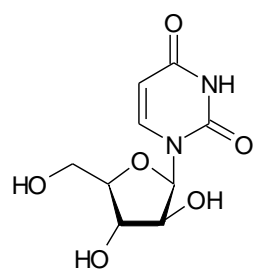
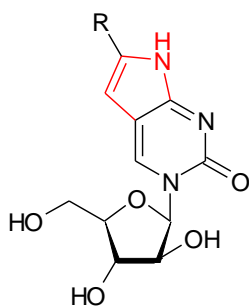
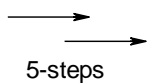


GRAPHICAL ABSTRACT:

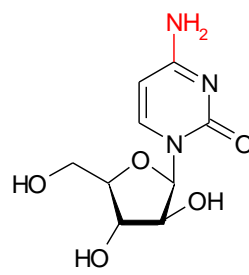


araU



bicyclic Cytarabine analogues

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



AraC

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 comparison to the mother compound. Of all the tested compounds, the derivative bearing a 4-*n*-pentylphenyl substituent exhibited the highest antiproliferative activity.

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 organs⁷⁻¹² 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 desirable, 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, Encycytabine (**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.

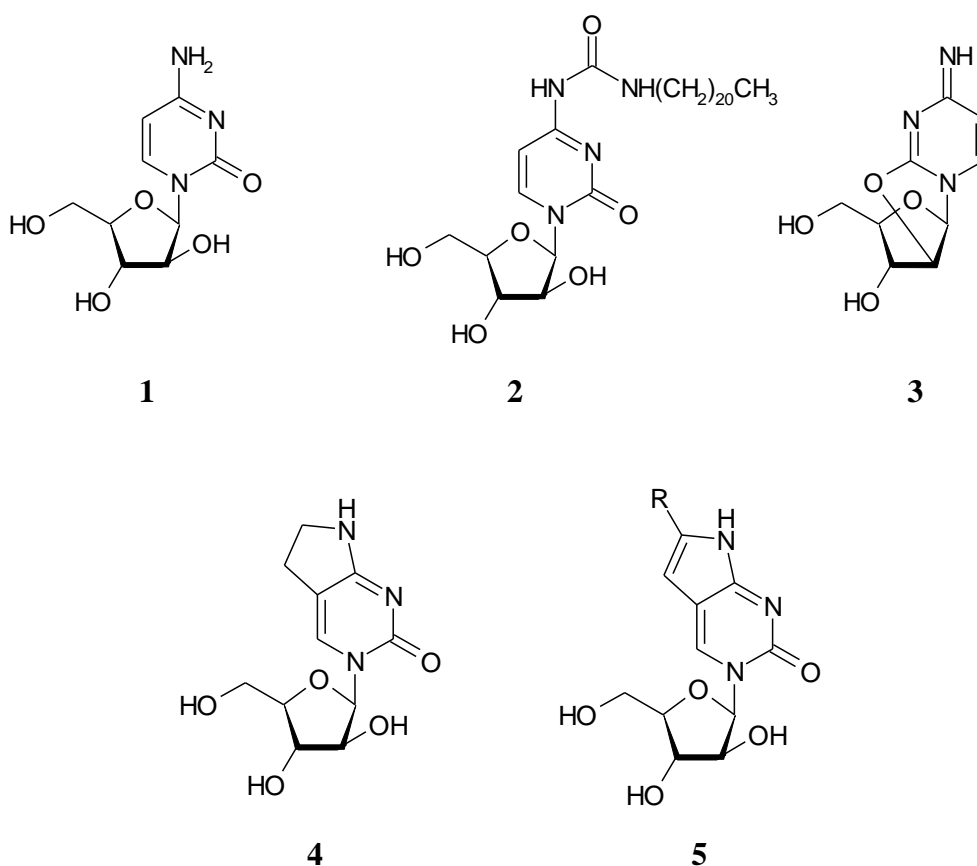


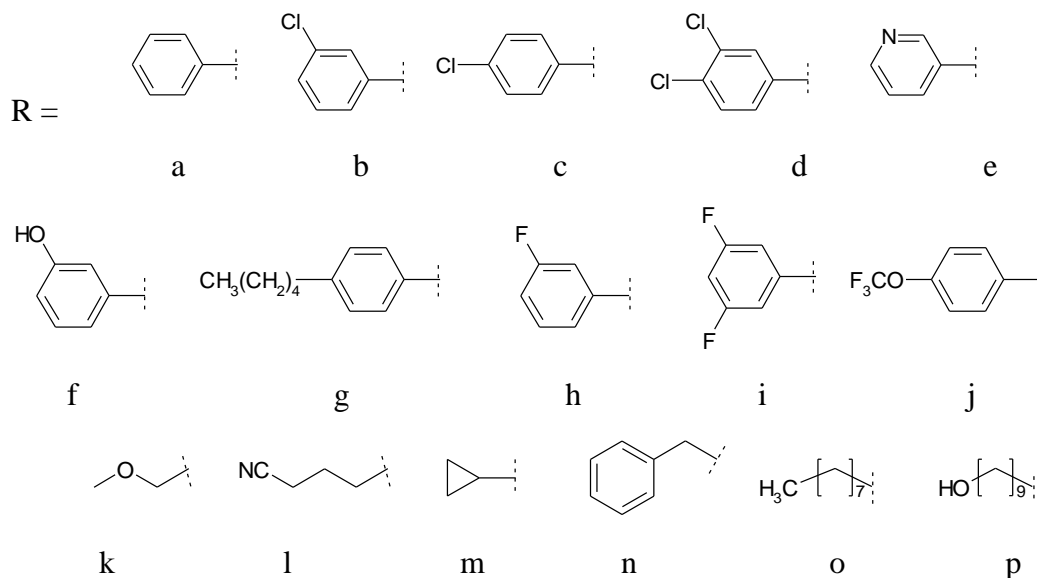
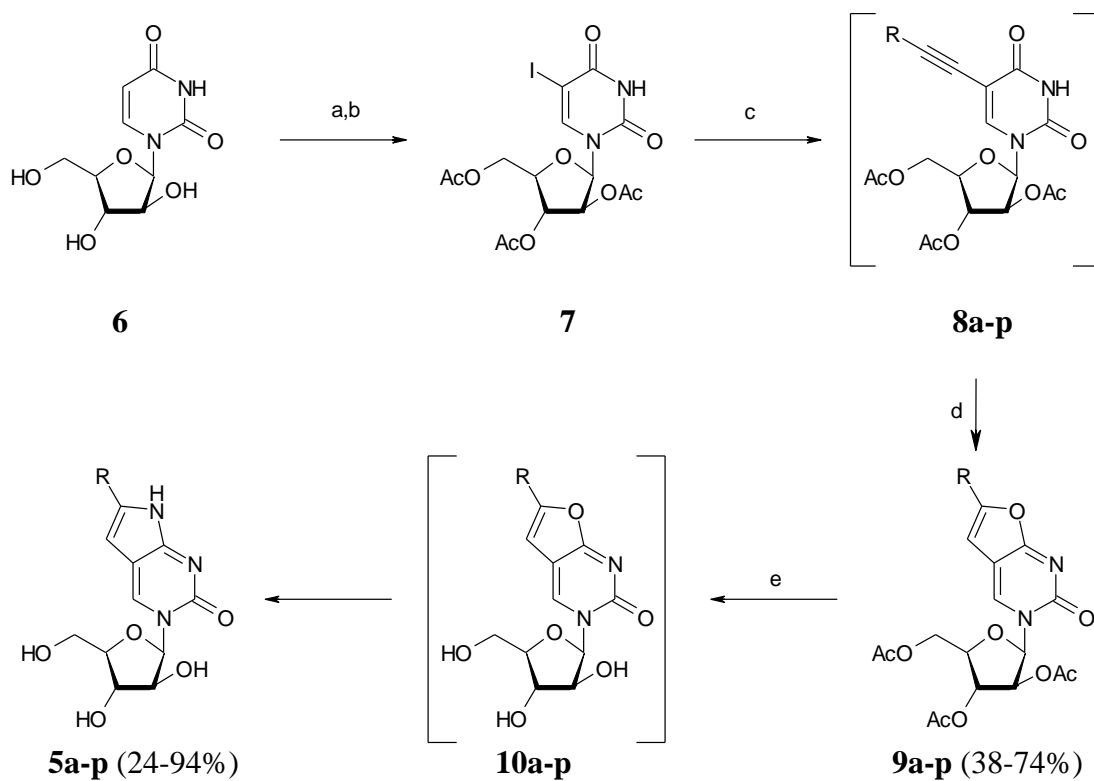
Fig.1 Cytarabine (1) and its derivatives 2-5

Nucleosides possessing bicyclic bases are known as efficient cytidine mimetics, forming stable Watson-Crick pairs with guanine 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} HBV²⁶ and the vaccinia virus (VACV).²⁷ They have not been, however, investigated as possible anticancer agents yet. BCNAs possessing an *arabinofuranosyl* ring and furo[2,3-*d*]pyrimidin-2(3*H*)-one as base were reported as potential antiviral 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*]pyrimidine³⁰ or thieno[5,4-*d*]pyrimidine³¹ bases exhibit remarkable *in vivo* and *in vitro* antileukemic 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 derivatives³²⁻³⁶ 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 convenient analogues of Cytarabine, possibly retaining the anticancer activity resulting from undergoing phosphorylation to appropriate triphosphates by cellular enzymes. Such triphosphates can inhibit DNA polymerases and terminate DNA chain elongation. Additionally, 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-arabinofuranosyl)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 I₂ in the presence of cerium ammonium nitrate,⁴² leading to formation of 1-(2,3,5-tri-*O*-acetyl- β -D-arabinofuranosyl)-5-iodouracil (**7**). Intermediate product **7** was then applied in a series of Sonogashira reactions using 16 alkynes, under Pd(PPh₃)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) Ac₂O, TEA, DMAP, MeCN, 20 h, rt b) (NH₄)₂Ce(NO₃)₆, I₂, MeCN, 3 h, 80 °C c) R-C≡CH, Pd(PPh₃)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% NH₄OH, 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 comparative studies of both methods were not reported. For this reason, we then tested both reagents for the last step of the synthesis; the removal of 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 reaction time is extended 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 NH₄OH were carried out at room temperature with prolonged reaction time (up to the 4 days in case of products **5o** and **5p** bearing long lipophilic 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 IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 conversion to appropriate 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-*arabinofuranosyl*) uracil, was applied to Sonogashira couplings with appropriate 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 amination reaction conditions, using aqueous versus methanolic ammonia solutions and traditional versus microwave heating revealed, that optimal conditions for this transformation could be achieved using methanolic 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 IC₅₀ values 68 and 61 μ M for HL-60 and Jurkat E6.1 respectively. Although the synthesised 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 60F₂₅₄, E. Merck). Column chromatography was performed on Silica Gel 60M (0.040-0.063 mm, E. Merck). The ¹H, ¹³C NMR and ¹⁹F spectra were recorded on Varian Unity Plus spectrometer (500 MHz) in MeOH-*d*₄, CDCl₃ and DMSO-*d*₆. Chemical shift values are reported in parts per million relative to SiMe₄ as internal reference. Fluorine ¹⁹F spectra were measured using CFCl₃ 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-arabinofuranosyl)-5-iodouracil (7)

5.00 g (20.49 mmol, 1 eq.) of 1-(β -D-arabinofuranosyl)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 CHCl₃ 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-arabinofuranosyl)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 MgSO₄. Analytically pure compound was obtained by crystallization from ethanol. Yield: 4.49 g (75%). Mp: 167.7-168.7 °C (lit. 168 °C). ¹H NMR (500 MHz, CDCl₃): 9.24 (s, 1H, NH), 7.92 (s, 1H, H₆), 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}) ¹³C NMR (125 MHz, CDCl₃): 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 C₁₅H₁₇IN₂O₉: 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-arabinofuranosyl)-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 alkyne (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:H₂O 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-arabinofuranosyl)-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); ¹H NMR (500 MHz, CDCl₃): 8.30 (s, 1H, H₄), 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, H₅), 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}) ¹³C NMR (125 MHz, CDCl₃): 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 C₂₃H₂₃N₂O₉: 471.13981, found: 471.13988.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-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); ¹H NMR (500 MHz, CDCl₃): 8.34 (s, 1H, H₄), 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, H₅), 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}) ¹³C NMR (125 MHz, CDCl₃): 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 C₂₃H₂₂³⁵ClN₂O₉: 505.10083, found: 505.10046.

3-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-6-(4-chlorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9c). Yield: 74%, colorless oil. [α]_D²⁰ +75.3 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.30 (s, 1H, H₄), 7.71 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 7.45 (d, *J* = 8.5 Hz, 2H, H_{Ar}), 6.79 (s, 1H, H₅), 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}). ¹³C NMR (125 MHz, CDCl₃): 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 C₂₃H₂₂³⁵ClN₂O₉: 505.10083, found: 505.10052.

3-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-6-(3,4-dichlorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9d). Yield: 72%, colorless oil. [α]_D²⁰ +71.7 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.35 (s, 1H, H₄), 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, H₅), 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}). ¹³C NMR (125 MHz, CDCl₃): 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 C₂₃H₂₀³⁵Cl₂N₂O₉: 539.06186, found: 539.06132.

3-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-6-(2-pyridyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9e). Yield: 56%, white crystals, mp.: 175.7-176.7 °C. [α]_D²⁰ +86.3 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.69-8.64 (m, 1H, H_{Ar}), 8.39 (s, 1H, H₄), 7.87-7.80 (m, 1H, H_{Ar}), 7.34-7.29 (m, 1H, H_{Ar}), 7.26 (s, 1H, H₅), 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}). ¹³C NMR (125 MHz, CDCl₃): 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 C₂₂H₂₂N₃O₉: 472.13506, found: 472.13462.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-6-(3-hydroxyphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9f). Yield: 43%, yellow crystals, mp.: 210.0-211.0 °C. [α]_D²⁰ +80.3 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.30 (s, 1H, H₄), 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, H₅), 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}). ¹³C NMR (125 MHz, CDCl₃): 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 C₂₃H₂₃N₂O₁₀: 487.13472, found: 487.13416.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-6-(4-*n*-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9g). Yield: 54%, colorless oil. [α]_D²⁰ +73.0 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.30 (s, 1H, H₄), 7.69 (d, $J = 8.0$ Hz, 2H, CH_{Ar}), 7.26 (d, $J = 8.0$ Hz, 2H, CH_{Ar}), 6.73 (s, 1H, H₅), 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}). ¹³C NMR (125 MHz, CDCl₃): 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 C₂₈H₃₂N₂O₉: 541.21806, found: 541.21734.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-6-(3-fluorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9h). Yield: 43%, white crystals, mp.: 226.4-227.0 °C. [α]_D²⁰ +75.0 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.34 (s, 1H, H₄), 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, H₅), 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}). ¹³C NMR (125 MHz, CDCl₃): 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. ¹⁹F NMR (471 MHz, CDCl₃): -111.98. HRMS (ESI): m/z [M+H]⁺ calcd for C₂₃H₂₂N₂O₉F: 489.13038, found: 489.13047.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-6-(3,5-difluorophenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9i). Yield: 66%, white crystals, mp.: 224.8-225.6 °C. [α]_D²⁰ +82.2 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.39 (s, 1H, H₄), 7.33-7.25 (m, 2H, H_{Ar}), 6.93-6.82 (m, 2H, H₅+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}). ¹³C NMR (125 MHz, CDCl₃): 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, $J\sim 3.6$ Hz), 137.9, 131.1 (t, $J\sim 10.5$ 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; ¹⁹F NMR (471 MHz, CDCl₃): -108.28. HRMS (ESI): m/z [M+H]⁺ calcd for C₂₃H₂₁N₂O₉F₂: 507.12096, found: 507.12116.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-6-(4-trifluoromethoxyphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9j). Yield: 43%, colorless oil. [α]_D²⁰ +56.6 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.34 (s, 1H, H₄), 7.82-7.76 (m, 2H, H_{Ar}), 7.31 (d, $J=8.5$ Hz, 2H, H_{Ar}), 6.82 (s, 1H, H₅), 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'b}), 4.43 (dd, $J_1=4.0$ Hz, $J_2=11.5$ Hz, 1H, H_{5'a}), 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}). ¹³C NMR (125 MHz, CDCl₃): 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; ¹⁹F NMR

NMR (471 MHz, CDCl₃): -58.13. HRMS (ESI): m/z [M+H]⁺ calcd for C₂₄H₂₁N₂O₁₀F₃: 555.12211, found: 555.12207.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-6-(methoxymethyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9k). Yield: 40%, yellow crystals, mp.: 108.5-110.1 °C. [α]_D²⁰ +106.8 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.27 (s, 1H, H₄), 6.50 (s, 1H, H₅), 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, CH₂MOM), 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}). ¹³C NMR (125 MHz, CDCl₃): 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 C₂₀H₂₄N₂O₉: 439.13472, found: 439.13485.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-6-(3-cyanopropyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9l). Yield: 65%, yellow crystals, mp.: 107.8 °C (decomposition). [α]_D²⁰ +105.6 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.21 (s, 1H, H₄), 6.42 (d, *J* = 3.5 Hz, 1H, H_{1'}), 6.32 (s, 1H, H₅), 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, CH₂Pr), 2.47 (d, *J* = 7.0 Hz, 2H, CH₂Pr), 2.16 (s, 3H, Me_{Ac}), 2.15 (s, 3H, Me_{Ac}), 2.12 (quintet, *J* = 7.0 Hz, 2H, CH₂Pr), 1.92 (s, 3H, Me_{Ac}). ¹³C NMR (125 MHz, CDCl₃): 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 C₂₁H₂₄N₃O₉: 462.15071, found: 462.15022.

3-(2,3,5-Tri-*O*-acetyl- β -D-arabinofuranosyl)-6-(cyclopropyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (9m). Yield: 31%, white crystals, mp.: 161.8-163.1 °C. [α]_D²⁰ +102.1 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CDCl₃): 8.07 (s, 1H, H₄), 6.43 (d, 1H, *J* = 3.5 Hz, H_{1'}), 6.15 (s, 1H, H₅), 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} + CH₂Pr), 1.08-0.97 (m, 4H, 2 x CH₂Pr). ¹³C NMR (125 MHz, CDCl₃): 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-arabinofuranosyl)-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); 1H 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-arabinofuranosyl)-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); 1H 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-arabinofuranosyl)-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); 1H 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});

¹³C NMR (125 MHz, CDCl₃): 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 C₂₆H₃₇N₂O₁₀: 537.24427, found: 537.24361.

General procedures for the synthesis of deprotected 6-aryl/6-heteroaryl 3H-pyrrolo[2,3-*d*]pyrimidin-2(7H)-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(3H)-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(3H)-one nucleosides **9** were dispersed in the 25% aqueous 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 additional 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-Arabinofuranosyl)-6-phenyl-3H-pyrrolo[2,3-*d*]pyrimidin-2(7H)-one (5a).

Yield: 90%, yellow crystals, m.p. 165.5- 167.0 °C. [α]_D²⁰ +77.8 (*c* 1.0, DMSO); ¹H NMR (500 MHz, CD₃OD): 8.62 (s, 1H, H₄), 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, H₅), 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'}). ¹³C NMR (125 MHz, CD₃OD): 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-Arabinofuranosyl)-6-(3-chlorophenyl)-3H-pyrrolo[2,3-*d*]pyrimidin-2(7H)-one (5b). Yield: 32%, yellow crystals, m.p. 212.0-213.3 °C. $[\alpha]_D^{20} +70.2$ (c 1.0, DMSO); 1H NMR (500 MHz, DMSO- d_6): 11.80 (s, 1H, NH), 8.46 (s, 1H, H₄), 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, H₅), 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-Arabinofuranosyl)-6-(4-chlorophenyl)-3H-pyrrolo[2,3-*d*]pyrimidin-2(7H)-one (5c). Yield: 32%, yellow crystals, m.p. 197.1-198.3 °C. $[\alpha]_D^{20} +67.4$ (c 1.0, DMSO); 1H NMR (500 MHz, DMSO- d_6): 11.80 (s, 1H, NH), 8.44 (s, 1H, H₄), 7.77-7.80 (m, 2H, H_{Ar}), 7.53-7.47 (m, 2H, H_{Ar}), 6.80 (s, 1H, H₅), 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-Arabinofuranosyl)-6-(3,4-dichlorophenyl)-3H-pyrrolo[2,3-*d*]pyrimidin-2(7H)-one (5d). Yield: 41%, yellow crystals, m.p. 226.5-227.4 °C. $[\alpha]_D^{20} +84.0$ (c 1.0, DMSO); 1H NMR (500 MHz, DMSO- d_6): 11.75 (wave, 1H, NH), 8.47 (s, 1H, H₄), 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, H₅), 6.23 (d, 1H, $J=3.5$ Hz, 1H, H_{1'}), 5.52 (wave, 2H, OH_{2'+OH3'}), 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-Arabinofuranosyl)-6-(2-pirydy)-3H-pyrrolo[2,3-d]pyrimidin-2(7H)-one

(5e). Yield: 46%, yellow solid, m.p. 188.5-189.1 °C. $[\alpha]_D^{20} +103.7$ (c 1.0, DMSO); 1H 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-Arabinofuranosyl)-6-(3-hydroksyphenyl)-3H-pyrrolo[2,3-d]pyrimidin-

2(7H)-one (5f). Yield: 74%, yellow solid, m.p. 231.5-232.4 °C. $[\alpha]_D^{20} +84.0$ (c 1.0, DMSO); 1H 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-Arabinofuranosyl)-6-(4-n-pentylphenyl)-3H-pyrrolo[2,3-d]pyrimidin-

2(7H)-one (5g). Yield: 55%, yellow solid, m.p. 124.3-125.9 °C. $[\alpha]_D^{20} +61.3$ (c 1.0, DMSO); 1H 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-Arabinofuranosyl)-6-(3-fluorophenyl)-3H-pyrrolo[2,3-*d*]pyrimidin-2(7H)-one (5h). Yield: 94%, yellow glass. $[\alpha]_{\text{D}}^{20} +84.3$ (*c* 1.0, DMSO); ^1H NMR (500 MHz, DMSO-*d*₆): 11.81 (s, 1H, NH), 8.48 (s, 1H, C₄), 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, H₅), 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 C₁₇H₁₇FN₃O₅: 326.11468, found: 326.11510.

3-(β -D-Arabinofuranosyl)-6-(3,5-difluorophenyl)-3H-pyrrolo[2,3-*d*]pyrimidin-2(7H)-one (5i). Yield: 60%, yellow crystals, decomposition at ~159.7 °C. $[\alpha]_{\text{D}}^{20} +77.7$ (*c* 1.0, DMSO); ^1H NMR (500 MHz, DMSO-*d*₆): 11.81 (s, 1H, NH), 8.50 (s, 1H, C₄), 7.58 (d, *J*=7.0 Hz, 2H, H_{Ar}), 7.21-7.15 (m, 1H, H_{Ar}), 6.98 (s, 1H, H₅), 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 C₁₇H₁₅F₂N₃O₅: 380.10525, found: 380.10551.

3-(β -D-Arabinofuranosyl)-6-(4-trifluoromethoxyphenyl)-3H-pyrrolo[2,3-*d*]pyrimidin-2(7H)-one (5j). Yield: 41%, yellow solid, m.p. 170.8-173.1 °C. $[\alpha]_{\text{D}}^{20} +72.0$ (*c* 1.0, DMSO); ^1H NMR (500 MHz, CD₃OD): 8.67 (s, 1H, H₄), 7.86-7.80 (m, 2H, H_{Ar}), 7.35 (d, *J*=8.0 Hz, 2H, H_{Ar}), 6.74 (s, 1H, H₅), 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, CD₃OD): 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 CF₃O group was not observed in carbon spectrum. ^{19}F NMR (471 MHz, CD₃OD): -59.45. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₈H₁₇F₃N₃O₆: 428.10640, found: 428.10636.

3-(β -D-Arabinofuranosyl)-6-methoxymethyl-3H-pyrrolo[2,3-d]pyrimidin-2(7H)-one (5k). Yield: 31%, white solid, m.p. 147.2 °C (decomposition). $[\alpha]_{\text{D}}^{20} +99.9$ (*c* 1.0, DMSO); ^1H 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 $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{18}\text{N}_3\text{O}_6$: 312.11901, found: 312.11905.

3-(β -D-Arabinofuranosyl)-6-(3-cyanopropyl)-3H-pyrrolo[2,3-d]pyrimidin-2(7H)-one (5l). Yield: 51%, yellow crystals, m.p. 124.8-126.9 °C. $[\alpha]_{\text{D}}^{20} +100.9$ (*c* 1.0, DMSO); ^1H 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 $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_5$: 312.11901, found: 312.11905.

3-(β -D-Arabinofuranosyl)-6-cyclopropyl-3H-pyrrolo[2,3-d]pyrimidin-2(7H)-one (5m). Yield: 46%, beige crystals, m.p. 148.8 °C (decomposition). $[\alpha]_{\text{D}}^{20} +100.5$ (*c* 1.0, DMSO); ^1H 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 $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{18}\text{N}_3\text{O}_5$: 308.12410, found: 308.12367.

3-(β -D-Arabinofuranosyl)-6-benzyl-3H-pyrrolo[2,3-d]pyrimidin-2(7H)-one (5n). Yield: 24%, yellow crystals, m.p. 180.8 °C (decomposition). $[\alpha]_{\text{D}}^{20} +78.9$ (*c* 1.0, DMSO); ^1H 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'}), 3.91-3.82 (m, 3H, H_{4'} + CH₂Bn), 3.65 (m, 2H, H_{5'}); ¹³C NMR (125 MHz, DMSO-d₆): 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 C₁₄H₁₈N₃O₅: 358.13975, found: 358.13915.

3-(β-D-Arabinofuranosyl)-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. [α]_D²⁰ +72.2 (*c* 1.0, DMSO); ¹H NMR (500 MHz, DMSO-d₆): 11.06 (s, 1H, NH), 8.22 (s, 1H, C₄), 6.20 (d, *J*=3.5 Hz, 1H, H_{1'}), 5.92 (s, 1H, H₅), 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, CH₂Oct), 1.65-1.55 (m, 2H, CH₂Oct), 1.34-1.18 (m, 10H, 5 x CH₂Oct), 0.86 (t, *J*=7.0 Hz, 3H, MeOct); ¹³C NMR (125 MHz, DMSO-d₆): 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 C₁₉H₂₉N₃O₅: 380.21800, found: 380.21783.

3-(β-D-Arabinofuranosyl)-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). [α]_D²⁰ +88.6 (*c* 1.0, DMSO); ¹H NMR (500 MHz, DMSO-d₆): 11.07 (s, 1H, NH), 8.22 (s, 1H, C₄), 6.20 (d, *J*=4.0 Hz, 1H, H_{1'}), 5.92 (s, 1H, H₅), 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, CH₂Non), 2.56-2.47 (m, 2H, CH₂Non), 1.66-1.55 (m, 2H, CH₂Non), 1.44-1.34 (m, 2H, CH₂Non), 1.33-1.18 (m, 10H, 5 x CH₂Non); ¹³C NMR (125 MHz, DMSO-d₆): 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 C₂₀H₃₁N₃O₆: 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), Wrocław, 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 % CO₂), 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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