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Bicyclic Cytarabine Analogues: Synthesis and Investigation of Antitumor Properties of Novel, 6-Aryl- and 6-Alkyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one Arabinosides

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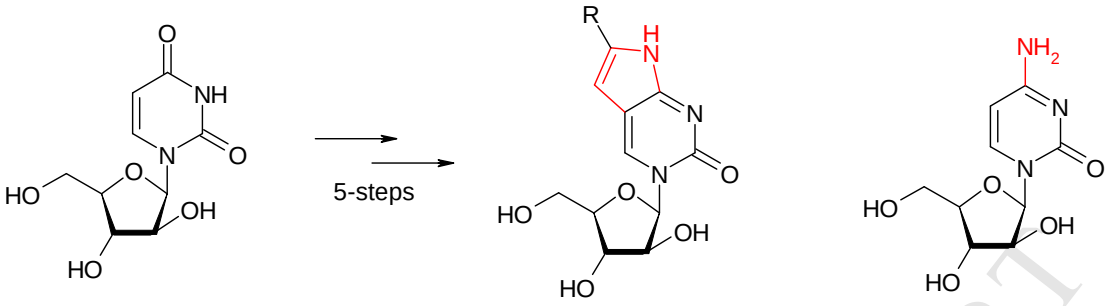
GRAPHICAL ABSTRACT:



araU

bicyclic Cytarabine analogues

R = Ph, substituted Ph, 2-pyridyl, benzyl, alkyl

AraC

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Bicyclic Cytarabine Analogues: Synthesis and Investigation of Antitumor Properties of Novel, 6-Aryl- and 6-Alkyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one Arabinosides

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Key words: bicyclic pyrimidine nucleoside analogues (BCNAs), arabinonucleosides, Cytarabine analogues, cytotoxicity

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ABSTRACT:

A series of sixteen hitherto unknown Cytarabine analogues bearing a bicyclic 3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one base modified with aryl, pyridyl, benzyl and alkyl substituents was prepared in a straightforward approach. This is the one of the few examples of the synthesis of pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one nucleosides and the first example of pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one nucleosides possessing arabinose moiety. For the first time, the conversion of the furopyrimidine arabinoside products to a series of novel pyrrolopyrimidines by ammonolysis reaction was thoroughly investigated using aqueous and methanolic reaction conditions under classical and micro-wave heating. This approach resulted in a small library of compounds, which were evaluated for their antiproliferative properties against HL-60 and Jurkat E6.1 cell lines. All synthesised compounds exhibited a weaker cytotoxic effect in comparision to the mother compound. Of all the tested compounds, the derivative bearing a 4-*n*-pentylphenyl substituent exhibited the highest antiproliferative activity.

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1. Introduction

Cytarabine ((1-β-D-arabinofuranosyl)cytosine), cytosine arabinoside, Ara-C, **1**, Fig. 1) is an effective drug widely used for the treatment of acute myelogenous leukemia and a lymphocytic leukemia.[1] Early experiments revealed a wide range of anticancer activity against numerous cell lines of sarcomas (Nakahara-Fukuoka sarcoma, reticulum cell sarcoma, ascites sarcoma-180), adenocarcinomas (adenocarcinoma-755, spontaneous mammary adenocarcinoma), and Ehrlich ascites carcinoma.[2-4] Cytarabine also exhibited remarkable synergistic effect with other anticancer drugs like Daunorubicin, Vinblastin and *cis*-diaminodichloroplatin (II) (CDDP).[5,6] However, the main disadvantages of antitumor therapy with Cytarabine are related to its acute and chronic toxicity to internal organs[7-12] and its poor bioavailability; it is quickly metabolized by cytidine deaminase to a pharmacologically inactive metabolite, uracil arabinoside (AraU). For this reason, any new approaches leading to improvement of the pharmacokinetics of Cytarabine are highly desirable and many efforts have been directed toward the discovery of new, cytidine deaminase-resistant Cytarabine analogues. It's highly desiderable, that they retain retain the antitumor activity exhibiting lower toxicity and better bioavailability then the mother compound. Such analogues include, but are not limited to, Enocytabine (**2**, N^4-behenoyl-1-(β-D-arabinofuranosyl)cytosine, BH-AC), which bears a highly lipophilic group at the 4-amino position of the cytosine moiety of Cytarabine (**1**)[13,14] and Ancitabine (**3**), a cyclonucleoside analogue possessing an additional covalent $O^{2'}$,2-linkage[15,16] in the structure. Krolikiewicz et al reported the synthesis of tetrahydro-2*H*-pyrrolo[2,3-*d*]pyrimidine analogue of Cytarabine **4**,[17] but this derivative was deprived of any biological activity. All decribed structures are represented in the Figure 1.

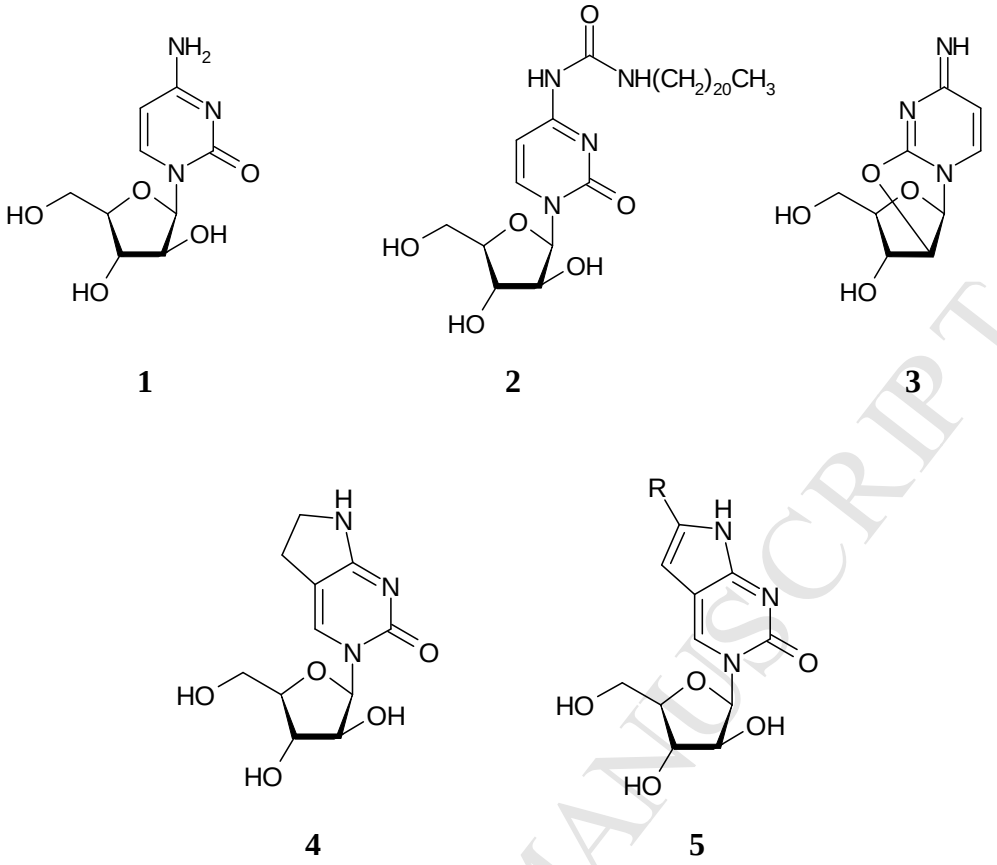


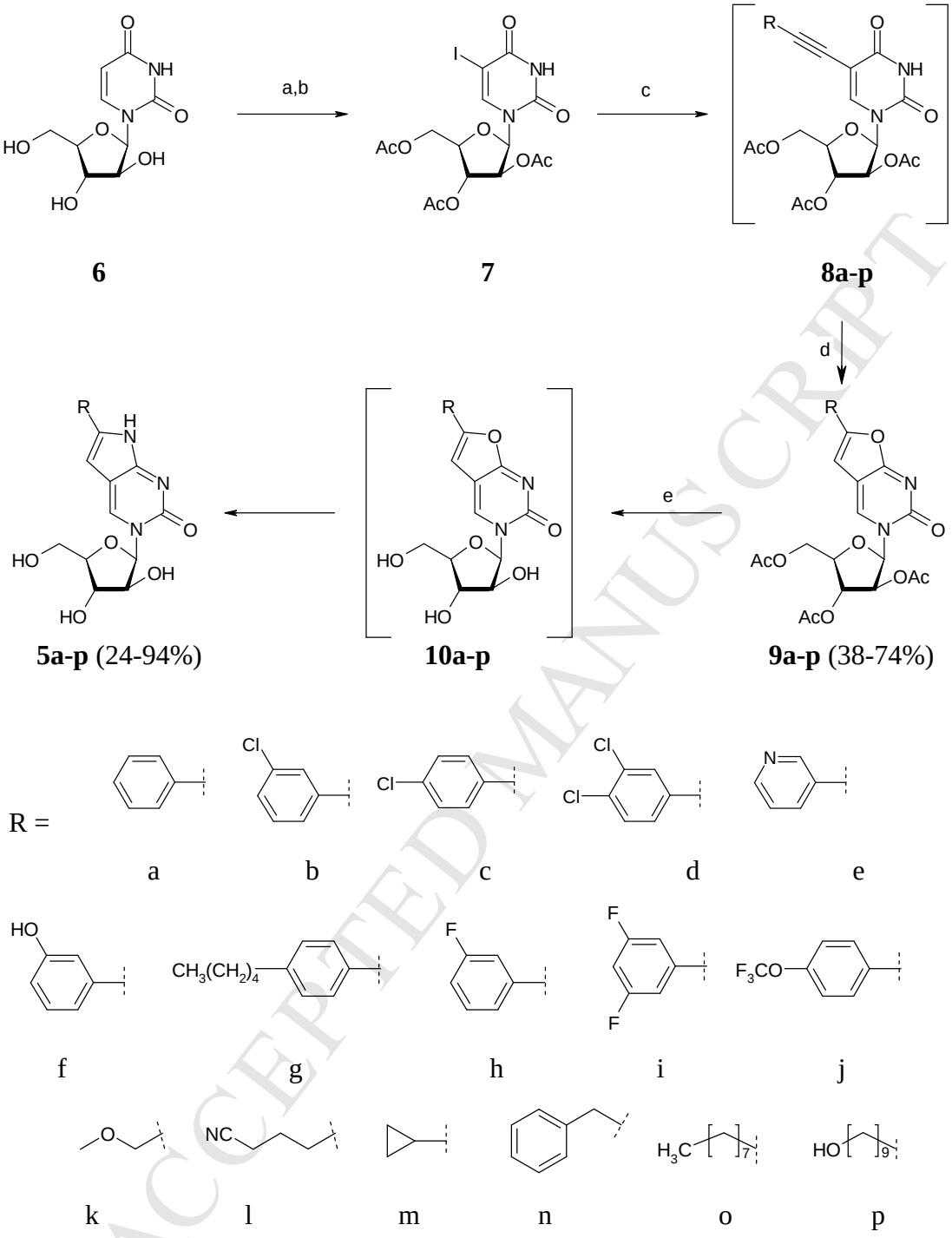
Fig.1 Cytarabine (**1**) and its derivatives **2**-**5**

Nucleosides possessing bicyclic bases are known as efficient cytidine mimetics, forming stable Watson-Crick pairs with guanidine in the DNA and RNA helices.[18,19] Bicyclic pyrimidine nucleoside analogues (BCNAs) are widely known for their antiviral activity against various RNA and DNA viruses such as: VZV,[20,21] HCMV,[22,23] HCV,[24,25] HBV[26] and the vaccinia virus (VACV).[27] They have not been, however, investigated as possible anticancer agents yet. BCNAs possessing an *arabino*furanosyl ring and furo[2,3-*d*]pyrimidin-2(3*H*)-one as base were reported as potential antivirial compounds, but exhibited weak activity against VZV and HCMV strains.[28] Gazivoda reported that 4′,5′-didehydro-L-ascorbic acid derivatives possessing bicyclic furo[2,3-*d*]pyrimidine bases exhibit pronounced cytostatic activity against malignant leukemia (L1210).[29] However, earlier reports revealed that bicyclic nucleosides possessing either oxazolo[5,4-*d*]pyrimidine[30] or thieno[5,4-*d*]pyrimidine[31] bases exhibit remarkable *in vivo* and *in vitro* antileucemic activity against L1210 cells, but there is very little additional literature data in that subject.

In our ongoing quest for the design and synthesis of new medicinally relevant heterocyclic derivatives[32-36] and compounds exhibiting antitumor activity,[37-40] we envisioned the possibility of synthesising novel, cytidine deaminase-resistant, Cytarabine analogues bearing a bicyclic, pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one base at the arabinose moiety (Fig. 1, **5**). There are only a few examples in the literature concerning the pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one nucleosides, and no examples of such compounds possessing arabinose moiety. We assumed, that designed pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one arabinosides could serve as covenient analogues of Cytarabine, possibly retaining the anticancer activity resulting from undergoing phosphorylation to appropriate triphosphates by cellural enzymes. Such triphosphates can inhibit DNA polymerases and terminate DNA chain elongantion. Addtionally, as an amine group is a part of pyrrole ring, these analogues would not serve as substrates for the cytidine deaminase. This in consequence, would improve bioavailability and extend the half-life of the compounds. We also assumed that the addition of lipophilic substituents to the pyrrole ring would increase the penetration of such compounds through the blood brain-barrier in reference to Cytarabine (**1**).

2. Results and Discussion

The synthesis of designed compounds was conducted starting from commercially available 1-(*β*-D-*arabino*furanosyl)uracil (**6**) protected by acetyl groups. Acetylation reaction was performed with acetic anhydride in the presence of triethylamine and a catalytic amount of DMAP (Scheme 1).[41] After crystallization from ethanol, the triacetate was subjected to the iodination reaction using I_2 in the presence of cerium ammonium nitrate,[42] leading to formation of 1-(2,3,5-tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-5-iodouracil (**7**). Intermediate product **7** was then applied in a series of Sonogashira reactions using 16 alkynes, under $Pd(PPh_3)_4$/CuI catalysis [26-27], which resulted in formation of 5-alkynyl derivatives **8** as the main products and bicyclic furanopyrimidines **9** as minor ones.[43] For complete cycloisomerisation, obtained mixtures of compounds **8** and **9** were heated for additional 12 h in 50-60 °C in the presence of one more equivalent of copper iodide.



Scheme 1. *Reagents and conditions:* a) Ac_2O, TEA, DMAP, MeCN, 20 h, rt b) $(NH_4)_2Ce(NO_3)_6$, I_2, MeCN, 3 h, 80 °C c) R-C≡CH, $Pd(PPh_3)_4$, CuI, TEA, DMF, 24h, rt d) CuI, TEA, DMF, 12 h, 50-60 °C e) 7N methanolic ammonia, 60 °C, 24-48 h, *mv* or 25% NH_4OH, rt, 1-4 d.

According to the literature data, transformation of the furo[2,3-*d*]pyrimidin-2(3*H*)-one nucleosides to the 3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one nucleosides

could be performed with aqueous or methanolic solutions of ammonia, but comperative studies of both methods were not reported. For this reason, we then tested both reagents for the last step of the synthesis; the removal the acetyl groups and concomitant conversion of the furane ring to the pyrrole ring. Treatment of compound **9** with 7N methanolic ammonia at room temperature led to the fast removal of the acetyl groups followed by rather slow conversion of the deprotected furo[2,3-*d*]pyrimidin-2(3*H*)-one nucleosides **10** to the 3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one nucleosides **5**.[33,44] We found that almost complete conversion of furane to the pyrrole ring could be performed under microwave heating at 60 °C. When the time reaction is extendeed to 24-48 h, the final compounds could be obtained with moderate to high yields (32-94%). Application of aqueous solution of ammonia slightly accelerated the transformation of the furane into the pyrrole ring, however it also led to formation of by-products, which in some cases were difficult to separate from the main product. When the reaction temperature was raised to 60 °C, the number of by-products increased, therefore most reactions with NH_4OH were carried out at room temperature with prolonged reaction time (up to the 4 days in case of products **5o** and **5p** bearing long liphophilic chains, which limited the solubility in the aqueous solutions).

All new Cytarabine analogues were tested for their anti-proliferative activity against HL-60 (human promyelocytic leukemia) and Jurkat E6.1 (human acute T cell leukemia) cells. Compound **5g** showed the highest anti-proliferative activity with IC_{50} values 68 and 61 µM for HL-60 and Jurkat E6.1 cells, respectively. Compounds **5a**, **5c-5f** and **5h-5j** showed rather poor anti-proliferative properties with IC_{50} values ranging from 95 to 203 µM. Compounds **5b**, **5k-5n** and **5p** did not influence the proliferation rate of HL-60 and Jurkat E6.1 cells. The reference IC_{50} values calculated for Cytarabine were 0.185 and 0.018 µM for HL-60 and Jurkat E6.1 cells, respectively. The significantly lower anti-proliferative properties of the synthesised compounds could be attributed to their different behaviour in the cell. It's likely, that they could undergo intracellular coversion to appropiate triphosphates, but would not be used as substrates in the DNA and RNA synthesis reactions. Therefore, they could not serve as agents stopping the synthesis of nucleic acids in the cell.

3. Conclusion

We have efficiently synthesised a series of sixteen hitherto unknown Cytarabine analogues bearing a bicyclic 3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one base modified with aryl, pyridyl, benzyl and alkyl substituents. This is the first example of synthesis of 3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one nucleosides possessing arabinose moiety. The key intermediate product, acetylated 5-iodo-3-(*β*-D-*arabino*furanosyl) uracil, was applied to Sonogashira couplings with appropiate terminal alkynes. The Sonogashira reaction products were usually accompanied by furo[2,3-*d*]pyrimidin-2(3*H*)-one by-products resulting from the metal-catalyzed cycloisomerization reaction, and were directly applied to the cycloisomerization step in the presence of copper(I) iodide. In the final step, the obtained furo[2,3-*d*]pyrimidin-2(3*H*)-one arabinosides were treated with the aqueous or methanolic ammonia, which resulted in the deprotection of the hydroxyl groups and transformation of furane ring into the pyrrole ring. A brief screening of amonolysis reaction conditions, using aqueous versus methanolic ammonia solutions and traditional versus microwave heating revealed, that optimal conditions for this transformation could be achived using metanolic ammonia under the microwave heating. The resulting library of compounds was evaluated for their anti-proliferative effect against HL-60 human promyelocytic leukemia cells and Jurkat E6.1 human acute T cell leukemia cells. Compound **5g**, bearing the 4-*n*-pentylphenyl substituent attached to the pyrrole ring, showed the highest anti-proliferative activity with IC_{50} values 68 and 61 μM for HL-60 and Jurkat E6.1 respctively. Although the synthetised compounds exhibited a rather weak cytotoxic effect on the investigated cell lines, the results and observed effects could help in the design of more active and selective inhibitors.

4. Experimental section

4.1. Chemistry

Commercially available chemicals were of reagent grade purity and used as received. The reactions were monitored by thin layer chromatography (TLC) using silica gel plates (Kieselgel $60F_{254}$, E. Merck). Column chromatography was performed on Silica Gel 60M (0.040-0.063 mm, E. Merck). The ^{1}H, ^{13}C NMR and ^{19}F spectra were recorded on Varian Unity Plus spectrometer (500 MHz) in MeOH-d_4, $CDCl_3$ and DMSO-d_6. Chemical shift values are reported in parts per million relative to $SiMe_4$ as internal reference. Fluorine ^{19}F spectra were measured using $CFCl_3$ as an internal reference. The resonance assignments are based on peak integration, peak

multiplicity and 2D correlation experiments. Multiplets were assigned as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), dt (doublet of triplet), ddd (doublet of doublet of doublet), m (multiplet), and bs (broad singlet). HRMS spectra were performed on LTQ Orbitrap Velos Thermo Scientific. The microwave-assisted reactions were performed in the CEM Discover SP microwave reactor, set to 80 Watt input power at 60-65 °C, in 35 mL glass tubes.

The synthesis of 1-(2,3,5-tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-5-iodouracil (7)

5.00 g (20.49 mmol, 1 eq.) of 1-(*β*-D-*arabino*furanosyl)uracil (**6**) was dispersed in the 40 mL of dry MeCN. Then 25 mg (0.20 mmol, 0.01 eq.) of DMAP, 11.55 mL (84.00 mmol, 4.1 eq.) of triethylamine and 7.80 mL (81.96 mmol, 4 eq.) of acetic anhydride were added. Reaction mixture was stirred at room temperature for 20 h. After this time all volatiles were evaporated under the reduced pressure. The residue was dissolved in $CHCl_3$ and washed twice with water. The combined organic layers were dried over magnesium sulphate and the solvent was removed on an evaporator. After crystallization from ethanol, 6.575 g (17.76 mmol, 87% yield) of 1-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)uracil were obtained. 3.50 g Of obtained (9.45 mmol, 1eq.) triacetate were dissolved in 150 mL of MeCN, then 2.59 g (4.72 mmol, 0.50 eq.) of ammonium cerium(VI) nitrate and 1.43 g (5.63 mmol, 0.6 eq.) of iodine was added. Obtained suspension was stirred in 80°C for 3 h, and then solvent was removed by evaporation under reduced pressure. The residue was dissolved in EtOAc and washed with brine, sodium thiosulfate solution and water. Combined organic phases were dried over $MgSO_4$. Analytically pure compound was obtained by crystallization from ethanol. Yield: 4.49 g (75%). Mp: 167.7-168.7 °C (lit. 168 °C). ^{1}H NMR (500 MHz, $CDCl_3$): 9.24 (s, 1H, NH), 7.92 (s, 1H, H_6), 6.31 (d, J = 4.0 Hz, 1H, $H_{1'}$), 5.41 (dd, J_1 = 2.0 Hz, J_2 = 4.0 Hz, 1H, $H_{2'}$), 5.13 (q, J = 2.0 Hz, 1H, $H_{3'}$), 4.46 (dd, J_1 = 6.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.42 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.25-4.19 (m, 1H, $H_{4'}$), 2.19 (s, 3H, Me_{Ac}), 2.16 (s, 3H, Me_{Ac}), 2.07 (s, 3H, Me_{Ac}) ^{13}C NMR (125 MHz, $CDCl_3$): 170.5, 169.6, 168.5, 159.5, 149.5, 145.0, 84.3, 80.7, 76.2, 74.4, 67.5, 62.5, 20.9, 20.7, 20.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{17}IN_2O_9$: 497.00515, found: 497.00439.

General procedure for the synthesis of protected 6-substituted furo[2,3-*d*]pyrimidin-2(3*H*)-one arabinosides (9)

1-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-5-iodouracil (**7**) (1 eq.) was dissolved in anhydrous DMF (5 mL by 1 mmol of nucleoside) under Ar atmosphere. To the obtained solution, the appropriate terminal alkine (3 eq.), triethylamine (1.9 eq.), copper iodide (0.2 eq.), and tetrakis(triphenylphosphine)palladium(0) (0.1 eq.) were added and reaction mixture was stirred at room temperature for 24 h. On the next day, the dark solution was heated to the 50-60 °C, then additional amount of copper iodide (1.0 eq) was added and stirring was continued for additional 12 hrs. Reaction mixture was diluted with ethyl acetate (v/v DMF:EtOAc 1:4), the organic phase was washed three times with water (v/v DMF:H_2O 1:4) and dried over magnesium sulphate. Volatiles were evaporated under reduced pressure and the oily residue was chromatographed using an appropriate eluent. Analytically pure compounds were obtained by crystallization from methanol or acetone.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-phenylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (9a). Yield: 38%, white crystals, mp.: 219.1-219.8 °C. $[\alpha]_D^{20}$ +89.9 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.30 (s, 1H, H_4), 7.81-7.75 (m, 2H, H_{Ph}), 7.49-7.45 (m, 2H, H_{Ph}), 7.45-7.39 (m, 1H, H_{Ph}), 6.80 (s, 1H, H_5), 6.46 (d, *J* = 4.0 Hz, 1H, $H_{1'}$), 5.67 (dd, J_1 = 1.0 Hz, J_2 = 4.0 Hz, 1H, $H_{2'}$), 5.11 (d, *J* = 1.0 Hz, 1H, $H_{3'}$), 4.54 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.43 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.35-4.29 (m, 1H, $H_{4'}$), 2.17 (s, 6H, 2 x Me_{Ac}), 1.92 (s, 3H, Me_{Ac}) ^{13}C NMR (125 MHz, $CDCl_3$): 171.9, 170.6, 169.6, 168.1, 156.1, 154.1, 136.5, 129.9, 128.9, 128.1, 124.9, 107.9, 97.5, 86.9, 81.6, 76.4, 73.9, 62.8, 20.8, 20.6, 20.4. IR (KBr cm^{-1}): 3475, 3088, 3040, 2936, 1757, 1679, 1610, 1574, 1490, 1421, 1387, 1373, 1351, 1236, 1207, 1179, 1126, 1107, 1048, 1005; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{23}N_2O_9$: 471.13981, found: 471.13988.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(3-chlorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9b). Yield: 70%, white crystals, mp.: 213.0-214.0 °C. $[\alpha]_D^{20}$ +81.3 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.34 (s, 1H, H_4), 7.77-7.73 (m, 1H, H_{Ar}), 7.68-7.63 (m, 1H, H_{Ar}), 7.43-7.34 (m, 2H, H_{Ar}), 6.83 (s, 1H, H_5), 6.45 (d, *J* = 3.5 Hz, 1H, $H_{1'}$), 5.67 (dd, J_1 = 1.5 Hz, J_2 = 3.5 Hz, 1H, $H_{2'}$), 5.11 (d, *J* = 1.5 Hz, 1H, $H_{3'}$), 4.55 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.43 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz,

1H, $H_{5',a}$), 4.34-4.29 (m, 1H, $H_{4'}$), 2.170 (s, 3H, Me_{Ac}), 2.166 (s, 3H, Me_{Ac}), 1.93 (s, 3H, Me_{Ac}) ^{13}C NMR (125 MHz, $CDCl_3$): 171.8, 170.7, 169.6, 168.0, 154.5, 154.0, 137.2, 135.1, 130.3, 129.9, 129.8, 124.9, 123.0, 107.6, 98.8, 87.1, 81.7, 76.4, 73.9, 62.8, 20.8, 20.7, 20.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{22}{}^{35}ClN_2O_9$: 505.10083, found: 505.10046.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(4-chlorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9c). Yield: 74%, colorless oil. $[\alpha]_D^{20}$ +75.3 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.30 (s, 1H, H_4), 7.71 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 7.45 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 6.79 (s, 1H, H_5), 6.45 (d, *J* = 3.5 Hz, 1H, $H_{1'}$), 5.73-5.62 (m, 1H, $H_{2'}$), 5.15-5.05 (m, 1H, $H_{3'}$), 4.55 (dd, J_1 = 7.5 Hz, J_2 = 11.5 Hz, 1H, $H_{5',b}$), 4.42 (dd, J_1 = 3.5 Hz, J_2 = 11.5 Hz, 1H, $H_{5',a}$), 4.36-4.26 (m, 1H, $H_{4'}$), 2.170 (s, 3H, Me_{Ac}), 2.166 (s, 3H, Me_{Ac}), 1.92 (s, 3H, Me_{Ac}). ^{13}C NMR (125 MHz, $CDCl_3$): 171.9, 170.7, 169.7, 168.1, 155.0, 154.0, 136.8, 135.9, 129.3, 126.7, 126.2, 107.8, 98.0, 87.0, 81.7, 76.4, 73.9, 62.8, 20.9, 20.7, 20.5; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{22}{}^{35}ClN_2O_9$: 505.10083, found: 505.10052.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(3,4-dichlorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9d) . Yield: 72%, colorless oil. $[\alpha]_D^{20}$ +71.7 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.35 (s, 1H, H_4), 7.85 (d, *J*=2.0 Hz, 1H, H_{Ar}), 7.61 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H, H_{Ar}), 7.53 (d, *J* = 8.5 Hz, 1H, H_{Ar}), 6.83 (s, 1H, H_5), 6.45 (d, *J* = 3.5 Hz, 1H, $H_{1'}$), 5.66 (dd, J_1 = 1.5 Hz, J_2 = 3.5 Hz, 1H, $H_{2'}$), 5.10 (d, *J* = 1.5 Hz, 1H, $H_{3'}$), 4.56 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5',b}$), 4.42 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5',a}$), 4.35-4.29 (m, 1H, $H_{4'}$), 2.17 (s, 3H, Me_{Ac}), 2.16 (s, 3H, Me_{Ac}), 1.92 (s, 3H, Me_{Ac}). ^{13}C NMR (125 MHz, $CDCl_3$): 171.8, 170.7, 169.7, 168.0, 153.9, 153.5, 137.4, 133.9, 133.5, 131.1, 128.1, 126.7, 124.0, 107.4, 99.1, 87.1, 81.8, 76.4, 73.9, 62.8, 20.9, 20.7, 20.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{20}{}^{35}Cl_2N_2O_9$: 539.06186, found: 539.06132.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(2-pyridyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9e). Yield: 56%, white crystals, mp.: 175.7-176.7 °C. $[\alpha]_D^{20}$ +86.3 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.69-8.64 (m, 1H, H_{Ar}), 8.39 (s, 1H, H_4), 7.87-7.80 (m, 1H, H_{Ar}), 7.34-7.29 (m, 1H, H_{Ar}), 7.26 (s, 1H, H_5), 6.47 (d, *J* = 4.0 Hz, 1H, $H_{1'}$), 5.67 (dd, J_1 = 1.5 Hz, J_2 = 4.0 Hz, 1H, $H_{2'}$), 5.12 (d, *J* = 1.5 Hz, 1H, $H_{3'}$), 4.53

(dd, J_1 = 6.5 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.45 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.36-4.30 (m, 1H, $H_{4'}$), 2.17 (s, 3H, Me_{Ac}), 2.16 (s, 3H, Me_{Ac}), 1.92 (s, 3H, Me_{Ac}). ^{13}C NMR (125 MHz, $CDCl_3$): 172.0, 170.5, 169.6, 168.1, 155.2, 154.0, 150.1, 147.1, 137.9, 137.1, 124.0, 120.2, 107.5 , 101.1, 86.9, 81.6, 76.3, 73.9, 62.7, 20.8, 20.7, 20.4; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{22}N_3O_9$: 472.13506, found: 472.13462.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(3-hydroxyphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9f). Yield: 43%, yellow crystals, mp.: 210.0-211.0 °C. $[\alpha]_D^{20}$ +80.3 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.30 (s, 1H, H_4), 7.75 (bs, 1H, OH), 7.59-7.55 (m, 1H, H_{Ar}), 7.31-7.22 (m, 2H, H_{Ar}), 6.97-6.92 (m, 1H, H_{Ar}), 6.77 (s, 1H, H_5), 6.47 (d, J = 4.0 Hz, 1H, $H_{1'}$), 5.69 (dd, J_1 = 1.5 Hz, J_2 = 4.0 Hz, 1H, $H_{2'}$), 5.11 (d, J = 1.5 Hz, 1H, $H_{3'}$), 4.53 (dd, J_1 = 6.5 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.43 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.35-4.30 (m, 1H, $H_{4'}$), 2.17 (s, 3H, Me_{Ac}), 2.16 (s, 3H, Me_{Ac}), 1.90 (s, 3H, Me_{Ac}). ^{13}C NMR (125 MHz, $CDCl_3$): 172.0, 170.8, 169.8, 168.2, 157.3, 156.7, 154.8, 136.3, 130.1, 129.2, 117.6, 116.8, 112.3, 108.6, 97.7, 87.1, 81.7, 76.4, 74.0, 62.8, 20.9, 20.7, 20.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{23}N_2O_{10}$: 487.13472, found: 487.13416.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(4-*n*-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9g). Yield: 54%, colorless oil. $[\alpha]_D^{20}$ +73.0 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.30 (s, 1H, H_4), 7.69 (d, J = 8.0 Hz, 2H, CH_{Ar}), 7.26 (d, J = 8.0 Hz, 2H, CH_{Ar}), 6.73 (s, 1H, H_5), 6.46 (d, J = 3.5 Hz, 1H, $H_{1'}$), 5.66 (dd, J_1 = 1.5 Hz, J_2=3.5 Hz, 1H, $H_{2'}$), 5.11 (d, J = 1.5 Hz, 1H, $H_{3'}$), 4.53 (dd, J_1 = 6.5 Hz, J_2=12.0 Hz, 1H, $H_{5'b}$), 4.44 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.36-4.30 (m, 1H, $H_{4'}$), 2.65 (t, J = 7.5 Hz, 2H, CH_{2chain}), 2.16 (s, 6H, Me_{Ac}), 1.92 (s, 3H, Me_{Ac}), 1.64 (q, J = 7.5 Hz, 2H, CH_{2chain}), 1.39-1.29 (m, 4H, 2 x CH_{2chain}), 0.90 (t, J = 7.0 Hz, 3H, CH_{3chain}). ^{13}C NMR (125 MHz, $CDCl_3$): 172.0, 170.6, 169.6, 168.0, 156.4, 154.1, 145.3, 136.0, 129.0, 125.6, 125.0, 108.1, 96.5, 86.9, 81.5, 76.4, 73.9, 62.8, 35.8, 31.4, 30.8, 22.4, 20.8, 20.6, 20.4, 13.9. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{28}H_{32}N_2O_9$: 541.21806, found: 541.21734.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(3-fluorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9h). Yield: 43%, white crystals, mp.: 226.4-227.0 °C**.** $[\alpha]_D^{20}$ +75.0 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.34 (s, 1H, H_4), 7.57 (d, J = 7.5

Hz, 1H, H_{Ar}), 7.49-7.38 (m, 2H, H_{Ar}), 7.11 (dt, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H, H_{Ar}), 6.83 (s, 1H, H_5), 6.46 (d, J = 3.5 Hz, 1H, $H_{1'}$), 5.67 (dd, J_1 = 1.5 Hz, J_2 = 3.5 Hz, 1H, $H_{2'}$), 5.11 (d, J = 1.5 Hz, 1H, $H_{3'}$), 4.55 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.43 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.35-4.30 (m, 1H, $H_{4'}$), 2.170 (s, 3H, Me_{Ac}), 2.166 (s, 3H, Me_{Ac}), 1.93 (s, 3H, Me_{Ac}). ^{13}C NMR (125 MHz, $CDCl_3$): 171.8, 170.7, 169.6, 168.0, 163.0 (d, J=246.6 Hz), 154.7 (d, J=3.4 Hz), 154.0, 137.1, 130.7 (d, J=8.3 Hz), 130.2 (d, J=8.8 Hz), 120.7 (d, J=2.9 Hz), 116.8 (d, J=21.5 Hz), 111.9 (d, J=23.9 Hz), 107.6, 98.7, 87.0, 81.7, 76.4, 73.9, 62.8, 20.9, 20.7, 20.4.^{19}F NMR (471 MHz, $CDCl_3$): −111.98. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{22}N_2O_9F$: 489.13038, found: 489.13047.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(3,5-difluorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9i).Yield: 66%, white crystals, mp.: 224.8-225.6 °C. $[\alpha]_D^{20}$ +82.2 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.39 (s, 1H, H_4), 7.33-7.25 (m, 2H, H_{Ar}), 6.93-6.82 (m, 2H, H_5+H_{Ar}), 6.45 (d, J = 3.5 Hz, 1H, $H_{1'}$), 5.67 (dd, J_1 = 1.5 Hz, J_2 = 3.5 Hz, 1H, $H_{2'}$), 5.10 (d, J = 1.5 Hz, 1H, $H_{3'}$), 4.56 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.42 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.35-4.29 (m, 1H, $H_{4'}$), 2.173 (s, 3H, Me_{Ac}), 2.165 (s, 3H, Me_{Ac}), 1.93 (s, 3H, Me_{Ac}).^{13}C NMR (125 MHz, $CDCl_3$): 171.7, 170.7, 169.6, 168.0, 163.3 (dd, J_1=12.7 Hz, J_2=249.5 Hz), 153.9, 153.4 (t, J~3.6 Hz), 137.9, 131.1 (t, J~10.5 Hz), 108.0 (m), 107.3, 105.1 (d, J=25.4 Hz), 100.0, 87.1, 81.8, 76.4, 73.9, 62.8, 20.9, 20.7, 20.4; ^{19}F NMR (471 MHz, $CDCl_3$): −108.28. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{21}N_2O_9F_2$: 507.12096, found: 507.12116.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(4-trifluorometoxyphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9j). Yield: 43%, colorless oil. $[\alpha]_D^{20}$ +56.6 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.34 (s, 1H, H_4), 7.82-7.76 (m, 2H, H_{Ar}), 7.31 (d, J=8.5 Hz, 2H, H_{Ar}), 6.82 (s, 1H, H_5), 6.46 (d, J=3.5 Hz, 1H, $H_{1'}$), 5.67 (dd, J_1=1.0 Hz, J_2=3.5 Hz, 1H, $H_{2'}$), 5.11 (d, J=1.0 Hz, 1H, $H_{3'}$), 4.55 (dd, J_1=7.0 Hz, J_2=11.5 Hz, 1H, $H_{5'a}$), 4.43 (dd, J_1=4.0 Hz, J_2=11.5 Hz, 1H, $H_{5'b}$), 4.35-4.29 (m, 1H, $H_{4'}$), 2.169 (s, 3H, Me_{Ac}), 2.167 (s, 3H, Me_{Ac}), 1.93 (s, 3H, Me_{Ac}) ^{13}C NMR (125 MHz, $CDCl_3$): 171.9, 170.6, 169.6, 168.0, 154.6, 154.0, 150.0 (q, J = 1.9 Hz), 137.0, 126.8, 126.5, 121.3, 120.3 (q, J = 257.8 Hz), 107.6, 98.3, 87.0, 81.7, 76.4, 73.9, 62.8, 20.8, 20.6, 20.4; ^{19}F

NMR (471 MHz, $CDCl_3$): −58.13. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{21}N_2O_{10}F_3$: 555.12211, found: 555.12207.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(methoxymethyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9k). Yield: 40%, yellow crystals, mp.: 108.5-110.1 °C. $[\alpha]_D^{20}$ +106.8 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.27 (s, 1H, H_4), 6.50 (s, 1H, H_5), 6.43 (d, *J* = 4.0 Hz, 1H, $H_{1'}$), 5.65 (dd, J_1 = 1.5 Hz, J_2 = 4.0 Hz, 1H, $H_{2'}$), 5.09 (d, *J* = 1.5 Hz, 1H, $H_{3'}$), 4.51 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.45 (s, 2H, CH_{2MOM}), 4.42 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.33-4.27 (m, 1H, $H_{4'}$), 3.48 (s, 3H, Me_{MOM}), 2.16 (s, 3H, Me_{Ac}), 2.15 (s, 3H, Me_{Ac}), 1.91 (s, 3H, Me_{Ac}). ^{13}C NMR (125 MHz, $CDCl_3$): 172.1, 170.6, 169.6, 168.0, 155.1, 154.0, 137.2, 106.6, 102.2, 86.9, 81.6, 76.4, 73.9, 66.5, 62.8, 58.9, 20.8, 20.7, 20.4; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{24}N_2O_9$: 439.13472, found: 439.13485.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(3-cyanopropyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9l). Yield: 65%, yellow crystals, mp.: 107.8 °C (decomposition). $[\alpha]_D^{20}$ +105.6 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.21 (s, 1H, H_4), 6.42 (d, *J* = 3.5 Hz, 1H, $H_{1'}$), 6.32 (s, 1H, H_5), 5.65 (dd, J_1 = 1.5 Hz, J_2 = 3.5 Hz, 1H, $H_{2'}$), 5.09 (d, *J* = 1.5 Hz, 1H, $H_{3'}$), 4.51 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.41 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.33-4.27 (m, 1H, $H_{4'}$), 2.88 (d, *J* = 7.0 Hz, 2H, CH_{2Pr}), 2.47 (d, *J* = 7.0 Hz, 2H, CH_{2Pr}), 2.16 (s, 3H, Me_{Ac}), 2.15 (s, 3H, Me_{Ac}), 2.12 (quintet, *J* = 7.0 Hz, 2H, CH_{2Pr}), 1.92 (s, 3H, Me_{Ac}). ^{13}C NMR (125 MHz, $CDCl_3$): 172.0, 170.6, 169.6, 168.0, 156.9, 154.0, 136.3, 118.6, 106.9, 100.6, 86.8, 81.5, 76.3, 73.8, 62.8, 27.0, 22.6, 20.8, 20.7, 20.4, 16.5. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}N_3O_9$: 462.15071, found: 462.15022.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(cyclopropyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9m). Yield: 31%, white crystals, mp.:161.8-163.1 °C. $[\alpha]_D^{20}$ +102.1 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.07 (s, 1H, H_4), 6.43 (d, 1H, *J* = 3.5 Hz, $H_{1'}$), 6.15 (s, 1H, H_5), 5.63 (d, *J* = 3.5 Hz, 1H, $H_{2'}$), 5.09 (s, *J* = 1.5 Hz, 1H, $H_{3'}$), 4.49 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.42 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.32-4.23 (m, 1H, $H_{4'}$), 2.16 (s, 3H, Me_{Ac}), 2.15 (s, 3H, Me_{Ac}), 1.86-1.97 (m, 4H, Me_{Ac} + CH_{cPr}), 1.08-0.97 (m, 4H, 2 x CH_{2cPr}). ^{13}C NMR (125 MHz, $CDCl_3$): 171.7, 170.5, 169.6, 168.0, 161.0, 154.1, 134.4, 107.8, 97.0, 86.7, 81.3, 76.3, 73.8, 62.8, 20.8,

20.6, 20.4, 9.3, 7.30, 7.27. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}N_3O_9$: 435.13967, found: 435.13981.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-benzylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (9n). Yield: 32%, yellow crystals, mp.: 87.6-90.7 °C. $[\alpha]_D^{20}$ +85.4 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.13 (s, 1H, H_4), 7.33-7.26 (m, 2H, H_{Ar}), 7.40-7.23 (m, 3H, H_{Ar}), 6.41 (d, J = 3.5 Hz, 1H, $H_{1'}$), 6.09 (s, 1H, H_5), 5.63 (dd, J_1 = 1.0 Hz, J_2 = 3.5 Hz, 1H, $H_{2'}$), 5.07 (d, J = 1.0 Hz, 1H, $H_{3'}$), 4.46 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.39 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.30-4.24 (m, 1H, $H_{4'}$), 3.99 (s, 1H, CH_{2Bn}), 2.15 (s, 3H, Me_{Ac}), 2.12 (s, 3H, Me_{Ac}), 1.90 (s, 3H, Me_{Ac}). ^{13}C NMR (125 MHz, $CDCl_3$): 172.2, 170.6, 169.6, 168.1, 158.8, 154.1, 135.8, 135.0, 129.0, 128.8, 127.3, 107.3, 100.0, 86.7, 81.4, 76.3, 73.8, 62.7, 34.8, 20.8, 20.6, 20.4. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{24}N_2O_9$: 485.15546, found: 485.15514.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-*n*-octylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (9o). Yield: 41%, white crystals, mp.: 118.5-119.5°C. $[\alpha]_D^{20}$ +89.0 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.12 (s, 1H, H_4), 6.44 (d, J = 3.5 Hz, 1H, $H_{1'}$), 6.17 (s, 1H, H_5), 5.64 (dd, J_1 = 1.5 Hz, J_2 = 3.5 Hz, 1H, $H_{2'}$), 5.09 (d, J = 1.5 Hz, 1H, $H_{3'}$), 4.49 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.41 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.32-4.25 (m, 1H, $H_{4'}$), 2.66 (t, J = 7.5 Hz, 2H, CH_{2Oct}), 2.16 (s, 3H, Me_{Ac}), 2.15 (s, 3H, Me_{Ac}), 1.91 (s, 3H, Me_{Ac}), 1.74-1.65 (m, 2H, CH_{2Oct}), 1.43-1.22 (m, 10H, 5 x CH_{2Oct}), 0.88 (t, J = 7.0 Hz, 3H, Me_{Oct}); ^{13}C NMR (125 MHz, $CDCl_3$): 171.0, 170.6, 169.6, 168.1, 160.3, 154.2, 135.3, 107.5, 98.8, 86.7, 81.4, 76.4, 73.9, 62.8, 31.8, 29.2, 29.1, 29.0, 28.3, 26.6, 22.6, 20.8, 20.7, 20.4, 14.0; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{35}N_2O_9$: 507.23371, found: 507.23298.

3-(2,3,5-Tri-*O*-acetyl-*β*-D-*arabino*furanosyl)-6-(9-hydroksy-*n*-nonyl)octylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (9p). Yield: 56%, colorless oil. $[\alpha]_D^{20}$ +83.8 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$): 8.12 (s, 1H, H_4), 6.44 (d, J = 3.5 Hz, 1H, $H_{1'}$), 6.17 (t, J_1 = 1.0 Hz, 1H, H_5), 5.64 (dd, J_1 = 1.5 Hz, J_2 = 3.5 Hz, 1H, $H_{2'}$), 5.09 (t, J = 1.5 Hz, 1H, $H_{3'}$), 4.50 (dd, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'b}$), 4.41 (dd, J_1 = 4.0 Hz, J_2 = 12.0 Hz, 1H, $H_{5'a}$), 4.32-4.25 (m, 1H, $H_{4'}$), 3.64 (t, J = 6.5 Hz, 2H, CH_{2Non}), 2.66 (t, J = 7.5 Hz, 2H, CH_{2Non}), 2.16 (s, 3H, Me_{Ac}), 2.15 (s, 3H, Me_{Ac}), 1.91 (s, 3H, Me_{Ac}), 1.70 (quintet, J = 7.5 Hz, 2H, CH_{2Non}), 1.62-1.52 (m, 2H, CH_{2Non}), 1.42-1.28 (m, 10H, 5 x CH_{2Non});

^{13}C NMR (125 MHz, $CDCl_3$): 172.2, 170.6, 169.7, 168.1, 160.5, 154.2, 135.2, 107.5, 98.6, 86.8, 81.4, 76.4, 73.9, 62.8, 62.9, 32.7, 29.3, 29.1, 28.9, 28.3, 26.6, 25.7, 20.8, 20.7, 20.4; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{37}N_2O_{10}$: 537.24427, found: 537.24361.

General procedures for the synthesis of deprotected 6-aryl/6-heteroaryl 3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one arabinosides 5

Procedure A was applied for the synthesis of **5a-5j** compounds, while procedure B was applied for the synthesis of **5k-5p** compounds.

A: Protected furo[2,3-*d*]pyrimidin-2(3*H*)-one nucleosides **9** were dissolved in the 7N methanolic ammonia (10 mL by 1 mmol of nucleoside) in a microwave tube and the reaction mixture was heated in 60-65 °C in the microwave synthesizer (80 Watt) for 24-48 h. Then the reaction mixture was moved to the round-bottom flask, silica gel was added and solvent was evaporated under the reduced pressure. The final compounds were purified by column chromatography on silica gel using 10%, then 20% methanol in chloroform as a mobile phase.

B: Protected furo[2,3-*d*]pyrimidin-2(3*H*)-one nucleosides **9** were dispersed in the 25% aquaous ammonia (24 mL by 1 mmol of nucleoside) and stirred at room temperature for 1 – 4 days. In case of the compound **5p**, the reaction mixture was heated at 50-60 °C and aditional portion of ammonia was added. After completion of the reaction, volatiles were evaporated under the reduced pressure and final compounds were purified by column chromatography on silica gel using 10%, then 20% methanol in chloroform as a mobile phase.

3-(*β*-D-*Arabino*furanosyl)-6-phenyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5a).

Yield: 90%, yellow crystals, m.p. 165.5- 167.0 °C. $[\alpha]_D^{20}$ +77.8 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, CD_3OD): 8.62 (s, 1H, H_4), 7.72-7.68 (m, 2H, H_{Ar}), 7.42-7.38 (t, *J*=7.3 Hz, 2H, H_{Ar}), 7.35-7.31 (m, 1H, H_{Ar}), 6.65 (s, 1H, H_5), 6.39 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 4.38 (dd, J_1=2.0 Hz, J_2=3.5 Hz, 1H, $H_{2'}$), 4.16 (t, *J*=2.0 Hz, 1H, $H_{3'}$), 4.10-4.06 (m, 1H, $H_{4'}$), 3.90 (d, *J*=4.5 Hz, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, CD_3OD): 160.5, 156.9, 141.8, 139.6, 132.0, 130.0, 129.6, 126.3, 111.5, 98.2, 90.2, 87.4, 78.3, 76.5, 62.9. IR (KBr cm^{-1}): 3700-2800, 1658, 1618, 1586, 1571, 1554, 1492, 1448, 1412, 1384, 1348,

1253, 1198, 1107, 1055; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{18}N_3O_5$: 344.12308, found: 344.12350.

3-(*β*-D-*Arabino*furanosyl)-6-(3-chlorophenyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5b). Yield: 32%, yellow crystals, m.p. 212.0-213.3 °C. $[\alpha]_D^{20}$ +70.2 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.80 (s, 1H, NH), 8.46 (s, 1H, H_4), 7.93-7.91 (m, 1H, H_{Ar}), 7.77 (dq, J_1=1.0 Hz, J_2=8.0 Hz, 1H, H_{Ar}), 7.46 (t, *J*=8.0 Hz, 1H, H_{Ar}), 7.38 (dq, J_1=1.0 Hz, J_2=8.0 Hz, 1H, H_{Ar}), 6.88 (s, 1H, H_5), 6.23 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.51 (d, *J*=4.0 Hz, 1H, $OH_{2'}$), 5.47 (d, *J*=5.0 Hz, 1H, $OH_{3'}$), 5.15 (t, *J*=5.5 Hz, 1H, $OH_{5'}$), 4.16-4.11 (m, 1H, $H_{2'}$), 3.99-3.95 (m, 1H, $H_{3'}$), 3.89 (dt, J_1=2.5 Hz, J_2=6.5 Hz, 1H, $H_{4'}$), 3.74-3.63 (m, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, DMSO-d_6): 159.7, 153.8, 139.0, 137.2, 133.8, 132.8, 130.7, 127.8, 124.5, 123.5, 108.1, 98.6, 87.9, 85.9, 76.4, 74.5, 61.2. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{17}N_3O_5Cl$: 378.08512, found: 378.08490.

3-(*β*-D-*Arabino*furanosyl)-6-(4-chlorophenyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5c). Yield: 32%, yellow crystals, m.p. 197.1-198.3 °C. $[\alpha]_D^{20}$ +67.4 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.80 (s, 1H, NH), 8.44 (s, 1H, H_4), 7.77-7.80 (m, 2H, H_{Ar}), 7.53-7.47 (m, 2H, H_{Ar}), 6.80 (s, 1H, H_5), 6.23 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.52 (d, *J*=4.0 Hz, 1H, $OH_{2'}$), 5.47 (d, *J*=5.5 Hz, 1H, $OH_{3'}$), 5.16 (t, *J*=5.5 Hz, 1H, $OH_{5'}$), 4.17-4.11 (m, 1H, $H_{2'}$), 4.00-3.96 (m, 1H, $H_{3'}$), 3.89 (dt, J_1=2.5 Hz, J_2=5.5 Hz, 1H, $H_{4'}$), 7.72-3.64 (bt, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, DMSO-d_6): 159.8, 153.8, 138.8, 137.6, 132.6, 129.6, 128.9, 126.6, 108.3, 97.9, 87.9, 85.9, 76.5, 74.5, 61.2. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{17}N_3O_5Cl$: 378.08512, found: 378.08462.

3-(*β*-D-*Arabino*furanosyl)-6-(3,4-dichlorophenyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5d). Yield: 41%, yellow crystals, m.p. 226.5-227.4 °C. $[\alpha]_D^{20}$ +84.0 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.75 (wave, 1H, NH), 8.47 (s, 1H, H_4), 8.09 (d, *J*=2.0 Hz, 1H, H_{Ar}), 7.80 (d, J_1=2.0 Hz, J_2=7.5 Hz, 1H, H_{Ar}), 7.67 (d, *J*=7.5 Hz, 1H, H_{Ar}), 6.92 (s, 1H, H_5), 6.23 (d, 1H, *J*=3.5 Hz, 1H, $H_{1'}$), 5.52 (wave, 2H, $OH_{2'}$+$OH_{3'}$), 5.16 (wave, 1H, $OH_{5'}$), 4.18-4.11 (m, 1H, $H_{2'}$), 4.01-3.94 (m, 1H, $H_{3'}$), 3.89 (dt, J_1=2.5 Hz, J_2=5.5 Hz, 1H, $H_{4'}$), 3.68 (d, *J*=5.5 Hz, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, DMSO-d_6): 159.7, 153.7, 139.2, 137.4, 131.8, 131.4, 131.0, 130.2, 126.4, 125.0,

108.2, 99.2, 87.9, 86.0, 76.4, 74.5, 61.2; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{17}N_3O_5Cl_2$: 412.04615, found: 412.04592.

3-(*β*-D-*Arabino*furanosyl)-6-(2-pirydyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5e). Yield: 46%, yellow solid, m.p. 188.5-189.1 °C. $[\alpha]_D^{20}$ +103.7 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.75 (s, 1H, NH), 8.64-8.59 (m, 1H, H_{Ar}), 8.51 (s, 1H, H_4), 7.92 (dt, J_1=1.0 Hz, J_2=8.0 Hz, 1H, H_{Ar}), 7.89-7.83 (m, 1H, H_{Ar}), 7.32 (ddd, J_1=1.0 Hz, J_2=4.5 Hz, J_3=8.0 Hz, 1H, H_{Ar}), 7.00 (s, 1H, H_5), 6.24 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.51 (d, *J*=4.0 Hz, 1H, $OH_{2'}$), 5.47 (d, *J*=5.5 Hz, 1H, $OH_{3'}$), 5.13 (t, *J*=5.5 Hz, 1H, $OH_{5'}$), 4.17-4.11 (m, 1H, $H_{2'}$), 4.01-3.95 (m, 1H, $H_{3'}$), 3.89 (dt, J_1=2.5 Hz, J_2=5.5 Hz, 1H, $H_{4'}$), 3.74-3.64 (m, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, DMSO-d_6): 159.4, 153.8, 149.5, 148.0, 139.7, 138.7, 137.1, 122.8, 119.8, 108.2, 99.9, 87.9, 86.0, 76.5, 74.5, 61.3; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{16}N_4O_5$: 345.11935, found: 345.11775.

3-(*β*-D-*Arabino*furanosyl)-6-(3-hydroksyphenyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5f). Yield: 74%, yellow solid, m.p. 231.5-232.4 °C. $[\alpha]_D^{20}$ +84.0 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.67 (s, 1H, NH), 9.60 (s, 1H, OH), 8.39 (s, 1H, C_4), 7.35-7.14 (m, 3H, H_{Ar}), 6.82-6.73 (m, 1H, H_{Ar}), 6.65 (s, 1H, H_5), 6.23 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.50 (d, *J*=4.0 Hz, 1H, $OH_{2'}$), 5.43 (d, *J*=5.5 Hz, 1H, $OH_{3'}$), 5.13 (t, *J*=5.0 Hz, 1H, $OH_{5'}$), 4.17-4.06 (m, 1H, $H_{2'}$), 4.02-3.95 (m, 1H, $H_{3'}$), 3.92-3.85 (m, 1H, $H_{4'}$), 3.75-3.63 (m, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, DMSO-d_6): 159.8, 157.7, 153.9, 139.1, 138.3, 129.9, 116.0, 115.4, 111.9, 108.3, 96.9, 87.8, 85.9, 76.5, 74.5, 61.3; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{16}N_4O_5$: 360.11901, found: 360.11752.

3-(*β*-D-*Arabino*furanosyl)-6-(4-*n*-pentylphenyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5g). Yield: 55%, yellow solid, m.p. 124.3-125.9 °C. $[\alpha]_D^{20}$ +61.3 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, CD_3OD): 8.58 (s, 1H, H_4), 7.58 (d, *J*=8.0 Hz, 2H, H_{Ar}), 7.20 (d, *J*=8.0 Hz, 2H, H_{Ar}), 6.58 (s, 1H, H_5), 6.39 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 4.38 (dd, J_1=3.5 Hz, J_2=2.0 Hz, 1H, $H_{2'}$), 4.16 (t, *J*=2.0 Hz, 1H, $H_{3'}$), 4.11-4.05 (m, 1H, $H_{4'}$), 3.90 (d, *J*=4.5 Hz, 2H, $H_{5'}$), 2.61 (t, *J*=7.5 Hz, 2H, CH_2), 1.62 (quintet, *J*=7.5 Hz, 2H, CH_2), 1.37-1.31 (m, 4H, 2 x CH_2), 0.90 (t, *J*=7.0 Hz, 3H, Me). ^{13}C NMR (125 MHz, CD_3OD): 160.5, 156.9, 144.9, 139.2, 130.0, 129.4, 126.2, 111.6, 97.4, 90.2, 87.3, 78.3, 76.5, 62.9, 36.6, 32.6, 32.2, 23.6, 14.4; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{27}N_3O_5$: 414.20235, found: 414.20177.

3-(*β*-D-*Arabino*furanosyl)-6-(3-fluorophenyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5h). Yield: 94%, yellow glass. $[\alpha]_D^{20}$ +84.3 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.81 (s, 1H, NH), 8.48 (s, 1H, C_4), 7.74-7.63 (m, 2H, H_{Ar}), 7.52-7.42 (m, 1H, H_{Ar}), 7.19-7.11 (m, 1H, H_{Ar}), 6.86 (s, 1H, H_5), 6.25 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.53 (d, *J*=4.0 Hz, 1H, $OH_{2'}$), 5.50 (d, *J*=5.0 Hz, 1H, $OH_{3'}$), 5.16 (t, *J*=5.5 Hz, 1H, $OH_{5'}$), 4.20-4.12 (m, 1H, $H_{2'}$), 4.05-3.96 (m, 1H, $H_{3'}$), 3.94-3.86 (m, 1H, $H_{4'}$), 3.81-3.75 (m, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, DMSO-d_6): 162.6 (d, *J*=243.0 Hz), 159.7, 153.9, 139.1, 137.6, 133.1 (d, *J*=8.4 Hz), 130.9 (d, *J*=8.6 Hz), 121.1 (d, *J*=2.3 Hz), 114.8 (d, *J*=21.3 Hz), 111.7 (d, *J*=23.1 Hz), 108.2, 98.6, 88.0, 86.0, 76.5, 74.6, 61.3; ^{19}F NMR (471 MHz, DMSO-d_6): −112.52. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{17}FN_3O_5$: 326.11468, found: 326.11510.

3-(*β*-D-*Arabino*furanosyl)-6-(3,5-difluorophenyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5i). Yield: 60%, yellow crystals, decomposition at ~159.7 °C. $[\alpha]_D^{20}$ +77.7 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.81 (s, 1H, NH), 8.50 (s, 1H, C_4), 7.58 (d, *J*=7.0 Hz, 2H, H_{Ar}), 7.21-7.15 (m, 1H, H_{Ar}), 6.98 (s, 1H, H_5), 6.24 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.56-5.48 (m, 2H, $OH_{2'}$+$OH_{3'}$), 5.15 (t, *J*=5.5 Hz, 1H, $OH_{5'}$), 4.21-4.13 (m, 1H, $H_{2'}$), 4.02-3.96 (m, 1H, $H_{3'}$), 3.94-3.87 (m, 1H, $H_{4'}$), 3.75-3.64 (m, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, DMSO-d_6): 162.8 (dd, J_1~13.9 Hz, J_2~245.2 Hz), 159.6, 153.8, 139.6, 136.5, 134.1 (t, *J*~10.8 Hz), 108.0 (m), 103.2 (t, *J*~26.2 Hz), 99.9, 88.1, 86.0, 76.5, 74.5, 61.2' ^{19}F NMR (471 MHz, DMSO-d_6): −109.61. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}F_2N_3O_5$: 380.10525, found: 380.10551.

3-(*β*-D-*Arabino*furanosyl)-6-(4-trifluorometoxyphenyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5j). Yield: 41%, yellow solid, m.p. 170.8-173.1 °C. $[\alpha]_D^{20}$ +72.0 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, CD_3OD): 8.67 (s, 1H, H_4), 7.86-7.80 (m, 2H, H_{Ar}), 7.35 (d, *J*=8.0 Hz, 2H, H_{Ar}), 6.74 (s, 1H, H_5), 6.39 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 4.36 (dd, J_1=2.5 Hz, J_2=3.5 Hz, 1H, $H_{2'}$), 4.14 (t, *J*=2.5 Hz, 1H, $H_{3'}$), 4.08 (dd, J_1=2.5 Hz, J_2=4.5 Hz, 1H, $H_{4'}$), 3.90 (d, *J*=4.5 Hz, 2H, $H_{5'}$). ^{13}C NMR (125 MHz, CD_3OD): 160.7, 157.0, 150.4, 140.3, 140.2, 131.3, 128.0, 122.6, 111.3, 99.3, 90.3, 87.5, 78.3, 76.5, 62.9, quarternary carbon in CF_3O group was not observed in carbon spectrum. ^{19}F NMR (471 MHz, CD_3OD): −59.45. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{17}F_3N_3O_6$: 428.10640, found: 428.10636.

3-(*β*-D-*Arabino*furanosyl)-6-methoxymethyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5k). Yield: 31%, white solid, m.p. 147.2 °C (decomposition). $[\alpha]_D^{20}$ +99.9 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, CD_3OD): 8.60 (s, 1H, H_4), 6.37 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 6.29 (s, 1H, H_5), 4.43 (s, 2H, CH_{2MOM}), 4.33 (dd, J_1=2.5 Hz, J_2=3.5 Hz, 1H, $H_{2'}$), 4.14 (t, *J*=2.5 Hz, 1H, $H_{3'}$), 4.08 (dd, J_1=2.5 Hz, J_2=4.5 Hz, 1H, $H_{4'}$), 3.87 (d, *J*=4.5 Hz, 2H, $H_{5'}$), 3.37 (s, 3H, Me_{MOM}); ^{13}C NMR (125 MHz, CD_3OD): 160.0, 156.9, 140.1, 139.7, 110.4, 101.3, 90.1, 87.4, 78.3, 76.4, 67.9, 62.9, 58.3; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{13}H_{18}N_3O_6$: 312.11901, found: 312.11905.

3-(*β*-D-*Arabino*furanosyl)-6-(3-cyanopropyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5l). Yield: 51%, yellow crystals, m.p. 124.8-126.9 °C. $[\alpha]_D^{20}$ +100.9 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, CD_3OD): 8.51 (s, 1H, H_4), 6.36 (d, *J*=4.0 Hz, 1H, $H_{1'}$), 6.11 (s, 1H, H_5), 4.35-4.31 (m, 1H, $H_{2'}$), 4.15-4.11 (m, 1H, $H_{3'}$), 4.08-4.03 (m, 1H, $H_{4'}$), 3.89-3.85 (m, 2H, $H_{5'}$), 2.78 (t, *J*=7.5 Hz, 2H, CH_{2Pr}); 2.50 (t, *J*=7.0 Hz, 2H, CH_{2Pr}); 2.06-1.97 (m, 2H, CH_{2Pr}); ^{13}C NMR (125 MHz, CD_3OD): 160.0, 156.9, 138.7, 120.7, 110.9, 99.3, 90.0, 87.3, 78.2, 76.5, 62.8, 25.1, 27.8, 16.8; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{19}N_4O_5$: 312.11901, found: 312.11905.

3-(*β*-D-*Arabino*furanosyl)-6-cyclopropyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5m). Yield: 46%, beige crystals, m.p. 148.8 °C (decomposition). $[\alpha]_D^{20}$ +100.5 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.06 (s, 1H, NH), 8.17 (s, 1H, C_4), 6.19 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.89 (s, 1H, H_5), 5.47 (d, *J*=3.5 Hz, 1H, $OH_{2'}$), 5.37 (d, *J*=5.0 Hz, 1H, $OH_{3'}$), 5.11 (bs, 1H, $OH_{5'}$), 4.11-4.04 (m, 1H, $H_{2'}$), 3.96-3.91 (m, 1H, $H_{3'}$), 3.87-3.82 (m, 1H, $H_{4'}$), 3.68-3.61 (m, 2H, $H_{5'}$), 1.92-1.82 (m, 1H, CH_{cPr}), 0.95-0.87 (m, 2H, CH_{2cPr}), 0.81-0.70 (m, 2H, CH_{2cPr}). ^{13}C NMR (125 MHz, DMSO-d_6): 159.1, 153.9, 143.9, 136.0, 108.0, 94.4, 87.6, 85.7, 76.5, 74.5, 61.2, 9.1, 7.6, 7.4; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{18}N_3O_5$: 308.12410, found: 308.12367.

3-(*β*-D-*Arabino*furanosyl)-6-benzyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5n). Yield: 24%, yellow crystals, m.p. 180.8 °C (decomposition). $[\alpha]_D^{20}$ +78.9 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.07 (s, 1H, NH), 8.26 (s, 1H, C_4), 7.36-7.26 (m, 4H, H_{Ph}), 7.26-7.18 (m, 5H, H_{Ph}), 6.20 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.91 (s, 1H, H_5), 5.46 (d, *J*=4.0 Hz, 1H, $OH_{2'}$), 5.36 (d, *J*=5.0 Hz, 1H, $OH_{3'}$), 5.12-5.03 (m, 1H,

$OH_{5'}$), 4.12-4.05 (m, 1H, $H_{2'}$), 3.97-3.92 (m, 1H, $H_{3'}$), 3.91-82 (m, 3H, $H_{4'}$ + CH_{2Bn}), 3.65 (m, 2H, $H_{5'}$); ^{13}C NMR (125 MHz, DMSO-d_6): 159.3, 153.9, 140.6, 138.3, 137.1, 128.7, 128.4, 126.5, 107.8, 97.7, 87.7, 85.8, 76.5, 74.5, 61.2, 33.7; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{18}N_3O_5$: 358.13975, found: 358.13915.

3-(*β*-D-*Arabino*furanosyl)-6-*n*-octyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5o).
Yield: 63%, white crystals, m.p. 110.5-112.7 °C. $[\alpha]_D^{20}$ +72.2 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.06 (s, 1H, NH), 8.22 (s, 1H, C_4), 6.20 (d, *J*=3.5 Hz, 1H, $H_{1'}$), 5.92 (s, 1H, H_5), 5.46 (d, *J*=4.0 Hz, 1H, $OH_{2'}$), 5.36 (d, *J*=5.5 Hz, 1H, $OH_{3'}$), 5.09 (t, *J*=5.0 Hz, 1H, $OH_{5'}$), 4.11-4.05 (m, 1H, $H_{2'}$), 3.98-3.91 (m, 1H, $H_{3'}$), 3.88-3.82 (m, 3H, $H_{4'}$), 3.65 (t, *J*=5.0 Hz, 2H, $H_{5'}$); 2.51 (t, *J*=7.5 Hz, 2H, CH_{2Oct}), 1.65-1.55 (m, 2H, CH_{2Oct}), 1.34-1.18 (m, 10H, 5 x CH_{2Oct}), 0.86 (t, *J*=7.0 Hz, 3H, Me_{Oct}); ^{13}C NMR (125 MHz, DMSO-d_6): 159.1, 153.9, 141.8, 136.5, 107.9, 96.3, 87.6, 85.7, 76.5, 74.5, 61.2, 31.3, 28.7, 28.6, 28.5, 27.6, 27.5, 22.1, 13.9; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{29}N_3O_5$: 380.21800, found: 380.21783.

3-(*β*-D-*Arabino*furanosyl)-6-(9-hydroksy-*n*-nonyl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one (5p).
Yield: 32%, white crystals, m.p. 196.3 °C (decomposition). $[\alpha]_D^{20}$ +88.6 (*c* 1.0, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): 11.07 (s, 1H, NH), 8.22 (s, 1H, C_4), 6.20 (d, *J*=4.0 Hz, 1H, $H_{1'}$), 5.92 (s, 1H, H_5), 5.47 (d, *J*=4.0 Hz, 1H, $OH_{2'}$), 5.37 (d, *J*=5.5 Hz, 1H, $OH_{3'}$), 5.10 (t, *J*=5.5 Hz, 1H, $OH_{5'}$), 4.06-4.12 (m, 1H, $H_{2'}$), 3.98-3.92 (m, 1H, $H_{3'}$), 3.88-3.82 (m, 3H, $H_{4'}$), 3.66 (t, *J*=5.5 Hz, 2H, $H_{5'}$); 3.41-3.32 (m, 2H, CH_{2Non}), 2.56-2.47 (m, 2H, CH_{2Non}), 1.66-1.55 (m, 2H, CH_{2Non}), 1.44-1.34 (m, 2H, CH_{2Non}), 1.33-1.18 (m, 10H, 5 x CH_{2Non}); ^{13}C NMR (125 MHz, DMSO-d_6): 159.1, 153.9, 141.9, 136.5, 108.0, 96.3, 87.6, 85.7, 76.5, 74.5, 61.2, 60.7, 32.5, 29.0, 28.7, 28.6, 27.6, 27.5, 25.5; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{31}N_3O_6$: 410.22856, found: 410.22831.

4.2 Biology - Cytotoxic Assays

Cell culture: HL-60 human promyelocytic leukemia cells and Jurkat E6.1 human acute T cell leukemia cells were obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.) and maintained in the Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (IIET, PAS), Wroclaw, Poland. Cells were maintained in RPMI-1640 GLUTAMAX

(Gibco, Scotland, UK) medium containing 100 U/mL penicillin, 100 μg/mL streptomycin (both from Polfa Tarchomin S.A., Warsaw, Poland) and supplemented with 10% fetal bovine serum (Sigma-Aldrich, Germany). Medium for HL-60 cells was additionally supplemented with 1 mM sodium pyruvate and 4.5 g/L glucose (both from Sigma-Aldrich, Germany).

Antiproliferative assays: Cells were plated on 96-well plates (Corning B.V. New York, USA) at a density of 1×10^4 cells per well in 100 μL of culture medium without FBS and antibiotics. After 24 h of incubation under standard conditions (37 °C in humid atmosphere with 5 % CO_2), cells were treated with cytarabine analogues suspended in 100 μL of culture medium at final concentrations: 100 – 10 – 1 – 0.1 μg/mL. After additional 72 h an MTT assay was applied as described earlier.[45] The optical densities of the samples were measured on a Synergy H4 Hybrid Reader (BioTek Instruments, USA). Two reference compounds were applied: cisplatin and cytarabine.

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