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Supporting Information

for

Anion exchange resins in phosphate form as versatile carriers for the reactions catalyzed by nucleoside phosphorylases

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Experimental details and NMR spectra of synthesized compounds

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1 – Table SI – 1. ^{1}H NMR spectral data for nelarabine, kinetin riboside, N^6-benzyladenosine, and cladribine in DMSO-d_6 (δ_{TMS}, ppm)**.**

Compound	*H-1′*	*H-2′* (lower field resonance)	*H-2″* (higher field resonance)	*H-3′*	*H-4′*	*H-5′*	*H-5″*	Others
Nelarabine	6.12 d	-	≈4.06 m	≈4.08	3.75 “q”	3.65 ddd	3.61 ddd	7.92 (s, H-8); 6.42 (s, 2H, N^2H_2); 5.60 (d, 1H, *J* = 5.36, 2′-O**H**); 5.49 (d, 1H, *J* = 3.48, 3′-O**H**); 5.05 (t, 1H, *J* = 5.29, 5′-O**H**) 3.96 (s, 3H, OC***H***$_3$)
Kinetin Riboside	5.96 d	4.68 dt	-	4.21 m	4.03 q	3.74 dt	3.62 ddd	8.45. (s, H-8); 8.31 (s, H-2) 7.60 (q, *J* = 0.7 & 1.65 Hz), 6.42 (q, *J* = 1.8 & 3.15 Hz),) & 6.29 (d, *J* = 3.0 Hz); (FF group) 8.38 (br. s, 6-NH) 5.50 (d, $J_{2',OH}$ = 6.20); 5.42 (q, $J_{5',OH}$ = 4.6 & 7.05; 5′-O**H**); 5.24 (d, $J_{3',OH}$ = 4.70; 3′-O**H**) 4.76 (br. s, 2H, -C***H***$_2$-Ph)
N^6-Benzyl-adenosine	5.89 d	4.61 “d”	-	4.15 br. m	3.96 q	3.67 dt	3.55 ddd	8.38. (s, H-8); 8.20 (s, H-2) 8.45 (br. s, 6-NH) 7.33 (d, ortho), 7.28 (td, meta) & 7.20 (tt, para); (Bzl) 5.43 (d, $J_{2',OH}$ = 6.20); 5.42 (q, $J_{5',OH}$ = 4.6 & 7.05; 5′-OH); 5.17 (d, $J_{3',OH}$ = 4.65; 3′-O**H**) 4.71 (br. s, 2H, -C***H***$_2$-Ph)
Cladribine	6.38 d	2.66 ddd	2.30 ddd	4.45-4.36 m	3.88 dt	3.62 ddd	3.53dt	8.38 (s, H-8); 7.85 (br.s, NH_2) 5.34 (d, *J* = 4.2 Hz; C3′-O**H**) 4.98 (t, *J* = 5.6 Hz; C5′-O**H**)

2 – Table SI – 2. ^{13}C NMR spectral data for nelarabine, kinetin riboside, N^6-benzyladenosine, and cladribine in DMSO-d_6 (δ_{TMS}, ppm)**.**

Compound	Chemical shifts (δ_{TMS}, ppm)									
	C1'	C2'	C3'	C4'	C5'	C2	C4	C5	C6	C8
Nelarabine [a])	84.20 (83.35)	75.47 75.29	75.29 75.47	83.35 (84.20)	60.95	153.98	159.74	113.16	160.52	139.06
Kinetin Riboside [b])	87.90	73.48	70.61	85.86	61.63	152.87	148.50	≈119.5	154.32	140.01
N^6-Benzyladenosine [c,d])	87.93	73.45	70.64	85.88	61.65	152.33	148.45	119.78	154.50	139.94
Cladribine	84.04	[e]	71.19	88.43	62.14	157.44	150.55	118.65	157.28	140.30

a) O***C***H$_3$ 54.05 ppm.

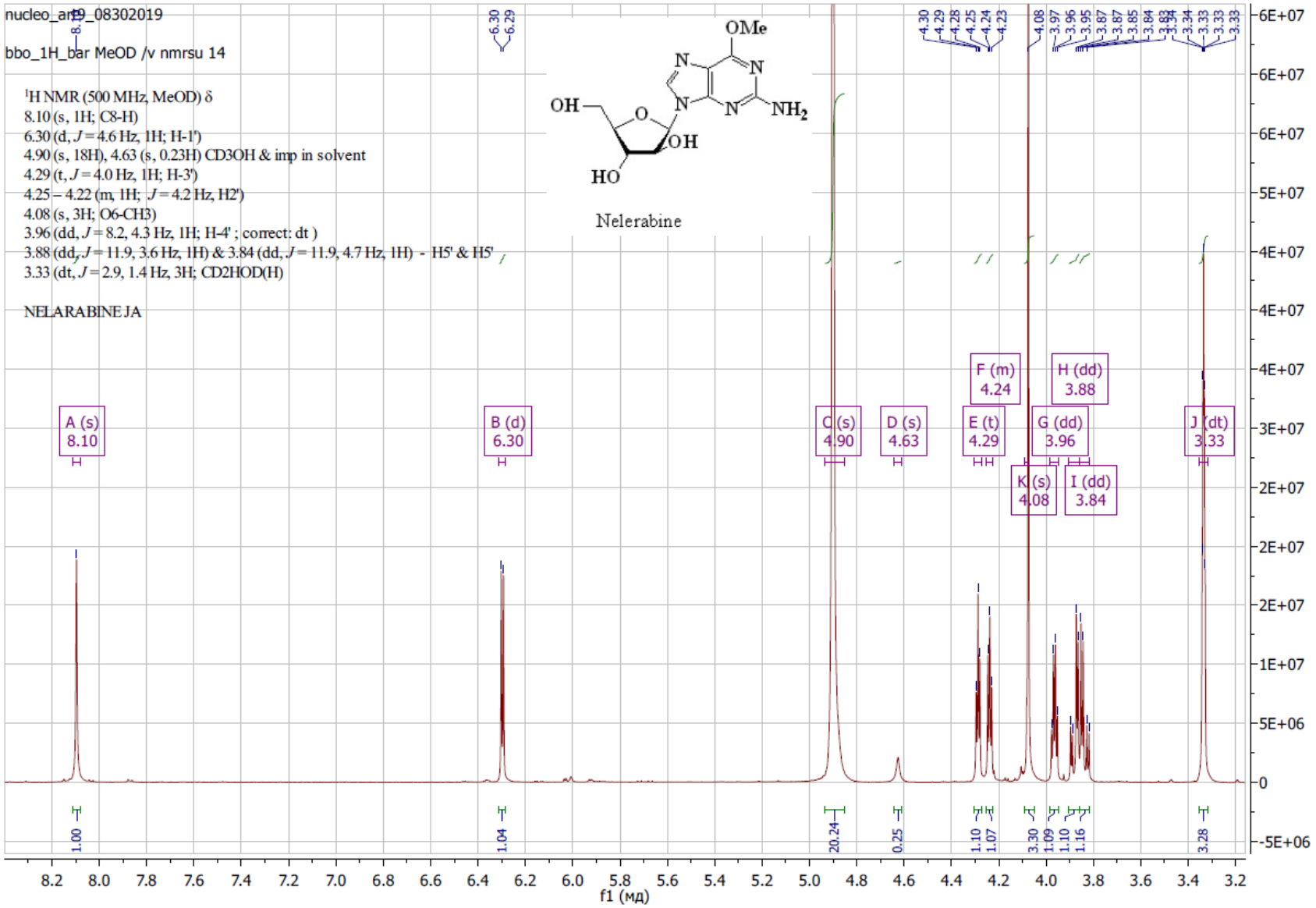
b) The ^{13}C resonances of KR (δ_{TMS}, ppm): 141.81 [correlates with a proton (q, *J*'s = 0.70 and 1.65 Hz) at 7.60 ppm]; 110.43 [correlates with a proton (q, *J*'s = 1.80 and 3.15 Hz) at 6.42 ppm]; 106.63 (correlates with a proton (d, *J* = 3.0 Hz) at 6.29 ppm]; the C1-*ipso* of the FF ring appeared at 152.87 ppm and the ***C***H$_2$ resonance is at 36.53 ppm.

c) The ^{13}C resonances of BzlNH (δ_{TMS}, ppm): 140.09 (*ipso*), 128.20 (*ortho*), 127.12 (*metha*), 126.60 (*para*), and 42.86 (***C***H$_2$).

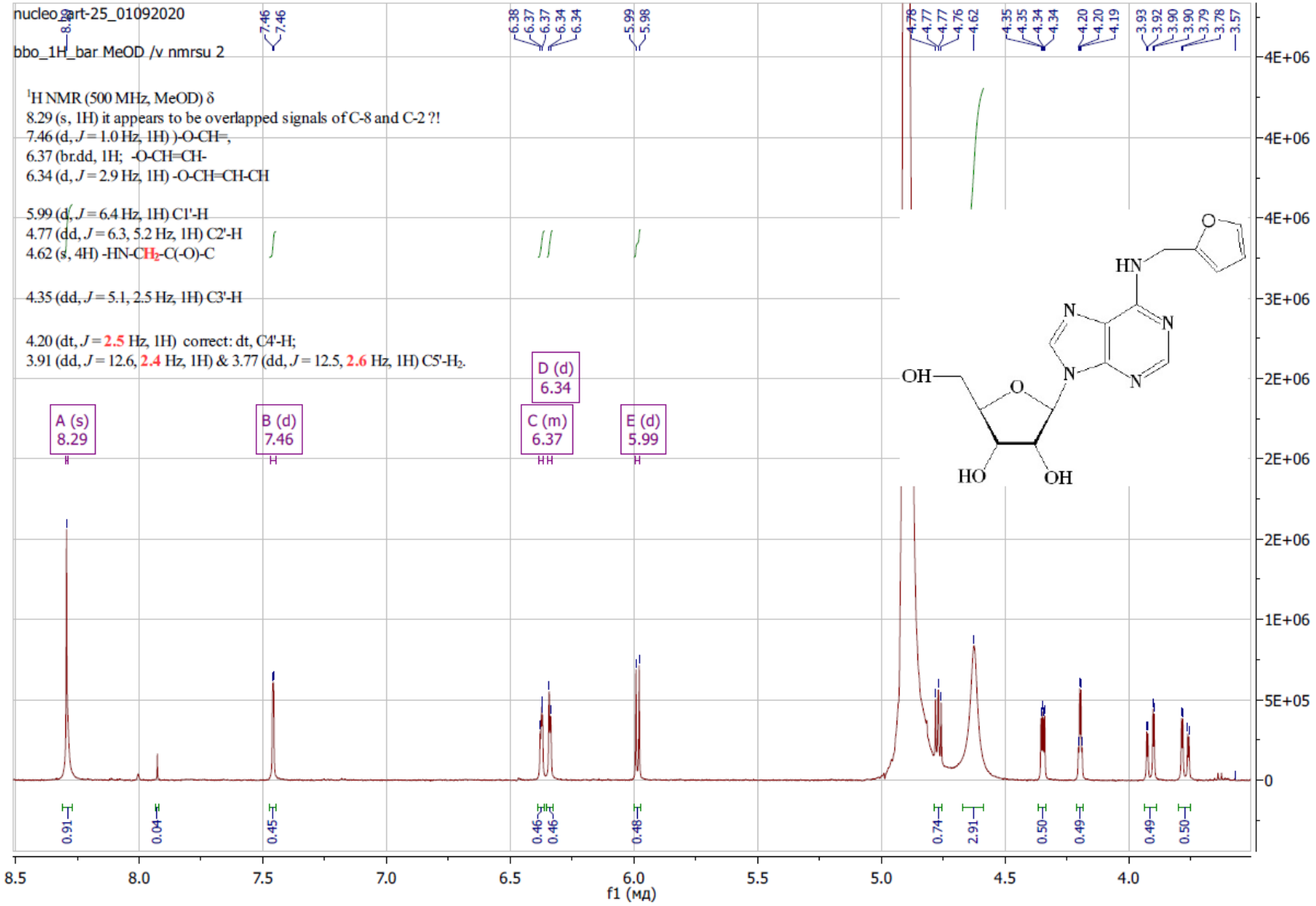
d) The ^{13}C-*ipso* resonance of the Bzl group is overlapped by the ^{13}C-8 resonance.

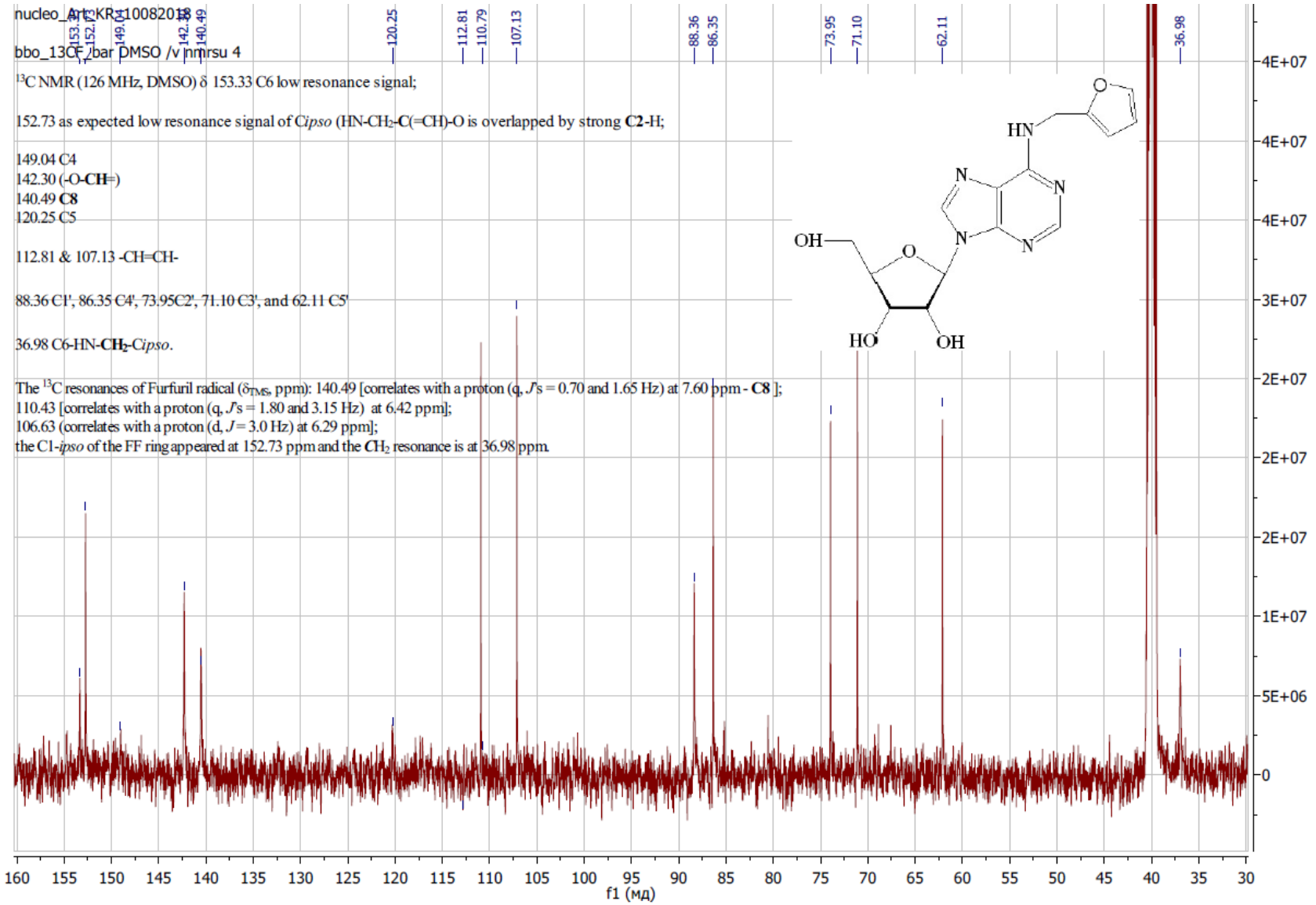
e) The resonance signal of C-2' is overlapped by DMSO

3 – **SI-3**. ^{1}H NMR spectrum of nelarabine in MeOD.



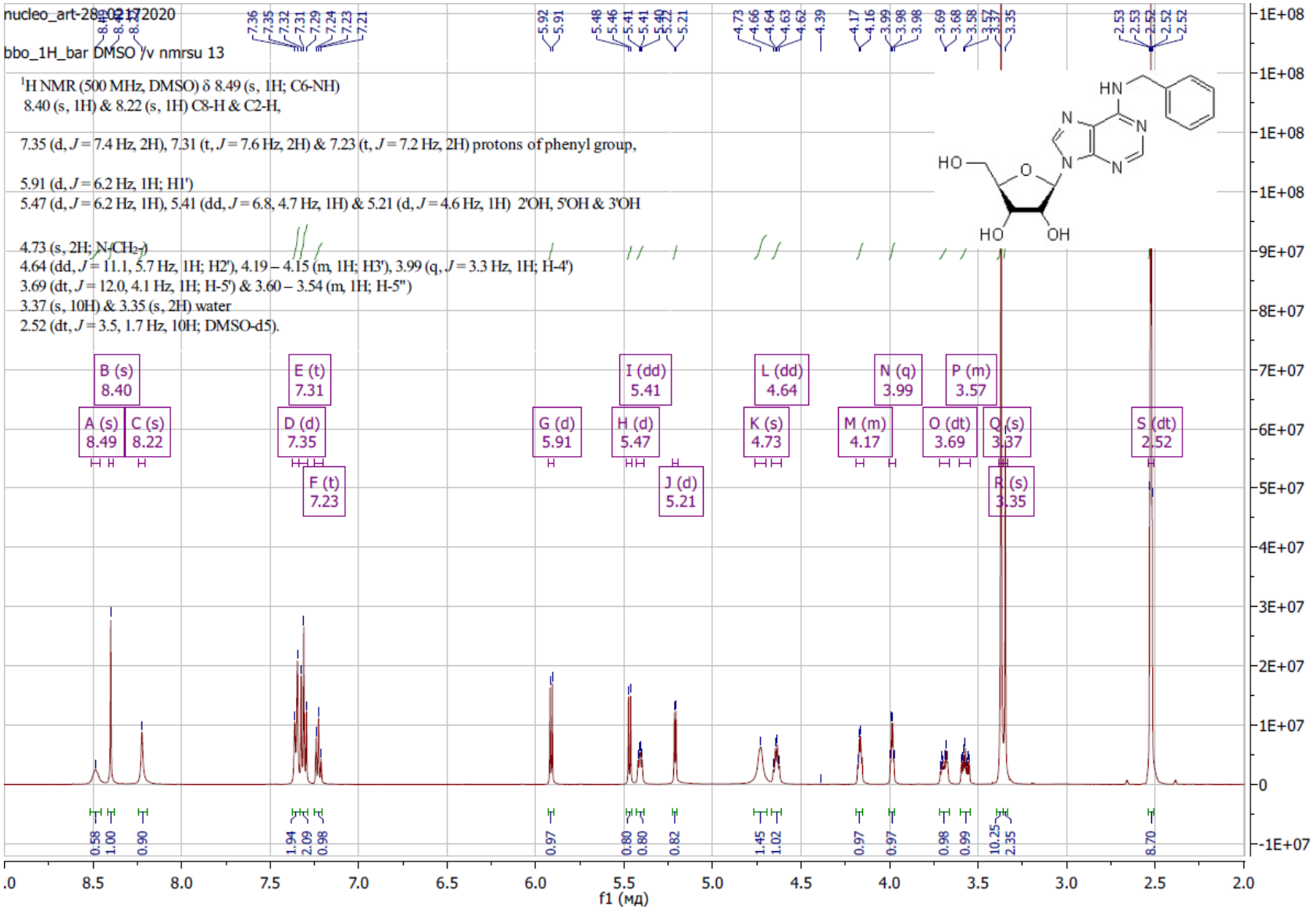
4 – **SI-4**. ^{1}H and ^{13}C NMR spectra of kinetin riboside in CD_5OD.

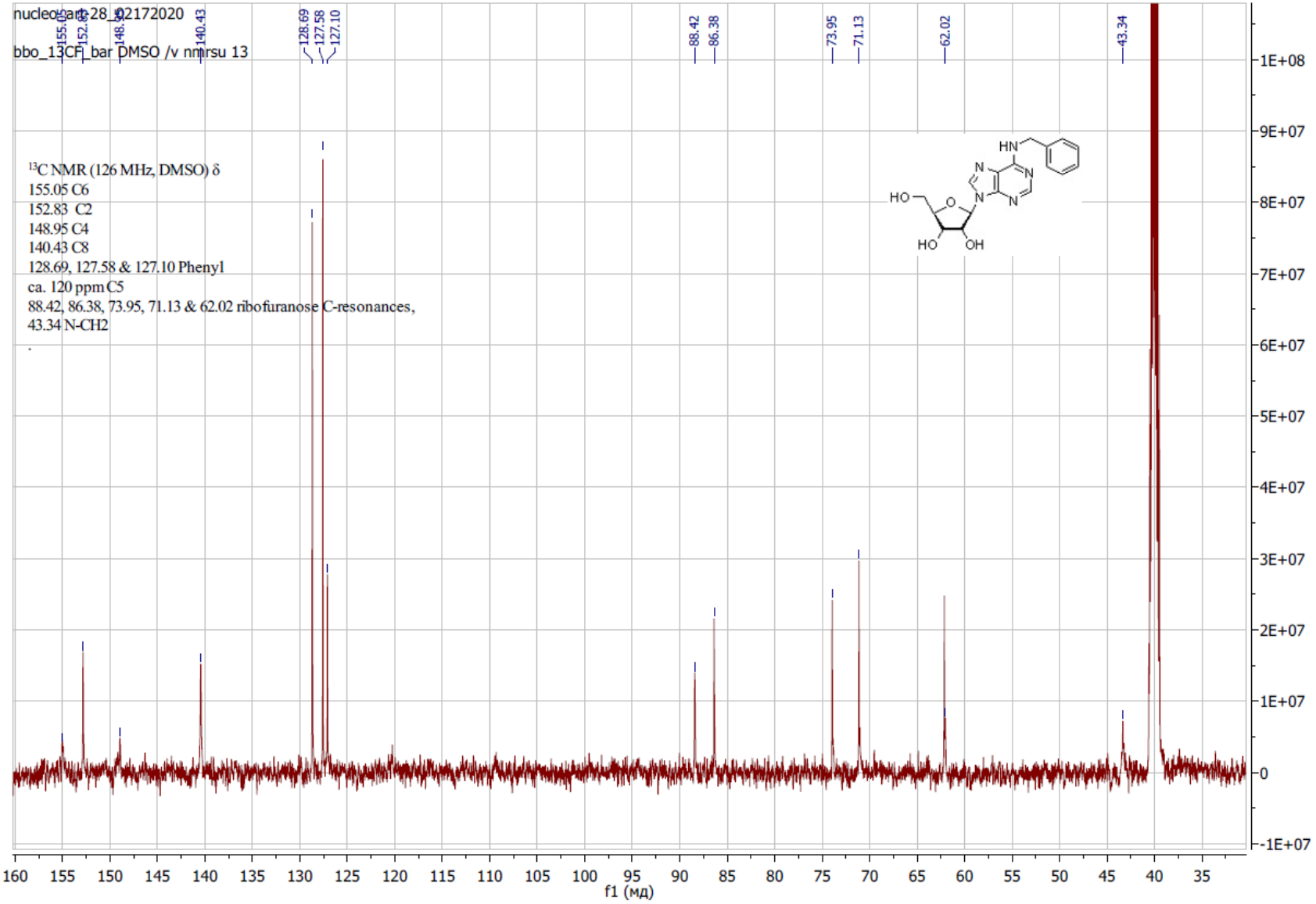



nucleo_Art_KR_10082018
bbo_13CF_/bar DMSO /v nmrsu 4
153.33
152.73
149.04
142.30
140.49
120.25
112.81
110.79
107.13
88.36
86.35
73.95
71.10
62.11
36.98
13C NMR (126 MHz, DMSO) δ 153.33 C6 low resonance signal;
152.73 as expected low resonance signal of Cipso (HN-CH2-C(=CH)-O is overlapped by strong C2-H;
149.04 C4
142.30 (-O-CH=)
140.49 C8
120.25 C5
112.81 & 107.13 -CH=CH-
88.36 C1', 86.35 C4', 73.95C2', 71.10 C3', and 62.11 C5'
36.98 C6-HN-CH2-Cipso.
The 13C resonances of Furfuril radical (δTMS, ppm): 140.49 [correlates with a proton (q, Js = 0.70 and 1.65 Hz) at 7.60 ppm - C8];
110.43 [correlates with a proton (q, Js = 1.80 and 3.15 Hz) at 6.42 ppm];
106.63 (correlates with a proton (d, J = 3.0 Hz) at 6.29 ppm];
the C1-ipso of the FF ring appeared at 152.73 ppm and the CH2 resonance is at 36.98 ppm.
HN
O
N
N
N
N
OH
O
HO
OH
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
f1 (мд)

5 – **SI-5**. Synthesis and ^{1}H and ^{13}C NMR spectra of N^6-benzyladenoside in DMSO-d_6.

***N*^{6}-Benzyladenosine**: To a heterogeneous reaction mixture containing guanosine (425 mg, 1.5 mmol) and N^6-benzyladenine (225 mg, 1 mmol) in 140 mL of 5.0 mM K-phosphate buffer (pH 7.0) was added *E. coli* PNP (140 IU) and the mixture was gently stirred at 40 °C for 20 h (some quantity of the starting heterocyclic base remained in the reaction mixture according to TLC). Then, guanosine (0.14 g, 0.5 mmol) was added and the heating continued for 6 h and the reaction mixture was left overnight at 4 °C. The solution was decanted from the precipitate formed and evaporated to dryness, the residue was mixed with one tee spoon of SiO_2 and put on the top of a standard column (2 × 22 cm) packed in DCE/MeOH 95:5 (vol). Elution with the same mixture of solvents gave 20 mg of N^6-benzyladenine and its riboside (0.22 g, yield 67%; purity 99.2% according to HPLC); mp 178-180 °C (from EtOH); UV (H_2O): λ_{max} 269 and 209 nm (ε 20,140 and 25,230), λ_{min} 232 nm (ε 2,720). λ_{max} 269 nm (ε 20,140); lit. data: (*1*) mp 182-183 °C (from EtOH); UV (EtOH), λ_{max} = 271 nm, (ε 4,290) [1]; (*2*) mp 177-179 °C (from EtOH)}. UV (MeOH): λ_{max} 268 nm (ε 20,850); λ_{min} 233 nm (ε 2,570) [2].


nucleo_art-28-02172020
bbo_1H_bar DMSO /v nmrsu 13
[1]H NMR (500 MHz, DMSO) δ 8.49 (s, 1H; C6-NH)
8.40 (s, 1H) & 8.22 (s, 1H) C8-H & C2-H,
7.35 (d, J = 7.4 Hz, 2H), 7.31 (t, J = 7.6 Hz, 2H) & 7.23 (t, J = 7.2 Hz, 2H) protons of phenyl group,
5.91 (d, J = 6.2 Hz, 1H; H1')
5.47 (d, J = 6.2 Hz, 1H), 5.41 (dd, J = 6.8, 4.7 Hz, 1H) & 5.21 (d, J = 4.6 Hz, 1H) 2'OH, 5'OH & 3'OH
4.73 (s, 2H; N-CH2-)
4.64 (dd, J = 11.1, 5.7 Hz, 1H; H2'), 4.19 – 4.15 (m, 1H; H3'), 3.99 (q, J = 3.3 Hz, 1H; H-4')
3.69 (dt, J = 12.0, 4.1 Hz, 1H; H-5') & 3.60 – 3.54 (m, 1H; H-5")
3.37 (s, 10H) & 3.35 (s, 2H) water
2.52 (dt, J = 3.5, 1.7 Hz, 10H; DMSO-d5).
B (s) 8.40
A (s) 8.49
C (s) 8.22
E (t) 7.31
D (d) 7.35
F (t) 7.23
G (d) 5.91
I (dd) 5.41
H (d) 5.47
J (d) 5.21
L (dd) 4.64
K (s) 4.73
N (q) 3.99
M (m) 4.17
P (m) 3.57
O (dt) 3.69
Q (s) 3.37
R (s) 3.35
S (dt) 2.52
HO
O
HO
OH
HN
N
N
N
N
f1 (мд)


nucleo_art_28_02172020
bbo_13CPI_bar DMSO /v nmrsu 13
155.05
152.83
148.95
140.43
128.69
127.58
127.10
88.42
86.38
73.95
71.13
62.02
43.34
13C NMR (126 MHz, DMSO) δ
155.05 C6
152.83 C2
148.95 C4
140.43 C8
128.69, 127.58 & 127.10 Phenyl
ca. 120 ppm C5
88.42, 86.38, 73.95, 71.13 & 62.02 ribofuranose C-resonances,
43.34 N-CH2
.
HN
N
N
N
N
HO
O
HO
OH
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35
f1 (мд)

6 – SI-6. Combined data on the enzymatic synthesis of nelarabine, kinetin riboside, N^6-benzyladenosine, and cladribine.

№	Product	Substrate(s)	Enzyme(s) (IU per 1 mmol of substrate)	Reactions condifions		Conver-sion (%)	Yield of pure product (%)	Refs
				Solution	Time (hrs) (Temp.;°C)			
1	Fludarabine	2F-Ade & Ara-C (1:1.4, mmol)	*E. coli* PNP (93.3) & cytidine deaminase 10 IU *E. coli* UP (8.7)	4 mM Phosphate buffer (pH 7.0)	864 (35)	-	14	[3]
2	Fludarabine	Ara-1Pi & 2F-Ado	*E. coli* PNP (360)	Water	3 (55)	94	77	[4]
3	Nelardbine	Ara-U & C^6OMe-Guo (2:1, mol)	*E. coli* PNP (37,778) *E. coli* UP (1,889)	10 mM Phosphate buffer (pH 7.4)	18 (60)	-	53 (for base taken in the reaction)	[5]
4	Nelarabine	Ara-U & C^6OMe-Guo (1.1:1, mol)	*E. coli* PNP (18,000) *E. coli* UP (1,020)	10 mM *K*-Pi buffer/n-PrOH (7% v/v; (pH 6.75)	624 (37)	-	17	[6]
5	Nelarabine	Chem.Ara-1Pi & C^6OMe-Guo (ca. 5:1, mol)	*E. coli* PNP (49)	Water	36 (45)	50	40	[4]
6	Nelarabine	Ara-1Pi & C^6OMe-Guo (ca. 1.7:1, mol)	*E. coli* PNP (ca. 1,400 prt Ara-1P & 2,333 per base)	Water	48 (47)	85	53 (HPLC purity 99.3%)	This study
7	Nelarabine (test experiment)	Ara-U & C^6OMe-Guo (1.5:1, mol)	*E. coli* PNP (10,000) *E. coli* UP (5,000)	5 mM *K*-Pi buffer (pH 8.00)	1 (23)	Base to nucleoside **44%** Ara-U to Ara-1Pi **≥95**	-	This study
8	Nelarabine	Crude Ara-1Pi & C^6OMe-Guo (ca. 2:1, mol)	*E. coli* UP (516 per Ara-U) *E. coli* PNP (1,484 per base)	Water	1st 72 (r.t.) 2nd 120 (50-55)	1st >95-97% 2nd *ca.* 60%	52 (HPLC purity 99%)	This study
9	Ara-1Pi	Ara-U (170 mg, 0.7 mmol)	*E. coli* UP (150)	Water	72 (40)	**≥97**	96	This study
10	Rib-1Pi	Uridin (63 mg, 0.258 mmol)	*E. coli* UP (387)	Water	60 (23)	**≥97**	96	This study
11	Kinetin Riboside Not optimized!	Crude Ara-1Pi & C^6OMe-Guo (ca. 2:1, mol)	*E. coli* UP (580) >95-97% *E. coli* PNP (342)	Water	1st 72 (r.t.) 2nd 144 (50)	1st >95-97% 2nd 72%	61 (HPLC purity 98%)	This study

12	Kinetin Riboside	Ara-1Pi & C^6OMe-Guo (ca.2:1, mol)	*E. coli* PNP (263)	Tris HCl 50 mM	2 h (r.t.)	>95-97%	93% (HPLC purity 98.5%)	This study
13	N^6-Benzyl-adenosine	Guanosine (1.5 + 0.5 mmol) & N^6-^{Bz}Ade (1 mmol)	*E. coli* PNP (120 IU; 80 & 120 units per Guo and base, respectively)	5 mM *K*-Pi buffer (pH 7.00)	20 + 6 h (40)	ca. 10% of base was recovered	67% (HPLC purity 99.2%)	This study
14	Cladribine	Dowex-*n*Pi dRib-1Pi ---------- dRib-1Pi & ^{2Cl}Ade (3: 1, mol)	*E. coli* UP (66.0) >95-97% ---------- *E. coli* PNP (1,800 per base)	Water	120 h (40 °C) ---------- 72 h (47 °C)	>95-97% ---------- 88%	93% ---------- -	This study
15	Cladribine	Thd (0.56 mmol) QAE Sephadex-*n*Pi & dRib-1Pi + ^{2Cl}Ade (0.059 mmol)	*E. coli* UP (89.0) >95-97% ---------- *E. coli* PNP (2,288 per base)	Water	120 h (40 °C) ---------- 96 h (40 °C)	>95-97% ---------- 81%	---------- 59%	This study

References and notes

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