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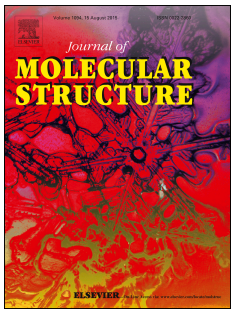


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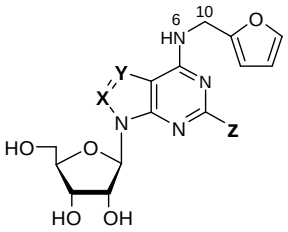
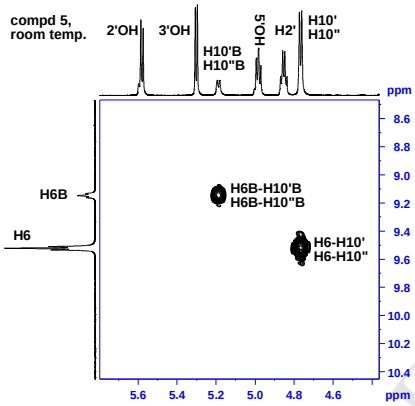



Kinetin riboside (**3**):
X = CH, Y = N, Z = H

Analogues:
**4** X = CH, Y = CH, Z = H
**5** X = N, Y = N, Z = H
**6** X = CH, Y = N, Z = $NH_2$
**7** X = CH, Y = N, Z = F



# Studies on structure of kinetin riboside and its analogues by variable-temperature NMR

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## Abstract

Kinetin riboside and its four base-modified analogues were synthesized and their structures in solution were examined by multinuclear 1D and 2D variable-temperature NMR techniques. At lowered temperature kinetin riboside was found to exist as a mixture of two distinct conformers resulting from the restricted rotation around the C6-$N^6$ bond. For 8-azakinetin riboside and 2-fluorokinetin riboside the presence of two rotamers was observed at room temperature.

*Keywords:* Kinetin riboside; Kinetin riboside analogues; Synthesis; Variable-temperature NMR; $^{15}$N NMR; Restricted rotation

## 1. Introduction

Multinuclear 1D and 2D NMR is a valuable tool for investigation of the dynamic intramolecular processes in adenine derivatives, among others of amino-imino tautomerism and rotation around C-N bond. It has been reported that the amino form of adenine is absolutely dominant in solution, however additional substitution may shift the tautomeric amino-imino equilibrium [1]. For $N^6$-alkoxy-9-alkyladenines and $N^6$-alkoxyadenosines the imino form is more favored in all cases where tautomerism has been clearly found to exist by $^1$H NMR in DMSO-$d_6$ at room temperature [2]. Modification of $N^6$-methoxy-9-methyladenine by introduction of selected substituents in its 2 position has been shown to shift the ratio of tautomers toward the amino tautomer as determined by $^1$H, $^{13}$C and $^{15}$N NMR in DMSO-$d_6$ [3,4]. The percent amino tautomer is much higher in solvents with increased dipole moment and with the ability to take part in hydrogen bonding [4].

On the other hand variable-temperature NMR technique has been used to demonstrate the restricted rotation of the C6-$N^6$ bond in $N^6$-substituted adenine derivatives. Martin and Reese have found that the spectrum of $N^6$,$N^6$-dimethyl-2',3'-*O*-isopropylideneadenosine in $CDCl_3$ exhibits two $N^6$-methyl signals at -60 °C and one signal at 40 °C [5]. They have interpreted it as an example of hindered internal rotation, with slow exchange occurring below, and fast exchange occurring above 0 °C. Engel and von Hippel have proposed the preferential *syn* conformation for $N^6$,9-dimethyladenine at low temperature in $CDCl_3$ [6]. Dodin et al. have postulated that for 9-alkyl-$N^6$-methyladenines the favored *syn* conformer is stabilized by $N^6$H-N7 hydrogen bonding, whereas the minor *anti* rotamer is stabilized by $N^6$H-N1 bonding but destabilized by the steric interaction of the methyl group [7]. These authors have observed a 1:1 ratio of both rotamers for 1-alkyl-4-methylaminopyrazolo[3,4-*d*]pyrimidines (9-alkyl-$N^6$-methyl-8-aza-7-deazaadenines) at -40 °C. Hindered rotation of C6-$N^6$ bond at room temperature has been reported for $N^6$,3-dialkyladenines in DMSO-$d_6$ and $CDCl_3$ [8]. Geometrical isomerism has been also found by means of $^1$H and $^{13}$C NMR in liquid ammonia containing potassium amide at -50 °C for the anions of adenine and 2-aminopurine [9].

Novotna et al. have reported the existence of amino and 1H-imino tautomeric forms for 2-chloro-$N^6$-furfuryladenine (Fig. 1: **1**) and 2-chloro-$N^6$-(5-methylfurfuryl)adenosine (**2**) at room temperature based on NMR studies performed in DMSO-$d_6$ and DMF-$d_7$ [10]. At increased temperature the spectra of these compounds show only a single set of signals attributed to a time-averaged contribution of both tautomers due to fast chemical exchange. The C2-non-substituted congener of **2**, $N^6$-furfuryladenosine (kinetin riboside, KR; **3**), has been shown to occur at room temperature only as amino tautomer. The abovementioned results motivated us to extend the described studies to **3** and its selected modified analogues, 7-deazakinetin riboside (7-deazaKR; Fig. 2: **4**), 8-azakinetin riboside (8-azaKR; **5**), 2-aminokinetin riboside (2-aminoKR; **6**) and 2-fluorokinetin riboside (2-fluoroKR; **7**) at various temperatures in order to have a broader view on their structures in solution.

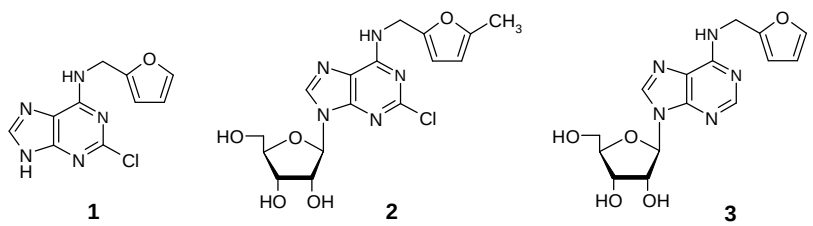



**Fig. 1.** Compounds discussed in ref. [10].

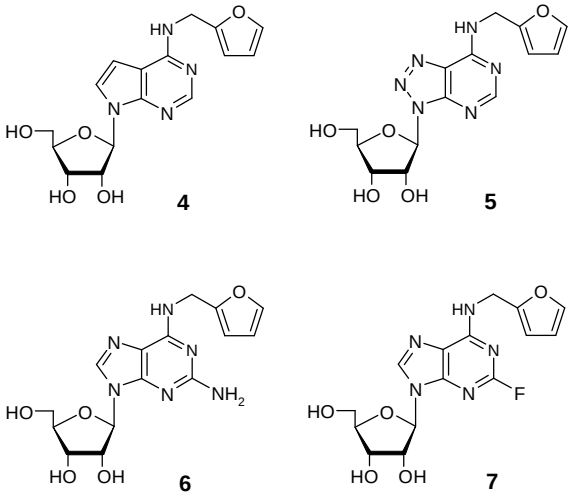



**Fig. 2.** Synthesized compounds discussed in this paper.

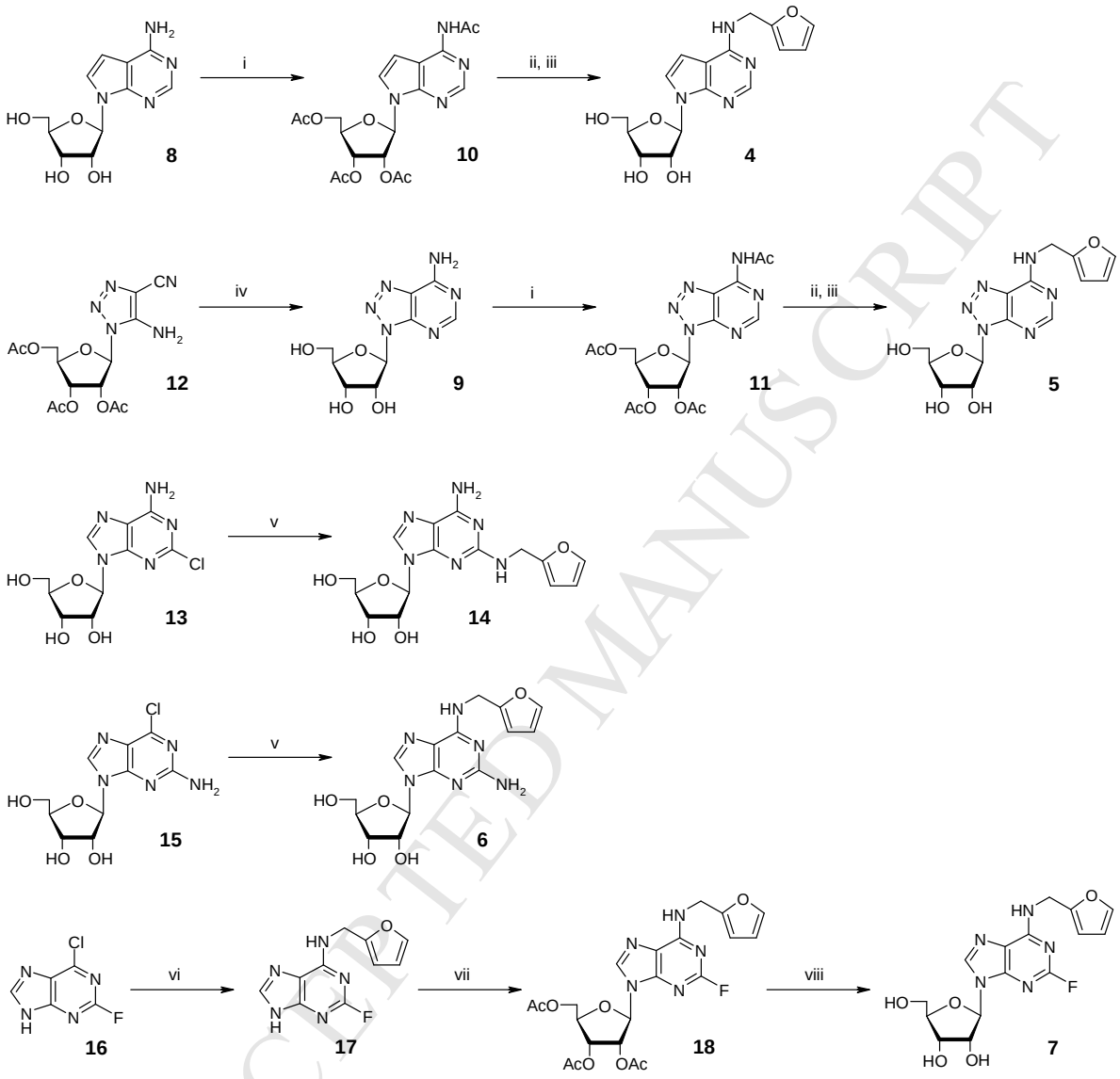
## 2. Results and discussion

### *2.1. Syntheses*

Kinetin riboside (**3**) was prepared by $N^6$-alkylation of *N*$^6$-acetyl-2',3',5'-tri-*O*-acetyladenosine with furfuryl alcohol under Mitsunobu conditions as described [11]. In a similar manner the analogous new compounds, 7-deazakinetin riboside (**4**) and 8-azakinetin riboside (**5**) were synthesized starting from 7-deazaadenosine (tubercidin; **8**) and 8-azaadenosine (**9**), which were converted into their tetraacetates (**10** and **11**, respectively) and subsequently subjected to Mitsunobu reaction followed by deacetylation of the product formed. Compound **9** was obtained by cyclization of 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carbonitrile (**12**) [12] with diethoxymethyl acetate and methanolic ammonia as elaborated earlier for the synthesis of related compounds [13].

2-Chloroadenosine (**13**) was reacted with furfurylamine and diisopropylethylamine according to the previously reported general procedure for the synthesis of $N^2$-substituted 2-aminoadenosines [14], but satisfactory yield of the resulting 2-(furfurylamino)adenosine (**14**) was achieved using larger excess of furfurylamine and with 2-methoxyethanol as solvent [15]. The synthesis of 2-aminokinetin riboside (**6**) was performed by the same method, making use of 2-amino-6-chloropurine riboside (**15**) as starting material. To obtain the hitherto undescribed 2-fluorokinetin riboside (**7**) 6-chloro-2-fluoropurine (**16**) was first treated with furfurylamine in the presence of triethylamine to afford 2-fluorokinetin (**17**) which was separated from the mixture containing also 6-chloro-2-(furfurylamino)purine. Compound **17**

was ribosylated and the resulting product **18** was afterwards deprotected to give the desired compound **7**. The synthetic route for the discussed compounds is outlined in Scheme 1.




**Scheme 1.** Synthesis of compounds **4**-**7** and **14**. Reagents and conditions: (i) $Ac_2O$, Pyr, rt → 60 °C, then imidazole, EtOH, rt; (ii) furfuryl alcohol, $Ph_3P$, DEAD, THF, rt; (iii) 25% $NH_4OH$, MeOH, $CH_2Cl_2$, rt; (iv) DEMA, 90 °C, then $NH_3$-MeOH, rt; (v) furfurylamine, DIPEA, 2-methoxyethanol, 125 °C; (vi) furfurylamine, $Et_3N$, MeOH, 60 °C; (vii) 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, p-TsOH, chlorobenzene, 145 °C; (viii) 25% $NH_4OH$, MeOH, $CHCl_3$, rt.

*2.2. NMR study*

Characteristic broadening of signals of the $CH_2$ group attached to the $N^6$ atom of adenosine has been reported previously by others and ascribed to a chemical exchange process connected to the restricted conformational change [16-18]. To our knowledge, however, studies at lower temperature in order to obtain separated signals of particular conformers have not been carried out as far. In the $^1$H NMR spectrum of kinetin riboside (**3**) in DMSO-$d_6$ recorded at room temperature we observe broad signal of $N^6$-C*H*$_2$ (specified as C10-*H*$_2$ in this paper) at 4.70 ppm and broad signal of $N^6$-*H* at 8.31 ppm (Table 1). In the $^1$H NMR spectrum of **3** in acetone-$d_6$ these protons are reflected by broad signals at 4.87 ppm and 7.42 ppm, respectively (Table 2). Decreasing the temperature causes the appearance of double signals starting from 0 °C. The spectrum of **3** in acetone-$d_6$ at -20 °C exhibits two sets of signals corresponding to major and minor components of the mixture (in a ratio of approximately 9:1, according to integration). We note the presence of two doublets of doublets of C10-*H*$_2$ (at 4.87 and 4.82 ppm) corresponding to the predominant conformer and two minor doublets of doublets (at 5.34 and 5.27 ppm). Besides, there are two triplets of $N^6$-*H* (major at 7.90 ppm and minor at 7.82 ppm), two singlets of C8-*H* (major at 8.23 ppm and minor at 8.31 ppm) and two singlets of C2-*H* (major at 8.27 ppm and minor at 8.13 ppm). The crucial assignment of $N^6$-*H* protons is supported by $^1$H-$^1$H COSY spectrum recorded at -20 °C showing distinct crosspeaks between the triplets at 7.90 ppm and at 7.82 ppm and the respective signals of C10-*H*$_2$ for both components of the mixture, but no crosspeaks between these triplets and the signals of C2-*H* (Fig. 3) are observed. Proton vicinal coupling constants ($^3J_{HH}$) between $N^6$-*H* and C10-*H*$_2$ are of 6.0 Hz and 6.7-6.9 Hz for major and minor component, respectively. The $^{13}$C spectrum of **3** recorded at -20 °C shows two sets of signals of *C*10, *C*6, *C*5, *C*2, *C*8, *C*11 and *C*14 (Table 2). In the $^1$H-$^{15}$N gHSQC spectrum of **3** at -20 °C two separated one-bond $^1$H-$^{15}$N correlation signals are detected: strong at 85.1 ppm and weak at 93.6 ppm. The spectrum confirmed the coupling of $N^6$ and $N^6$-*H* for the major component, but indicated also the coupling of $N^6$ and $N^6$-*H* for the minor one (Fig. 4). This means that both components are in one tautomeric form – the amino form. Additionally, $^1$H-$^{15}$N gHMBC spectrum reveals the long range correlations of *N*1 with $N^6$-*H* and C2-*H* at 231.4 ppm, thus unambiguously supporting for aromatic character of *N*1 (Supplementary data: Fig. 16). These results in turn clearly show the restricted rotation around the C6-$N^6$ bond in the molecule of **3** resulting in the existence of *syn* and *anti* rotamers at temperature below 0 °C (Fig. 5). Comparison of the $^1$H NMR spectra recorded between 35 °C and -30 °C in steps of 5

°C (Supplementary data: Fig. 67) reveals that hydroxyl protons of the ribose moiety undergo downfield shift of frequency resonance by *ca* 0.42-0.68 ppm because of increased hydrogen bonding with the solvent. However in the case of $N^6$-*H* the observed broad singlet at 35 °C is shifted downfield by 0.69 ppm and at -30 °C gives undeniable triplet for both components of the mixture. This effect results from slowing down intermolecular proton exchange leading to the split of $N^6$-*H* signal with observable coupling to nearby protons. Analogously, the same effect of splitting the signal depending on temperature is observed for C10-$H_2$. It is noteworthy that C2-*H* or C8-*H* signals of minor conformer are shifted upfield or downfield respectively, compared with C2-*H* or C8-*H* of major component due to the anisotropic shielding/deshielding effects of the aromatic ring of furan.

**Table 1**
Selected $^{1}H$, $^{13}C$ and $^{15}N$ NMR chemical shifts for compounds **3**-**7** in DMSO-$d_6$ at room temperature; δ in ppm (J in Hz)

| | **3** (KR) | **4** (7-deazaKR) | **5** (8-azaKR) | **6** (2-aminoKR) | **7** (2-fluoroKR) |
|---|---|---|---|---|---|
| $N^6$-*H* | 8.31 brs | 8.00 t (6.0) | 9.52 t (5.8, 6.0)[a]<br>9.14 t (6.4, 6.5)[b] | 7.72 brs | 8.88 t (5.1, 5.0)[a]<br>8.61 brs[b] |
| C10-$H_2$ | 4.70 brs | 4.70 d (6.0) | 4.76 d (5.9)[a]<br>5.19 d (6.7)[b] | 4.63 brs | 4.62 d (8.9)[a]<br>5.16 m[b] |
| C8-*H* | 8.38 s | 7.36 d (4.0) | – | 7.93 s | 8.38 brs[a]<br>8.40 brs[b] |
| C7-*H* | – | 6.66 d (4.0) | – | – | – |
| C12-*H* | 6.23 d (3.2) | 6.27 d (3.2) | 6.31 d (3.0)[a]<br>6.33 d (2.9)[b] | 6.24 d (3.2) | 6.26 d (2.8)[a]<br>6.28 brs[b] |
| C14-*H* | 7.53 dd (2.0, 0.8) | 7.57 brs | 7.56 d (0.9)[a]<br>7.58 brs[b] | 7.53 dd (2.0, 0.8) | 7.56 brs[a]<br>7.54 brs[b] |
| C2-*H* | 8.24 s | 8.15 s | 8.45 s[a]<br>8.34 s[b] | – | – |
| *C*10 | 36.49 | 36.58 | 36.70 | 36.24 | 37.23 |
| *C*6 | 154.31 | 155.77 | 154.16[a]<br>155.59[b] | 154.63 | 155.78 (20.7) |
| *C*5 | 119.82 | 103.52 | 124.63[a]<br>123.83[b] | 113.73 | 118.00 |
| *C*2 | 152.25 | 151.20 | 156.84[a]<br>156.49[b] | 159.92 | 158.48 (204.5) |
| *C*4 | 148.58 | 149.48 | 148.57[a]<br>149.98[b] | 151.08 | 150.02 (20.1) |
| *C*8 | 140.01 | 122.37 | – | 136.18 | 140.09[a]<br>139.75[b] |
| *C*11 | 152.86 | 152.87 | 151.65 | 153.31 | 151.98[a]<br>152.44[b] |
| *C*12 | 106.65 | 106.80 | 107.29[a]<br>107.46[b] | 106.62 | 107.11 |
| *C*14 | 141.83 | 141.90 | 142.19[a]<br>142.65[b] | 141.65 | 142.09[a]<br>142.36[b] |
| *C*1’ | 87.90 | 87.54 | 89.84[a]<br>89.52[b] | 86.97 | 87.53 |
| $N^6$ | 86.1 | 86.6 | 96.3[a]<br>103.4[b] | 83.2 | 92.5[a]<br>99.5[b] |
| *N*7 | 240.3 | – | nd[c] | 241.0 | 241.3 |
| *N*9 | 170.3 | 154.6 | 238.3 | 166.4 | 171.8 |
| *N*1 | nd[c] | 226.4 | 230.4 | nd[c] | 195.3 |
| *N*3 | nd[c] | 224.4 | 216.5 | nd[c] | nd[c] |
| C2-*F* | – | – | – | – | 50.60[a]<br>52.62[b] |

[a] Major component. [b] Minor component. [c] Not detected.

**Table 2**
Selected $^{1}H$, $^{13}C$ and $^{15}N$ NMR chemical shifts for compounds **3**, **4** and **6** in acetone-$d_6$ at room and low temperatures; δ in ppm (J in Hz)

| | **3** (KR) | | **4** (7-deazaKR) | | **6** (2-aminoKR) | |
|---|---|---|---|---|---|---|
| | 25 °C | -20 °C | 25 °C | -20 °C | 25 °C | -20 °C |
| $N^6$-*H* | 7.42 brs | 7.90 t (6.0, 6.0)[a]<br>7.82 t (6.7, 6.9)[b] | 7.24 brs | 7.56 t<br>(5.4, 5.4) | 7.12 brs | 7.63 t (6.0, 6.1)[a]<br>7.26 t (5.8, 6.7)[b] |
| C10-$H_2$ | 4.87 brs[c] | 4.87 dd (6.1, 15.7)[a]<br>4.82 dd (6.1, 15.8)[a]<br>5.34 dd (6.9, 15.9)[b]<br>5.27 dd (6.8, 15.9)[b] | 4.83 dd<br>(5.7, 16.5)<br>4.79 dd<br>(5.6, 16.5) | 4.82 dd<br>(5.6, 15.6)[d]<br>4.77 dd<br>(5.5, 15.3)[d] | 4.74 brs | 4.72 d (6.0)[a]<br>5.23 d (6.6)[b] |
| C8-*H* | 8.17 s | 8.23 s[a]<br>8.31 s[b] | 7.27 d<br>(3.6) | 7.30 d (3.6) | 7.74 s | 7.81 s[a]<br>7.88 s[b] |
| C7-*H* | — | — | 6.60 d<br>(3.6) | 6.60 d (3.7) | — | — |
| C2-*H* | 8.26 s | 8.27 s[a]<br>8.13 s[b] | 8.19 s | 8.18 s | — | — |
| *C*10 | 37.70 | 37.28[a]<br>40.22[b] | 37.95 | 37.44 | 37.46 | 37.02 |
| *C*6 | 155.98 | 155.57[a]<br>156.31[b] | 157.45 | 157.07 | 156.30 | 155.84[a]<br>156.58[b] |
| *C*5 | 122.14 | 121.89[a]<br>120.85[b] | 106.06 | 105.95 | 116.43 | 115.97[a]<br>114.81[b] |
| *C*2 | 152.99 | 152.74[a]<br>152.53[b] | 151.97 | 151.63 | 160.70 | 160.44[a]<br>160.04[b] |
| *C*8 | 141.67 | 141.85[a]<br>141.54[b] | 125.64 | 126.01 | 138.70 | 138.75[a]<br>nd[e] |
| *C*11 | 153.83 | 153.46[a]<br>153.92[b] | 154.02 | 153.57 | 154.08 | 153.73[a]<br>154.39[b] |
| *C*14 | 142.85 | 142.76[a]<br>143.04[b] | 142.83 | 141.85 | 142.71 | 142.61[a]<br>142.81[b] |
| $N^6$ | 83.9 | 85.1[a]<br>93.6[b] | 84.6 | 85.5 | 82.3 | 83.1[a]<br>90.4[b] |
| *N*7 | 238.2 | 237.8[a]<br>241.9[b] | — | — | 237.6 | 238.0 |
| *N*9 | 168.5 | 168.6[a]<br>167.4[b] | 152.6 | 152.4 | 165.0 | 165.7 |
| *N*1 | 231.5 | 231.4 | 226.3 | 225.6 | nd[e] | 197.6 |
| *N*3 | 221.1 | 220.2 | 222.1 | 220.9 | nd[e] | nd[e] |

[a] Major component. [b] Minor component. [c] Signal partially overlapped with signal of C2'-*H*. [d] Signal overlapped with signal of C2'-O*H*. [e] Not detected.

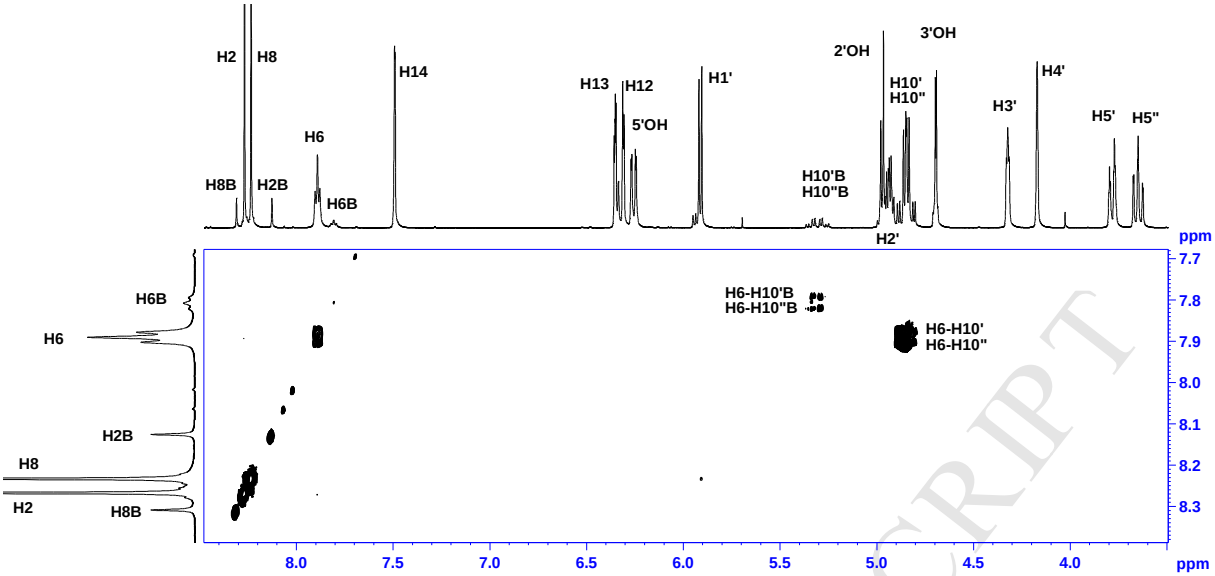



**Fig 3.** Fragment of $^{1}$H-$^{1}$H COSY spectrum of compound **3** in acetone-$d_6$ recorded at -20 °C.

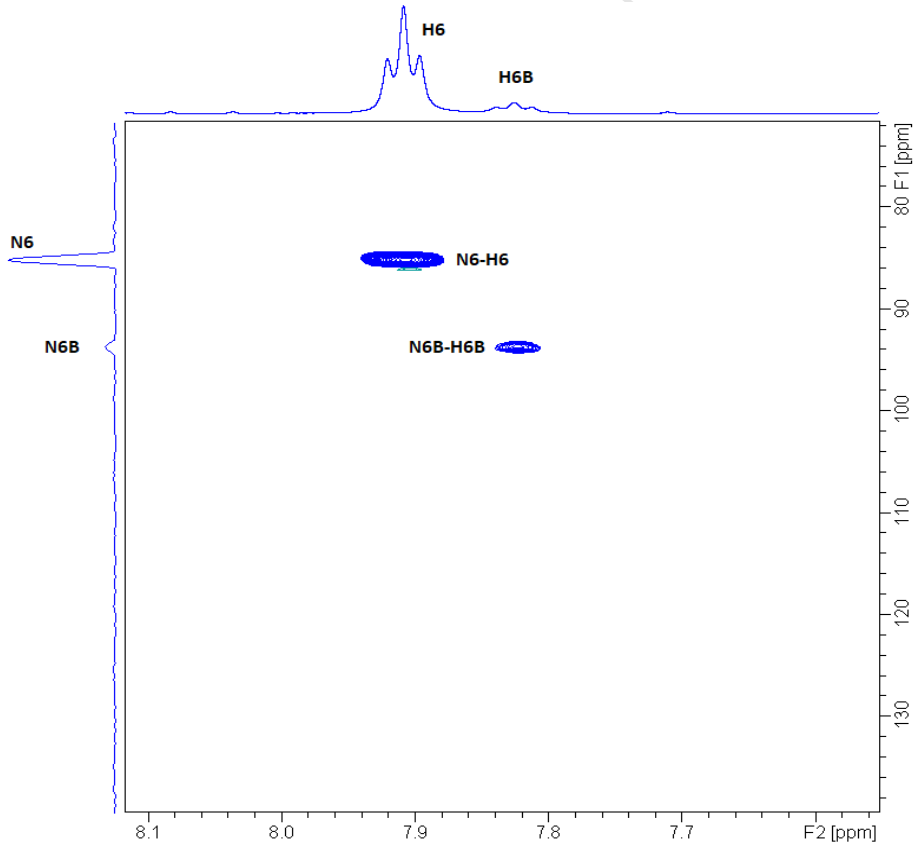



**Fig. 4.** Fragment of $^{1}$H-$^{15}$N gHSQC spectrum of compound **3** in acetone-$d_6$ recorded at -20 °C

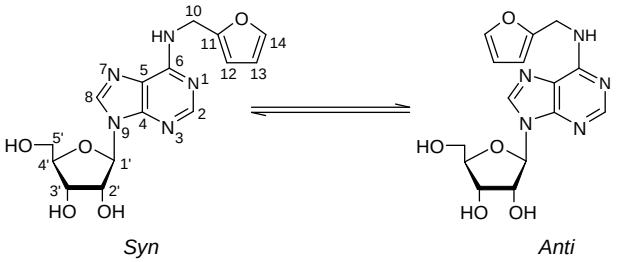



**Fig. 5.** Rotameric equilibrium of **3** at lowered temperature and numbering of atoms.

The $^{1}$H NMR spectra of 8-azakinetin riboside (**5**) and 2-fluorokinetin riboside (**7**) in DMSO-$d_6$ recorded at room temperature (Table 1) exhibit two sets of signals corresponding to major and minor components of the mixture (approximately 80% and 20%, respectively, according to integration). The latter compound, bearing the electron-withdrawing substituent in position 2 resembles the described previously [10] analogue **2**. We observe two doublets of C10-$H_2$ for **5** (major at 4.76 ppm and minor at 5.19 ppm) and two signals of C10-$H_2$ for **7** (major doublet at 4.62 ppm and minor one at 5.16 ppm). Signals of N$^6$-*H* occur as two triplets for **5** (major at 9.52 ppm and minor at 9.14 ppm), whereas for **7** as major triplet at 8.88 ppm and minor broad signal at 8.61 ppm. In the $^{1}$H-$^{1}$H COSY spectrum of **5** and **7** recorded at 25 °C crosspeaks between both signals of N$^6$-*H* and the respective signals of C10-$H_2$ are present (Fig. 6). Proton vicinal coupling constants ($^{3}J_{HH}$) between N$^6$-*H* and C10-$H_2$ are of 5.8-6.0 Hz and 6.4-6.5 Hz for major and minor component of **5**, respectively. For **5** there are two signals of C2-*H* (major at 8.45 ppm and minor at 8.34 ppm), of C12-*H* (major at 6.31 ppm and minor at 6.33 ppm) and of C14-*H* (major at 7.56 ppm and minor at 7.58 ppm), whereas for **7** of C8-*H* (major at 8.38 ppm and minor at 8.40 ppm), of C12-*H* (major at 6.26 ppm and minor at 6.28 ppm) and of C14-*H* (major at 7.56 ppm and minor at 7.54 ppm). The $^{13}$C spectrum of **5** recorded at room temperature shows two sets of signals of *C*6, *C*5, *C*2, *C*4, *C*12, *C*14 and *C*1'. The analogous spectrum of **7** shows two sets of signals of *C*8, *C*11 and *C*14. Splitting of signals is also detected at room temperature in $^{1}$H-$^{15}$N gHSQC spectrum for **5** (*N*$^6$: major signal at 96.3 ppm and pronounced minor one at 103.4 ppm) and for **7** (*N*$^6$: major signal at 92.5 ppm and slightly visible minor one at 99.5 ppm), and in $^{19}$F spectrum for **7** (C2-*F*: major signal at 50.60 ppm and minor one at 52.62 ppm). All these separated signals coalesce upon heating to 80 °C (Table 3). As in the case of **3** at -20 °C, the $^{1}$H-$^{15}$N gHSQC spectrum of **5** recorded at room temperature indicated the coupling of *N*$^6$ and N$^6$-*H* for both components (Fig. 7), suggesting the restricted rotation around the C6-N$^6$ bond and the existence of two rotamers of **5**. The coupling of *N*$^6$ and N$^6$-*H* for the minor component is also observed, although less evidently, for compound **7**. Analysis of the $^{1}$H NMR spectra recorded for **5** and

**7** between 20 °C and 80 °C in steps of 5 °C reveals that signals of hydroxyl protons of ribose moiety undergo upfield shift of frequency resonance by *ca* 0.31-0.37 ppm due to breaking hydrogen bonds with the solvent (Supplementary data: Fig. 69 and 70). The observed coupled nuclei *i.e.* $N^6$-*H* and C10-$H_2$ for **5** and **7** are gradually broadened loosing their splitting pattern and $N^6$-*H* is shifted upfield by 0.41-0.47 ppm. The changes of frequency resonances of C2-*H* and C8-*H* of minor component for **5** and **7**, respectively, are the same as in the case of **3**.

**Table 3**
Selected $^1$H, $^{13}$C and $^{15}$N NMR chemical shifts for compounds **5** and **7** in DMSO-$d_6$ at room and increased temperatures; δ in ppm (J in Hz)

| | **5** (8-azaKR) | | **7** (2-fluoroKR) | |
|---|---|---|---|---|
| | 25 °C | 80 °C | 25 °C | 80 °C |
| $N^6$-*H* | 9.52 t (5.8, 6.0)[a]<br>9.14 t (6.4, 6.5)[b] | 9.14 brs | 8.88 t (5.1, 5.0)[a]<br>8.61 brs[b] | 8.44 brs |
| C10-$H_2$ | 4.76 d (5.9)[a]<br>5.19 d (6.7)[b] | 4.83 brs | 4.62 d (8.9)[a]<br>5.16 m[b] | 4.77[d] |
| C2-*H* | 8.45 s[a]<br>8.34 s[b] | 8.40 brs | — | — |
| *C*6 | 154.16[a]<br>155.59[b] | 151.40 | 155.78 (20.7) | 155.67 |
| *C*5 | 124.63[a]<br>123.83[b] | nd[c] | 118.00 | 117.65 |
| *C*2 | 156.84[a]<br>156.49[b] | 156.21 | 158.48 (204.5) | 158.12 |
| *C*4 | 148.57[a]<br>149.98[b] | nd[c] | 150.02 (20.1) | nd[c] |
| *C*8 | — | — | 140.09[a]<br>139.75[b] | 139.52 |
| *C*11 | 151.65 | 151.40 | 151.98[a]<br>152.44[b] | 151.82 |
| *C*14 | 142.19[a]<br>142.65[b] | 141.69 | 142.09[a]<br>142.36[b] | 141.56 |
| *C*1’ | 89.84[a]<br>89.52[b] | 89.76 | 87.53 | 87.55 |

[a] Major component. [b] Minor component. [c] Not detected. [d] Signal overlapped with signal of C5’-O*H*.

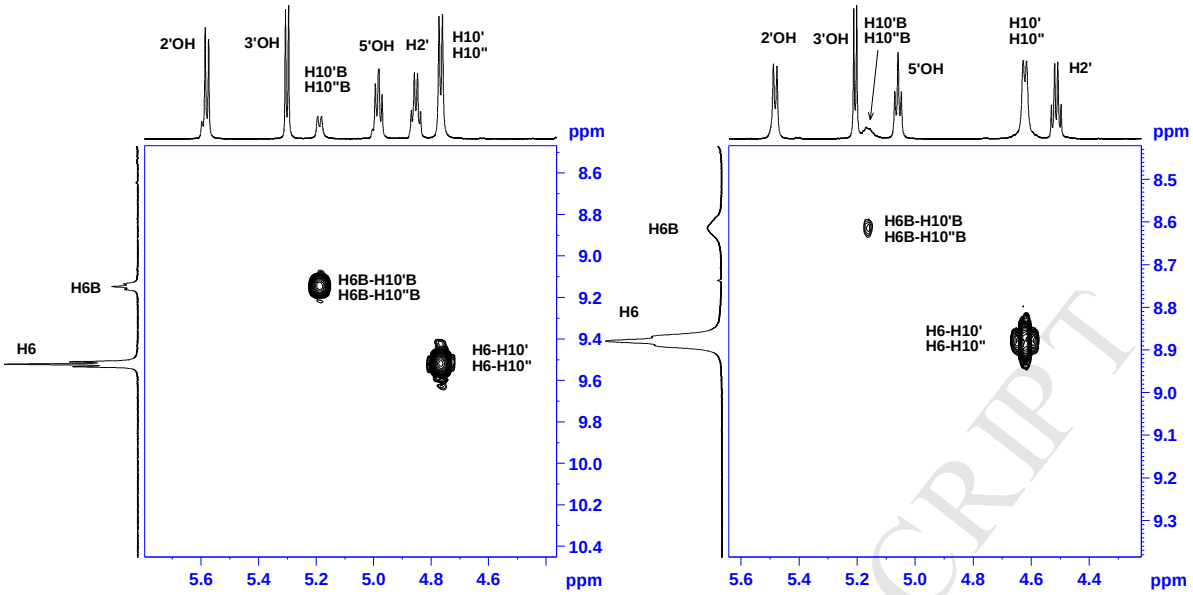



**Fig. 6.** Fragments of $^{1}$H-$^{1}$H COSY spectra of compounds **5** (left) and **7** (right) in DMSO-$d_6$ recorded at room temperature

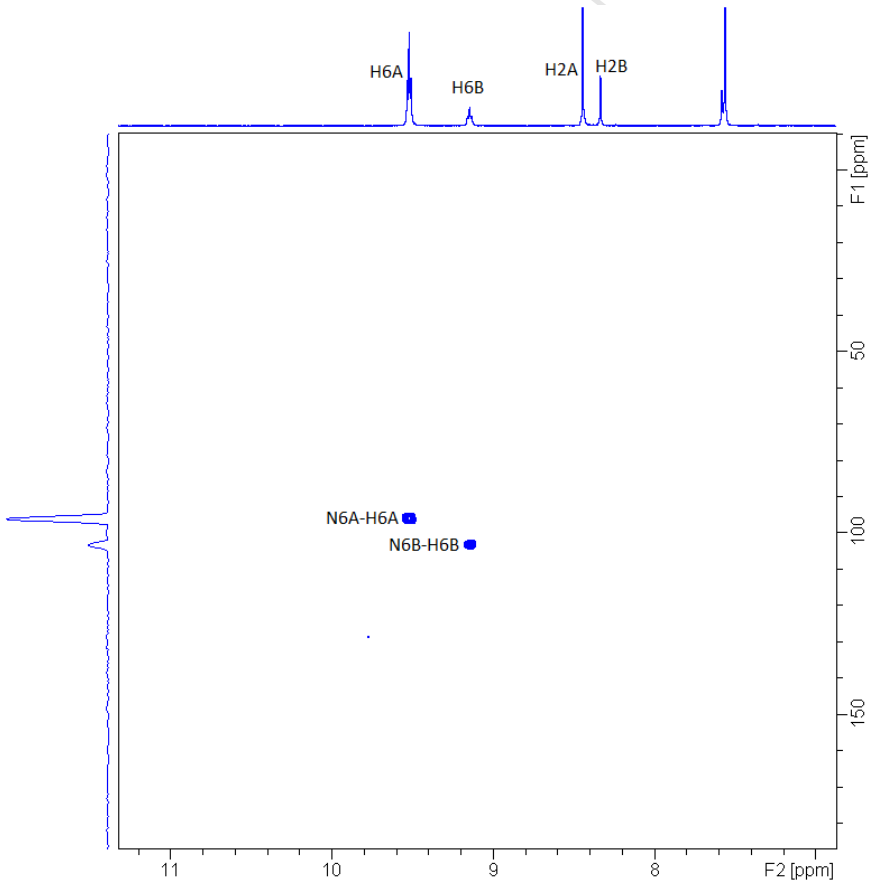



**Fig. 7.** Fragment of $^{1}$H-$^{15}$N gHSQC spectrum of compound **5** in DMSO-$d_6$ recorded at room temperature

There is only one set of signals in $^{1}H$, $^{13}C$ and $^{1}H$-$^{15}N$ gHSQC NMR spectra of 7-deazakinetin riboside (**4**) and 2-aminokinetin riboside (**6**) in DMSO-$d_6$ at room temperature (Table 1). The $^{1}H$ spectra show a doublet of C10-$H_2$ at 4.70 ppm for **4** and a broad signal of C10-$H_2$ at 4.63 ppm for **6**. The signal of $N^6$-*H* occurs at 8.00 ppm (triplet) for **4** and at 7.72 ppm (broad singlet) for **6**. In acetone-$d_6$ the signal of C10-$H_2$ for **4** appears at 4.83 ppm as two doublets of doublets, whereas for **6** at 4.74 ppm as a broad singlet (Table 2). The signal of $N^6$-*H* is detected for **4** at 7.24 ppm (broad singlet) and for **6** at 7.12 ppm (broad singlet). The splitting of signals is observed upon lowering the temperature in the case of **6**. At -20 °C for **6** there are two doublets of C10-$H_2$ (major at 4.72 ppm and minor at 5.23 ppm), two triplets of $N^6$-*H* (major at 7.63 ppm and minor at 7.26 ppm) and two singlets of C8-*H* (major at 7.81 ppm and minor at 7.88 ppm). The $^{1}H$ NMR spectrum of 2-(furfurylamino)adenosine (**14**) in DMSO-$d_6$ at room temperature is significantly different from that of its regioisomer **6**. The signal of $N^2$-C$H_2$ for **14** occurs at 4.45 ppm and, contrary to $N^6$-C$H_2$ of **6**, is a partially ovelapping doublet of doublets. The signal of $N^2$-*H* (triplet at 6.59 ppm) is shifted upfield in comparison with $N^6$-*H* of **6**. These sharp signals observed for **14** indicate that internal rotation around the exocyclic C2-$N^2$ bond in the case of this compound is not restricted.

In literature there has appeared a brief remark postulating that the presence of two rotamers around the C-N bond in purine derivatives might be misinterpreted as a tautomeric equilibrium [1]. Our results seem to reveal the existence of the restricted rotation in kinetin riboside (**3**), which is manifested by broad signals of $N^6$-*H* and $N^6$-C$H_2$ in the $^{1}H$ NMR spectrum at room temperature and by separated signals in the $^{1}H$, $^{13}C$ and $^{1}H$-$^{15}N$ gHSQC NMR spectra when recorded at lowered temperature. Significantly less visible double set of signals at -20 °C is also observed for compound **6**. Two sets of signals corresponding to both rotamers occur at room temperature for nitrogen-enriched compound **5**, and for compound **7** possessing the electron-withdrawing substituent in position 2. On the other hand for compound **4** with nitrogen deficiency in position 7 we do not observe double set of signals that could indicate decisive effect of nitrogen N7 on restricted rotation along C6-$N^6$ bond as in the case of other discussed compounds. Our suggestions are in some contrast to the conclusions formulated by Novotna et al. [10], the latter concerning however other compounds, namely **1** and **2**. In turn, our hypothesis has been confirmed by others who have reported the restricted rotation around the exocyclic C-N bond in 8-azaadenine derivatives *i.e.* the compounds related to **5**, 9-(3-chlorophenyl)-$N^6$-cyclopentyl-2-methylthio-8-azaadenine [19] and 9-substituted $N^6$-arylhydrazone-2-propylthio-8-azaadenines [20].

## 3. Conclusions

Four base-modified analogues of kinetin riboside were prepared making use of various synthetic methodologies. Their structures as well as the structure of kinetin riboside were examined in solution using 1D and 2D multinuclear NMR techniques. Separation of signals as a result of restricted rotation around the C6-$N^6$ bond was demonstrated at lowered temperature for kinetin riboside and 2-aminokinetin riboside, whereas at room temperature for 8-azakinetin riboside and 2-fluorokinetin riboside. The key experimental evidence for the existence of two rotamers was provided by: i/ $^1$H-$^1$H COSY spectra showing for both of them crosspeaks between the triplets of $N^6$-*H* and the respective signals of C10-$H_2$; ii/ $^1$H-$^{15}$N gHSQC spectra confirming for both the couplings of $N^6$ and $N^6$-*H*. For 7-deazakinetin riboside the double sets of signals were not observed due to the lack of nitrogen in the 7 position thus suggesting its crucial role for rotation restriction along C6-$N^6$ bond.

## 4. Experimental

### *4.1. General methods*

1D and 2D $^1$H, $^{13}$C and $^{15}$N NMR spectra were recorded on a Bruker Avance III 500 MHz spectrometer, equipped with 5 mm broad-band multinuclear (PABBO) probe in DMSO-$d_6$ at 298 K. 1D $^{19}$F NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer. Chemical shifts (δ) for $^1$H and $^{13}$C NMR were reported in ppm relative to the tetramethylsilane (TMS) peak. For $^{31}$F and $^{15}$N NMR δ were reported in ppm, relative to trichlorofluoromethane and liquid $NH_3$, respectively. Mass spectra were recorded using Thermo Scientific QExactive mass spectrometer. Thin-layer chromatography (TLC) was carried out on Merck precoated 60 $F_{254}$ silica gel plates, while column chromatography on Merck silica gel 60H (40-63 μm). Anhydrous pyridine was prepared by drying with KOH and distillation with $P_2O_5$. 7-Deazaadenosine, 6-chloro-2-fluoropurine, 2-chloroadenosine, 2-amino-6-chloropurine riboside and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose were purchased from Carbosynth. Other reagents were purchased from Sigma-Aldrich, Merck or Fluorochem.

### *4.2. 7-Amino-3-(β-D-ribofuranosyl)-3H-[1,2,3]triazole[4,5-d]pyrimidine (8-azaadenosine, **9**)*

5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carbonitrile [12] (**12**; 801 mg, 2.18 mmol) was heated in diethoxymethyl acetate (4 ml) at 90 °C for 7h. The

solution was evaporated and the resulting syrup was dissolved in methanolic ammonia (4 ml). The mixture was allowed to react at room temperature overnight and the obtained suspension was evaporated to afford crude material which was then dried under vacuum at 50 °C to remove acetamide (415 mg, 71% yield). $^1$H NMR spectrum of **9** as in lit. [21].

*4.3. General procedure for acetylation reactions of compounds **8** and **9** (based on lit. [11])*

To a suspension of **8** (266 mg, 1.0 mmol) or **9** (268 mg, 1.0 mmol) in anhydrous pyridine (2 ml) was added acetic anhydride (1.02 g, 10 mmol). The mixture was stirred at room temperature overnight, then at 60 °C overnight. The reaction was cooled down and quenched by addition of EtOH. The volatiles were evaporated, the residue was co-evaporated with toluene and finally dissolved in EtOH (2.5 ml). Imidazole (53 mg, 0.78 mmol) was added and the solution was stirred at room temperature for 8 h. The solution was diluted with EtOAc (5 ml) and washed with brine (2 x 5 ml). The organic layer was dried with $Na_2SO_4$ and evaporated. The residue was subjected to chromatography on silica gel column using $CH_2Cl_2$-MeOH (98:2→95:5) to give solid foam of **10** (369 mg, 85% yield) or **11** (240 mg, 55% yield). $^1$H NMR data of 4-acetylamino-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine (**10**) and of 7-acetylamino-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3H-[1,2,3]triazole[4,5-*d*]pyrimidine (**11**) are given in Supplementary data section.

*4.4. General procedure for converting tetraacetates **10** and **11** into **4** and **5** (based on lit. [11])*

To a solution of **10** (347 mg, 0.8 mmol) or **11** (349 mg, 0.8 mmol) in anhydrous THF (5 ml) were added $Ph_3P$ (316 mg, 1.2 mmol), furfuryl alcohol (118 mg, 1.2 mmol) and DEAD (as 40% solution in toluene; 209 mg, 1.2 mmol). The mixture was stirred at room temperature overnight. This was sufficient to achieve complete conversion of **11**, but in the case of **10** the addition one more of the same portion of all reagents and reacting for 7 days was necessary. The mixture was evaporated and the residue was chromatographed on silica gel column with $CH_2Cl_2$-MeOH (99:1). The resulting partly purified material was next treated with $CH_2Cl_2$ (6 ml), MeOH (12 ml) and $NH_4OH$ (6 ml), and stirred at room temperature overnight. It was then evaporated and co-evaporated twice with toluene. The residue was subjected to column chromatography with $CH_2Cl_2$-MeOH (97:3→9:1) to give the desired product as colorless oil.

*4.5. 4-Furfurylamino-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine (7-deazakinetin riboside, **4**)*

Because the attempts to crystallize compound **4** were unsuccessful, its analytical sample was prepared by heating at 55 °C under vacuum for 15 h to remove acetamide. $^{1}H$, $^{13}C$ and $^{15}N$ NMR data of **4** are given in Table 1, Table 2 and in Supplementary data section. HRMS $[M+H]^+$ calcd for $C_{16}H_{19}N_4O_5$: 347.1355; found 347.1344.

*4.6. 7-Furfurylamino-3-β-D-ribofuranosyl-3H-[1,2,3]triazole[4,5-d]pyrimidine (8-azakinetin riboside, **5**)*

Rechromatography on silica gel column with EtOAc-MeOH (95:5) afforded solid **5**, which was then heated at 55 °C under vacuum for 15 h to remove acetamide. $^{1}H$, $^{13}C$ and $^{15}N$ NMR data of **5** are given in Table 1, Table 3 and in Supplementary data section. HRMS $[M+H]^+$ calcd for $C_{14}H_{17}N_6O_5$: 349.1260; found 349.1249.

*4.7. 2-(Furfurylamino)adenosine (**14**)*

To a solution of **13** (241 mg, 0.8 mmol) in 2-methoxyethanol (3 ml) was added furfurylamine (699 mg, 7.2 mmol) and DIPEA (931 mg, 7.2 mmol), and the whole was heated at 125 °C for 72 h, then evaporated. The residue was subjected to chromatography on silica gel column with $CH_2Cl_2$-MeOH (92:8→9:1) which gave **14** as the yellowish oily material. It was next rechromatographed with EtOAc-MeOH (9:1) to obtain white solid (194 mg, 67% yield). $^{1}H$ NMR spectrum of **14** as in lit. [15]. $^{13}C$ NMR data are given in Supplementary data section.

*4.8. 2-Amino-$N^6$-furfuryladenosine (**6**)*

To a solution of **15** (241 mg, 0.8 mmol) in 2-methoxyethanol (3 ml) was added furfurylamine (699 mg, 7.2 mmol) and DIPEA (931 mg, 7.2 mmol) and the whole was heated at 125 °C for 48 h, then evaporated. The residue was chromatographed on silica gel column with $CH_2Cl_2$-MeOH (95:5→92:8) and rechromatographed with EtOAc-MeOH (9:1) to give **6** as white solid (278 mg, 96% yield). It was next crystallized from EtOAc. $^{1}H$, $^{13}C$ and $^{15}N$ NMR data of **6** are given in Table 1, Table 2 and in Supplementary data section. HRMS $[M+H]^+$ calcd for $C_{15}H_{19}N_6O_5$: 363.1417; found 363.1401.

*4.9. 2-Fluoro-$N^6$-furfuryladenosine (**7**)*

To a solution of **16** (400 mg, 2.32 mmol) in anhydrous MeOH (17 ml) was added furfurylamine (225 mg, 2.32 mmol) and $Et_3N$ (293 mg, 2.9 mmol). The mixture was stirred at 60 °C for 48 h, then concentrated under vacuum. The precipitated product **17** was separated, washed with cooled MeOH and dried to afford 234 mg of solid which was not further

purified. The mixture of crude **17** (200 mg, 0.86 mmol), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (280 mg, 0.88 mmol) and p-toluenesulfonic acid monohydrate (17.5 mg, 0.09 mmol) in chlorobenzene (5 ml) was heated at 145 °C for 3 h. It was evaporated and the oily residue was next chromatographed on silica gel column with hexane-EtOAc (2:1→1:3) to isolate 119 mg of **18** as solid foam. This material was treated with $CHCl_3$-MeOH-25% $NH_4OH$ (1:2:2, 5 ml), the resulting solution was kept at room temperature overnight and evaporated. The deacetylated product **7** was purified by column chromatography with $CHCl_3$-MeOH (9:1), then the obtained foam was dried under vacuum at 55 °C for 8 h to remove the remaining acetamide (66 mg of **7**, 9% yield from **16**). The solid sample of **7** precipitated from its solution in MeOH-chloroform-toluene (1:2:2, 5 ml), then it was dried under vacuum. $^{1}H$, $^{13}C$, $^{15}N$ and $^{19}F$ NMR data of **7** are given in Table 1, Table 3 and in Supplementary data section. HRMS $[M+H]^+$ calcd for $C_{15}H_{17}N_5O_5F$: 366.1214; found 366.1202.

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## Supplementary data

Supplementary data associated with this article can be found in the online version at

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Four analogues of kinetin riboside (KR) were synthesized.

Multinuclear NMR was used to study the structure of KR and its analogues in solution.

At lowered temperature KR exists as the mixture of two conformers.

The existence of two forms was found at room temperature for 8-azaKR and 2-fluoroKR.

Conformations of studied compounds result from restricted rotation around C6-$N^6$ bond.