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[C0033]

# SYNTHESIS OF 1-(2'-DEOXY-ß-D-RIBOFURANOSYL)-1*H*-IMIDAZO[4,5-*d*]PYRIDAZINE-4,7(5*H*,6*H*)-DIONE: A POTENTIALLY BENEFICIAL BUILDING BLOCK FOR ANTISENSE APPLICATIONS

Huan-Ming Chen and Ramachandra S. Hosmane*

*Laboratory for Drug Design and Synthesis*
*Department of Chemistry & Biochemistry*
*University of Maryland, Baltimore County*
*1000 Hilltop Circle, Baltimore, Maryland 21250, USA*

* Phone: 410-455-2520 Fax: 410-455-1148 E-Mail: hosmanw@umbc.edu

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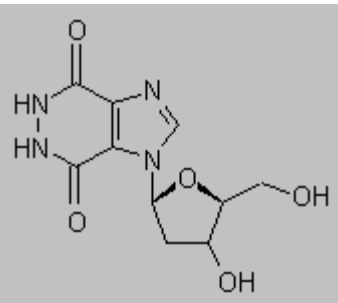
## ABSTRACT

*Synthesis of the title compound,1-(2'-deoxy-ß-D-ribofuranosyl)-1H-imidazo[4,5-d]pyridazine- -4,7(5H,6H)-dione (**1**), is reported. It was synthesized in five steps, commencing with methyl 1-(ß-D-ribofuranosyl)imidazo-4,5-dicarboxylate (**2**). The 3',5'-hydroxyl groups of **2** was protected with a bis-silylating agent to form **3**, which was then converted into the corresponding 2'-thionocarbonate derivative **5**. The reduction of the latter with tri-n-butyltin hydride (to form **6**), followed by silyl deprotection with tetra-n-btylammonium fluoride, afforded 7. Treatment of the latter with hydrazine hydrate yielded the target nucleoside **1**.*

## INTRODUCTION

The target nucleoside, 1-(2'-deoxy-ß-D-ribofuranosyl)-1*H*-imidazo[4,5-d]pyridazine-4,7(5*H*,6*H*)-dione (**1**), can be considered an analogue of purine nucleoside in which a pyridazine moiety replaces a pyrimidine in fusion to an imidazole ring. Our molecular modeling studies[1] suggest that nucleoside **1** has some unique structural features that would make it an attractive building block for oligonucleotides for potential antisense and triple-helical applications. These features



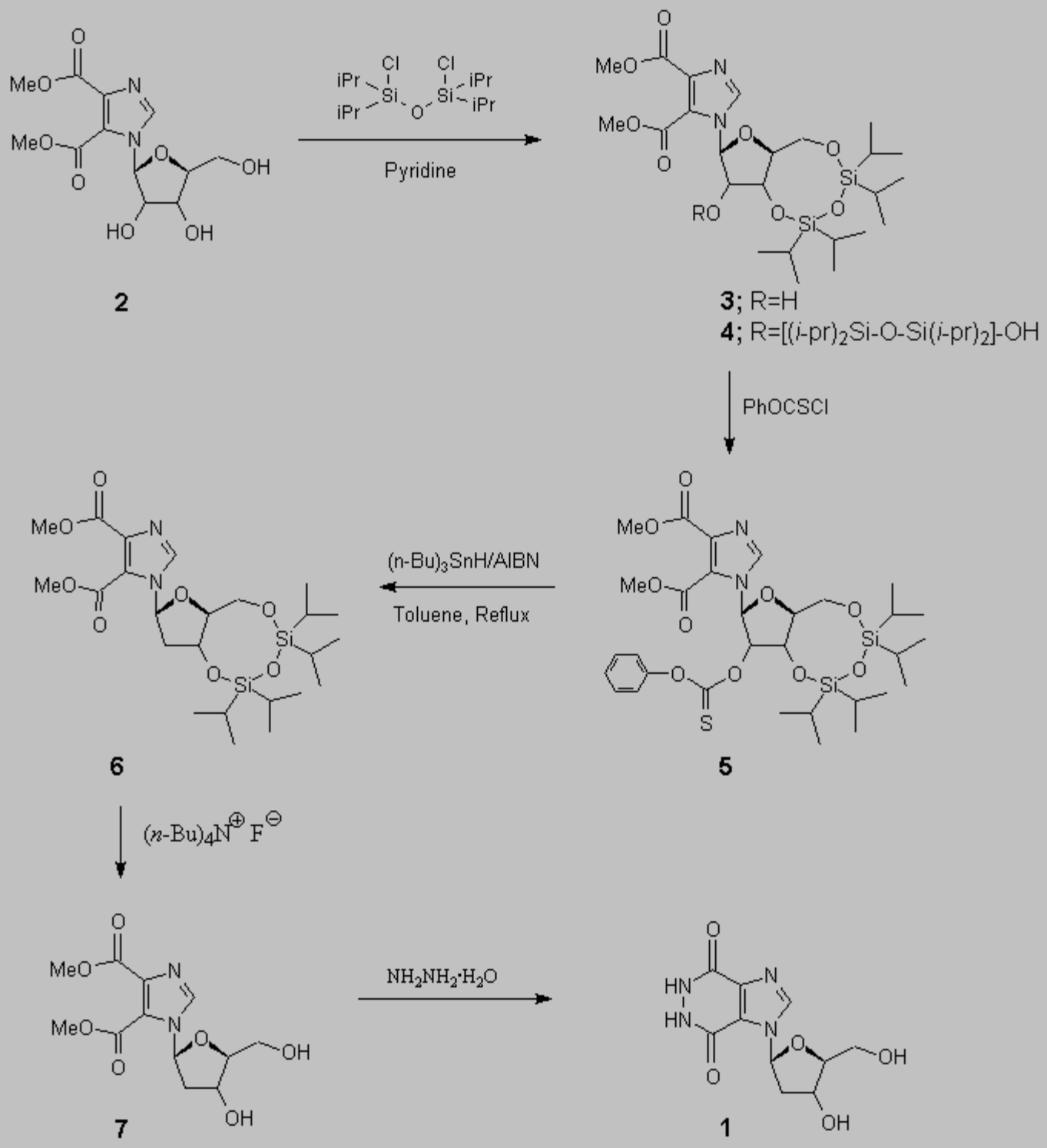

**1**

include, but are not limited to, its potential capability to base-pair with cytidine forming three H-bonds, like guanosine, but with a significantly shortened sugar-sugar (C-1' to C-1') distance, which in turn might lead to a decreased interstrand span and consequently somewhat compressed double-helix. The study of effect of such a compression on the double-helical or triple-helical conformations, interactions, and stability would be interesting and rewarding.

The synthesis of the ribose analogue of **1**, containing a 2'-OH group in place of 2'-H, has long been reported in the literature, both by imidazole ring-closure[2f] as reported here, as well as by glycosylation of the parent heterocyclic base, imidazo[4,5-*d*]pyridazine-4,7(5*H*,6*H*)-dione.[2b] Surprisingly, however, the target 2'-deoxy analogue **1**, which is a necessary building block for the DNA double-helix or the triple-helical strand, is as yet unknown. We report herein the synthesis of **1** in five steps starting from methyl 1-(-D-ribofuranosyl)imidazo-4,5-dicarboxylate (**2**)[2].

Nucleoside **2** (**Scheme I**) was selectively protected at the 3',5'-position using the Markiewicz reagent[3] 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane to form the corresponding 3',5'-O-(1,1,3,3-tetraisopropyl-disiloxan-1,3-diyl) (TIPDS) derivative **3** as the major product, along with a small amount of the completely silyl-protected derivative **4**. Functionalization of **3** at O-2' with phenyl chlorothionocarbonate (phenoxythiocarbonyl chloride), employing 4-(dimethylamino)pyridine (DMAP) as a catalyst, afforded the 3',5'-O-TIPDS-protected 2'-O-(phenoxythiocarbonyl) ester (**5**). Free radical-mediated Barton deoxygenation[4] with tributylstannane (tributyltin hydride), employing ,'-azobis(isobutyronitrile) (AIBN) as a radical initiator in toluene at reflux, gave satisfactory conversion into the 3',5'-TIPDS-protected-2'-deoxynucleoside (**6**). The use of excess tributylstannane makes the conversion almost quantitative, and the extended reaction time doesn't give any by-products. The silyl deprotection of **6** was achieved by treatment with tetra-*n*-butylammonium fluoride, which gave the required imidazole precursor, methyl 1-(2'-deoxy--D-erythro-pentofuranosyl)-4,5-

## SCHEME I




**2**

**3**; R=H
**4**; R=[(*i*-pr)$_2$Si-O-Si(*i*-pr)$_2$]-OH

**5**

**6**

**7**

**1**

imidazoledicarboxylate (**7**). It was discovered that **7** was unstable in solution, and was completely decomposed in 2-3 days to give the corresponding aglycon methyl imidazole-4,5-dicarboxylate. The ring-closure of **7** with hydrazine hydrate provided the target nucleoside 1-(*2*'-deoxy--D-ribofuranosyl)-1*H*-imidazo[4,5-*d*]pyridazine-4,7(5*H*,6*H*)-dione (**1**). The structure of the latter was consistent with its spectroscopic and microanalytical data.

## EXPERIMENTAL SECTION

1HNMR spectra were recorded on a General Electric QE-300 (300 MHz) instrument. The spectral data are reported in the following format: chemical shift (all relative to Me4Si as an internal reference standard unless otherwise indicated), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, b = broad ), integration, coupling constants, exchangeability after D2O addition, and assignment of resonances. Elemental Microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia. The mass spectra were recorded at the Mass Spectral Facility, Department of Biochemistry, Michigan State University. Thin layer chromatography was performed on Merck Kieselgel 60 GF254 plates ( 0.2 mm thickness). Melting points were determined on a Thomas-Hoover capillary melting point apparatus, and are uncorrected.

***Methyl1-[(3',5'-O-(1,1,3,3,-Tetraisopropyldisiloxan-1,3-diyl))-ß-D-ribofuranosyl]-4,5-imidazole-dicarboxylate (3) and Methyl1-[((2'-O-(3-Hydroxy-1,1,3,3,-tetraisopropyldisiloxyl)-3',5'-O- (1,1,3,3-tetraisopropyl disiloxan-1,3-diyl))-ß-D-ribofuranosyl]-4,5-imidazoledicarboxylate (4)***

To a solution of dry methyl 1--D-ribofuranosyl-4,5-imidazoledicarbo xylate[2] (**2**) (500 mg, 1.58 mmol) in dry pyridine (10 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[7] (550 mg, 1.75 mmol), and the mixture was stirred for 4 h at ambient temperature with protection from moisture. Volatile materials were evaporated *in vacuo*, and the residue was dissolved in chloroform. The chloroform solution was washed twice with cold water and dried over anhydrous sodium sulfate. The residue after evaporation was purified by silica gel flash chromatography, eluting with chloroform to give **3** and **4** as a colorless liquids, whose yield, spectral and analytical data are given below.

Compound **3:** Yield 800 mg (91%), Rf 0.38 (chloroform/ methanol, 30:1); $^{1}$H-NMR ($CDCl_3$) 8.10 (s, 1H, imidazole), 6.11 (s, 1H, 1'-H), 4.44 (dd, 1H, *J*=4.2 and 9.0 Hz, 3'-H), 4.26 (d, 1H, $J_{gem}$=13.5 Hz, 5'-H), 4.18 (d, 1H, *J*=4.2 Hz, 2'-H), 4.16 (dd, 1H, *J*=9.0 and 2.4 Hz, 4'-H), 4.03 (dd, 1H, $J_{gem}$=13.5 Hz, *J5',4'*=2.4 Hz, 5'-H), 3.95 (s, 3H, $OCH_3$), 3.94 (s, 3H, $OCH_3$), 2.89 (brs, 1H, 2'-OH, exchangeable with $D_2O$), 1.05 (m, 28H, isopropyl groups); $^{13}$C-NMR ($CDCl_3$, 75.48 MHz) 12.45 (CHSi), 12.82 (CHSi), 12.90 (CHSi), 13.35 (CHSi), 16.81 ($CCH_3$), 16.87 ($CCH_3$), 16.97 ($CCH_3$), 16.97 ($CCH_3$), 17.26 ($CCH_3$), 17.26 ($CCH_3$), 17.32 ($CCH_3$), 17.42 ($CCH_3$), 52.50 ($OCH_3$), 52.78 ($OCH_3$), 59.91 (C-5'), 68.43 (C-3'), 76.82 (C-2'), 81.87 (C-4'), 91.54 (C-1'), 122.83 (C-4 or 5), 137.26 (C-2), 138.57 (C-5 or 4), 160.26 (C=O), 162.91 (C=O).

*Anal. Calcd. for $C_{24}H_{42}N_2O_9Si_2$ (MW 558.78): C, 51.59; H, 7.58; N, 5.01. Found: C, 51.50; H, 7.57; N, 5.04.*

Compound **4**: Yield 120 mg (9%), Rf 0.45 (chloroform/ methanol, 30:1); $^{1}$H-NMR ($CDCl_3$) 8.37 (s, 1H, imidazole), 6.06 (s, 1H, 1'-H), 4.48 (d, 1H, *J*=3.3 Hz, 2'-H), 4.35 (dd, 1H, *J*=3.3 and 9.6 Hz, 3'-H), 4.31 (d, 1H, $J_{gem}$=13.8 Hz, 5'-H), 4.27 (dd, 1H, *J*=9.6 and 2.4 Hz, 4'-H), 4.02 (dd, 1H, *Jgem*=13.8 Hz, $J_{5',4'}$=2.4 Hz, 5'-H), 3.93 (s, 3H, $OCH_3$), 3.93 (s, 3H, $OCH_3$), 1.03 (m, 56H, isopropyl groups); $^{13}$C-NMR ($CDCl_3$, 75.48 MHz) 12.51 (CHSi), 12.91 (CHSi), 13.05 (CHSi), 13.05 (CHSi), 13.24 (CHSi), 13.35 (CHSi), 13.39 (CHSi), 13.48 (CHSi), 16.59 ($CCH_3$), 16.78 ($CCH_3$), 16.81 ($CCH_3$), 16.81 ($CCH_3$), 17.06 ($CCH_3$), 17.06 ($CCH_3$), 17.06 ($CCH_3$), 17.23 ($CCH_3$), 17.26 ($CCH_3$), 17.26 ($CCH_3$), 17.30 ($CCH_3$), 17.37 ($CCH_3$), 17.42 ($CCH_3$), 17.51 ($CCH_3$), 17.55 ($CCH_3$), 17.65 ($CCH_3$), 52.56 ($OCH_3$), 53.17 ($OCH_3$), 59.56 (C-5'), 68.47 (C-3'), 77.95 (C-2'), 81.44 (C-4'), 92.15 (C-1'), 121.16 (C-4

or 5), 138.64 (C-2), 139.48 (C-5 or 4), 161.49 (C=O), 163.11 (C=O).

*Anal.* Calcd. for $C_{36}H_{70}N_2O_{11}Si_4$ (MW 819.30): C, 52.78; H, 8.61; N, 3.42. Found: C, 52.45; H, 8.89; N, 2.93.

***Methyl 1-[(2'-O-Phenoxythiocarbonyl)-3',5'-O-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl)-ß-D-erythro-pentofuranosyl)]-4,5-imidazoledicarboxylate (5)***

To a solution of methyl 1-(3',5'-O-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl)-*ß*-D-erythropentofuranosyl)--4,5-imidazoledicarboxylate (**3**) ( 560 mg, 1 mmol) and DMAP (250 mg, 2.05 mmol) in 15 mL of dried acetonitrile was added phenoxy thiocarbonyl chloride (200 µL, 250 mg, 1.45 mmol). The solution was stirred for 16 h at ambient temperature, evaporated to dryness *in vacuo*. The residue was applied to column chromatography, eluted with chloroform to give a colorless oily product (660 mg, 95%). Rf 0.60, chloroform/ methanol (30:1); $^1$H-NMR ($CDCl_3$) 8.16 (s, 1H, imidazole), 7.43 (t, 2H, J=7.8 Hz, $Ph_{meta(3,5)}$-H), 7.31 (t, 1H, J=7.8 Hz, $Ph_{para(4)}$-H), 7.13 (d, 2H, J=7.8 Hz, $Ph_{ortho(2,6)}$-H), 6.38(s, 1H, 1'-H), 6.01 (d, 1H, J=4.5 Hz, 2'-H), 4.61 (dd, 1H, J=4.5 and 9.3 Hz, 3'-H), 4.31 (d, 1H, $J_{gem}$=13.5 Hz, 5'-H), 4.16 (dd, 1H, J=9.3 and 2.4 Hz, 4'-H), 4.05 (dd, 1H, $J_{gem}$=13.5 Hz, $J_{5',4'}$=2.4 Hz, 5'-H), 3.93 (s, 3H, $OCH_3$), 3.91 (s, 3H, $OCH_3$), 1.06 (m, 28H, isopropyl groups); $^{13}$C-NMR ($CDCl_3$, 75.48 MHz) 12.80 (CHSi), 12.91 (CHSi), 13.37 (CHSi), 13.49 (CHSi), 16.87 ($CCH_3$), 16.94 ($CCH_3$), 16.96 ($CCH_3$), 17.08 ($CCH_3$), 17.24 ($CCH_3$), 17.27 ($CCH_3$), 17.34 ($CCH_3$), 17.44 ($CCH_3$), 52.49 ($OCH_3$), 52.74 ($OCH_3$), 59.29 (C-5'), 67.69 (C-3'), 82.39 (C-4'), 85.10 (C-2'), 89.40 (C-1'), 121.68 (Ph-$C_{2,6}$), 126.69 (Ph-$C_4$), 129.57 (Ph-$C_{3,5}$), 137.13 (C-2), 138.93 (C-5 or 4), 139.24 (C-4 or 5), 153.47 (Ph-$C_1$), 159.79 (C=O), 162.76 (C=O), 193.70 (C=S).

*Anal.* Calcd. for $C_{31}H_{46}N_2O_{10}SSi_2$ (MW 694.94): C, 53.58; H, 6.67; N, 4.03; S, 4.61. Found: C, 53.88; H, 7.47; N, 3.53; S, 3.27.

***Methyl 1-(2'-Deoxy-3',5'-O-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl)-ß-D-erythropentofuranosyl)- -4,5-imidazoledicarboxylate (6)***

A solution of methyl1-(2'-O-phenoxythiocarbonyl-3',5'-O-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl)- ß-D-erythropentofuranosyl)-4,5-imidazoledicarboxylate (**5**) (695 mg, 1 mmol) and AIBN (32 mg, 0.2 mmol) in 30 mL of dried toluene was purged with oxygen-free nitrogen for 30 min. Tributylstannane (400 µL, 433 mg, 1.49 mmol) was added and the solution was refluxed for 3 h. Tlc showed a single spot with very slightly lower Rf value than that of starting material. The solvent was evaporated and pure product as a colorless liquid was obtained by column chromatography (Hexane / ethyl acetate, 3:1) in almost quantitative yield. Rf 0.12, hexane/ethyl acetate (3 :1); $^1$H-NMR ($CDCl_3$) 8.09 (s, 1H, imidazole), 6.33(dd, 1H, J=7.4 and 1.2 Hz, 1'-H), 4.54 (dt, 1H, J=10.2 and 7.4 Hz, 3'-H), 4.11 (dd, 1H, $J_{gem}$=13.2 Hz, $J_{5',4'}$=2.4 Hz, 5'-H), 4.06 (dd, 1H, $J_{gem}$=13.2 Hz, $J_{5',4'}$=3.0 Hz, 5'-H), 3.93 (s, 3H, $OCH_3$), 3.91 (s, 3H, $OCH_3$), 3.82 (dt, 1H, J=8.2 and 2.6 Hz, 4'-H), 2.64 (ddd, 1H, J=13.2, 10.2 and 7.4 Hz, 2'-H), 2.35 (ddd, 1H, J=13.2, 7.4 and 1.2 Hz, 2'-H), 1.06 (m, 28H, isopropyl groups); $^{13}$C-NMR ($CDCl_3$, 75.48

MHz) 12.54 (CHSi), 12.94 (CHSi), 13.04 (CHSi), 13.43 (CHSi), 16.86 ($CCH_3$), 16.97 ($CCH_3$), 16.97 ($CCH_3$), 17.09 ($CCH_3$), 17.31 ($CCH_3$), 17.31 ($CCH_3$), 17.39 ($CCH_3$), 17.49 ($CCH_3$), 41.70 (C-2'), 52.34 ($OCH_3$), 52.50 ($OCH_3$), 60.48 (C-5'), 67.52 (C-3'), 85.25 (C-4'), 86.02 (C-1'), 136.41 (C-4 or 5), 137.12 (C-2), 138.41 (C-5 or 4), 160.37 (C=O), 163.04 (C=O);

*Anal.* Calcd. for $C_{24}H_{42}N_2O_8Si_2$ (MW 542.7764): C, 53.11; H, 7.80; N, 5.16. Found: C, 53.39; H, 7.83; N, 4.91.

***Methyl 1-(2'-Deoxy--D-erythropentofuranosyl)-4,5-imidazoledicarboxylate (7)***

Addition of 1M tetrabutylammonium fluoride-THF (2 mL, 2 mmol) to an ice-cooled solution of methyl 1-(2'-deoxy-3',5'-O-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl)--D-erythro-pentofuranosyl)-4,5-imidazoledicarboxylate (**6**) (543 mg, 1 mmol) in 10 mL of dried THF. The reaction was stopped after 45 min stirring at 0 $^oC$. The solvent was evaporated in vacuo and pure product as foam was obtained by column chromatography (chloroform / methanol, 10:1) in 75 % yield. Rf 0.20, chloroform/ methanol (10:1); $^1H$-NMR ($CDCl_3$) 8.51 (s, 1H, imidazole), 6.38 (dd, 1H, J=6.2 and 3.4 Hz, 1'-H), 4.82 (brs, 1H, OH, exchangeable with $D_2O$), 4.54 (dd 1H, J=12.8 and 6.6 Hz, 3'-H), 4.15 (brs, 1H, OH, exchangeable with $D_2O$), 3.98 (m, 3H, 4',5'-H), 3.91 (s, 3H, $OCH_3$), 3.89 (s, 3H, $OCH_3$), 2.56 (ddd, 1H, J=13.6, 12.8 and 6.2 Hz, 2'-H), 2.35 (ddd, 1H, J=13.6, 6.6 and 3.4 Hz, 2'-H); $^{13}C$-NMR ($CDCl_3$, 75.48 MHz) 42.49 (C-2'), 52.42 ($OCH_3$), 52.73 ($OCH_3$), 60.62 (C-5'), 68.91 (C-3'), 87.03 (C-4'), 87.21 (C-1'), 136.50 (C-4 or 5), 136.86 (C-5 or 4), 137.68 (C-2), 160.40 (C=O), 163.05 (C=O);

*Anal.* Calcd. for $C_{12}H_{16}N_2O_7$ (MW 300.2676): C, 48.00; H, 5.37; N, 9.33. Found: C, 48.21; H, 5.68; N, 9.45.

***1-(2'-Deoxy--D-ribofuranosyl)-1H-imidazo[4,5-d]pyridazine-4,7(5H,6H)-dione (1)***

A solution of methyl 1-(2'-deoxy-b-D-ribofuranosyl)imidazole-4,5-dicarboxylate (**7**) (0.15 g, 0.5 mmol) and 99% hydrazine was refluxed 6 hour. The excess hydrazine was removed by distillation *in vacuo* and the residue coevaporated several times with water. The crystalline residue was recrystallized from methanol to give white crystals (0.1g, 75%). mp. >250 °C; Rf, 0.34 (chloroform/methanol/30% ammonium hydroxide, 2:2:1); $^1H$ NMR (DMSO-$d_6$): d 8.13 (s,1H, imidazole), 6.58 (dd, 1H, J=*9.6* and1.8 Hz, 1'-H), 4.33 (m, 1H, 4'-H), 3.85 (m, 1H, 3'-H), 3.58 (m, 2H, 5'-H), 2.34 (dt, 1H, J=13.6 and 6.3 Hz, 2'-H), 2.08 (d, 1H, J=13.6 Hz, 2'-H). ms: (FAB) m/z 310 ($MH^+$).

*Anal. Calcd. For $C_{10}H_{12}N_4O_5 \cdot H_2O$ (286.2438): C, 41.96; H, 4.93; N, 19.57. Found: C, 41.98; H, 4.92; N, 19.56.*

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