, 3h, 63-90%.

Alternatively, by protecting the sugar moiety before ammoniolysis, diester **7a** was transformed with iodine in acetone at ambient temperature into 2',3'-*O*-isopropylidene derivative **7b** which was ammoniolyzed quantitatively into monoamide **8b**. Dehydration of **8b** by trifluoroacetic anhydride (TFAA) and pyridine in THF[16] provided pyrazolylacetonitrile **10b** with a specific sharp band at 2263 cm^{-1} in the IR spectrum. Treatment of **10b** with liquid ammonia at 130°C did not yield the desired 3-deazaguanosine analog **2b**. We anticipated that the unprotected 5'-OH group has unfavorably influenced the final ring closure, and further protection with *tert*-butyldimethylsilyl (TBDMS) group was considered. No reaction of **10b** with TBDMS-chloride in pyridine was observed. Since the protection with much more reactive corresponding trifluoromethylsulfonate (triflate) was not possible due to its known reactivity towards CN group,[17] the overall synthetic pathway was started at the step of diester **7b**. With TBDMS-triflate, **7b** was converted into 5'-*O*-TBDMS pyrazolylacetate **7c**. The latter was, after one-day ammoniolysis and subsequent dehydration of

monoamide **8c**, transformed into the corresponding acetonitrile **10c**, similar to the conversion of 5'-*O*-nonsubstituted acetate **7b** to acetonitrile **10b**. The ring closure of acetonitrile **10c** and deprotection of 5'-O in liquid ammonia at 130°C afforded 6-amino-3-(2,3-*O*-isopropyl-idene-β-D-ribofuranosyl)-1H-pyrazolo[4,3-*c*]pyridine-4(5H)-one (**2b**) which was isolated by extraction with water in 62% yield as a fluffy material (**Scheme 2**). ^{1}H-NMR data of the aglycone part of the molecule (with δ 5.43, 5.72 and 10.15 belonging to H-7, NH_2 and NH, respectively) were in an excellent agreement with those of 7-amino-1H-pyrazolo[4,3-*c*]pyridine-4(5H)-one (δ 5.41, 5.78 and 10.5).[18] Five aromatic resonances at δ 102.37, 147.34, 149.21, 149.66 and 157.97 were found in the ^{13}C-NMR spectrum in addition to 68.95 ppm which correlated with H-7. A bathochromic shift of **2b** (λ_{max} 286 nm in MeOH) in comparison to **10b** (λ_{max} 250 nm in MeOH) was observed in the UV spectrum. When **10c** was treated with methanolic ammonia at 60°C only deprotection of the 5'-O, furnishing **10b**, was observed.

Acetonitriles **10b** and **10c** were fully deprotected with Dowex H$^+$ in MeOH. The resulting **10a** was readily closed in liquid ammonia at 150°C to pyrazolo[4,3-*c*]pyridine C-ribonucleoside **2a**. It underwent isopropylidenation to give **2b** identical (TLC, IR, ^{1}H- and ^{13}C-NMR) to pyrazolo[4,3-*c*]pyridine 2',3'-*O*-isopropylidene ribofuranoside **2b** obtained by the ring closure of **10c**. Acetylation of **2a** (Ac_2O, Et_3N, DMAP)[14] provided the 2',3',5'-tri-*O*-acetyl derivative **2c**.

Additional proof for **2a-b** was obtained from HRMS of their derivatized analogs. Microscale transformation of **2a** and **2b** with bis(trimethylsilyl)acetamide, TMSCl and pyridine[19] provided hexa- and tetra(trimethylsilyl) derivatives, respectively. By the use of GCMS technique, mass spectra with the two most intensive peaks at m/z 714(M$^+$, 100) and 395(B+30, 84), and 610(M$^+$, 32) and 395(B+30, 100), respectively, were obtained.

EXPERIMENTAL SECTION

General. Materials and methods. Toluene and pyridine were dried by refluxing over KOH and BaO, respectively and then distilled; benzene and xylene were dried over $CaCl_2$ and then distilled; DMF was dried over BaO and distilled under reduced pressure. MeCN was distilled over CaH_2, THF over $LiAlH_4$. Ac_2O and TFAA were distilled prior to use. Ion exchanger Dowex H$^+$ (50WX8, Fluka) was washed with MeOH prior to use. Other solvents and reagents were of commercial purity. Evaporations were conducted with rotary evaporator under reduced pressure. Flash chromatography was carried out on Silica gel 60 (40-63 μm, Merck) and analytical TLC on precoated plates Silica gel 60 F_{254} (Merck). The spots were visualized by irradiation with UV light (254 nm) or by spraying with either 3.5% phosphomolibdic acid in EtOH or sat. aq. $(NH_4)_2SO_4$ and heating.

Melting points were determined on Kofler apparatus and are uncorrected. IR spectra were obtained using Bio-Rad FTS 15/80 spectrophotometer. Mass spectra were recorded on VG Autospec Q spectrophotometer at Jozef Stefan Institute, Ljubljana. Microanalyses were performed at Department of Chemistry, University of Ljubljana and at Department of Chemistry, Karl-Franz University, Graz. Optical rotations were measured on Perkin Elmer 241 MC polarimeter. NMR spectra were recorded using Varian VXR-300 instrument (^{1}H at 299.94 and ^{13}C at 75.43 MHz). Me_4Si and solvents were used as internal references for ^{1}H and ^{13}C ($CDCl_3$, 77.00 ppm; Me_2SO-d_6, 39.50 ppm; Me_2CO-d_6, δ_{CD3} 29.80) measurements, respectively. Chemical shifts and coupling constants were obtained from the first order analysis of the spectra. The spectra were assigned by means oh the corresponding ^{1}H-^{1}H and ^{13}C-^{1}H chemical shift correlated spectra.

2-(1,3-Dimethoxycarbonyl-1-propen-2-yl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole (5a). To an ice cold mixture of **3a**[21] (26.72 g, 52 mmol) in benzene (250 ml) DMAL[20] (8.9 g, 57 mmol) was added dropwise at such a rate that the temperature of the mixture was kept bellow 7°C. After an overnight stirring, the ice bath was removed and the mixture allowed to warm to rt and evaporated. The residue was dissolved in EtOAc (500 ml) and washed with 1N aq. HCl (2 x 100 ml), water and brine and dried (Na_2SO_4). Chromatography on silica gel (450 g, CH_2Cl_2) with CH_2Cl_2 (5 l) yielded **5a** (21.4 g, 62%) as a white crisp foam. ^{1}H NMR ($CDCl_3$): δ 3.68, 3.80(2s, 6H, 2Me), 4.56(d, 2H, C<u>H</u>$_2$COOMe; J_{gem} = 2.0 Hz), 4.64(m, 1H, H-5'), 4.81(m, 1H, H-5"), 4.83(m, 1H, H-4'), 5.71(d, 1H, H-1'), 6.05(t, 1H, H-3'), 6.16(t, 1H, H-2'), 7.06(s, 1H, CH=C), 7.39, 7.54, 8.04(3m, 15H, 3Ph); $J_{1'2'}$ = 5.2 Hz. ^{13}C ($CDCl_3$): δ 33.57 (<u>C</u>H$_2$COOMe), 52.13, 52.50(2COO<u>Me</u>), 63.78(C-5'), 72.53(C-3'), 74.80(C-2'), 74.93 (C-1'), 80.59(C-4'), 113.50(<u>C</u>H=C), 128.22, 128.60, 129.28, 129.53, 132.85, 132.91, 133.25, 133.32 and 133.36(3Ph), 142.53(CH=<u>C</u>), 163.77(C-5), 164.78, 164.87, 164.96, 165.80 and 167.89(3<u>C</u>OPh, 2<u>C</u>OOMe). MS m/z 520(M$^+$-N_2-PhCOOH). Anal. Calcd for $C_{34}H_{30}N_4O_{11}$: C, 60.90; H, 4.51; N, 8.35. Found: C, 61.15; H, 4.44; N, 8.39.

1- and 2-(1,3-Dimethoxycarbonyl-1-propen-2-yl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole (4a and 5a). Into a hot (120°C) solution of tetrazole **3a** (25.7 g, 50 mmol) in DMF (250 ml) DMCP[20] (9.8 g, 50 mmol) was added dropwise and the reaction mixture stirred for 4h. N,N-Dimethylformamidinium chloride was filtered off and the filtrate evaporated. The residue was applied to a silica gel column (500 g in CH_2Cl_2) and eluted with CH_2Cl_2 (7 l) to give mixture of **4a** and **5a** (22.32 g, 62%) in an 1/6 ratio as a white foam. The mixture was used in reactions in xylene without any further separation.

4a: ^{1}H NMR ($CDCl_3$): δ 3.63, 3.78(2s, 6H, 2COOMe), 4.18, 4.70(2d, 2H, $C\underline{H}_2$COOMe, J_{gem} = 17.4 Hz), 4.57(dd, 1H, H-5'), 4.76(dd, 1H, H-5"), 4.82(m, 1H, H-4'), 5.57(d, 1H, H-1'), 6.19(dd, 1H, H-3'), 6.48(dd, 1H, H-2'), 6.64(s, 1H, CH=C), 7.33, 7.56, 7.97(3m, 15H, 3Ph); $J_{1'2'}$ = 4.0, $J_{2'3'}$ = 5.4, $J_{3'4'}$ = 6.3, $J_{4'5'}$ = 4.2, $J_{4'5"}$ = 3.6, $J_{5'5"}$ = 12.3 Hz. ^{13}C NMR ($CDCl_3$): δ 36.57($\underline{C}H_2$COOMe), 52.38, 52.56(2COO$\underline{Me}$), 63.25(C-5'), 72.34(C-3'), 72.68(C-1'), 74.64(C-2'), 81.23(C-4'), 120.97($\underline{C}$H=C), 128.34, 128.40, 129.16, 129.71, 129.80, 133.23, 133.53, 133.61 and 133.65(3Ph), 141.78(CH=$\underline{C}$), 151.94(C-5), 164.41, 165.00, 165.13, 166.10 and 168.31(3$\underline{CO}$Ph, 2$\underline{CO}$OMe). HRMS (FAB$^+$) m/z calcd for $C_{34}H_{31}N_4O_{11}$ 671.199, found 671.199(MH^+). Anal. Calcd for$C_{34}H_{30}N_4O_{11}$: C, 60.90; H, 4.51; N, 8.35. Found: C, 60.84; H, 4.67; N, 8.07. (Pure **4a** was obtained after the thermolysis of **4a/5a** mixture in xylene; *vide infra*.)

1- and 2-(1,2-Dimethoxycarbonylethenyl)-5-[5-*O*-benzoyl-2,3-*O*-(1,1,3,3-tetra-isopropyldisyloxane-1,3-diyl)-β-D-ribofuranosyl]tetrazole (4b and 5b). A mixture of tetrazole **3b**[21] (1.54 g, 3 mmol) in toluene (25 ml) and DMAD (0.43 g, 3 mmol) was stirred at rt for one day. DMAD was added again in two portions (0.43 g, 3 mmol and 0.2 g, 1.4 mmol) in one day interval and then mixture left stirring for another day. The evaporated residue was purified by chromatography on silica gel (200 g, CH_2Cl_2) with CH_2Cl_2 (2 l) to provide mixture of **4b-Z**, **4b-E** and **5b-E** in ratio 47:3:50 as a pale yellow oil (0.77 g, 40%). **4b-Z** crystallized (mp 105-107°C from MeOH) in few days and was separated from **4b-E** and **5b-E** by washing with MeOH. The mixture of **4b** and **5b** was used as such in further reaction.

4b-Z: ^{1}H NMR ($CDCl_3$): δ 1.03-1.13(m, 28H, 4*i*-Pr), 3.63, 3.70(2s, 6H, 2COOMe), 4.19-4.29(m, 2H, H-3', H-5'), 4.35(m, 1H, H-4'), 4.44(dd, 1H, H-5"), 5.07(dd, 1H, H-2'), 5.27(d, 1H, H-1'), 7.08(s, 1H, CH=C), 7.45, 7.59, 8.01(3m, 5H, Ph); $J_{1'2'}$ = 2.7, $J_{2'3'}$ = 4.3 Hz. ^{13}C NMR ($CDCl_3$): δ 12.38, 12.50, 12.60, 13.10, 16.63, 16.67, 16.88, 17.02, 17.09 (4*i*Pr), 52.37, 53.31(2COO$\underline{Me}$), 64.17(C-5'), 74.11(C-3'), 76.22(C-2'), 77.87(C-1'), 80.92(C-4'), 127.90, 129.08, 129.16, 132.69(Ph), 128.05($\underline{C}$H=C), 132.96(CH=$\underline{C}$), 153.85(C-5), 160.51, 161.59(2$\underline{CO}$OMe), 165.57($\underline{CO}$Ph). MS m/z 647(M^+-*i*Pr). Anal. Calcd for $C_{31}H_{46}N_4O_{10}Si_2$: C, 53.89; H, 6.71; N, 8.11. Found: C, 54.13; H, 6.98; N, 7.96.

4b-E*: ^{1}H NMR ($CDCl_3$): δ 1.06(m, 28H, 4*i*-Pr), 3.76, 3.82(2s, 6H, 2COOMe), 4.42-4.69(m, 4H, H-5', H-5", H-4', H-3'), 5.14(d, 1H, H-1'), 5.25(m, 1H, H-2'), 6.66(s, 1H, CH=C), 7.50-7.60, 7.63-7.72, 7.90-7.95(3m, 5H, Ph); $J_{1'2'}$ = 2.6 Hz. ^{13}C NMR ($CDCl_3$): δ 12.60-13.32, 16.87-17.36(4*i*Pr), 52.70, 53.57(2COO$\underline{Me}$), 63.30(C-5'), 73.90 (C-3'), 76.27(C-2'), 76.77(C-1'), 82.02(C-4'), 126.42($\underline{C}$H=C), 128.27-129.26, 133.13 (Ph), 133.17(CH=$\underline{C}$), 153.62(C-5), 160.32, 163.03, 165.88(2$\underline{CO}$OMe, $\underline{CO}$Ph).

5b-E*: ^{1}H NMR ($CDCl_3$): δ 1.06(m, 28H, 4*i*Pr), 3.85, 4.03(2s, 6H, 2COOMe), 4.45-4.52(m, 2H, H-4', H-5'), 4.59-4.64(m, 2H, H-3', H-5"), 4.84(t, 1H, H-2'), 5.32(d, 1H, H-1'), 6.86(s, 1H, CH=C), 7.40-7.48, 7.53-7.61, 7.99-8.06(3m. 5H, Ph); $J_{1'2'} = J_{2'3'}$ = 4.6 Hz. ^{13}C NMR ($CDCl_3$): δ 12.65-13.37, 16.91-17.40(4*i*Pr), 52.69, 53.88(2COOMe), 64.52(C-5'), 74.32(C-3'), 76.97(C-2'), 77.16(C-1'), 82.57(C-4'), 112.35(CH=C), 128.31, 128.50, 129.62, 133.03(Ph), 139.04(CH=C), 162.11, 163.59, 166.08, 166.23(C-5, 2COOMe, COPh). *^{1}H and ^{13}C NMR data were obtained from the spectra of **4b-Z**, **4b-E** and **5b-E** mixture.

2-(3-Hydroxy-1-methoxycarbonyl-1-propen-2-yl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazole (5c). A mixture of tetrazole 3a (1.5 g, 2.9 mmol) and MHB (0.53 g, 4.6 mmol) was stirred at 4°C for 3 d. Another portion of MHB (0.34 g, 3 mmol) was added and stirred for 2 d. The evaporated residue was applied to a silica gel column (250 g, CH_2Cl_2/EtOAc, 5:1) and eluted with CH_2Cl_2/EtOAc, 5:1 to give 5c (1 g, 55%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 2.66(t, 1H, OH), 3.67(s, 3H, COOMe), 4.62(dd, 2H, CH_2OH; ^3J = 6.5 Hz, ^4J = 1.4 Hz), 4.64(m, 1H, H-5'), 4.76-4.84(m, 2H, H-4', H-5"), 5.68(d, 1H, H-1'), 6.01(t, 1H, H-3'), 6.12(t, 1H, H-2'), 6.33(t, 1H, CH=C), 7.40, 7.53, 8.03(3m, 15H, 3Ph); $J_{1'2'}$ = 5.0, $J_{2'3'}$ = 5.1, $J_{3'4'}$ = 5.3 Hz. ^{13}C NMR ($CDCl_3$): δ 52.25(COOMe), 61.54(CH_2OH), 63.95(C-5'), 72.60(C-3'), 75.01(C-2'), 75.13(C-1'), 80.41(C-4'), 115.26(CH=C), 128.35, 128.40, 128.66, 128.73, 129.40, 129.68, 129.71, 133.15, 133.51, 133.56(3Ph), 142.79(CH=C), 163.45(C-5), 164.13(COOMe), 165.16, 165.26, 166.24(3COPh). Anal. Calcd for $C_{32}H_{28}N_4O_{10}$: C, 61.14; H, 4.49; N, 8.91. Found: C, 61.23; H, 4.60; N, 8.70.

Methyl 2-[5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-methoxycarbonyl-1H-pyrazolyl-3]-acetate (6a).

Method A. A mixture of **4a** and **5a** (11.5 g, 17.2 mmol) of a 1/6 ratio (*vide supra*) in xylene (300 ml) was heated at 160°C for 2 d. Pyrazole **6a** was filtered with suction (5.1 g, 52%; mp 150-1°C from EtOH). The filtrate was evaporated, applied to a silica gel column (250 g, CH_2Cl_2) and eluted with CH_2Cl_2/EtOAc (20:1) to give **4a** (1.02 g) as a white crisp foam.

Method B: A solution of **5a** (10.24 g, 15.3 mmol) in xylene (250 ml) was heated at 160°C for 22 h. The resulting pyrazole **6a** (6.88 g, 70%) was identical (TLC, NMR) to **6a** obtained by Method A.

^{1}H NMR ($CDCl_3$): δ 3.72, 3.74(2s, 6H, 2COOMe), 4.01(s, 2H, CH_2COOMe), 4.68 (dd, 1H, H-5'), 4.76(m, 1H, H-4'), 4.85(dd, 1H, H-5"), 5.80(dd, 1H, H-3'), 5.88(d, 1H, H-1'), 6.07(dd, 1H, H-2'), 7.31, 7.42, 7.55, 7.86, 8.06(5m, 15H, 3Ph); $J_{1'2'}$ = 3.1, $J_{2'3'}$ = 5.1, $J_{3'4'}$ = 7.2, $J_{4'5'}$ = 2.9, $J_{4'5"}$ = 5.9, $J_{5'5"}$ = 11.6 Hz. ^{13}C NMR ($CDCl_3$): δ 32.33 CH_2COOMe), 50.91, 51.86(2COOMe), 63.85(C-5'), 71.58(C-3'), 75.46(C-2'), 76.15(C-

1'), 78.67(C-4'), 108.46(C-4), 127.90, 128.00, 128.01, 128.33, 128.74, 128.93, 129.29, 129.39, 129.50, 132.91, 133.00(3Ph), 145.72, 146.94(C-3, C-5), 162.86(COOMe), 164.60, 164.82, 166.48(3COPh), 170.16(CH_2COOMe). Anal. Calcd for $C_{34}H_{30}N_2O_{11}$: C, 63.55; H, 4.71; N, 4.36. Found: C, 63.66; H, 4.92; N, 4.49.

Dimethyl {5-[5-*O*-benzoyl-2,3-*O*-(1,1,3,3-tetraisopropyldisyloxane-1,3-diyl)-β-D-ribofuranosyl]-1H-pyrazol-3,4-diyl}dicarboxylate (6b). A mixture of **4b** and **5b** (0.5 g, 0.72 mmol; *vide supra*) in xylene (20 ml) was heated at reflux temperature for 1h. The evaporated residue was chromatographed on silica gel (20 g, CH_2Cl_2) with CH_2Cl_2 (1.5 l) to give **4b** (0.09 g) and with CH_2Cl_2/EtOAc (20:1, 1l) to give pyrazle **6b** (0.21 g, 88%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 1.06(m, 28H, 4*i*Pr), 3.82, 3.95(2s, 6H, 2COOMe), 4.21(dd, 1H, H-3'), 4.43(dt, 1H, H-4'), 4.51(m, 2H, H-2', H-5'), 4.84(H-5''), 5.47(d, 1H, H-1'), 7.45, 7.59, 8.05(3m, 5H, Ph), 11.7(br s, 1H, NH); $J_{1'2'}$ = 2.9, $J_{2'3'}$ = 4.2, $J_{3'4'}$ = 6.9, $J_{4'5'}$ = 2.6, $J_{4'5''}$ = 7.1, $J_{5'5''}$ = 12.1 Hz. ^{13}C NMR ($CDCl_3$): δ 12.77, 12.81, 13.08, 13.45, 17.00, 17.06, 17.19, 17.27, 17.36(4*i*Pr), 51.86, 52.54(2COOMe), 65.10(C-5'), 74.60(C-3'), 78.60(C-2'), 79.81(C-1'), 81.09(C-4'), 110.75(C-4), 128.57, 129.27, 129.76, 133.58(Ph), 143.75, 147.21(C-3, C-5), 162.11, 162.62(2COOMe), 167.61(COPh). Calcd for $C_{31}H_{46}N_2O_{10}Si_2$: C, 56.17; H, 6.99; N, 4.23. Found: C, 55.95; H, 7.01; N, 4.40.

Methyl 2-[4-methoxycarbonyl-5-(β-D-ribofuranosyl)-1H-pyrazolyl-3]-acetate (7a). A mixture of pyrazole **6a** (6.88 g, 10.7 mmol) in methanolic methylate (90 ml, 0.3 N) was stirred at rt for 2 h, neutralized with Dowex H$^+$ and filtered. The filtrate was coevaporated several times with water to remove MeOBz. Pyrazole **7a** (2.69 g, 76%) was obtained as a white crisp foam. ^{1}H NMR (Me_2SO-d_6, D_2O): δ 3.68, 3.77(2s, 6H, 2COOMe), 3.69(dd, 1H, H-5'), 3.85(dd, 1H, H-5''), 3.90(s, 2H, CH_2COOMe), 3.95(m, 1H, H-4'), 4.05(t, 1H, H-3'), 4.10(t, 1H, H-2'), 5.32(d, 1H, H-1'); $J_{1'2'}$ = 3.7, $J_{4'5'}$ = 3.4, $J_{4'5''}$ = 2.8, $J_{5'5''}$ = 12.3 Hz. ^{13}C NMR (Me_2SO-d_6): δ 33.69(CH_2COOMe), 50.98, 51.63 (2COOMe), 60.42(C-5'), 69.66(C-3'), 75.95(C-2'), 77.52(C-1'), 83.02(C-4'), 107.69(C-4), 147.34, 147.83(C-3, C-5), 163.34(COOMe), 170.52(CH_2COOMe). IR (KBr): 1738 (CH_2COOMe), 1695(ArCOOMe) cm^{-1}. HRMS m/z calcd for $C_{13}H_{18}N_2O_8$ 330.1060, found 330.1063(M$^+$). Anal. Calcd for $C_{13}H_{18}N_2O_8$: C, 47.28; H, 5.49; N, 8.48. Found: C, 47.35; H, 5.44; N, 8.39. An analytical sample was purified by chropmatography on silica gel with CH_2Cl_2/MeOH (5:1).

Methyl 2-[5-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]-acetate (7b). A mixture of pyrazolylacetate **7a** (2.69 g, 8.2 mmol) in a solution of iodine in acetone (0.9 g I_2/150 ml acetone) was overnight stirred at rt. 5% Aq. $Na_2S_2O_3$ (24 ml) was added and the mixture extracted with $CHCl_3$ (150 + 80 ml). Combined extracts were washed with brine (50 ml), dried (Na_2SO_4) and evaporated to

give **7b** (3.0 g, 99%) as a white foam ^{1}H NMR ($CDCl_3$): δ 1.35, 161(2s, 6H, CMe_2), 3.74(dd, 1H, H-5'), 3.75, 3.84(2s, 6H, 2COOMe), 4.00(dd, 1H, H-5"), 4.01(s, 2H, $C\underline{H}_2COOMe$), 4.32(m, 1H, H-4'), 4.75(dd, 1H, H-2'), 4.85(dd, 1H, H-3'), 5.71(d, 1H, H-1'); $J_{1'2'}$ = 2.4, $J_{2'3'}$ = 6.3, $J_{3'4'}$ = 3.9, $J_{4'5'}$ = 3.2, $J_{4'5"}$ = 3.6, $J_{5'5"}$ = 11.9 Hz. ^{13}C NMR ($CDCl_3$): δ 25.55, 27.46($C\underline{M}e_2$), 32.41($\underline{C}H_2COOMe$), 51.39, 52.40(2COO$\underline{M}e$), 62.47 (C-5'), 80.17(C-1'), 80.78(C-3'), 86.32(C-4'), 86.74(C-2'), 108.43(C-4), 113.46($\underline{C}Me_2$), 145.05, 151.03(C-3, C-5), 163.56(Ar$\underline{C}OOMe$), 170.61 ($CH_2\underline{C}OOMe$). IR (film, NaCl): 1742($CH_2\underline{C}OOMe$), 1715($\underline{C}OOMe$) cm^{-1}. HRMS m/z calcd for $C_{16}H_{22}N_2O_8$ 370.1380, found 370.1390(M^+). Anal. Calcd for $C_{16}H_{22}N_2O_8$ x H_2O: C, 49.53; H, 6.23; N, 7.22. Found: C, 49.70; H, 6.05; N, 7.37. An analytical sample was purified by chromatography on silica gel with CH_2Cl_2/MeOH (20:1).

Methyl 2-[5-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]-acetate (7c). To a solution of pyrazolylacetate **7b** (3.0 g, 8.1 mmol) in CH_2Cl_2 (100 ml) were added Et_3N (1.13 ml, 8.1 mmol) and TBDMS-triflate (1.85 ml, 8.1 mmol) and stirred at rt for 7 h. The mixture was diluted with $CHCl_3$ (100 ml) and washed with water (3 x 25 ml), brine (25 ml), dried (Na_2SO_4) and evaporated to give **7c** (3.55 g, 91%) as a pale yellow stiff syrup. ^{1}H NMR ($CDCl_3$): δ 0.15, 0.17(2s, 6H, $SiMe_2$), 0.93(s, 9H, *t*-Bu), 1.33, 1.58(2s, 6H CMe_2), 3.69, 3.79(2s, 6H, 2COOMe), 3.74(dd, 1H, H-5'), 3.90(s, 2H, $C\underline{H}_2COOMe$), 4.01(dd, 1H, H-5"), 4.25(m, 1H, H-4'), 4.67(dd, 1H, H-3'), 4.72(dd, 1H, H-2'), 5.70(d, 1H, H-1'); $J_{1'2'}$ = 1.7, $J_{2'3'}$ = 5.7, $J_{3'4'}$ = 4.0, $J_{4'5'}$ = 3.9, $J_{4'5"}$ = 2.9, $J_{5'5"}$ = 11.5 Hz. ^{13}C NMR ($CDCl_3$): δ -5.32($SiMe_2$), 18.54($\underline{C}Me_3$), 25.61, 27.49($C\underline{M}e_2$), 25.99($C\underline{M}e_3$), 33.92($\underline{C}H_2COOMe$), 51.21, 52.00(2COO$\underline{M}e$), 63.35(C-5'), 79.70, 80.14, 85.86, 86.66(C-1', C-2', C-3', C-4'), 107.95(C-4), 113.46($\underline{C}Me_2$), 148.16, 148.58(C-3, C-5), 163.67(Ar$\underline{C}OOMe$), 170.98 ($CH_2\underline{C}OOMe$). IR (film, NaCl): 1745($CH_2\underline{C}OOMe$), 1718($\underline{C}OOMe$) cm^{-1}. HRMS m/z calcd for $C_{21}H_{33}N_2O_8Si$ 469.201, found 469.200(M^+-Me).

Monoamide 8 from diester 7. *General Method.* A mixture of pyrazolylacetate **7** in 27% methanolic ammonia (8 ml/1 mmol) was shaken at rt in a pressure bottle for 1-2 d and then evaporated to give acetamide **8** in quantitative yield. **8** was used in further reaction without purification.

2-[4-Methoxycarbonyl-5-(β-D-ribofuranosyl)-1H-pyrazolyl-3]-acetamide (8a). Reaction time with **7a** (1 g, 3 mmol): 2 d. Yield after freeze drying 0.95 g, fluffy compound. ^{1}H NMR (Me_2SO-d_6, 80 °C): δ 3.57(dd, 1H, H-5'), 3.69(s, 2H, $C\underline{H}_2CONH_2$), 3.72(dd, 1H, H-5"), 3.73(s, 3H, Me), 3.83(m, 1H, H-4'), 4.02(m, 1H, H-3'), 4.09(m, 1H, H-2'), 5.23(d, 1H, H-1'), 6.4-7.7(br, 3H, NH, NH_2),; $J_{1'2'}$ = 3.9, $J_{4'5'}$ = 3.8, $J_{4'5"}$ = 3.6, $J_{5'5"}$ = 11.9 Hz. ^{13}C NMR (Me_2SO-d_6, 80 °C): δ 33.69($\underline{C}H_2CONH_2$), 50.55(Me), 61.09(C-5'), 70.15(C-3'), 75.31(C-2'), 78.08(C-1'), 83.37(C-4'), 108.11(C-4), 146.11,

149.26(C-3, C-5), 163.54(COOMe), 170.36($CONH_2$). IR (KBr): 1674($CONH_2$), 1696(COOMe) cm^{-1}. HRMS (FAB^+) m/z calcd for $C_{12}H_{18}N_3O_7$, 316.1152, found 316.1145(MH^+). Anal. Calcd for $C_{12}H_{17}N_3O_7$: C, 45.72; H, 5.44; N, 13.33. Found: C, 46.12; H, 5.74; N, 12.98. An analytical sample was purified by chromatography on silica gel with CH_2Cl_2/MeOH (10:1, 5:1).

2-[5-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]-acetamide (8b). Reaction time with **7b** (0.61 g, 1.65 mmol): 2d. Yield 0.59 g, colorless foam. ^1H NMR (Me_2CO-d_6): δ 1.32, 1.54(2s, 6H, CMe_2), 3.17(br s, 1H, OH-5'), 3.56(dd, 1H, H-5'), 3.79(m, 4H, COOMe, H-5"), 3.89(s, 2H, CH_2CONH_2), 4.14(m, 1H, H-4'), 4.81(m, 2H, H-2', H-3'), 5.54(d, 1H, H-1'), 6.72, 7.17(2 br s, 3H, NH, NH_2),; $J_{1'2'}$ = 2.7 Hz. ^{13}C NMR (Me_2CO-d_6): δ 25.68, 27.38(CMe_2), 34.26(CH_2CONH_2), 51.28 (COOMe), 62.82(C-5'), 80.60(C-3'), 82.09(C-2'), 86.85(C-1'), 86.90(C-4'), 109.07(C-4), 113.85(CMe_2), 164.55(COOMe), 172.18($CONH_2$). IR (film, NaCl): 1698($CONH_2$), 1676(COOMe) cm^{-1}. HRMS m/z calcd for $C_{15}H_{21}N_3O_7$, 355.1380, found 355.1390(M^+). Anal. Calcd for $C_{15}H_{21}N_3O_7$: C, 50.70; H, 5.96; N, 11.83. Found: C, 50.65; H, 6.08; N, 11.60. An analytical sample was purified by chromatography on silica gel with CH_2Cl_2/MeOH (50:1, 20:1).

2-[5-(5-*O*-*Tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]-acetamide (8c). Reaction time with 7c (3.5 g, 7.5 mmol): 1 d. Yield 3.1 g, colorless foam. ^1H NMR (Me_2CO-d_6): δ 0.05(s, 6H, $SiMe_2$), 0.88 (s, 9H, *t*-Bu), 1.32, 1.52(2s, 6H CMe_2), 3.77(m, 2H, H-5', H-5"), 3.78(s, 3H, COOMe), 3.86(s, 2H, CH_2COOMe), 4.10(m, 1H, H-4'), 4.73(dd, 1H, H-3'),. 5.03(br s, 1H, H-2'), 5.48(d, 1H, H-1'), 6.57, 7.00(2 br s, 3H, NH, NH_2); $J_{1'2'}$ = 3.2 Hz. ^{13}C NMR (Me_2CO-d_6): δ -5.26, -5.22($SiMe_2$), 18.81(CMe_3), 25.67, 27.69(CMe_2), 26.21(CMe_3), 34.23 (CH_2CONH_2), 51.23(COOMe), 64.48(C-5'), 80.60(C-1'), 83.23(C-3'), 85.74(C-2'), 86.87(C-4'), 109.36(C-4), 113.76(CMe_2), 164.59(COOMe), 171.53($CONH_2$). IR (film, NaCl): 1709(COOMe), 1679($CONH_2$) cm^{-1}. HRMS m/z calcd for $C_{20}H_{32}N_3O_7Si$ 454.201, found 454.200(M^+-Me).

3-(β-D-Ribofuranosyl)-1H-pyrazolo[4,3-*c*]pyridine-4,6(5H,7H)-dione (1). A mixture of monoamide **8a** (0.99 g, 3 mmol) in methanolic sodium methylate (1M, 6 ml) was stirred at reflux temperature for 2 h and then neutralized with glacial acetic acid (0.34 ml). Filtration and washing with water provided **1** (0.58 g, 61%) as a white solid; mp 294-6°C (from water). ^1H NMR (Me_2SO-d_6): δ 3.50(dd, 1H, H-5'), 3.65(dd, 1H, H-5"), 3.82(m, 1H, H-4'), 3.91(br s, 2H, H-7a, H-7b), 3.98(m, 1H, H-3'), 4.18(br s, 1H, H-2'), 5.07(br s, 1H, H-1'), 10.85(br s, 1H, NH). ^{13}C NMR (Me_2SO-d_6): δ 31.4(br, C-7), 61.50(C-5'), 70.80(C-3'), 75.0(br, C-2'), 76.4(br, C-1'), 84.60(C-4'), 106.70(C-3a), 142.5, 144.5(2 br, C-3, C-7a), 161.65, 170.6(C-4, C-6). IR (KBr): 1702, 1682(2CO)cm^{-1}

[1]. Anal. Calcd for $C_{11}H_{13}N_3O_6$: C, 46.65; H, 4.63; N, 14.84. Found: C, 46.55; H, 4.48; N, 14.77.

N-acetyl 2-[5-(2,3,5-*O*-acetyl-β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]-acetamide (9). A mixture of monoamide **8** (120 mg, 0.4 mmol) in MeCN, DMAP (2 mg, 0.02 mmol), Et_3N (0.28 ml, 2 mmol) and Ac_2O (0.18 ml, 2.4 mmol) was stirred at rt for 2 h. The evaporated residue was treated with water and extracted with $CHCl_3$ (2 x 7 ml) and the combined organic layers washed with sat. aq. $NaHCO_3$ (3 x 3 ml), dried (Na_2SO_4). The evaporated residue was purified on silica gel column (10 g, CH_2Cl_2/MeOH, 20:1) with CH_2Cl_2/MeOH (20:1, 0.5 l) to provide tetraacetyl derivative **9** (102 mg, 58%) as a foam. ^{1}H NMR ($CDCl_3$): δ 2.06, 2.15, 2.16, 2.34(4s, 12H, 4COMe), 3.80(s, 3H, COOMe), 4.10(d, 2H, C<u>H</u>$_2$CONHAc, J_{gem} = 7.6 Hz), 4.17(dd, 1H, H-5'), 4.38(m, 1H, H-4'), 4.47(dd, 1H, H-5''), 5.25(dd, 1H, H-3'), 5.56(d, 1H, H-1'), 5.62(dd, 1H, H-2'), 9.23(s, 1H, NH;); $J_{1'2'}$ = 2.7, $J_{2'3'}$ = 4.9, $J_{3'4'}$ = 7.7, $J_{4'5'}$ = 2.3, $J_{4'5''}$ = 4.5, $J_{5'5''}$ = 11.6 Hz. ^{13}C NMR ($CDCl_3$): δ 20.42, 20.55, 20.84, 25.21(4CO<u>Me</u>), 36.31(<u>C</u>H$_2$CONHAc), 51.64(COO<u>Me</u>), 63.21(C-5'), 70.38, 74.97(C-2', C-3'), 77.57, 78.11(C-1', C-4'), 108.72(C-4), 146.71, 146.84(C-3, C-5), 163.62(<u>C</u>OOMe), 168.91, 169.32, 169.56, 171.05(4<u>C</u>OMe), 172.00(<u>C</u>ONHAc). HRMS m/z calcd for $C_{20}H_{25}N_3O_{11}$ 438.150, found 438.149(M$^+$). Anal. Calcd for $C_{20}H_{25}N_3O_{11}$: C, 49.69; H, 5.21; N, 8.69. Found: C, 49.88; H, 5.44; N, 8.55.

2-[5-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]-acetonitrile (10b). Into an ice-cold mixture of **8b** (0.57 g, 1.6 mmol) in THF (5 ml) and pyridine (0.54ml, 6.8 mmol) TFAA (0.51 ml, 3.6 mmol) was added at such a rate that the temperature was kept below 5°C. After an overnight stirring at rt the reaction mixture was diluted with CH_3Cl (30 ml), washed with water (10 ml) and sat. aq. $NaHCO_3$ (2 x 5 ml), dried (Na_2SO_4) and evaporated to yield **10b** (0.43 g, 63%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 1.30, 1.52(2s, 6H, CMe_2), 3.60(m, 2H, H-5', H-5''), 3.76(s, 3H, COOMe), 4.01(m, 1H, H-4'), 4.12(s, 2H, CH_2CN), 4.69(m, 2H, H-2', H-3'), 5.05(dd, 1H, OH-5'; ^3J = 6.8 and 5.3 Hz), 5.36(d, 1H, H-1'); $J_{1'2'}$ = 3.4 Hz. ^{13}C NMR ($CDCl_3$): δ 17.01(<u>C</u>H$_2$CN), 25.45, 27.36(C<u>Me</u>$_2$), 51.24(COO<u>Me</u>), 61.02(C-5'), 77.93, 80.83(C-3', C-2'), 85.06, 85.30(C-1', C-4'), 107.39(C-4), 113.44(<u>C</u>Me$_2$), 117.57(CN), 144.63, 146.31 (C-3, C-5), 162.82(CO). IR (KBr): 2263(CN), 1711(CO) cm^{-1}. UV (MeOH) λ_{max} 224(sh, ε = 148), 250(149), 270(sh, 151). HRMS m/z calcd for $C_{15}H_{20}N_3O_6$ 338.1352, found 338.1380(MH$^+$). Anal. Calcd for $C_{15}H_{19}N_3O_6$ x 0.5 H_2O: C, 52.02; H, 5.82; N, 12.13. Found: 51.95; H, 5.77; N, 11.72. An analytical sample was purified by chromatography on silica gel with CH_2Cl_2/MeOH (50:1).

2-[5-(5-*O*-*Tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]acetonitrile (10c). Into an ice-cold solution of

acetamide **8c** (3g, 6.4 mmol) in THF (70 ml) and pyridine (2.26 ml, 28 mmol) TFAA (2 ml, 14 mmol) was added dropwise over 1 h. Reaction mixture was left stirring overnight at rt and then diluted with $CHCl_3$ (150 ml), washed with water (6 x 30 ml), dried (Na_2SO_4) and evaporated. The oily residue was chromatographed on silica gel column (250 g, CH_2Cl_2) with CH_2Cl_2 (4 l) to give **10c** (1.53 g, 53%) as a colorless glassy compound. ^{1}H NMR ($CDCl_3$): δ 0.16, 0.19(2s, 6H, $SiMe_2$), 0.94(s, 9H, *t*-Bu), 1.35, 1.59(2s, 6H, CMe_2), 3.75(dd, 1H, H-5'), 3.87(s, 3H, COOMe), 3.95(d, 2H, CH_2CN; J_{gem} = 7.3 Hz), 4.04(dd, 1H, H-5"), 4.27(m, 1H, H-4'), 4.68(m, 2H, H-3', H-2'), 5.71(d, 1H, H-1'); $J_{1'2'}$ = 1.2, $J_{4'5'}$ = 2.7, $J_{4'5''}$ = 2.7, $J_{5'5''}$ = 11.7 Hz. ^{13}C NMR ($CDCl_3$): δ -5.49, -5.46 ($SiMe_2$), 17.42($\underline{C}H_2CN$), 18.42($\underline{C}Me_3$), 25.48, 27.38($C\underline{Me}_2$), 25.84($C\underline{Me}_3$), 51.46(COO$\underline{Me}$), 63.40 (C-5'), 79.59(C-1'), 80.13, 86.58(C-2', C-3'), 85.86(C-4'), 107.34(C-4),113.44($\underline{C}Me_2$),116.48(CN), 144.61, 148.59(C-3, C-5), 163.01(CO). IR (film, NaCl): 2258(CN), 1717(CO) cm^{-1}. HRMS m/z calcd for $C_{20}H_{30}N_3O_6Si$ 436.190, found 436.189 (M+). Anal. Calcd for $C_{20}H_{30}N_3O_6Si$: C, 55.85; H, 7.37; N, 9.30. Found: C, 55.55; H, 7.48; N, 9.11.

2-[5-(β-D-ribofuranosyl)-4-methoxycarbonyl-1H-pyrazolyl-3]-acetonitrile (10a). A mixture of acetonitrile **10b** (0.5 g, 1.5 mmol) in MeOH (50 ml) and Dowex H$^+$ (5 g) was overnight stirred at rt, filtered and evaporated to give acetonitrile **10a** (0.4 g, 91%) with mp 73-5°C (from acetone/EtOH). ^{1}H NMR (Me_2CO-d_6): δ 3.85(s, 3H, COOMe), 3.90(m, 1H, H-5'), 4.00-4.11(m, 2H, H-4', H-5"), 4.07(s, 2H, CH_2CN), 4.16(br s, 1H, H-3'), 4.24-4.34(br, 2H, H-2', OH-5'), 4.8(br s, 1H, OH-3'), 5.2(br, 1H, OH-2'), 5.46(d, 1H, H-1'), 12.9(br, 1H, NH); $J_{1'2'}$ = 6.8 Hz. ^{13}C NMR (Me_2CO-d_6): δ 17.49($\underline{C}H_2CN$), 51.63(COO$\underline{Me}$), 61.22(C-5'), 70.22(C-2'), 77.60(C-3'), 80.01(C-1'), 83.39(C-4'), 107.69(C-4), 117.55(CN), 145.6, 149.9(2 br, C-3, C-5), 164.05(CO). IR (KBr): 3390(OH), 2263(CN), 1708(CO) cm^{-1}. MS (FAB$^-$) m/z 298(MH^-). Anal. Calcd for $C_{12}H_{15}N_3O_6$ x 0.5 H_2O: C, 47.07; H, 5.26; N, 13.70. Found: C, 47.02; H, 5.21; N, 13.72.

3-(β-D-Ribofuranosyl)-6-amino-1H-pyrazolo[4,3-*c*]pyridine-4(5H)-one (2a). A mixture of pyrazolylacetonitrile **10a** (0.3 g, 1 mmol) and liquid ammonia (10 ml) was heated at 160°C in a stainless steel high-pressure vessel for 3 h and then evaporated with MeOH to solid residue. The residue was treated with hot water (20 ml), filtered and freeze dried to give **2a** (0.25 g, 89%) as a pale brown fluffy compound. ^{1}H NMR (Me_2SO-d_6): δ 3.51(m, 1H, H-5'), 3.63(dd, 1H, H-5"), 3.84(m, 1H, H-4'), 4.09(m, 1H, H-3'), 4.35(m, 1H, H-2'), 4.88(d, 1H, H-1'), 5.37(s, 1H, H-7), 5.53(s, 2H, NH_2), 10.0, 12.1(2br, 2H, 2NH); $J_{1'2'}$ = 6.8 Hz. ^{13}C NMR (Me_2SO-d_6): δ 62.30(C-5'), 69.30(C-7), 71.28(C-3'), 74.54(C-2'), 78.41(C-1'), 85.72(C-4'), 102.52(C-3a), 148.51, 149.06, 149.89(C-3, C-6, C-7a), 158.22(C-4). IR (KBr): 1652(CO) cm^{-1}. Anal. Calcd for

$C_{11}H_{14}N_4O_5$: C, 46.81; H, 5.00; N, 19.85. Found: C, 47.17; H, 4.83; N, 19.80. An analytical sample was purified by filtering of its water solution through a membrane filtered and subsequent freeze drying.

3-(2,3-*O*-Isopropilidene-β-D-ribofuranosyl)-6-amino-1H-pyrazolo[4,3-*c*]-pyridine-4(5H)-one (2b). A mixture of acetonitrile **10c** (0.45 g, 1 mmol) and liquid ammonia (10 ml) was heated at 130°C in a stainless steel high-pressure vessel for 3 h andthen evaporated to solid residue. The residue was partitioned between water (80 ml) and $CHCl_3$ (50 ml). Water layer was separated from organic, filtered and freeze dried to give **2b** (0.2 g, 63%) as a pale pink fluffy compound $[\alpha]_{23}^{D}$ -41.1 (c 0.42, Me_2SO). ^1H NMR (Me_2SO-d_6): δ 1.29, 1.50(2s, 6H, CMe_2), 3.47(dd, 1H, H-5'), 3.58(dd, 1H, H-5"), 4.03(m, 1H, H-4'), 4.80(dd, 1H, H-3'), 4.96(d, 1H, H-1'), 5.03(dd, 1H, H-2'), 5.34(s, 1H, H-7), 5.73(s, 2H, NH_2), 10.15, 12.34(2 br s, 2H, 2NH); $J_{1'2'}$ = 5.3, $J_{2'3'}$ = 6.4, $J_{3'4'}$ = 3.1, $J_{4'5'}$ = 3.8, $J_{4'5''}$ = 4.5, $J_{5'5''}$ = 11.7 Hz. ^{13}C NMR (Me_2SO-d_6): δ 25.43, 27.48($C\underline{Me}_2$), 61.90 (C-5'), 68.93(C-7), 79.99(C-1'), 82.07(C-3'), 82.91(C-2'), 83.82(C-4'), 102.37(C-3a), 113.41($\underline{C}Me_2$), 147.34, 149.21, 149.66(C-3, C-6, C-7a), 157.97(C-4). IR (KBr): 1634 (CO) cm^{-1}. UV (MeOH) λ_{max} 226(sh, ε = 151), 268(184), 284(sh, 184). HRMS (EI) m/z calcd for $C_{14}H_{18}N_4O_5$ 322.1277, found 322.1279; (FAB$^+$) m/z calcd for $C_{14}H_{19}N_4O_5$ 323.1355, found 323.1361(MH^+). Anal. Calcd for $C_{14}H_{18}N_4O_5$ x CH_3OH: C, 50.84; H, 6.25; N, 15.81. Found: C, 50.70; H, 6.33; N, 15.71. An analytical sample was purified by chromatography on silica gel with $CHCl_3$ and $CHCl_3$/MeOH (50:1, 20:1, 10:1).

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-6-amino-1H-pyrazolo[4,3-*c*]-pyridine-4(5H)-one (2c). A mixture of **2a** (0.2 g, 0.7 mmol), Et_3N (0.4 ml, 2.8 mmol), Ac_2O (0.27 ml, 2.8 mmol) and DMAP (10 mg, 0.08 mmol) in MeCN (9 ml) was stirred at rt for 3 h and then evaporated several times with MeOH. The residue was chromatographed on silica gel column (50 g, CH_2Cl_2) with CH_2Cl_2 (0.5 l), CH_2Cl_2/MeOH (50:1, 0.1 l, 30:1, 0.1 l, 20:1, 0.2 l) to give **2c** (0.1 g, 35%) with mp 256-60°C (from water). ^1H NMR (Me_2SO-d_6): δ 2.01, 2.03, 2.07(3s, 9H, 3Me), 4.09(dd, 1H, H-5'), 4.17(dd, 1H, H-4'), 4.34(dd, 1H, H-5"), 5.19(d, 1H, H-1'), 5.32(s, 1H, H-7), 5.42(t, 1H, H-3'), 5.69(s, 2H, NH_2), 5.75(t, 1H, H-2'), 10.16(s, 1H, NH), 12.41(s, 1H, NH); $J_{1'2'}$ = 6.2, $J_{2'3'}$ = 6.0, $J_{3'4'}$ = 5.5, $J_{4'5'}$ = 5.9, $J_{4'5''}$ = 3.8, $J_{5'5''}$ = 11.2 Hz. ^{13}C NMR (Me_2SO-d_6): δ 20.33, 20.42, 20.56 (3Me), 63.49(C-5'), 68.64(C-7), 71.60(C-3'), 73.13(C-2'), 76.20(C-1'), 79.03(C-4'), 102.79(C-3a), 145.88, 149.32, 149.41(C-3, C-6, C-7a), 157.78(C-4), 169.44, 169.56, 170.13(3CO). IR (KBr): 3461, 3352(2NH), 1746, 1666, 1646(3CO), cm^{-1}. HRMS (EI) m/z calcd for $C_{17}H_{20}N_4O_8$ 408.1297, found 408.1281(M^+). Anal. Calcd for $C_{17}H_{20}N_4O_8$: C, 50.00; H, 4.94; N, 13.72. Found: C, 50.06; H, 5.15; N, 13.42.

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REFERENCES

(1) (a)Piccirilli, J. A.; Krauch, T.; Moroney, S. E.; Benner, S. A. *Nature*, **1990**, *343*, 33. (b) Voegel, J. J.; Benner, S. A. *J. Am. Chem. Soc.* **1994**, *116*, 6929.

(2) (a) Moffat, A. S. *Science*, **1992**, *252*, 1374. (b) Letai, A. G.; Palladino, M. A.; Fromm, E.; Rizzo, V.; Fresco, J. R. *Biochemistry*, **1988**, *27*, 9108.

(3) Bhattacharya, B. K.; Revankar, G. R. *Nucleosides & Nucleotides*, **1991**, *10*, 729

(4) Koh, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 1470.

(5) Prhavc, M.; Townsend, L. B.; Kobe, J. *Nucleosides & Nucleotides*, **1991**, *10*, 729.

(6) Moody, C. J.; Rees, C. W.; Young, R. G. *J. Chem. Soc. Perkin Trans. 1*, **1991**, 329.

(7) Moody, C. J.; Rees, C. W.; Young, R. G. *Ibid.* **1991**, 323.

(8) Sauer, D. R.; Schneller, S. W. *Nucleosides & Nucleotides*, **1989**, *8*, 1079.

(9) Earl, R. A.; Townsend, L. B. *Can. J. Chem.* **1980**, *58*, 2550.

(10) Earl, R. A.; Townsend, L. B. *Org. Synth.* Coll. Vol. 7, 334.

(11) Farkas, J.; Sorm, F. *Collect. Czech. Chem. Commun.* **1972**, *37*, 2798.

(12) Robins, R. K.; Horner, J. K.; Greco, V. G.; Noell, C. W.; Beams, C. G. Jr. *J. Org. Chem.* **1963**, *28*, 304.

(13) Cook, P. D.; Rousseau, R. J.; Mian, A. M.; Meyer, R. B. Jr.; Dea, P.; Ivanovics, G.; Streeter, D. G.; Witkowski, J. T.; Stout, M. G.; Simon, L. N.; Sidwell, R. W.; Robins, R. K. *J. Am. Che. Soc.* **1975**, *97*, 2916.

(14) Matsuda, A.; Sinozaki, M.; Suzuki, M.; Watanabe, K.; Miyasaka, T. *Synthesis*, **1986**, 385.

(15) Kartha, K. P. R. *Tetrahedron Lett.* **1986**, *27*, 3415

(16) Campagna, F.; Carotti, A.; Casini, G. *Tetrahedron Lett.* **1977**, 1813.

(17) Emde, H.; Domsch, D.; Feger, H.; Frick, U.; Goetz, A.; Hergott, H. H.; Hofmann, K.; Kober, W.; Kraegeloh, K.; Oesterle, T.; Steppen, W.; West, W.; Simchen, G. *Synthesis*, **1982**, 1.

(18) Ehler, K. W.; Robins, R. K.; Meyer, R. B. Jr. *J. Med Chem.* **1977**, *20*, 317.

(19) Pang, H.; Schram, K. H.; Smith, D. L.; Gupta, S. P.; Townsend, L. B.; McCloskey, J. A. *J. Org. Chem.* **1982**, *47*, 3923.

(20) DMAL and DMAP were prepared by the literature procedure: Bryson, T. A.; Dolak, T. M. *Org. Synth.* Coll. Vol. 6, 505.

(21) Kobe, J.; Prhavc, M.; Hohnjec, M.; Townsend, L. B. *Nucleosides & Nucleotides*, **1994**, *13*, 2209.

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