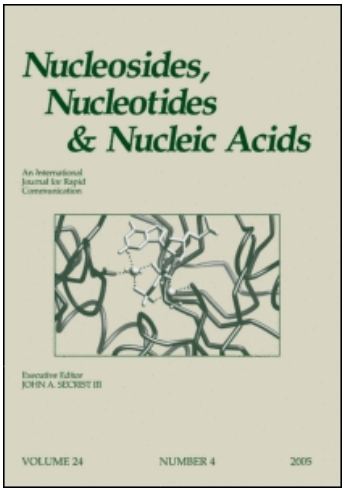

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Synthesis, Cytotoxic Effect and Antiviral Activity of 1-(β-D-Arabinofuranosyl)-5-Bromo-N⁴-Substitutedcytosine and 1-(β-D-Arabinofuranosyl)-5-Bromo-4-Methoxypyrimidin-2(1*h*)-One Derivatives

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SYNTHESIS, CYTOTOXIC EFFECT AND ANTIVIRAL ACTIVITY OF 1-(β-D-ARABINOFURANOSYL)-5-BROMO-N^4-SUBSTITUTED CYTOSINE AND 1-(β-D-ARABINOFURANOSYL)-5-BROMO-4-METHOXYPYRIMIDIN-2(1*H*)-ONE DERIVATIVES

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ABSTRACT: A convenient and mild synthesis of 5-bromo-N^4-substituted-1-(β-D-arabinofuranosyl)cytosine and 5-bromo-O^4-methyl-1-(β-D-arabinofuranosyl)pyrimidin-2(1*H*)-one derivatives by selective oxyfunctionalization of the corresponding 4-thionucleosides with 3,3-dimethyldioxirane is reported. The cytotoxicity and the antiviral activity against parainfluenza 1 (Sendai virus) of all new synthesized products are also reported.

Introduction

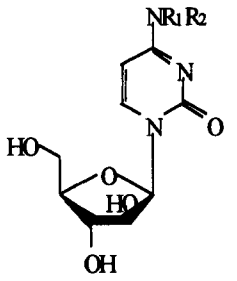
A number of purine and pyrimidine 1-(β-D-arabinofuranosyl)nucleoside derivatives in which the 2'-hydroxyl group has the opposite configuration to that of a ribonucleoside show important antiviral and antitumor properties.[1] Among them, 1-(β-D-arabinofuranosyl)cytosine (ara-C, cytarabine) is one of the most effective drugs for the treatment of acute myeloblastic leukemia.[2] For this reason, several ara-C analogues modified either on the sugar residue or on the cytosine ring have been synthesized to study their use in chemotherapy.[3] Two main approaches can be used for the modification of the heterocyclic moiety: in the first approach a suitable substituent can be introduced in the C-5 position;[4] in the second approach N-alkyl or N-aryl substituents can be introduced selectively on the C-4 position.

N^4-alkyl- and aryl-derivatives of ara-C have been previously prepared starting from suitable nitrogen nucleophiles and 1-(β-D-arabinofuranosyl)pyrimidines, characterized by the presence of a good leaving group at C-4 position. Vorbruggen's trimethylsilyl procedure, by which it should be possible to convert unprotected 1-(β-D-arabinofuranosyl)uracil (ara-U) directly to ara-C derivatives,[5] and multi-step procedure based on 4-chloro-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)pyrimidin-2(1*H*)-one and 4-alkylmercapto-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)pyrimidin-2(1*H*)one as reactive intermediates,[6,7] have all been used. However, the reaction conditions are rather drastic, an autoclave treatment is required with volatile nitrogen nucleophiles, and moisture-sensitive syrups which cannot crystallize are obtained in the case of 4-chloro reactive intermediates.

Moreover, Reese and his co-workers[8] have reported the use of 4-(1,2,4-triazol-1-yl)- and 4-(3-nitro-1,2,4-triazol-1-yl)-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl) pyrimidin-2(1H)-ones as crystalline valuable intermediates in the synthesis of derivatives of ara-C. This multi-step procedure generally requires mild experimental conditions, but in the case of aniline derivatives as nucleophiles the reaction proceeded much slower and was best carried out in boiling pyridine solution.

Recently, in the course of our studies on the organic chemistry of nucleic acids and their components, we have reported a general and convenient method for the synthesis of purine and pyrimidine nucleosides by oxidative functionalization of suitable thionucleosides with 3,3-dimethyldioxirane[9] (DMD).[10] This procedure, which works at room temperature under mild and strictly neutral conditions, allows the site-specific introduction of oxygen or nitrogen nucleophiles directly on the C-4 position of the pyrimidine ring, and on the C-6, and C-8 positions of the purine ring.[11] The versatility of the C-4 nucleophilic substitution in the oxidation of 4-thiopyrimidine nucleosides has been recently illustrated by the efficient synthesis of N^4-substituted-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)cytosine derivatives **1a-1d** also in the presence of highly hindered aromatic amines as like as 2,6-dimethylaniline (product **1d**).

In this paper we describe the efficient synthesis of 5-bromo-N^4-substituted-1-(β-D-arabinofuranosyl)cytosine and 5-bromo-O^4-methyl-1-(β-D-arabinofuranosyl)pyrimidin-2(1*H*)-one derivatives, in which both C-4 and C-5 positions of the cytosine ring have both been modified, by selective oxidation of the corresponding 4-thionucleosides with



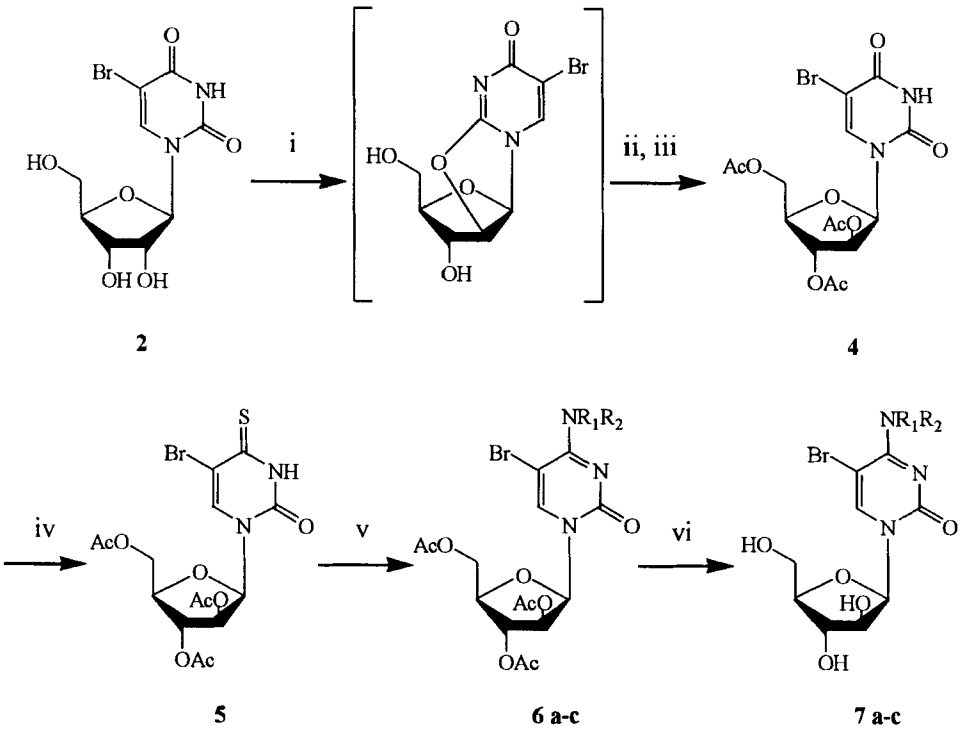
a : R_1=H, R_2=Me
b : R_1=H, R_2=Et
c : R_1=H, R_2=*p*-Me-Ph
d : R_1=H, R_2=*o*-di-Me-Ph

1 a-d

DMD. The cytotoxicity and the antiviral activity against parainfluenza 1 (Sendai virus) of all new synthesized products, and the precedently not described cytotoxicity and antiviral activity of compounds **1a-1d,** are also reported.

Results and Discussion

5-Bromo-N^4-substituted-1-(β-D-arabinofuranosyl)cytosines **7a-7c** were prepared starting from commercially available 5-bromouridine **2**. According to the procedure of Moffat and his co-workers[12] compound **2** was treated with diphenyl carbonate in the presence of sodium bicarbonate in hexamethylphosphoric triamide at 150°C to give 5-bromo-2,2'-anhydro-1-(β-D-arabinofuranosyl)uracil **3** in nearly quantitative yield. Compound **3** was not isolated but was hydrolyzed by heating with triethylamine at 70°C and then treated with an excess of acetic anhydride in pyridine to give 5-bromo-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)uracil **4** in 75% overall yield for the three steps (Scheme 1). According to the procedure reported by Fox and his co-workers[13], compound **4** was treated with an excess of P_2S_5 in dioxane at 120°C to give 5-bromo-4-thio-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)uracil **5** in 70% yield. Treatment of **5** with a freslhly prepared solution of DMD (0.9 M acetone solution, 1.5 equiv./mol.) in CH_2Cl_2 at 25°C in the presence of amines (ammonia, methylamine, pyrrolidine) as nucleophiles afforded 4-alkylamino-5-bromo-1-(β-D-2',3',5'-tri-O-acetylarabino-furanosyl)pyrimidin-2(1*H*)-one derivatives **6a-6c** in 73%, 71%, and 68% yield, respectively (Scheme 1). Deprotection of compounds **6a-c** with an excess of ammonia in methanol afforded 5 bromoarabinosylcytidine (5-Br-ara-C) **7a** and N^4-alkylamino-5-bromo-1-(β-D-arabinofuranosyl)cytosine **7b-7c** in 90%, 84%, and 89% yields, respectively (Scheme 1).

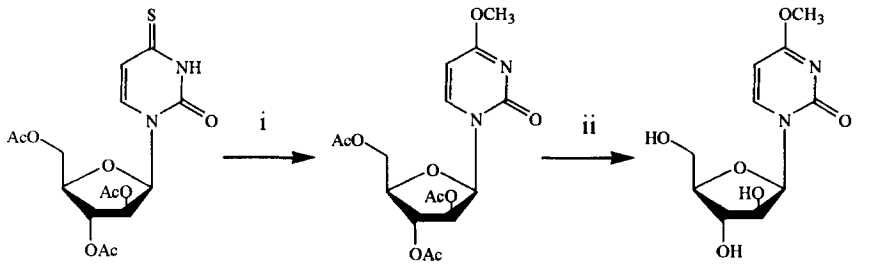


a : R_1=R_2=H. b : R_1=H, R_2=Me. c : R_1=R_2= -$CH_2(CH_2)CH_2$-.

Scheme 1: i. Diphenylcarbonate, hexamethylphosphorictriamide, $NaHCO_3$, reflux. ii. Triethylamine, 70°C. iii. Pyridine, acetic anhydride, 25°C. iv. P_2S_5, dioxane, reflux. v. Nitrogen nucleophile (ammonia, methylamine, pyrrolidine), DMD, CH_2Cl_2, 25°C. vi. NH_3/MeOH 2N, dioxane, 25°C.

The synthesis of 5-bromo-4-methoxy-1-(β-D-arabinofuranosyl)pyrimidin-2(1*H*)-one **9** as a representative model of 5-substituted-O^4-alkylpyrimidine nucleoside derivatives was of interest in light of the isolation from DNA of O^4-alkylated pyrimidine nucleosides (which were generally overlooked because the usual acid and alkaline conditions used to release the nucleosides from polynucleotides led to their dealkylation or depyrimidination[14]).

5-Bromo-4-methoxy-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)pyrimidin-2(1*H*)-one **8** was selectively obtained in 76% yield when compound **5** was allowed to react with DMD in the presence of a large excess of dry methanol (Scheme 2). Deprotection



5 8 9

Scheme 2: i.Dry MeOH, DMD, CH_2Cl_2, 25°C. ii. NH_3/MeOH 2N, dioxane, 25°C.

of **8** with an excess of ammonia in methanol afforded 5-bromo-O^4-methyl-1-(β-D-arabino furanosyl)pyrimidin-2(1*H*)-one **9** in 88% yield (Scheme 2).

The cytotoxic effects of deprotected arabino nucleosides **1a-d**, **7a-c**, and **9**, were evaluated using murine fibroblast cell line (3T3 cells), plasmocytoma murine cell line (NSO cells), and human lymphoblastoid cell line (Daudi cells). Results reported in Table 1 are referred to human lymphocytes and are representative of all others cell lines studied. Data have been obtained both by analysis of tritiated thymidine incorporation and by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test to analyze both proliferation and DNA synthesis in cell cultured in the presence of different compounds. All products show potent cytotoxic effects. In particular, compounds **1a-1d** show an IC50 value< 0.5 μg ml^{-1}, and compounds **9** and **7b** show an IC50< 10 μg/ml (1.6 μg ml^{-1} and 7.8 μg ml^{-1}, respectively). Compounds **7a** and **7c** are relatively less cytotoxic with an IC50<40 μg ml^{-1} (33 μg $ml^{-1}$and 31 μg ml^{-1}, respectively) (Table 1).

Compounds **1a-1d**, **7a-7c**, and **9**, have been assayed for antiviral activity on parainfluenza 1 (Sendai) virus, an enveloped virus with nonsegmented negative-strand RNA genome employed as in *vivo* experimental model of parainfluenza virus infection, or for in *vitro* studies of new antiviral agents. Virus replication was evaluated, according to the procedure previously reported,[15] by measuring the haemagglutinating units in the supernatant of the infected Madin Darby canine kidney cells (MDCK cells). None of the products, with the exception of **1b**, was found to be effective in inhibiting

TABLE 1: Cytotoxicity of compounds **1a-d**, **7a-c**, and **9** (against human lymphocytes).

Conc. μg/ml	**1a**	**1b**	**1c**	**1d**	**7a**	**7b**	**7c**	**9**
1000	230±1	293±10	339±47	308±24	239±28	335±41	254±29	409±45
500	109±8	326±17	394±48	273±11	503±77	390±47	582±43	407±23
250	294±29	315±25	364±70	297±25	1083±114	810±81	1167±94	412±51
125	323±40	383±48	400±26	491±80	6371±517	2503±119	5173±438	349±87
62	399±48	462±53	454±52	427±51	8824±763	3989±513	7425±648	637±52
30	409±20	899±108	593±47	731±49	45193±999	11419±991	41568±998	894±438
15	793±52	1117±93	1218±147	1348±999	72283±987	25834±987	71180±999	2308±210
7.5	1207±114	3363±240	3270±150	3381±209	118037±98	49690±999	109323±99	6368±579
3.7	1967±206	4093±442	4146±831	3996±428	123408±87	84580±890	121084±89	21806±99
1.7	2037±203	5956±634	5007±437	5394±607	119203±82	119350±99	124293±99	45190±98
IC50	<0.5 μg/ml	<0.5 μg/ml	<0.5 μg/ml	<0.5 μg/ml	33 μg/ml	7.8 μg/ml	31 μg/ml	1.6 μg/ml

Human normal lymphocytes 200000 cells/well stimulated with PHA 5 μg/ml. Tritiated thymidine (1 μCi/well) was added and cells were harvested 24 hrs later. Values are reported as counts/min. (CPM); blank is 419±86 CPM; cells growing without any compound gave 112099±13920 CPM.

virus replication at all the doses used in the experiments. Compound **1b**, at the dose of 50 μg ml^{-1}, was found to produce a 50% decrease in viral titre, but a high toxic effect on uninfected cells has been found at the dose of 100 μg ml^{-1}. Thus, the therapeutic index is too low for an eventual clinical use. The latter data are in accord with the consideration that, although ara-C has antiviral activity against a wide variety of viruses, its cytotoxic activity prevents its use as an antiviral agent.[16]

Experimental

NMR spectra were recorded on a Bruker (200 MHz) spectrometer and are reported in δ values. Microanalyses were performed on a C. Erba 1106 analyzer. Mass spectra were recorded on a VG 70/250S spectrometer with an electron beam of 70 eV (for compounds containing bromine the average of the two isotopic M^+ peaks is given). All solvents were reagent grade and were redistilled and dried according to standard procedures. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was

purchased from Sigma. It was dissolved at a concentration of 5 mg/ml in sterile PBS at room temperature and the solution was further sterilised by filtration and stored at 4°C in a dark bottle. SDS was obtained from Sigma and N,N-dimethylformamide (DMF) was purchased from Fluka. Lysis buffer was prepared as follows: 20% w/v of SDS was dissolved at 37°C in a solution of 50% of DMF and demineralised water; pH was adjusted to 4.7 by adding 2.5% of an 80% acetic acid and 2.5% 1 N HCl. Chromatographic purifications were performed on colums packed with Merck silica gel 60, 230-400 mesh for flash technique.

Starting materials: commercially available 5-bromouridine **2** (Aldrich, Co.) was used without further purification. N^4-substituted-1-(β-D-2',3',5'-tri-O-acetylarabino-furanosyl)cytosine derivatives **1a-d** were prepared as reported in ref. 11.

5-Bromo-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)uracil (4). A solution of 5-bromouridine (**2**) (5.2 g, 16 mmol), diphenyl carbonate (0.22 mmol) and sodium bicarbonate (1.3 mmol) in hexamethylphosphoramide (15 ml) was refluxed for 0.5 h to obtain 5-bromo-2,2'-anhydro-1-(β-D-arabinofuranosyl)uracil **3** in nearly quantitative yield. After cooling, the reaction mixture dissolved in water (150 ml) was washed with $CHCl_3$ (90 ml). The organic phase was dried (Na_2SO_4), evaporated and heated in triethylamine (8 ml) at 70 °C for 5 h. The crude reaction mixture was treated with an excess of acetic anhydride in pyridine solution at 25 °C for 24 h. The residue was evaporated and purified by flash-chromatography using CH_2Cl_2/CH_3OH to give **4** (5.39 g, 75%), oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): 2.04 (s, 6H); 2.15 (s, 3H); 4.31 (m, 3H); 5.28 (m, 2H); 6.05 (d, 1H); 7.78 (s, 1H). ^{13}C-NMR ($CDCl_3$, 200 MHz): 20.27 (CH_3); 20.90 (CH_3); 62.83 (CH_2); 69.94 (CH); 72.99 (CH); 80.08 (CH); 87.21 (CH); 97.85 (C); 138.48 (CH); 149.66 (C); 158.70 (C); 169.51 (C); 169.55 (C); 170.03 (C). Anal. Calcd for $C_{15}H_{17}BrN_2O_9$: C, 40.10%; H, 3.81%; N, 6.23%. Found: C, 40.15%; H, 3.78%; N, 6.16%. MS m/z (21%) 449 [M^+].

5-Bromo-4-thio-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)uracil (5): A solution of 5-bromo-1-(β-D-2',3',5'-tri-O-acetyl-arabinofuranosyl)uracil (2.45 g, 5.4 mmol) and P_2S_5 (1.3 g, 2.85 mmol) in dioxane (15 ml) was refluxed for 2 h. After cooling, the reaction mixture dissolved in $CHCl_3$ (70 ml) was washed with water (40 ml). The separated organic phase was dried (Na_2SO_4) and evaporated. The residue was

purified by flash-chromatography using CH_2Cl_2/CH_3OH to give **5** (1.76 g, 70%), oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): 1.49 (s, 6H); 1.57 (s, 3H); 4.68 (m, 3H); 5.33 (m, 1H); 5.80 (s, 1H); 6.61 (s, 1H); 9.50 (br s, 1H). ^{13}C-NMR ($CDCl_3$, 200 MHz): 20.41 (CH_3); 20.87 (CH_3); 62.91 (CH_2); 69.87 (CH); 72.84 (CH); 80.02 (CH); 87.76 (CH); 108.44 (C); 134.30 (CH); 147.43 (C); 169.56 (C); 170.09 (C); 185.59 (C). Anal. Calcd for $C_{15}H_{17}BrN_2O_8S$: C, 38.70%; H, 3.65%; N, 6.02%. Found: C, 38.72%; H, 3.68%; N, 6.09%. MS m/z (19%) 465 [M^+].

Synthesis of N^4-alkyl-5-bromo-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)-cytosine derivatives (6a-6c) and 5-bromo-4-methoxy-1-(β-D-2',3',5'-tri-O-acetyl-arabinofuranosyl)pyrimidin-2(1*H*)-one (8). General procedure: The reactions were carried out by adding freshly prepared solution of DMD (0.09 M acetone solution, 1.5 equiv./mol.) to solutions of **5** (1 mmol) in CH_2Cl_2 in the presence of amines (ammonia, methylamine, and pyrrolidine; 1.5 mmol) and dry methanol (1.5 mmol) at 25 °C. The reaction mixture was evaporated and the residue was purified by flash-chromatography using a CH_2Cl_2/CH_3OH to give the title compounds **6a-c** and **8** in good yields.

5-Bromo-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)cytosine (6a). (327 mg, 73%), oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): 2.05 (s, 6H); 2.08 (s, 3H); 4.30 (m, 3H); 5.39 (m, 2H); 5.88 (d, 1H); 7.31 (s, 1H). ^{13}C-NMR ($CDCl_3$, 200 MHz): 20.46 (CH_3); 20.72 (CH_3); 63.01 (CH_2); 69.94 (CH); 73.43 (CH); 79.11 (CH); 89.86 (CH); 95.98 (C); 140.98 (CH); 155.61 (C); 166.07 (C); 169.58 (C); 169.67 (C); 170.39 (C). Anal. Calcd for $C_{15}H_{18}BrN_3O_8$: C, 40.18%; H, 4.01%; N, 9.33%. Found: C, 40.29%; H, 4.01%; N, 9.37%. MS m/z (21%) 448 [M^+].

5-Bromo-N^4-Methyl-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)cytosine (6b). (328 mg, 71%), oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): 2.03 (s, 6H); 2.05 (s, 3H); 2.93 (s, 3H); 4.29 (m, 3H); 5.33 (m, 2H); 5.77 (d, 1H); 7.28 (s, 1H). ^{13}C-NMR ($CDCl_3$, 200 MHz): 20.44 (CH_3); 20.71 (CH_3); 63.27 (CH_2); 70.21 (CH); 73.19 (CH); 79.30 (CH); 88.01 (CH); 96.35 (C); 138.69 (CH); 155.32 (C); 164.16 (C); 169.62 (C); 170.22 (C). Anal. Calcd for $C_{16}H_{18}BrN_3O_8$: C, 41.75%; H, 3.94%; N, 9.13%. Found: C, 41.62%; H, 4.30%; N, 9.17%. MS m/z (22%) 460 [M^+].

5-Bromo-4-(pyrrolidin-1-yl)-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)-pyrimidin-2(1*H*)-one (6c). (341 mg, 68%), oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): 2.04 (s,

6H); 2.08 (s, 3H); 2.14 (m, 4H); 3.46 (m, 4H); 4.28 (m, 3H); 5.26 (m, 2H); 5.71 (d, 1H); 7.37 (s, 1H). ^{13}C-NMR ($CDCl_3$, 200 MHz): 20.45 (CH_3); 20.71 (CH_3); 24.48 (CH_2); 24.57 (CH_2); 46.86 (CH_2); 47.04 (CH_2); 63.29 (CH_2); 70.25 (CH); 73.14 (CH); 79.31 (CH); 87.59 (CH); 93.80 (C); 139.40 (CH); 155.17 (C); 161.02 (C); 169.60 (C); 170.11 (C). Anal. Calcd for $C_{19}H_{24}BrN_3O_8$: C, 45.43%; H, 4.81%; N, 8.36%. Found: C, 45.53%; H, 4.73%; N, 8.36%. MS m/z (20%) 502 [M^+].

4-Methoxy-1-(β-D-2',3',5'-tri-O-acetylarabinofuranosyl)pyrimidin-2(1H)-one (8). (292 mg, 76%), oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): 2.11 (s, 6H);.2.28 (s, 3H); 4.11 (s, 3H); 4.40 (m, 1H); 4.55 (m, 2H); 5.20 (m, 1H); 5.68 (q, 1H); 6.08 (d, 1H); 6.52 (d, 1H); 7.88 (H, d, 1H). ^{13}C-NMR ($CDCl_3$, 200 MHz): 20.23 (CH_3); 20.42 (CH_3); 20.50 (CH_3); 54.37 (CH_3); 62.74 (CH_2); 74.05 (CH); 76.44 (CH); 80.93 (CH); 85.66 (CH); 94.10 (CH); 143.23 (CH); 155.09 (C); 168.31 (C); 169.82 (C); 170.74 (C); 172.12 (C). Anal. Calcd for $C_{16}H_{20}N_2O_9$: C, 50.00%; H, 5.24%; N, 7.28%. Found: C, 50.12%; H, 4.78%; N, 8.21%. MS m/z (18%)384,[M^+].

Deacetylation of arabino nucleoside derivatives 6a-6c, and 8. General procedure: The reactions were carried out by adding an excess of ammonia (5.0 equiv./mol., 2 N methanol solution) to solutions of the required substrate (1mmol) in 5 ml of CH_2Cl_2 at 25°C. The rection mixture was evaporated and the residue was purified by flash-chromatography using CH_2Cl_2/CH_3OH to give the deprotected derivatives **7a-7c** and **9** in good yields.

1-(β-D-Arabinofuranosyl)-5-bromocytosine (7a). (290 mg, 90%), oil; ^{1}H-NMR (CD_3OD, 200 MHz): 3.92 (m, 2H); 4.08 (m, 1H); 4.22 (m, 2H); 5.95 (d, 1H); 8.10 (s, 1H). ^{13}C-NMR (CD_3OD, 200 MHz): 61.95 (CH_2); 70.72 (CH); 76.18 (CH); 85.81 (CH); 92.39 (CH); 95.86 (C); 143.11 (CH); 158.51 (C); 167.70 (C). Anal. Calcd for $C_9H_{12}BrN_3O_5$: C, 33.55%; H, 3.75%; N, 13.04%. Found: C, 33.77%; H, 3.72%; N, 13.16%.MS m/z (21%) 322, [M^+].

1-(β-D-Arabinofuranosyl)-5-bromo-N^4-methylcytosine (7b). (282 mg, 84%), oil; ^{1}H-NMR (CD_3OD, 200 MHz): 2.97 (s, 3H); 3.88 (m, 2H); 4.11 (m, 1H); 4.25 (m, 2H); 5.98 (d, 1H); 8.05 (s, 1H). ^{13}C-NMR (CD_3OD, 200 MHz): 27.76 (CH_3); 62.06 (CH_2); 70.83 (CH); 76.09 (CH); 85.80 (CH); 92.29 (CH); 96.83 (C); 141.32 (CH);

158.65 (C); 165.46 (C). Anal. Calcd for $C_{10}H_{14}BrN_3O_5$: C, 35.73%; H, 4.19%; N, 12.50%. Found: C, C, 35.84%; H, 4.16%; N, 12.56%.MS m/z (20%) 336, [M^+].

1-(β-D-Arabinofuranosyl)-5-bromo-4-(pyrrolidin-1-yl)pyrimidin-2(1H)-one (7c). (335 mg, 89%), oil; ^{1}H-NMR (CD_3OD, 200 MHz): 2.07 (m, 4H);.3.54 (m, 4H); 3.93 (m, 2H); 4.03 (m, 1H); 4.20 (m, 2H); 6.03 (d, 1H); 8.12 (s, 1H). ^{13}C-NMR (CD_3OD, 200 MHz): 25.66 (CH_2); 26.45 (CH_2); 47.71 (CH_2); 61.99 (CH_2); 70.75 (CH); 76.21 (CH); 85.82 (CH); 87.59 (CH); 92.44 (C); 142.42 (CH); 158.21 (C); 162.082 (C). Anal. Calcd for $C_{13}H_{18}BrN_3O_5$: C, 41.50%; H, 4.82%; N, 11.17%. Found: C, 41.58%; H, 4.78%; N, 11.24%. MS m/z (22%) 376 [M^+].

1-(β-D-Arabinofuranosyl)-4-methoxypyrimidin-2(1H)-one (9). (213 mg, 88%), oil; ^{1}H-NMR (CD_3OD, 200 MHz): 3.99 (m, 2H); 4.16 (s, 3H); 4.21 (m, 2H); 4.52 (m, 1H); 5.20 (m, 1H); 6.33 (d, 1H); 6.42 (d, 1H).^{13}C-NMR (CD_3OD, 200 MHz): 54.80 (CH_3); 62.74 (CH_2); 76.55 (CH); 78.09 (CH); 87.10 (CH); 89.14 (CH); 95.82 (CH); 146.53 (CH); 158.27 (C); 173.72 (C). Anal. Calcd for $C_{10}H_{14}N_2O_6$: C, 46.51%; H, 5.46%; N, 10.84%. Found: C, 46.12%; H, 5.50%; N, 11.0%. MS m/z (21%) 258, [M^+].

Cell lines: All cell lines were obtained from ATCC. The cells were cultured in RPMI 1640 supplemented with 5% FCS, 0.1 mM glutamine, 1% penicillin and streptomycin. Cells were grown in Nunc clone plastic bottles (TedNunc, Roskilde, Denmark) and split twice weekly at different cell densities according to standard procedures. 3T3 cells were grown as monolayer and were split by using trypsin. Perypheral blood mononuclear cells (MNC) were separated from heparinized whole blood obtained from healthy donor on a Ficoll-Hypaque gradient as previously described.[17] MNC thus obtained were washed twice with RPMI 1640 supplemented with 10% FCS, glutamine and antibiotics, suspended at 200000 viable cells/ml in medium containing, as mitogen, 5 μg/ml PHA (Sigma) and used in toxicity tests.

Toxicity tests: Cell were plated at different concentrations on flat botton 96 well microplates (0.1 ml/well). Lymphocytes were plated out at 20000 cells/well; 3T3 cells (murine fibroblast line) were plated at 10000 cells/well; NSO cells (plasmocytoma murine cell line) were plated out at 3000 cells/well; Daudi cells (human lymphoblastoid cell line) were plated at 3000 cells/well. 12 hours after plating, different concentrations of each compound were added to each well. After 48 hours, MTT assay was performed

to analyze cytotoxicity of the different compounds. Some experiments were performed by using confluent cells: compounds were added on 3T3 monolayer 3 days after plating. Tests were then run as described above.

Proliferation assay: Same cells (as above) have been used in thyimidine incorporation assay as previously described.[18] In brief, cell lines in exponential phase were washed twice in RPMI 1640 with 50 units/ml penicillin, 50 micrograms/ml streptomycin and 2 mM L-glutamine (all reagents from Flow Laboratories, Milan, Italy), supplemented with 10% endotoxin free foetal calf serum (Hyclone Laboratories, Logan, UT) and suspended at a concentration of 200,000 cell/ml; cells were then plated out in 96/well microtiter plates at a final concentration of 20,000 cells/well in presence of serial dilution of different compounds in DMSO. Control samples with DMSO alone were also performed. Each point was performed in quadruplicate. One or two days later, 1 microCi/well tritiated thymidine (Amersham International, plc, Amersham, U.K.) in 20 microliters culture medium was added to each well. Eight hours later, cells were harvested by a semiautomated cell harvester. Radioactivity was measured as counts per minute (CPM) in a liquid scintillator counter (LKB Wallac, Turku, Finland). Each experiment was performed not less than three times with consistent results.

MTT/formazan extraction procedure: 20 µl of the 5 mg/ml stock solution of MTT were added to each well; after 2 hours of incubation at 37°C, 100 µl of the extraction buffer were added. After an overnight incubation at 37°C, the optical densitied at 570 nm were measured using a Titer-Tech 96-well multiscanner, employing the extraction buffer as the blank.

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