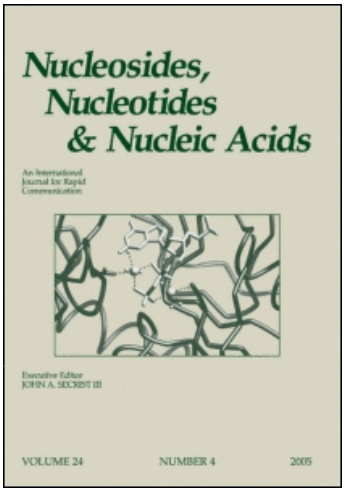

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Synthesis of a Novel Pyrazolo[1, 5-*C*]Pyrimidine *C*-Nucleoside and Conformational Analysis By NMR Spectroscopy

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SYNTHESIS OF A NOVEL PYRAZOLO[1,5-*c*]PYRIMIDINE *C*-NUCLEOSIDE AND CONFORMATIONAL ANALYSIS BY NMR SPECTROSCOPY

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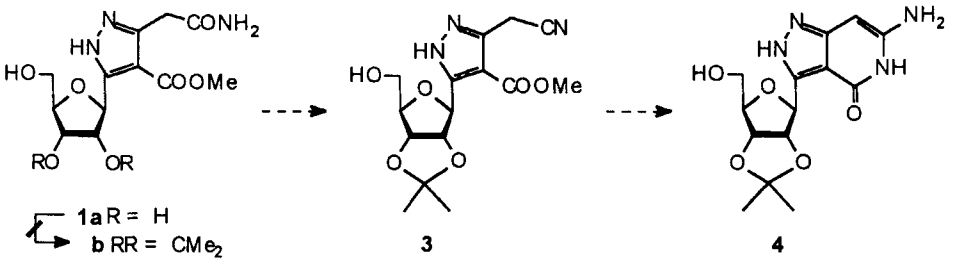
ABSTRACT: Isopropylidenation of [4-methoxycarbonyl-5-(β-D-ribofuranosyl)-1*H*-pyrazol-3-yl]acetamide (**1a**) followed by the acidic cleavage of the sugar acetonide afforded 3-methoxycarbonyl-7,7-dimethyl-2-(β-D-ribofuranosyl)-4*H*,7*H*-pyrazolo[1,5-*c*] pyrimidine-5(6*H*)-one (**2b**), the structure of which was established unequivocally by X-ray structure analysis of the monocrystals. Compounds **1a** and **2b** have 75% and 70% preference for the N-type puckering between C3'-*endo* and C3'-*endo*-C4'-*exo* forms, and a great preference of 69% and 74% for γ^+ rotamers in solution, respectively.

Introduction

Several base modified nucleosides have been reported to act as antiviral and anticancer agents[1] most likely due to their capability to mimic natural counterparts in structure and function. Numerous purine and pyrimidine analogues have been incorporated into oligonucleotides; purine analogues however normally resulted in destabilisation of duplex.[2,3] They are important as triplex forming oligonucleotides that can be targeted to mixed sequences.[4] The sequence specificity for binding is achieved through the formation of Hoogsten pairing of pyrimidine- and purine-like bases of the third strand with the purine bases of the duplex thus forming parallel and antiparallel motifs of triplex DNA, respectively.[5] Thus nonnatural base could recognise GC base pairs, without protonation in a pyrimidine-motif forming a triple helical complex possessing a suitable donor-acceptor hydrogen bonding pattern.[4,6] Besides, the



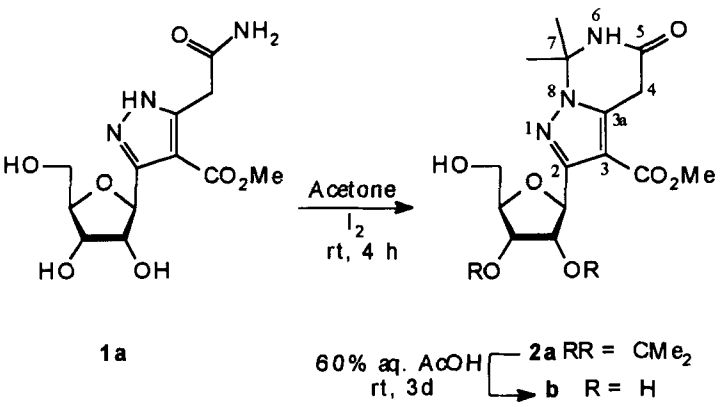
Scheme 1

oligonucleotides containing simple azole enhanced the triplex forming ability considerably at nonhomopurine targets exhibiting selectivity. Pyrazole-2'-deoxyribonucleoside was of particular interest suggesting specific interaction and it could be viewed as a starting point in the design of novel pyrazoles with suitable exocyclic groups.[7]

Results and Discussion

In order to simplify isolation and purification of the acetonitrile intermediate we wanted to transform [4-methoxycarbonyl-5-(β-D-ribofuranosyl)-1*H*-pyrazol-3-yl]acetamide (**1a**) into the 2',3'-*O*-isopropylidene derivative **1b** (Scheme 1) prior to dehydration. The use of an isopropylidene group was crucial because the protection should be stable in basic conditions and readily removable after the ring-closure to pyrazolo[4,3-*c*]pyridine **4**.

When the monoamide **1a** reacted with acetone in the presence of catalytic amount of iodine[8] a product was isolated (70% yield) which could not undergo further dehydration to the corresponding acetonitrile **3**. This fact and spectroscopic data (HRMS, ^{1}H and ^{13}C NMR) revealed the presence of two isopropylidene groups in the molecule, one of them definitely forming the anticipated dioxolane ring on the ribose moiety with methyl signals at δ 1.32 and 1.52 ppm in ^{1}H NMR spectrum. Among three additional options the possible isopropylidene bridge between 5'-O and either of the ring nitrogens was rejected on the basis of spectroscopic data. The second set of methyl signals at 1.80 and 1.81 ppm belonged to an *N,N'*-isopropylidene moiety.[9] The reaction of amide functions with acetone is known in peptide synthesis to give cyclic structures, *e.g.* 2,2,4-trialkyl-5-



Scheme 2

oxoimidazolidine derivatives (*N,N'*-isopropylidene dipeptides),[10] whereas the formation of *N,O*-isopropylidene moiety was accomplished in the case of neighbouring OH and NH groups.[11] The formation of a six membered ring is certainly favoured in either of the remaining possibilities affording more stable lactame structure.

NMR analysis employing 2D techniques (^{1}H-^{13}C and ^{1}H-^{15}N HSQC and HMBC) further disproved the assumed *N,O*-isopropylidene linkage and unambiguously confirmed the structure **2a** (Scheme 2).

First, sugar C-4' (87.56 ppm) and C-5' (63.46 ppm) correlated with a proton of primary hydroxy group (dd, 4.19 ppm) thus revealing the presence of free 5'-OH group. Second, crosspeaks between both pyrazole N-8 (-162.9 ppm) and amide N-6 (-249.4 ppm) nitrogen atoms and isopropylidene methyl groups (1.80 and 1.81 ppm) were found in ^{1}H-^{15}N HMBC spectrum. In addition, we have observed correlations between pyrazole N-8 nitrogen and methylene protons H-4a and H-4b (s, 3.91 ppm) and also between pyrazole N-1 (-92.7 ppm) and sugar H-1' (d, 5.50 ppm).

Furthermore, the structure of this novel *C*-nucleoside was deduced by single-crystal X-ray diffraction (Figure 1). The title nucleoside **2a** possesses a C-glycosidic bond in the range of formycin derivatives.[12] There are two slightly different molecules of **2a** in the asymmetric unit of the crystal unit cell. The glycosidic torsion angle [O4'-C1'-C2-N1] (χ = 27.17° and 20.90°) is in the *anti* range, while the *C*-nucleosides mostly prefer the *syn* conformations.[12] Especially those with 5-membered base rings are controlled by

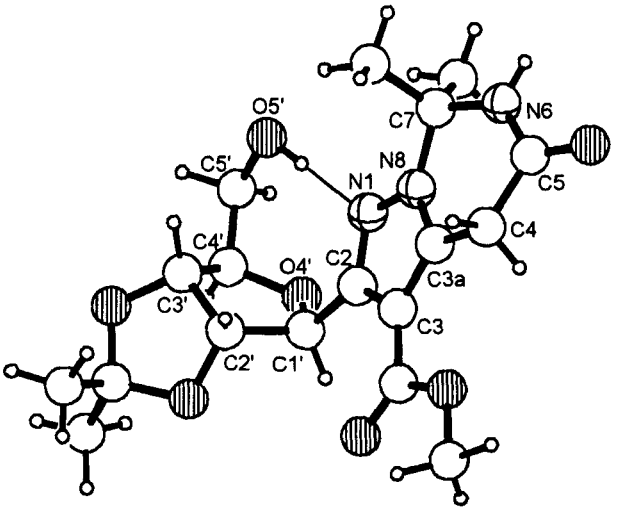


Figure 1: PLUTON[28] drawing of **2a** in the solid state with the numbering scheme.

intramolecular hydrogen bonds[12] between 5'-OH and the carbonyl or hydroxyl groups of the base. In our case the intramolecular hydrogen bond between O-5' and N-1 of 2.89 and 2.90 Å, where the nitrogen atom acts as the acceptor and apparently stabilises the *anti* conformation. The sugar puckering in solution was also investigated and is presented in second part of this paper.

The above interesting results prompted further investigation of the preparation, stability and deprotection of the novel tetrahydropyrimidine ring. Using the "classical method" for acetonide formation[13] by reaction of nucleoside **1a** in acetone with *p*-toluenesulfonic acid as a catalyst, diisopropylidene derivative **2a** was formed in quantitative yield as well. Different stability of the two CMe_2 groups was demonstrated by treatment of the compound **2a** in 60% aqueous AcOH. After three days at ambient temperature 2',3'-*O*-isopropylidene group was cleaved and the pyrazolo[1,5-*c*]pyridine *ribo*-nucleoside **2b** was isolated in quantitative yield (Scheme 2), whilst at 78 °C a mixture of products was formed. The half-life of the condensed dihydropyrimidine ring under this conditions was 1 day as determined from ^{1}H NMR spectrum of the reaction mixture. Similarly, treatment of **2a** with either DOWEX H^+ in aqueous MeOH or trifluoroacetic acid in aqueous THF at room temperature affected 2',3'-*O*-acetonide only thus forming diol **2b**. However at 55 °C in CF_3COOH, dihydropyrimidine ring was

hydrolysed and acetamide **1a** was found to be the only product of the reaction (Scheme 2).

Conformational Analysis of 1a and 2b in Solution.

(i) Conformational equilibrium of ribofuranosyl moiety. The analysis of solution conformation of ribofuranosyl moiety in **1a** and **2b** is based on three coupling constants ($^3J_{1'2'}$, $^3J_{2'3'}$, $^3J_{3'4'}$) acquired at 353 K and 298 K, respectively. The precise values for proton-proton coupling constants were obtained through the simulation and iteration procedure and are given in the experimental section. We attempted to describe the experimental $^3J_{HH}$ data in terms of a two-state North (N) ⇄ South (S) pseudorotational equilibrium as is normally done for ribosfuranose rings. The Altona-Sundaralingam parameters are the phase angle of pseudorotation (P), which defines part of the ring which is most puckered, and the puckering amplitude (Ψ_m), which indicates the extent of puckering.[14] The North range ($0° < P < 36°$) is centred around P = 18° (C3'-*endo*), whereas the South ($144° < P < 180°$) is centred around P = 162° (C2'-*endo*).[15] The values of Ψ_m in the crystal structures are found in a range from 30° to 46°.[15] The two-state dynamic N ⇄ S pseudorotational equilibrium in nucleosides and nucleotides in solution is known to be controlled by the relative strengths of anomeric and gauche effects.[16] The heterocyclic base is involved in the O4'-C1'-N1/9 anomeric effect (*i.e.* orbital mixing of one of the lone pairs on O4' with the σ^* of the glycosyl bond) which drives N ⇄ S pseudorotational equilibrium towards N. The steric effect of the nucleobase which drives N ⇄ S pseudorotational equilibrium towards S (*i.e.* pseudoequatorial orientation) has been evaluated from the energetics in *C*-[17,18] and carba *C*-nucleosides.[19] The 3'-OH groups in 2'-deoxynucleosides drive the N ⇄ S equilibrium towards S-type conformation through the tendency to adopt a *gauche* orientation of the [O4'-C4'-C3'-O3'] fragment.[16] The 2'-OH in ribonucleosides is involved in three *gauche* interactions which compete for the drive of N ⇄ S equilibrium: (i) [O4'-C1'-C2'-O2'] drives towards N, (ii) [N1/9-C1'-C2'-O2'] drives towards S, and (iii) [O3'-C3'-C2'-O2'] which adopts *gauche* orientation in both N and S-type sugar conformations.[16]

The experimental $^3J_{HH}$ coupling constants were interpreted in terms of a two-state N ⇄ S pseudorotational equilibrium with the help of the computer program PSEUROT,[20] which calculates the best fit of the conformational parameters for the two-state N ⇄ S equilibrium to the experimental time-averaged vicinal proton-proton coupling constants.

Table 1. Conformational Equilibria of *C*-nucleosides **1a** and **2b** in solution.

	N ⇄ S pseudorotational equilibrium							Conformational equilibrium across C4'-C5' bond[d]		
	P_N	Ψ_m^N	P_S	Ψ_m^S	x_N	$J^{expt} - J^{calcd}$	r.m.s. error	%γ^+	%γ^t	%γ^-
1a[a]	27°	39°	162°[c]	39°[c]	0.70	<0.1	0.01	69	26	5
2b[b]	24°	39°	162°[c]	39°[c]	0.75	<0.1	0.02	74	22	4

[a] $^3J_{HH}$ at 353 K in DMSO-d6 were used in the analysis. We note that it was not possible to extract individual coupling constant from ^{1}H NMR spectrum of **1a** in D_2O in temperature range from 298 K to 343 K. [b] $^3J_{HH}$ at 298 K in D_2O were used in the analysis. [c] The value was kept fixed in the calculation. [d] The reversed assignment of $^3J_{4'5'}$ and $^3J_{4'5''}$ shows: 70% γ^+, 13% γ^t, 17% γ^- for **1a**, and 75% γ^+, 13% γ^t, 12% γ^- for **2b**.

In our iterative procedure two of the five parameters (P and Ψ_m of the assumed minor conformer) were constrained to average values since only three experimental coupling constants are available. The results show that both **1a** and **2b** exhibit a predominance of 70% and 75%, respectively for the N-type puckering (Table 1).

The high preference for N-type sugar conformations in **1a** and **2b** can be interpreted by the competing steric effects of the base, which drive N ⇄ S equilibrium towards S (pseudoequatorial aglycon),[19,21] and relatively stronger $n_{O4'} \rightarrow \sigma^*_{C1'-Csp2}$ interactions, which drive N ⇄ S equilibrium towards N (pseudoaxial aglycon), where orbital overlap is optimal.[17,18,21]

(ii) Conformational equilibrium across C4'-C5' bond. The orientation of the 4'-CH_2OH group in relation to the furanose ring in **1a** and **2b** has been determined by the interpretation of the experimental $^3J_{4'5'}$ and $^3J_{4'5''}$ coupling constants in terms of the conformational equilibrium between three staggered rotamers.[22] The downfied of the two protons was assigned to be H5'.[23] The quantitative evaluation of the $^3J_{4'5'}$ and $^3J_{4'5''}$ coupling constants has shown that γ^+ rotamers greatly predominate by 69% in **1a** and by 74% in **2b**. The high preference for γ^+ (and γ^t) rotamers in **1a** and **2b** is due to the *gauche* effect between C5'-O5' and C4'-O4' bonds. It should be noted that reversed assignment results in only 5% change in the estimation of the populations of the rotamers along C4'-C5' bond **(Table 1)**.

Conclusions

The reaction of *ribo* 2-(2*H*-pyrazol-3-yl)acetamide **1a** with acetone and either iodine or p-toluenesulfonic acid yielded after acidic hydrolysis at ambient temperature corresponding pyrazolo[1,5-*c*]pyrimidine derivative **2b**. Treatment with aqueous CF_3COOH at 55 °C caused opening of fused didydropyrimidine thus forming starting pyrazolylacetamide **1a**. The interpretation of $^3J_{HH}$ coupling constants for **1a** and **2b** has shown that their constituent ribofuranosyl moieties exhibit a 75% and 70% preference for the N-type puckering between C3'-*endo* and C3'-*endo*-C4'-*exo* forms, respectively. The analysis of experimental $^3J_{4'5'}$ and $^3J_{4'5''}$ coupling constants in **1a** and **2b** showed a great preference of 69% and 74% for γ^+ rotamers, respectively.

Experimental

NMR spectroscopy. NMR spectra were recorded on Varian Unity Inova 600 (^{1}H at 600.139 MHz, ^{15}N at 60.815 MHz) or Varian VXR 300 (^{1}H at 299.942 MHz, ^{13}C at 75.439 MHz) spectrometers at the National NMR Center of Slovenia. Sample concentration was 3 mg in 0.6 ml of D_2O (99.9% D), DMSO-d6 (99.8% D) or acetone-d6 (99.9% D). TMS was used as internal reference in ^{1}H and ^{13}C, whereas acetonitrile was used as external reference for ^{15}N (δ^{15}N = -135.8 ppm relative to nitromethane). The sample temperature was set at 298K for **2b** and at 353K for **1a** and controlled to approximately ±0.5 K.

2-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-3-methoxycarbonyl-7,7-Dimethyl-4*H*,7*H* -pyrazolo[1,5-*c*]pyrimidine-5(6*H*)-one (2a). *Method A*: A mixture of acetamide **1a**[6] (0.5 g, 1.6 mmol) in a solution of I_2 (0.17 g) in acetone (20 mL) was stirred for 4 h at rt. Then 5% aqueous $Na_2S_2O_3$ (6 mL) was added and the mixture extracted with $CHCl_3$ (2 x 15 mL). The combined organic layers were washed with water (2 x 10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated to yield **2a** (0.45 g, 71%) as a crisp foam which upon treatment with MeOH afforded amorphous product; mp 191-3 °C (colourless needles, from EtOH).

Method B: Ethyl orthoformate (50 μL, 0.3 mmol) was added at rt to a well-stirred suspension of acetamide **1a** (26 mg, 0.08 mmol) in acetone (0.3 mL) containing *p*-toluenesulfonic acid monohydrate (15 mg, 0.08 mmol). After an overnight stirring at rt the mixture was poured into 25% aqueous ammonia (5 mL) and extracted with CH_2Cl_2 (15 mL). Organic layer was washed with water (5 mL) and brine (5 mL) and dried

(Na_2SO_4). Evaporation of solvent yielded solid **2a** (30 mg, 95%) identical to the product of method A. ^{1}H NMR ($(CD_3)_2CO$) δ 1.32, 1.53 (2s, 6H, $OCMe_2$) 1.80, 1.81 (2s, 6H, $NCMe_2$), 3.54 (ddd, 1H, H-5"; $J_{5',5''}$ = 11.7, $J_{5'',5'\text{-OH}}$ = 7.8, $J_{5'',4'}$ = 7.6 Hz), 3.68 (ddd, 1H, H-5'; $J_{5',5'\text{-OH}}$ = 4.7, $J_{5',4'}$ = 4.6 Hz), 3.81 (s, 3H, COOMe), 3.91 (s, 2H, H-4a, H-4b), 4.15 (m, 1H, H-4'), 4.19 (dd, 1H, OH-5'), 4.82 (dd, 1H, H-3'; $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.2 Hz), 4.99 (dd, 1H, H-2'; $J_{2',1'}$ = 3.2 Hz), 5.50 (d, 1H, H-1'), 7.98 (broad, 1H, NH). ^{13}C NMR ($(CD_3)_2CO$) δ 25.69, 27.74 ($OC\underline{Me}_2$), 30.14, 30.16 ($NC\underline{Me}_2$), 31.32 (C-4), 51.48 (COO<u>Me</u>), 63.46 (C-5'), 74.29 ($N\underline{C}Me_2$), 81.27 (C-1'), 83.11 (C-3'), 86.43 (C-2'), 87.56 (C-4'), 108.55 (C-3), 113.74 ($O\underline{C}Me_2$), 141.39 (C-3a), 154.35 (C-2), 163.81 (<u>C</u>OOMe), 165.24 (C-5). ^{15}N NMR ($(CD_3)_2CO$) δ -92.7 (N-1), -162.9 (N-8), -249.4 (N-6). HRMS m/z calcd for $C_{18}H_{25}N_3O_7$ 395.1693, found 395.1700 (M^+). Anal. calcd for $C_{18}H_{25}N_3O_7$: C, 54.68; H, 6.37; N, 10.63. Found: C, 54.92; H, 6.43; N, 10.57.

3-methoxycarbonyl-7,7-dimethyl-2-(β-D-ribofuranosyl)-4*H*,7*H*-pyrazolo[1,5-*c*] pyrimidine-5(6*H*)-one (2b). Compound **2a** (20 mg, 0.05 mmol) was stirred in 60% aqueous acetic acid (0.8 mL) for 3 d at r.t. Reaction mixture was evaporated, 3 times with MeOH and purified on silica gel column with MeOH-CH_2Cl_2 (1:20, 1:5) to yield after freeze drying white fluffy **2b** (15 mg, 84%). ^{1}H NMR ($(CD_3)_2SO$), δ 1.65, 1.66 (2s, 6H, CMe_2), 3.46 (ddd, 1H, H-5"; $J_{5',5''}$ = 11.6, $J_{5'',4'}$ = 4.6 Hz), 3.63 (ddd, 1H, H-5'; $J_{5',4'}$ = 3.5 Hz), 3.75 (s, 3H, COOMe), 3.79 (m, 1H, H-4'), 3.87 (s, 2H, H-4a, H-4b), 4.01 (ddd, 1H, H-3', $J_{3',4'}$ = 6.6, $J_{3',2'}$ = 5.2 Hz), 4.14 (ddd, 1H, H-2'), 4.69 (dd, 1H, 5'-OH; $J_{5'\text{-OH},5''}$ = 7.3, $J_{5'\text{OH},5'}$ = 3.9 Hz), 4.83 (d, 1H, 3'-OH; $J_{3'\text{-OH},3'}$ = 6.5 Hz), 4.99 (d, 1H, OH-2'; $J_{2'\text{-OH},2'}$ = 5.2 Hz), 5.18 (d, 1H, H-1'; $J_{1',2'}$ = 3.5 Hz), 8.90 (s, 1H, NH). ^{13}C NMR ($(CD_3)_2SO$) δ 29.54 ($C\underline{Me}_2$), 30.62 (C-4), 51.23 COO<u>Me</u>), 61.72 (C-5'), 70.31 (C-3'), 72.73 (C-7), 74.52 (C-2'), 78.54 C-1'), 83.47 (C-4'), 107.02 (C-3), 140.04 (C-3a), 153.01 C-2), 162.81 (<u>C</u>OOMe), 164.70 (C-5). ^{15}N NMR (D_2O) δ –102.6 (N-1), –166.3 (N-8), –244.1 (N-6). HRMS FAB (glycerol) m/z calcd for $C_{15}H_{22}N_3O_7$ 356.1458, found 356.1467 (MH^+). Anal. calcd for $C_{15}H_{21}N_3O_7$ x H_2O: C, 48.25; H, 6.20; N, 11.25. Found: C, 47.91; H, 5.96; N, 11.08.

[4-Methoxycarbonyl-5-(β-D-ribofuranosyl)-1*H*-pyrazol-3-yl]acetamide (1a). CF_3COOH (20 μL) was added into a suspension of **2a** (15 mg, 0.04 mmol) in THF/H_2O (0.25 mL, 2/1). The mixture was stirred for 1 d at 55 °C and evaporated to yield glassy compound which was by TLC and ^{1}H NMR analysis identical to the acetamide **1a** used as starting material for **2a**.

Table 2. X-ray structure data

Empirical formula	$C_{18}H_{25}N_3O_7$
Formula weight	395.41
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	monoclinic, P 2_1
Unit cell dimensions	a = 6.202(2) Å b = 33.077(3) Å c = 9.460(2) Å β = 91.47(3)°
Volume	1940.0(8) $Å^3$
Z, Calculated density	4, 1.354 Mg/m^3
Absorption coefficient	0.105 mm^{-1}
F(000)	840
Crystal size	0.21 x 0.22 x 0.34 mm
θ range for data collection	2.48 to 26.20°
Index ranges	-7<=h<=7, -40<=k<=40, -11<l<=11
Reflections collected / unique	11556 / 6590 [R(int) = 0.0393]
Completness to 2θ = 26.20°	85.3%
Refinement method	Full-matrix least squares on F^2
Data / restraints / parameters	6590 / 1 / 706
Goodness-of-fit on F^2	0.947
Final R indices [I>2σ(I)]	R1 = 0.0341, wR2 = 0.0684
R indices (all data)	R1 = 0.0547, wR2 = 0.0731
Extinction coefficient	0.054(2)
Largest diff. peak and hole	0.122 and -0.154 e. $Å^{-3}$

Conformational analysis of $^3J_{HH}$. The conformational analysis of the ribofuranosyl moiety in **1a** and **2b** has been performed with the use of computer program PSEUROT[20] which finds the best fit between experimental and calculated $^3J_{HH}$. The input consists of the parameters P_1 - P_6 for the generalized Karplus-Altona equation,[24] the λ electronegativities of the four substituents, A and B parameters, the experimental $^3J_{HH}$ and the initial guesses of the geometries of the starting conformers and their respective populations. The following λ electronegativity values were used: 0.0 for H, 0.45 for *C*-aglycone, 1.26 for OH, 1.27 for O4', 0.62 for C1', C2', C3' and C4' and 0.68 for C5'. As there are only three experimental $^3J_{HH}$ to define the N ⇄ S pseudorotational equilibrium and the S-type conformers in **1a** and **2b** are populated by less than 30% we have constrained their geometry during the iteration procedure to P_S = 162° and Ψ_m^S = 39°.

The best fit between J^{expt} and J^{calcd} was obtained with root-mean-square error below 0.02 Hz and $\Delta J^{max} < 0.1$ Hz.

X-ray structure analysis. X-ray crystallographic data are presented in Table 2. The X-ray data were collected on Kappa CCD Nonius diffractometer with MoKα radiation (λ = 0.71073 Å) with a graphite monochromator. The structure was solved routinely applying SHELXS-86[25] and the initial model was refined on F^2 using fullmatrix least-squares techniques with anisotropic displacement parameters for nonhydrogens.[26] All hydrogens were found in the final difference electron density map and were fully refined with isotropic thermal displacement parameters in the final stage of refinement. The absolute configuration was assumed from chemical considerations and NMR. All computations were performed on PC486/16 with the programs SHELXS-97,[26] NRCVAX[27] and PLUTON98.[28] Scattering factors were taken from the usual source.[29]

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