Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 3065–3081
Bioorganic & Medicinal Chemistry

Novel selective human mitochondrial kinase inhibitors: Design, synthesis and enzymatic activity

Nunzia Ciliberti,[a] Stefano Manfredini,[a,*] Angela Angusti,[g] Elisa Durini,[a] Nicola Solaroli,[a,b] Silvia Vertuani,[a] Lisa Buzzoni,[a] Maria Cruz Bonache,[a] Efrat Ben-Shalom,[c] Anna Karlsson,[b] Ann Saada[d,e] and Jan Balzarini[f]

[a]*Department of Pharmaceutical Sciences, University of Ferrara, Italy*
[b]*Division of Metabolic Diseases, Department of Laboratory Medicine Karolinska Institute, Novum, S-141 86 Stockholm, Sweden*
[c]*Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel*
[d]*Metabolic Disease Unit, Hadassah Medical Center, Jerusalem, Israel*
[e]*Ben Gurion University of the Negev, Jerusalem, Israel*
[f]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*
[g]*Biotechnology Research Institute (BRI) NRC-CNRC 6100 Royalmount Avenue, Montreal, Que., Canada H4P 2R2*

Received 14 June 2006; accepted 31 January 2007
Available online 2 February 2007

Abstract—Selective and effective TK2 inhibitors can be obtained by introduction of bulky lipophilic chains (acyl or alkyl entities) at the 2′ position of araT and BVaraU, nucleoside analogues naturally endowed with a low TK2 affinity. These derivatives showed a competitive inhibitory activity against TK2 in micromolar range. BVaraU nucleoside analogues, modified on the 2′-*O*-acyl chain with a terminal *N*-Boc amino-group, conserved or increased the inhibitory activity against TK2 (**7l** and **7m** IC_{50}: 6.4 and 3.8 μM, respectively). The substitution of an ester for a carboxamide moiety at the 2′ position of araT afforded a consistent reduction of the inhibitory activity (**25**, IC_{50}: 480 μM). On the contrary, modifications at 2′-OH position of araC and araG, have provided inactive derivatives against TK2 and dGK, respectively. The biological activity of a representative compound, 2′-*O*-decanoyl-BVaraU, was also investigated in normal human fibroblasts and was found to impair mitochondrial function due to TK2 inhibition.

1. Introduction

Deoxyribonucleoside kinases (dNKs) are key enzymes in the salvage pathway of mammalian cells.[1] dNKs catalyze the conversion of the nucleosides derived from DNA degradation, or from the extra-cellular environment, into the corresponding deoxyribonucleoside monophosphates. Human dNKs, thymidine kinase 1 (TK1), thymidine kinase 2 (TK2), deoxycytidine kinase (dCK), and deoxyguanosine kinase (dGK), have different sub-cellular localization and variable substrate specificities.[2–4] TK2 and dGK are key enzymes in the mitochondrial nucleoside salvage pathway and are crucial in the maintenance of balanced mitochondrial deoxyribonucleotide pool especially in non-replicating tissues.[5] Mutated TK2 and dGK are associated with decreased copy number of mitochondrial DNA (mtDNA) and mitochondrial respiratory chain (MRC) dysfunction.[6,7]

TK2 is homologous with dCK, dGK, the recently discovered multisubstrate deoxyribonucleoside kinase from *Drosophila melanogaster* (*Dm*-dNK) and herpes simplex virus type 1 thymidine kinase (HSV-1 TK).[8] TK2 is able to activate several antiviral nucleoside analogues such as (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), 1-β-D-arabinofuranosyl-5-(2-bromovinyl)uracil (BVaraU), 3′-azido-2′-,3′-dideoxythymidine (AZT), and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU).[9–13] Thus, treatment with these analogues may be accompanied with mitochondrial toxicity of terminally differentiated cells (AZT and FIAU).[14,15] Because of this it has been suggested to exploit TK2 in gene therapy against malignancies.[16,17]

Keywords: Design; Synthesis; Nucleoside analogs; Human TK2 inhibitors.
* Corresponding author. Tel.: +39 0532 291292; fax: +39 0532 291296; e-mail: s.manfredini@unife.it

0968-0896/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2007.01.049

The development of potent and selective inhibitors of TK2 may be helpful to unravel a variety of metabolic processes, including the role of TK2 in the metabolic activation of some antiviral and anticancer nucleoside analogues, the role of TK2 in the maintenance of the mitochondrial dNTP pool, mtDNA synthesis and repair, and in the homeostasis of mitochondria. Also, specific TK2 inhibitors may elucidate the differential activities of cytosolic versus mitochondrial thymidine kinase in different cell lines or in the different phases of the cellular replication cycle.

So far only few literature reports have described selective and effective TK2 inhibitors. Among these, the 5′-*O*-substituted derivatives of thymidine, BVDU, and their acyclic analogues are able to selectively inhibit the mitochondrial enzyme, therefore their structural features can contribute to desiging new TK2 inhibitors.[18–20] Also some ribofuranosylnucleoside analogues highly functionalized at the 3′-position are unexpectedly able to inhibit the mitochondrial enzyme. This is in complete disagreement with the available data that the enzyme recognize, as an efficient substrate, only natural deoxyribonucleosides and unnatural deoxyribonucleoside analogues.[21]

Recently, we have described the inhibitory activity against TK2 of a series of 2′-*O*-alkylester and ether derivatives of arabinofuranosyl nucleosides. The bulky lipophylic entities introduced at the 2′-OH of 1-β-D-arabinofuranosylthymine (araT) and BVaraU, both not preferential substrates for the TK2 enzyme, converted the araT and BVaraU nucleoside analogues to selective and purely competitive TK2 inhibitors in the micromolar concentration range.[22,23]

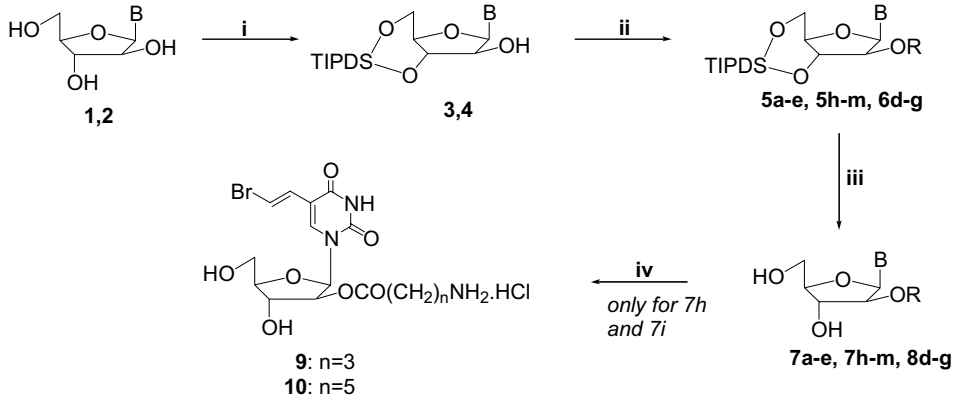
Intrigued by these interesting preliminary results, further investigations were performed to complete the series of 2′-*O*-acyl/alkyl substituted arabinofuranosyl nucleosides and to synthesize a new class of compounds. In this study we report in detail: (a) the synthetic approaches applied to achieve the desired chemical structures; (b) the inhibitory effect of synthesized derivatives on phosphorylation by deoxyribonucleoside kinases; (c) the biological effect of one of the most effective TK2 inhibitors (**7e**) in tissue culture.

Preliminary data aimed to identify potential dGK inhibitors, able to provide information about substrate-specificity and the physiological role of the mitochondrial enzyme will be also reported.

2. Chemistry

2′-*O*-Acyl derivatives of BVaraU (compounds **5a**, **b**, **d**, and **e**) and araT (compounds **6d–g**) were prepared introducing the suitable acyl chain, in refluxing pyridine on the 3′,5′-*O*-TIPDS protected arabinonucleosides **3**[24] and **4**, as previously reported by us (compound **5c**.[24]) Compound **4** was synthesized as described for the derivative **3**.

2′-*O*-(*N*-Boc-aminoalkanoyl) derivatives of BVaraU (compounds **5h–m**) were obtained in quantitative yield by reaction of the *N*-Boc amino acid derivatives with 3′,5′-*O*-TIPDS protected BVaraU (**3**), in presence of DMAP in CH_2Cl_2 at room temperature. The TIPSD cleavage, was performed, following the procedure described by us for compounds **7c**, **e**,[24] by treatment with NH_4F and Dowex® H^+ form in CH_3OH, to give the final products **7a**, **b**, **d**, **h–m**, and **8d–g** in 30–90% yield (Scheme 1).



TIPDS: tetraisopropyldisiloxane-1,3-diyl.

a:R= -COBn; **b**:R= -CO(p-OCH_3-Bn); **c**:R= -$COCH_2OCH_3$; **d**:R= -$CO(CH_2)_6CH_3$; **e**:R= -$CO(CH_2)_8CH_3$; **f**:R= -$CO(CH_2)_3CH_3$; **g**:R= -$CO(CH_2)_{10}CH_3$; **h**:R= -$CO(CH_2)_2CH_2$NH-Boc; **i**:R= -$CO(CH_2)_4CH_2$NH-Boc; **l**:R= -$CO(CH_2)_6CH_2$NH-Boc; **m**:R= -$CO(CH_2)_{10}CH_2$NH-Boc;

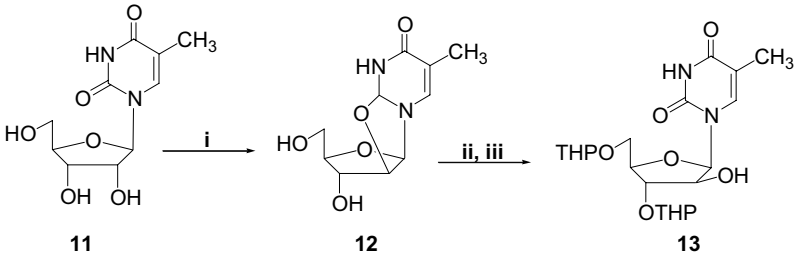
Scheme 1. Reagents and conditions: (i) $TIPDSCl_2$, pyridine, room temperature; (ii) RCOCl, pyridine, reflux or *N*-Boc-aa, DMAP, DCC, CH_2Cl_2, 0 °C to room temperature; (iii) NH_4F, CH_3OH, Dowex® H^+ form 50W × 2 50–100 mesh, room temperature; (iv) HCl (g) Et_2O, 0 °C.

The Boc protecting group was removed from compounds **7h** and **7i** by bubbling HCl in diethyl ether at 0 °C: after the crystallization step, the hydrochloride salts **9** and **10** were obtained in yield greater than 90% (Scheme 1).

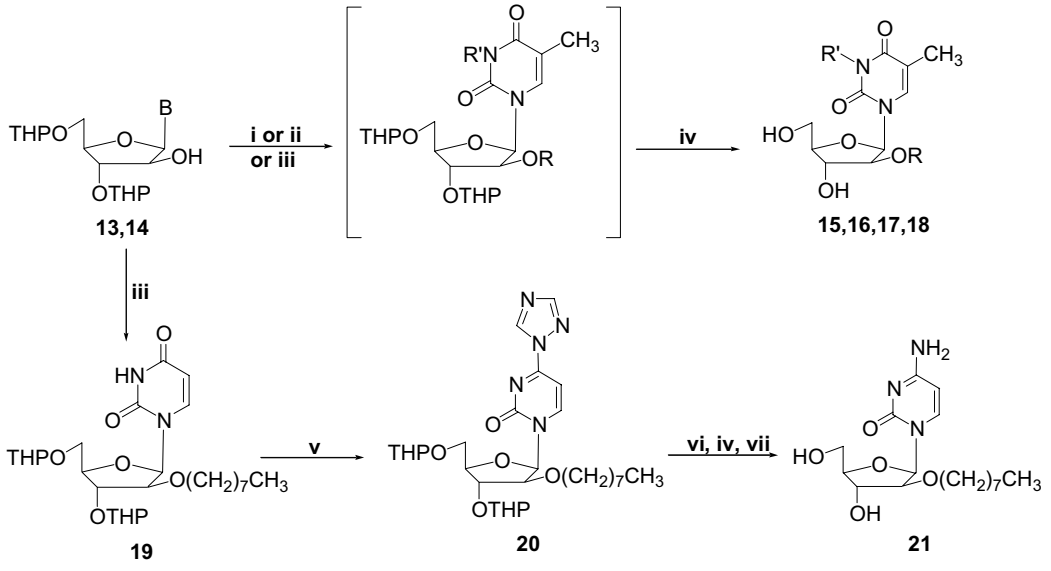
Due to the low reactivity of the alkylating reagents, the 2′-*O*-alkyl ethers of araT (**15-18**) were obtained in applying different synthetic conditions that required the synthesis of the unnatural nucleoside 1-β-D-ribofuranosylthymine (**11**).[25,26] To this end, the protection of 3′ and 5′ hydroxyl groups was carried out using 1,2-dihydropyrane (DHP) on the 2,2′-anhydro-1-(β-D-arabinofuranosyl)thymine intermediate (**12**).[27] The next treatment with 0.1 M KOH in EtOH (Scheme 2)[28] allowed to obtain the starting compound **13** for the alkylation reactions in good overall yield (75%).

The compound **15** was obtained by reaction of compound **13** with benzyl bromide and NaH in THF followed by the treatment of the crude mixture with *p*-TsOH monohydrate in methanol at room temperature (overall yield 52%) (Scheme 3). In this case, alkylation at N-3 was not observed (see below) in the above described reaction conditions.

No product formation was observed, even under refluxing conditions, when the less reactive octyl bromide was used as alkylating agent. In attempt to obtain the expected product we modified the reaction conditions using alkyl bromide in a 1:1 mixture of THF–DMSO to obtain, as the solely product, the 3′,5′ THP–protected N^3-alkylated derivative. Crude mixture of this latter compound was next deprotected, as show in Scheme 3, providing the final compound **16** in 58% overall yield.



Scheme 2. Reagents and conditions: (i) $(PhO)_2CO$, $NaHCO_3$, DMF, 150 °C; (ii) DHP, *p*-TsOH·H_2O, CH_3CN, room temperature; (iii) 0.1 M KOH, EtOH 95%, room temperature.



Scheme 3. Reagents and conditions: (i) NaH, alkyl bromide, THF, room temperature or reflux; (ii) K_2CO_3, octyl bromide, THF–DMSO, room temperature; (iii) NaH, $I(CH_2)_7CH_3$, THF reflux; (iv) CH_3OH, *p*-TsOH·H_2O, room temperature; (v) $POCl_3$, 1,2,4-triazole, TEA, 0 °C to room temperature; (vi) dioxane and 30% NH_4OH (1:1 mixture) room temperature; (vii) Dowex® OH^- form 1 × 2 200, H_2O.

Aiming to obtain a more reactive alkylating agent, the octyl bromide was next converted in the corresponding iodide derivative under standard conditions (NaI, CH_3CN) and then used in high excess with NaH in refluxing THF. The crude mixture obtained, subjected to the THP deprotection step, gave the expected 2′-*O*-alkylated compound **17** and the N^3- and 2′-*O*-bis-alkylated **18** in 55% and 19% overall yield the respectively (molar ratio **17/18** = 3:1). The 2′-*O*-octyl derivative of 1-β-D-arabinofuranosylcytosine (AraC) (**21**) was prepared from the 3′,5′-*O*-tetrahydropyranyl-araU (**14**)[28] following and adapting the procedure described in the literature[29] (Scheme 3).

The synthesis of the 2′-carbamoyl derivative of araT (**25**) was performed starting from compound **22** obtained as reported.[30] The next reduction, carried out in standard conditions (Pd/C under H_2 atmosphere), gave the formation of the amino group on the sugar ring and, completely unexpected, concomitant cleavage of the benzoyl group at N-3. The derivative **23** thus obtained was next reacted with activated decanoic acid that was obtained using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBT). The resulting compound **24** was then deprotected to give the expected **25** (Scheme 4).

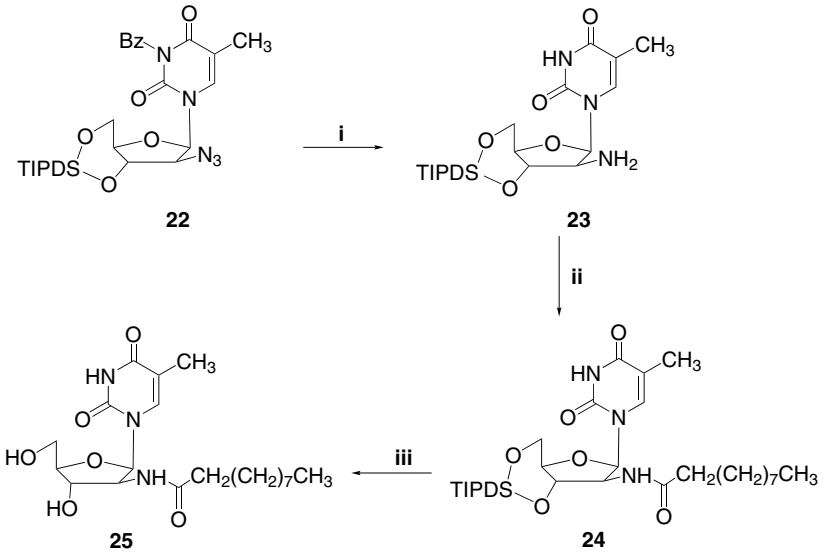
The preparation of 2′-*O*-acyl derivatives of 9-β-D-arabinofuranosyl guanosine (araG) was carried out starting from the 3′,5′-*O*-TIPDS derivative **26** in turn obtained modifying a previously reported synthesis.[31] The next protection of the 2-amino group (**27**), performed applying the methodology reported for guanosine derivatives,[32] allowed the selective introduction of the acyl chain on the 2′ hydroxyl group by using octanoyl chloride in refluxing pyridine, or valeric anhydride in DMF. In satisfactory yield (61–77%) were obtained the 2′-*O*-octanoyl and 2′-*O*-pentanoyl araG derivatives (compounds **28a** and **28b**). The bis-acylated derivative of araG (**31**) was obtained from the intermediate **26**, under the same reaction conditions used for compounds **5** and **6**, avoiding protection of $-NH_2$ at the purinic base.

Compounds **28a**, **28b**, and **31** were next deprotected at the sugar ring as previously described, obtaining compounds **29a**, **29b**, and **32**, respectively. Finally compounds **29a** and **29b** were further deprotected at the 2-amino group following and modifying the method described by Davisson et al.[32] providing the derivatives **30a** and **30b** in good yield (66–97%) (Scheme 5).

3. Biological results and discussion

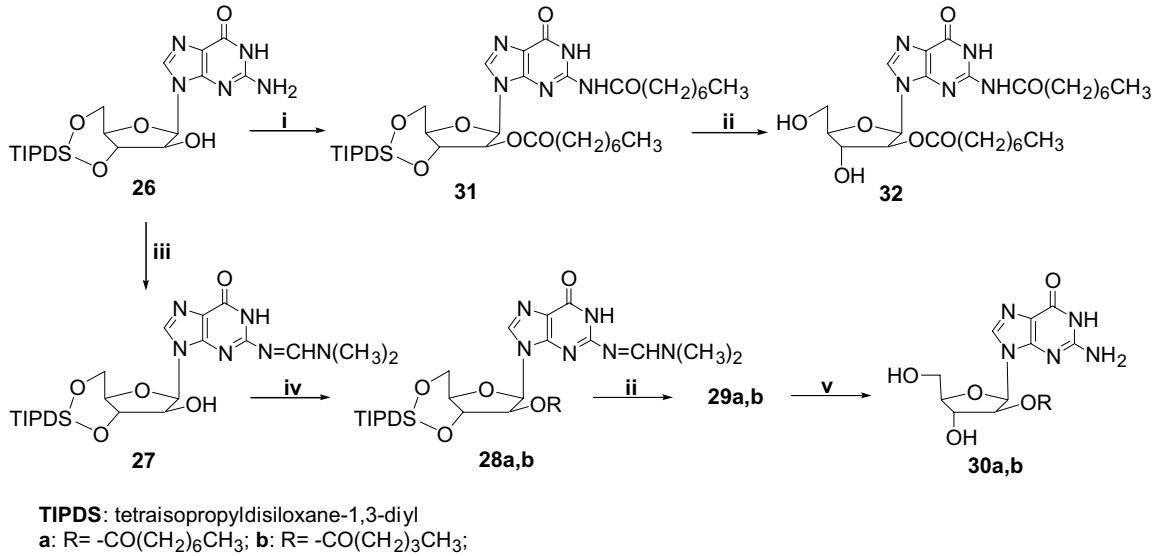
We started the present study with several aims: (a) to design selective TK2 inhibitors; (b) to gain insights into structure–activity relationships (SAR) of the inhibitors for TK2 versus other nucleoside kinases; (c) to reveal whether the newly designed TK2 inhibitors can be uptaken by intact mitochondria; (d) to investigate the impact of TK2 inhibitors on mitochondrial (dys) function.

New synthetic work has been directed to modify the more interesting BVaraU and araT acyl derivatives by introducing an additional hydrophilic (NH_2) function on the acyl moiety (compounds **9** and **10**), or by the isosteric substitution of the pre-existing 2′-ester function of **8e** with a carboxamido moiety (compound **25**). The selected modifications introduced on the active molecules new features useful to investigate the TK2 pocket



TIPDS: tetraisopropyldisiloxane-1,3-diyl.

Scheme 4. Reagents and conditions: (i) H_2, Pd/C, dry CH_3OH; (ii) EDC, HOBT, $CH_3(CH_2)_8COOH$, dry CH_2Cl_2; (iii) NH_4F, dry CH_3OH, Dowex® H^+ form 50Wx2 (50–100), room temperature.



Scheme 5. Reagents and conditions: (i) octanoyl chloride, pyridine, reflux; (ii) NH_4F, CH_3OH, Dowex® H^+ form 50Wx2 (50–100 mesh), room temperature; (iii) CH_3CN, DIPEA, *N*,*N*-dimethylformamide dimethyl acetal; (iv) octanoyl chloride, pyridine, TEA reflux or DMF, valeric anhydride, 4-DMAP, room temperature; (v) CH_3OH/H_2O 2:1, TFA.

with a greater detail. To further investigate the TK2 mitochondrial enzyme, the general approach, based on the introduction of a lipophilic chain at 2′-position of arabinofuranosyl derivatives, was applied also to araC (**21**).[33] Similarly, the approach was also extended to araG in order to explore dGK inhibitors. Furthermore, inhibitory effects of dThd phosphorylation by deoxyribonucleoside kinases from different origin were studied in order to characterize the specificity of the different compounds.

AraT shows (Table 1) preferential inhibition of HSV-1 TK and varicella-zoster virus thymidine kinase (VZV-TK), but also *Dm*-dNK efficiently recognizes araT as an alternative substrate. In contrast, mitochondrial TK2 has at least a 10-fold lower affinity for araT (IC_{50}: 285 μM) and cytosolic TK1-catalyzed dThd phosphorylation was not measurably inhibited in the presence of 1000 μM araT (Table 1). Interestingly, introduction of acyl or alkyl entities at the 2′-position of araT markedly decreases the affinity for all nucleoside kinases except for TK2 where a substantially increased activity was noticed. In fact, the 2′-*O*-decanoyl and 2′-*O*-dodecanoyl derivatives (compounds **8e** and **8g**) showed pronounced affinity for TK2 (IC_{50}: 27–28 μM) (Table 1). Different results were obtained when the acyl chain was introduced on the 2′-amino-2′-deoxy derivative of araT (compound **25**, 480 μM). The isosteric substitution of the 2′-ester function, present in compound **8e**, by a 2′-carboxamido moiety (**25**) produced in fact a consistent reduction of the inhibitory activity. The results indicate possible steric requirements for the activity, indeed the partial double bond character in the amide causes the linkage to be more stable than esters and makes that portion of the molecule more rigid. Thus the molecule may not fit equally well into the TK2 binding pocket. Alternatively, a hydrogen accepting atom (O) instead of a hydrogen donating (N) atom may be required at the 2′ position of the pentose ring.

Table 1. Inhibitory effects of araT derivatives on 2 μM [*methyl*-^{3}H]dThd phosphorylation by deoxyribonucleoside kinases from different origin

Compound	IC_{50}[a] (μM)				
	TK1	TK2	HSV-1TK	*Dm*-dNK	VZV-TK
AraT	>1000	285 ± 1	24 ± 3.1	65 ± 28	17 ± 8.7
8d[23]	>1000	120 ± 14	>1000	>1000	>1000
8e[23]	>1000	27 ± 2.3	⩾1000	872	>1000
8f[23]	>1000	>1000	>1000	>1000	>1000
8g[23]	⩾1000	28 ± 2	⩾1000	>1000	>1000
15[23]	>1000	801 ± 71	>1000	>1000	>1000
16	>500	>500	>500	>500	>500
17[23]	>1000	120 ± 14	>1000	>1000	>1000
18	Nd	>1000	>1000	>1000	>1000
25	Nd	480	>500	>500	>500

[a] 50% inhibitory concentration or compound concentration required to inhibit the enzyme-catalysed phosphorylation of 2 μM [*methyl*-^{3}H] dThd by 50%.

When similar substituents were introduced in BVaraU, a comparable phenomenon was observed as with the araT derivatives (Table 2). Indeed, the pronounced affinity of BVaraU for HSV-1 TK, VZV-TK (IC_{50}: 3.6–3.2 μM), and *Dm*-dNK (IC_{50}: 28 μM) is markedly decreased (10- to >100-fold), whereas TK2 affinity was substantially increased (IC_{50}: 6.3–6.8 μM) for the 2′-*O*-octanoyl and 2′-*O*-decanoyl derivatives (compounds **7d** and **7e**).

Again, there was no appreciable activity against TK1. Long-chain substituents seemed to be better than short-chain substituents. This was also the case for the 2′-*O*-octanoyl (**7l**) and 2′-*O*-dodecanoyl (**7m**) derivatives of BVaraU with a terminal NH-Boc moiety that showed an IC_{50} of 6.4 and 3.8 μM, respectively, for TK2, whereas the 2′-*O*-hexanoyl (**7i**) and 2′-*O*-butanoyl (**7h**) NH-Boc derivatives were ~10-fold less effective. Also, the 2′-*O*-butanoyl (**9**) and 2′-*O*-hexanoyl (**10**) derivatives with a terminal amino moiety, markedly lost affinity for TK2 with respect to the corresponding NH-Boc protected derivatives (**7h** and **7i**). These data indicate that masking of the hydrophilic amino group is very important to achieve activity.

Since TK2 is not only endowed with dThd kinase activity but also with dCyd kinase activity, the 2′-octyl derivative of araC was also synthesized (**21**), but no measurable affinity for TK2 was found (Table 3).

Finally, 2′-*O*-acyl-araG derivatives (**30a**, **b** and **32**) were synthesized to explore their activity against the araG mitochondrial enzyme besides the previously mentioned enzymes. None of these compounds were inhibitory to any of the nucleoside kinases evaluated (Table 4).

In order to assess the biological activity of the TK2 inhibitors on human tissue we tested 2′-*O*-decanoyl-BVaraU (**7e**) on isolated human fibroblasts mitochondria rather than recombinant enzymes. The IC_{50} against TK2 was 40 and 97 μM with dThd and dCyd, as substrates, respectively, indicating that higher concentrations are needed for 'in organello' inhibition than in vitro experiments using recombinant TK2. This occurrence might be explained in view of a possible partial hydrolysis mediated by esterases. Indeed, although this class of compounds has been tested against pig liver esterase[25] and became stable, we cannot exclude sensitivity towards mitochondrial esterases. Still the inhibition was specific since control experiments showed that dGK activity was not inhibited by this compound (IC_{50} > 1000 μM). Moreover, this compound did not affect cytochrome *c* oxidase or succinate dehydrogenase activities.

We also tested the effect (Fig. 1) of 2′-*O*-decanoyl-BVaraU (**7e**) on mitochondrial function by employing a selective medium lacking glucose, with a minimal amount of galactose sufficient to sustain the pentose phosphate pathway but not ATP production by glycolysis: the ATP production and cell viability are therefore solely dependent on the MRC in this medium. In order to sustain MRC deficient cells, a permissive medium, containing glucose pyruvate and uridine is employed.[34] We determined the viability of normal fibroblasts in

Table 2. Inhibitory effects of BVaraU derivatives on 2 μM [*methyl*-^{3}H]dThd phosphorylation by deoxyribonucleoside kinases from different origin

Compound	IC_{50}[a](μM)				
	TK1	TK2	HSV-1TK	*Dm*-dNK	VZV-TK
BVaraU	>500	43 ± 5.8	3.6 ± 1.5	28 ± 10	3.2 ± 1.1
7a	Nd	74 ± 3	247 ± 54	419 ± 68	268 ± 2.0
7b	Nd	44 ± 14	68 ± 3	182 ± 98	39 ± 12
7c[24]	>1000	402 ± 234	84 ± 1.8	215 ± 73	35 ± 1.5
7d	>1000	6.3 ± 0.5	⩾1000	178	718 ± 59
7e[24]	>1000	6.8 ± 0.7	>1000	163	845 ± 30
7h	Nd	55 ± 6	485 ± 20	133 ± 80	130
7i	Nd	61 ± 8	⩾500	77 ± 24	277 ± 1
7l	Nd	6.4 ± 2.6	447 ± 8	74 ± 31	349 ± 1
7m	Nd	3.8 ± 0.2	>500	⩾500	>500
9	>500	187 ± 14	17 ± 1.0	160 ± 60	3.6 ± 0.4
10	>500	> 500	322 ± 83	>500	94 ± 22

[a] 50% inhibitory concentration or compound concentration required to inhibit the enzyme-catalyzed phosphorylation of 2 μM [*methyl*-^{3}H] dThd by 50%.

Table 3. Inhibitory effects of araC derivatives on 2 μM [*methyl*-^{3}H]dThd phosphorylation by deoxyribonucleoside kinases from different origin

Compound	IC_{50}[a](μM)				
	TK1	TK2	HSV-1TK	*Dm*-dNK	VZV-TK
AraC[23]	>1000	>1000	>1000	5319	>1000
21[23]	>1000	>1000	>1000	>1000	>1000

[a] 50% inhibitory concentration or compound concentration required to inhibit the enzyme-catalyzed phosphorylation of 2 μM [*methyl*-^{3}H] dThd by 50%.

Table 4. Inhibitory effects of araC derivatives on 2 μM [*methyl*-^{3}H]dThd phosphorylation by deoxyribonucleoside kinases from different origin

Compound	IC_{50}[a] (μM)					
	TK1	TK2	HSV-1 TK	*Dm*-dNK	VZV-TK	dGK
AraG	>500	>500	>500	>500	>500	133
30a	>500	>500	>500	>500	>500	>500
30b	>500	>500	>500	>500	>500	>500
32	>500	>500	>500	>500	>500	>500

[a] 50% inhibitory concentration or compound concentration required to inhibit the enzyme-catalyzed phosphorylation of 2 μM [*methyl*-^{3}H]dThd by 50%.

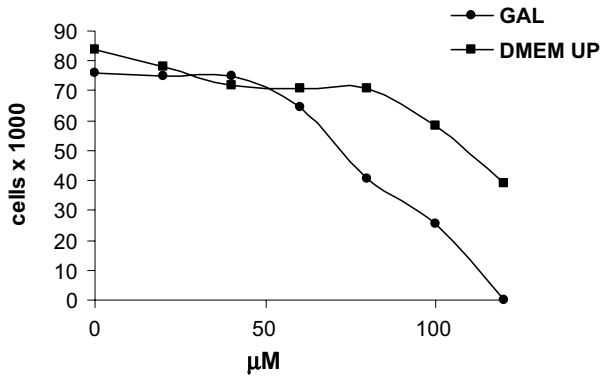


Figure 1. The effect of 2′-*O*-decanoyl-BVaraU (**7e**) on mitochondrial function. Stationary, normal fibroblasts were treated with various concentrations of 2′-*O*-decanoyl-BVaraU (**7e**) in selective medium (GAL) and permissive medium (DMEM UP). Viability count was performed after 1 week of treatment.

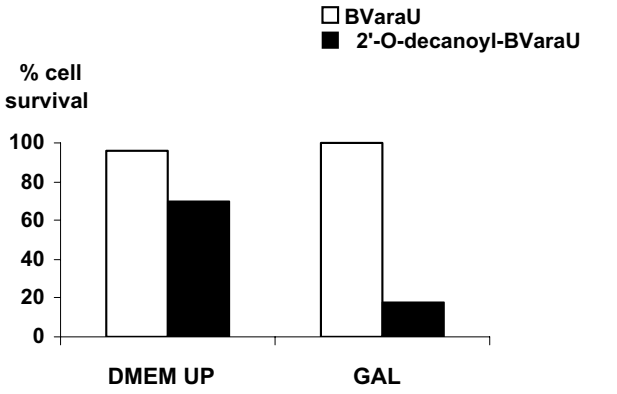


Figure 2. The effect of 2′-*O*-decanoyl-BVaraU (**7e**) and BVaraU on mitochondrial function. Stationary, normal fibroblasts were treated with 100 μM of 2′-*O*-decanoyl-BVaraU (**7e**) and BVaraU in selective medium (GAL) and permissive medium (DMEM UP). Viability count was performed after 1 week treatment.

both media in order to differentiate between the specific effect on MRC and a potential general toxic effect. After 1 week treatment, for concentrations up to 60 μM, the compound **7e** did not alter the cellular viability, regardless of the medium, indicating that this compound at low concentrations is non-toxic and does not alter MRC function in whole cells. Between 60 and 100 μM the viability was impaired in selective medium (GAL), while it was near normal in permissive medium (DMEM UP). This demonstrates that, at these concentrations, **7e** specifically inhibits MRC function. At concentrations higher than 100 μM a generally toxic effect was observed, as the survival was impaired also in the permissive medium (Fig. 1). This toxic effect was not due to solvent as control experiments showed cells treated with solvent only were not affected (data not shown).

Differently, as show in Figure 2, at 100 μM the mother compound BVaraU did not impair the mitochondrial function.

2′-*O*-Decanoyl-BVaraU-treated cells, grown in permissive medium, were also assayed for the MRC enzymes cytochrome *c* oxidase (COX, complex IV) and succinate dehydrogenase (SDH, Complex II). Since COX contain subunits encoded by the mtDNA while SDH encoded solely in the nucleus, COX activity represents an mtDNA-dependent MRC activity, while SDH activity serves as an mtDNA-independent control. Treatment with increasing amount of 2′-*O*-decanoyl-BVaraU (**7e**) resulted in decreased COX activity and COX/SDH ratios (Fig. 3). Notably the effect of this compound was much less on cells with mutated TK2.[15]

Finally we determined the mtDNA content by real time PCR in fibroblasts treated, for 1 week, with 100 μM of 2′-*O*-decanoyl-BVaraU (**7e**). The mtDNA relatively to nuclear DNA was decreased to 50% of that of untreated cells, confirming that the mitochondrial dysfunction is a consequence of mtDNA depletion induced by TK2 inhibition.

4. Conclusion

We have demonstrated that nucleoside analogues such as araT and BVaraU bearing bulky lipophylic substituents at the 2′ position of the sugar can be efficiently recognised by TK2 in a highly selective manner. This finding suggests that TK2, in contrast with the HSV-1 TK, VZV-TK, and *Dm*-dNK, must contain a lipophilic pocket or cleft in which the 2′-substituents may fit. Since there is no crystal structure available for TK2, it is currently unclear how this lipophylic pocket is created in the presence of the test compounds. However, the presence of such a putative pocket may be a useful tool to rationally design new selective substrate/inhibitors of the mitochondrial TK2, not affecting TK1 or any of the other nucleoside kinases. We have also demonstrated that 2′-*O*-decanoyl-BVaraU (**7e**) is able to inhibit the TK2 enzyme ‘in organello.’

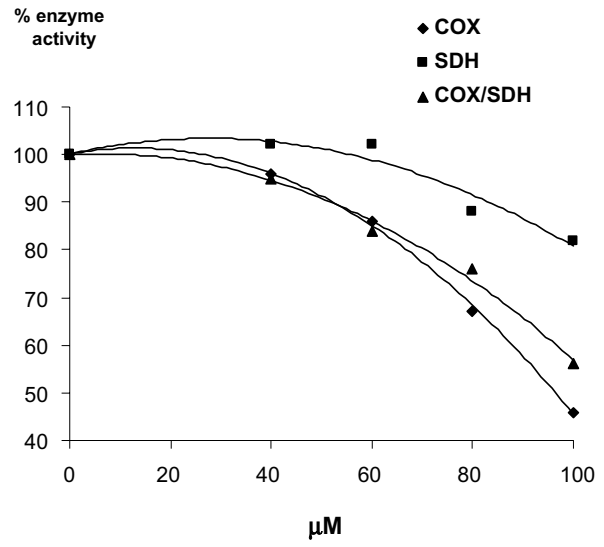


Figure 3. The effect of 2′-*O*-decanoyl-BVaraU (**7e**) on MRC activity. Stationary, normal fibroblasts were treated with various concentrations of 2′-*O*-decanoyl-BVaraU (**7e**) in permissive medium for one week. Cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH) activites were assayed and COX/SDH ratios were calculated.

The compound was able to penetrate whole cells, enter the mitochondria, and subsequently inhibit MRC (mitochondrial respiratory chain) activity at micromolar concentrations. Therefore TK2 inhibitors must be carefully used, as an excess may cause mtDNA depletion with subsequent mitochondrial dysfunction.

5. Experimental

5.1. Chemistry

Reaction courses were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated Macherey-Nagel durasil-25, with detection under a 254-nm UV lamp and/or by spraying the plates with 10% H_2SO_4/CH_3OH and heating. Column chromatography was performed with Macherey-Nagel 0.063–0.2 mm/70–230 mesh silica gel. MALDI-TOFMS (Matrix-assisted laser desorption ionization time-of-flight) spectra were obtained on a Hewlett–Packard HPG2025A mass spectrometer operative on a positive linear mode. Nuclear magnetic resonance spectra were determined in DMSO-d_6, D_2O and $CDCl_3$ solution with a Brucker AC-200 spectrometer or Varian MERCURY*plus* 400 MHz and chemical shifts are presented in ppm from internal tetramethylsilane as a standard. Melting points were determined by Kofler melting point apparatus (Thermovar, C. Reichert AG, Vienna) and are uncorrected. Microanalysis, unless indicated, was in agreement with calculated values within ±0.4%. All drying operations were performed over anhydrous sodium sulfate or magnesium sulfate; room temperature varied between 22 and 25 °C.

5.1.1. 1-[3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (4). To a suspension of dried araT (258 mg, 1 mmol) in dry pyridine (10 mL) under argon atmosphere, was dropwise added 1,3-dichloro-1,1,3,3 tetraisopropyldisiloxane ($TPDSCl_2$, 320 μl, 1 mmol) and the mixture was stirred at room temperature for 20 h. The pyridine was evaporated and coevaporated three times with EtOH and the residue was portioned between EtOAc and H_2O. The organic phase was washed with cold HCl 1N (2 × 20 mL), H_2O (20 mL), saturated $NaHCO_3$ (20 mL), and brine (20 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The resulting residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 90/10, v/v) to give **4** (465 mg, 93%) as a colorless solid (mp >250 °C, dec).

^{1}H NMR ($CDCl_3$) δ (ppm): 0.98–1.18 (m, 28H, TIPDS); 1.90 (s, 3H, CH_3); 3.40–3.58 (m, 1H, H4′); 3.70–3.81 (m, 1H, H3′); 3.92–4.32 (m, 3H, H5′, H5″, H2′); 4.50–4.61 (m, 1H, OH2′); 6.05 (d, 1H, J = 6.3 Hz, H1′); 7.49 (d, 1H, J = 1.1 Hz, H6); 9.22 (br s, 1H, NH).

MALDI-TOFMS: *m/z* 501.7 Da $(M+H)^+$; 523.7 Da $(M+Na)^+$; 539.0 Da $(M+K)^+$. $C_{22}H_{40}N_2O_7Si_2$ requires 500.24.

5.2. General procedure for the acylation of compounds 3[24] and 4. Synthesis of the compounds 5a, b, d, e and 6d–g

The acylation of compounds **3** and **4** (0.5 mmol) was performed with the appropriate acyl chloride (1 mmol) in pyridine, as previously reported by us for compound **5c**.[24]

5.2.1. 1-[2′-*O*-Phenylacetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (5a). Yield 47%; white foam.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.94–1.28 (m, 28H, TIPDS); 3.51 (s, 2H, CH_2CO); 3.76–4.00 (m, 3H, H4′, H5′ and H5″); 4.39–4.41 (m, 1H, H3′); 5.57–5.60 (m, 1H, H2′); 6.19 (d, 1H, J = 6.5 Hz, H1′); 6.60 (d, 1H, J = 13.5 Hz, vinyl); 6.75–6.98 (m, 5H, Ph); 7.38 (d, 1H, vinyl); 7.26 (s, 1H, H6); 8.12 (br s, 1H, NH).

MALDI-TOFMS: *m/z* 731.2 Da $(M+Na)^+$; 747.4 Da $(M+K)^+$. $C_{31}H_{45}BrN_2O_8Si_2$ requires 708.19.

5.2.2. 1-[2′-*O*-(4-Methoxy)-phenylacetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (5b). Yield 22%; white foam.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.92–1.20 (m, 28H, TIPDS); 3.49 (s, 2H, CH_2CO); 3.79–4.07 (m, 6H, CH_3, H4′, H5′ and H5″); 4.41–4.43 (m, 1H, H3′); 5.57–5.59 (m, 1H, H2′); 6.17 (d, 1H, J = 6.6 Hz, H1′); 6.59 (d, 1H, J = 13.5 Hz, vinyl); 6.77, 7.02 (AB system, 4H, J_{AB} = 8.6 Hz, Ph); 7.37 (d, 1H, vinyl); 7.27 (s, 1H, H6); 8.10 (br s, 1H, NH).

MALDI-TOFMS: *m/z* 762.0 Da $(M+Na)^+$; 777.3 Da $(M+K)^+$. $C_{32}H_{47}BrN_2O_9Si_2$ requires 738.20.

5.2.3. 1-[2′-*O*-Octanoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabino-furanosyl]-5(*E*)-(2-bromovinyl)uracil (5d). Yield 90%; yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.81 (t, 3H, J = 6.7 Hz, CH_3); 0.94–1.15 (m, 8H, 4 × CH_2); 1.18–1.30 (m, 28H, TIPDS) 1.35–1.65 (m, 2H, CH_2CH$_2$CO); 2.10–2.21 (m, 2H, CH_2CO); 3.70–3.81 (m, 1H, H4′); 3.92–4.15 (m, 2H, H5′, H5″); 4.30–4.41 (m, 1H, H3′); 5.51–5.62 (m, 1H, H2′); 6.15 (d, 1H, J = 6.4 Hz, H1′); 6.59 (d, 1H, J = 13.6 Hz, vinyl); 7.35 (d, 1H, J = 13.6 Hz, vinyl); 7.46 (s, 1H, H6); 9.25 (br s, 1H, NH).

MALDI-TOFMS: m/z 739.9 Da $(M+Na)^+$; 755.7 Da $(M+K)^+$.$C_{31}H_{53}BrN_2O_8Si_2$ requires 716.25.

5.2.4. 1-[2′-*O*-Decanoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (5e). Yield 69%; colourless solid (mp: 235–238 °C).

^{1}H NMR ($CDCl_3$) δ (ppm): 0.88 (t, 3H, J = 6.6 Hz, CH_3); 1.02–1.17 (m, 12H, 6 × CH_2); 1.23–1.26 (m, 28H, TIPDS); 1.42–1.58 (m, 2H, CH_2CH_2CO); 2.21 (t, 2H, J = 7.4 Hz, CH_2CO); 3.79–3.98 (m, 1H, H4′); 4.06–4.12 (m, 2H, H5′, H5″); 4.39–4.50 (m, 1H, H3′); 5.58–5.69 (m, 1H, H2′); 6.23 (d, 1H, J = 6.3 Hz, H1′); 6.66 (d, 1H, J = 13.6 Hz, vinyl); 7.43 (d, 1H, J = 13.6 Hz, vinyl); 7.52 (s, 1H, H6); 9.86 (br s, 1H, NH).

MALDI-TOFMS: m/z 768.1 Da $(M+Na)^+$; 783.4 Da $(M+K)^+$. $C_{33}H_{57}BrN_2O_8Si_2$ requires 744.28.

5.2.5. 1-[2′-*O*-Octanoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (6d). Yield 50%; yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.88 (t, 3H, J = 6.5 Hz, CH_3); 1.00–1.19 (m, 10H, 5 × CH_2); 1.21–1.41 (m, 28H, TIPDS); 1.43–1.78 (m, 2H, CH_2CO); 1.92 (s, 3H, CH_3); 3.76–3.81 (m, 1H, H4′); 3.95–4.28 (m, 2H, H5′, H5″); 4.40–4.52 (m, 1H, H3′); 5.50–5.60 (m, 1H, H2′); 6.23 (d, 1H, J = 6.5 Hz, H1′); 7.29 (s, 1H, H6); 8.12 (br s, 1H, NH).

MALDI-TOFMS: m/z 650.0 Da $(M+Na)^+$; 665.3 Da $(M+K)^+$. $C_{30}H_{54}N_2O_8Si_2$ requires 626.34.

5.2.6. 1-[2′-*O*-Decanoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (6e). Yield 58%; yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.78–0.89 (m, 3H, CH_3); 1.00–1.18 (m, 28H, TIPDS); 1.14–1.19 (m, 14H, 7 × CH_2); 1.85 (s, 3H, CH_3); 2.01–2.48 (m, 2H, CH_2CO); 3.65–3.74 (m, 1H, H5″); 3.83–4.45 (m, 3H, H5′, H4′, H3′); 5.42–5.51 (m, 1H, H2′); 6.17 (d, 1H, J = 6.4 Hz, H1′); 7.20 (s, 1H, H6); 8.50 (br s, 1H, NH).

MALDI-TOFMS: m/z 677.5 Da $(M+Na)^+$; 693.7 Da $(M+K)^+$. $C_{32}H_{58}N_2O_8Si_2$ requires 654.37.

5.2.7. 1-[2′-*O*-Pentanoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (6f). Yield 68%; yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.77 (t, 3H, J = 5.5 Hz, CH_3); 0.87–1.12 (m, 28H, TIPDS); 1.15–1.65 (m, 4H, 2 × CH_2); 1,85 (s, 3H, CH_3); 2.10–2.23 (m, 2H, CH_2CO); 3.69–3.78 (m, 1H, H4′); 3.90–4.12 (m, 2H, H5′, H5″); 4.34–4.45 (m, 1H, H3′); 5.42–5.53 (m, 1H, H2′); 6.16 (d, 1H, J = 6.6 Hz, H1′); 7.22 (s, 1H, H6); 8.03 (br s, 1H, NH).

MALDI-TOFMS: m/z 607.5 Da $(M+Na)^+$; 623.8 Da $(M+K)^+$. $C_{32}H_{48}N_2O_8Si_2$ requires 584.86.

5.2.8. 1-[2′-*O*-Dodecanoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (6g). Yield 95%; yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.87 (t, 3H, J = 6.1 Hz, CH_3); 0.89–1.13 (m, 28H, TIPDS); 1.12–1.38 (m, 16H, 8 × CH_2); 1.39–1.71 (m, 2H, CH_2CH$_2$CO); 1.85 (s, 3H, CH_3); 2.09–2.37 (m, 2H, CH_2CO); 3.69–3.88 (m, 1H, H4′); 3.90–4.15 (m, 2H, H5′, H5″); 4.41–4.47 (m, 1H, H3′); 5.43–5.58 (m, 1H, H2′); 6.16 (d, 1H, J = 6.5 Hz, H1′); 7.24 (s, 1H, H6); 9.15 (br s, 1H, NH).

MALDI-TOFMS: m/z 683.8 Da $(M+H)^+$; 705.4 Da $(M+Na)^+$; 721.4 Da $(M+K)^+$. $C_{34}H_{62}N_2O_8Si_2$ requires 682.40.

5.3. General procedure for the synthesis of the *N*-Boc-aminoacyl derivatives 5h–m

To a stirred solution of the selected *N*-Boc amino acid derivative (0.25 mmol) in dry CH_2Cl_2 (3.5 mL), compound **3** (150 mg, 0.25 mmol) and DMAP (3 mg, 0.03 mmol) were added under argon atmosphere. To the solution, cooled at 0 °C, was then added *N*,*N*′-dicyclohexylcarbodiimide (DCC, 68 mg, 0.33 mmol) and the resulting mixture was reacted at 25 °C for 3–5 h. After this period, the *N*,*N*′-dicyclohexylurea (DCU), deriving by the reaction, was filtered off and the solvent was evaporated to dryness. The resulting residue was dissolved in EtOAc (15 mL), washed with HCl 0.5 N (10 mL), saturated $NaHCO_3$ (10 mL), and finally with brine (10 mL). After drying over anhydrous Na_2SO_4, the solvent was evaporated to give, in all cases, the pure 2′-*O*-aminoacyl derivatives as white foams, in almost quantitative yield (98–100%).

5.3.1. 1-[2′-*O*-(*N*-Boc-4-aminobuanoyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (5h). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.99–1.12 (m, 28H, TIPDS); 1.49 (s, 9H, *t*-Bu); 1.75–1.81 (m, 2H, CH_2CH_2CO); 2.25–2.30 (m, 2H, CH_2CO); 3.05–3.11 (m, 1H, C*H*H–N); 3.22–3.29 (m, 1H, CH*H*–N); 3.86–3.88 (m, 1H, part of ABX system, H4′); 4.01 (dd, 1H, part of ABX system, J_{AB} = 13.6 Hz, J_{AX} = 2.4 Hz, H5′); 4.17–4.20 (m, 1H, part of ABX system, H5″); 4.31–4.35 (m, 1H, H3′); 4.80 (br s, 1H, NH–Boc); 5.74–5.76 (m, 1H, H2′); 6.14 (d, 1H, J = 6.0 Hz, H1′); 6.68 (d, 1H, J = 13.6 Hz, vinyl); 7.47 (d, 1H, vinyl); 7.68 (s, 1H, H6); 8.68 (br s, 1H, NH).

MALDI-TOFMS: m/z 798.9 Da $(M+Na)^+$; 814.1 Da $(M+K)^+$. $C_{32}H_{54}BrN_3O_{10}Si_2$ requires 775.25.

5.3.2. 1-[2′-*O*-(*N*-Boc-6-aminohexanoyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (5i). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.97–1.15 (m, 28H, TIPDS); 1.46 (s, 9H, *t*-Bu); 1.45–1.89 (m, 6H, 3 × CH_2); 2.19–2.24 (m, 2H, CH_2CO); 3.01–3.19 (m, 2H, CH_2–N); 3.80–3.85 (m, 1H, part of ABX system, H4′); 4.02, 4.15 (2H, part of ABX system, J_{AB} = 13.4 Hz, J_{AX} = 2.6 Hz, J_{BX} = 1.8 Hz, H5′ and H5″); 4.36–4.45 (m, 1H, H3′); 4.82 (br s, 1H, NH–Boc), 5.62–5.69 (m, 1H, H2′); 6.21 (d, 1H, *J* = 6.4 Hz, H1′); 6.66 (d, 1H, *J* = 13.6 Hz, vinyl); 7.43 (d, 1H, *J* = 13.6 Hz, vinyl); 7.53 (s, 1H, H6); 9.39 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 826.4 Da $(M+Na)^+$; 842.7 Da $(M+K)^+$. $C_{34}H_{58}BrN_3O_{10}Si_2$ requires 803.28.

5.3.3. 1-[2′-*O*-(*N*-Boc-8-aminooctanoyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (5l). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.00–1.16 (m, 28H, TIPDS); 1.30–1.97 (m, 10H, 5 × CH_2); 1.44 (s, 9H, *t*-Bu); 2.16–2.25 (m, 2H, CH_2CO); 3.05–3.11 (m, 2H, CH_2–N); 3.77–3.84 (m, part of ABX system, 1H, H4′); 4.02, 4.13 (part of ABX system, 2H, J_{AB} = 13.4 Hz, J_{AX} = 3.0 Hz, J_{BX} = 2.2 Hz, H5′ and H5″); 4.38–4.47 (m, 1H, H3′); 4.76 (br s, 1H, NH–Boc), 5.55–5.62 (m, 1H, H2′); 6.22 (d, 1H, *J* = 6.4 Hz, H1′); 6.64 (d, 1H, *J* = 13.6 Hz, vinyl); 7.41 (d, 1H, vinyl); 7.45 (s, 1H, H6); 9.80 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 855.0 Da $(M+Na)^+$; 871.2 Da $(M+K)^+$; 894.1 Da $(M+Na+K)^+$. $C_{36}H_{62}BrN_3O_{10}Si_2$ requires 831.32.

5.3.4. 1-[2′-*O*-(*N*-Boc-12-aminododecanoyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (5m). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.00–1.16 (m, 28H, TIPDS); 1.21–1.52 (m, 16H, 8 × CH_2); 1.44 (s, 9H, *t*-Bu); 1.62–1.78 (m, 2H, CH_2); 2.22–2.29 (m, 2H, CH_2CO); 3.04–3.14 (m, 2H, CH_2–N); 3.87–4.05 (m, 3H, H5′, H5″, H4′); 4.36–4.41 (m, 1H, H3′); 4.61 (br s, 1H, NH-Boc); 5.24–5.30 (m, 1H, H2′); 6.30 (d, 1H, *J* = 5.8 Hz, H1′); 6.66 (d, 1H, *J* = 13.6 Hz, vinyl); 7.39 (d, 1H, vinyl); 7.74 (s, 1H, H6); 9.31 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 810.4 Da $(M+Na)^+$; 926.2 Da $(M+K)^+$. $C_{40}H_{70}BrN_3O_{10}Si_2$ requires 887.38.

5.4. General procedure for the deprotection of TIPDS. Synthesis of compounds 7a, b, d, h–m and 8d–g

The TIPDS cleavage was carried out by treatment of the protected compound (0.3 mmol) in methanol (10 mL) with Dowex® H^+ form resin (0.25 g) and ammonium fluoride (0.44 g, 1.2 mmol) as previously reported by us (compounds **7c** and **e**).[24]

5.4.1. 1-(2′-*O*-Phenylacetyl-β-D-arabinofuranosyl)-5(*E*)-(2-bromovinyl)uracil (7a). Yield 69%; white foam.

^{1}H NMR (DMSO-d_6) δ (ppm): 3.51–3.69 (m, 4H, CH_2CO, H5′ and H5″); 3.79–3.82 (m, 1H, H4′); 4.12–4.14 (m, 1H, H3′); 5.12–5.18 (m, 1H, H2′); 5.12–5.23 (m, 1H, OH5′); 5.76–5.83 (m, 1H, OH3′); 6.12 (d, 1H, *J* = 5.7 Hz, H1′); 6.86 (d, 1H, *J* = 13.5 Hz, vinyl); 7.07–7.25 (m, 5H, Ph); 7.27 (d, 1H, *J* = 13.6 Hz, vinyl); 7.87 (s, 1H, H6); 11.55 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 489.9 Da $(M+Na)^+$. $C_{19}H_{19}BrN_2O_7$ requires 466.04. Anal. ($C_{19}H_{19}BrN_2O_7$) C, H, N.

5.4.2. 1-[2′-*O*-(4-Methoxyphenylacetyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (7b). Yield 67%; white foam.

^{1}H NMR (DMSO-d_6) δ (ppm): 3.27 (s, 3H, CH_3); 3.44 (s, 2H, CH_2CO); 3.60–3.64 (m, 2H, H5′ and H5″); 3.79–3.82 (m, 1H, H4′); 4.14–4.16 (m, 1H, H3′); 5.16–5.20 (m, 1H, OH5′); 5.17–5.21 (m, 1H, H2′); 5.85 (br s, 1H, OH3′); 6.12 (d, 1H, *J* = 5.3 Hz, H1′); 6.85 (d, 1H, *J* = 13.5 Hz, vinyl); 7.77, 7.00 (AB system, 4H, J_{AB} = 8.6 Hz, Ph); 7.27 (d, 1H, *J* = 13.6 Hz, vinyl); 7.86 (s, 1H, H6); 11.55 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 497.6 Da $(M+H)^+$; 520.0 Da $(M+Na)^+$. $C_{20}H_{21}BrN_2O_8$ requires 496.05. Anal. ($C_{20}H_{21}BrN_2O_8$) C, H, N.

5.4.3. 1-(2′-*O*-Octanoyl-β-D-arabinofuranosyl)-5(*E*)-(2-bromovinyl)uracil (7d). Yield 58%; yellow foam.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.81 (t, 3H, *J* = 6.5 Hz, CH_3); 1.10–1.35 (m, 8H, 4 × CH_2); 1.50–1.69 (m, 2H, CH_2CH_2CO); 2.27 (t, 2H, *J* = 7.2 Hz, CH_2CO); 3.48–3.50 (m, 1H, H4′); 3.78–4.01 (m, 2H, H5′, H5″); 4.28–4.38 (m, 1H, H3′); 5.13–5.26 (m, 1H, H2′); 5.12–5.50 (m, 1H, OH5′); 5.70 (br s, 1H, OH3′); 6.20 (d, 1H, *J* = 5.7 Hz, H1′); 6.60 (d, 1H, *J* = 13.6 Hz, vinyl); 7.31 (d, 1H, *J* = 13.6 Hz, vinyl); 7.60 (s, 1H, H6); 9.38 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 475.3 Da $(M+H)^+$; 497.3 Da $(M+Na)^+$; 536.4 Da $(M+K)^+$. $C_{19}H_{27}BrN_2O_7$ requires 474.10. Anal. ($C_{19}H_{27}BrN_2O_7$) C, H, N.

5.4.4. 1-[2′-*O*-(*N*-Boc-4-aminobuanoyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (7h). Yield: 75%; white foam.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.36 (s, 9H, *t*-Bu); 1.46–1.53 (m, 2H, *CH*$_2$CH$_2$CO); 2.09–2.15 (m, 1H, C*H*HCO); 2.20–2.26 (m, 1H, CH*H*CO); 2.84–2.86 (m, 2H, CH_2–N); 3.63–3.70 (m, 2H, H5′ and H5″); 3.79–3.83 (m, 1H, H4′); 4.09–4.13 (m, 1H, H3′); 5.16–5.22 (m, 2H, H2′ and OH5′); 5.81 (br s, 1H, OH3′); 6.13 (d, 1H, *J* = 4.8 Hz, H1′); 6.80 (bt, 1H, *J* = 5.6 Hz, NH–Boc); 6.88 (d, 1H, *J* = 13.2 Hz, vinyl); 7.26 (d, 1H, vinyl); 8.00 (s, 1H, H6); 11.64 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 556.5 Da $(M+Na)^+$; 573.0 Da $(M+K)^+$. $C_{20}H_{28}BrN_3O_9$ requires 533.10. Anal. ($C_{20}H_{28}BrN_3O_9$) C, H, N.

5.4.5. 1-[2′-*O*-(*N*-Boc-6-aminohexanoyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (7i). Yield: 78%; white foam.

^{1}H NMR (DMSO) δ (ppm): 1.05–1.13 (m, 2H, CH_2); 1.23–1.37 (m, 4H, $2\times CH_2$); 1.35 (s, 9H, *t*-Bu); 2.09–2.15 (m, 1H, C*H*H–CO); 2.18–2.24 (m, 1H, CH*H*–CO); 2.80–2.85 (m, 2H, CH_2–N); 3.60–3.71 (m, 2H, H5′, H5″); 3.77–3.81 (m, 1H, H4′); 4.10–4.11 (m, 1H, H3′); 5.09–5.18 (m, 1H, OH5′); 5.16–5.18 (m, 1H, H2′); 5.79 (d, 1H, J = 4.0 Hz, OH3′); 6.13 (d, 1H, J = 5.2 Hz, H1′); 6.71 (t, 1H, J = 6.0 Hz, NH–Boc); 6.89 (d, 1H, J = 13.6 Hz, vinyl); 7.26 (d, 1H, J = 13.6 Hz, vinyl); 7.93 (s, 1H, H6); 11.63 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 584.5 Da $(M+Na)^+$; 601.2 Da $(M+K)^+$. $C_{22}H_{32}BrN_3O_9$ requires 561.13. Anal. ($C_{22}H_{32}BrN_3O_9$) C, H, N.

5.4.6. 1-[2′-*O*-(*N*-Boc-8-aminooctanoyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (7l). Yield: 71%; white foam.

^{1}H NMR ($CDCl_3$) δ (ppm): 1.20–1.24 (m, 6H, $3\times CH_2$); 1.45 (s, 9H, *t*-Bu); 1.43–1.67 (m, 4H, $2\times CH_2$); 2.21–2.30 (m, 2H, CH_2CO); 3.04–3.14 (m, 2H, CH_2–N); 3.88–4.07 (m, 3H, H5′, H5″, H4′); 4.37–4.43 (m, 1H, H3′); 4.79 (br s, 1H, NH–Boc); 5.24–5.27 (m, 1H, H2′); 6.30 (d, 1H, J = 6.0 Hz, H1′); 6.67 (d, 1H, J = 13.8 Hz, vinyl); 7.39 (d, 1H, J = 13.8 Hz, vinyl); 7.77 (s, 1H, H6); 9.21 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 613.0 Da $(M+Na)^+$; 629.2 Da $(M+K)^+$, 652.2 Da $(M+Na+K)^+$. $C_{24}H_{36}BrN_3O_9$ requires 589.16. Anal. ($C_{24}H_{36}BrN_3O_9$) C, H, N.

5.4.7. 1-[2′-*O*-(*N*-Boc-12-aminododecanoyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil (7m). Yield: 100%; white foam.

^{1}H NMR ($CDCl_3$) δ: 1.21–1.52 (m, 16H, $8\times CH_2$); 1.44 (s, 9H, *t*-Bu); 1.62–1.78 (m, 2H, CH_2); 2.22–2.29 (m, 2H, CH_2CO); 3.04–3.14 (m, 2H, CH_2–N); 3.87–4.05 (m, 3H, H5′, H5″, H4′); 4.36–4.41 (m, 1H, H3′); 4.61 (br s, 1H, NH–Boc); 5.24–5.30 (m, 1H, H2′); 6.30 (d, 1H, J = 5.8 Hz, H1′); 6.66 (d, 1H, J = 13.6 Hz, vinyl); 7.39 (d, 1H, J = 13.6 Hz, vinyl); 7.74 (s, 1H, H6); 9.31 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 669.0 Da $(M+Na)^+$; 685.2 Da $(M+K)^+$ $C_{28}H_{44}BrN_3O_9$ requires 645.23. Anal. ($C_{28}H_{44}BrN_3O_9$) C, H, N: H calcd 6.86; found 6.83.

5.4.8. 1-(2′-*O*-Octanoyl-β-D-arabinofuranosyl)thymine (8d). Yield 50%; white foam.

^{1}H NMR (DMSO) δ (ppm): 0.85 (t, 3H, J = 6.8 Hz, CH_3); 1.08–1.28 (m, 8H, $4\times CH_2$); 1.30–1.10 (m, 2H, CH_2CH$_2$CO); 1.77 (s, 3H, CH_3); 2.02–2.34 (m, 2H, CH_2CO); 3.57–3.69 (m, 2H, H5′, H5″); 3.71–3.82 (m, 1H, H4′); 4.08–4.18 (m, 1H, H3′); 5.02–5.19 (m, 2H, OH5′, H2′); 5.72–5.83 (m, 1H, OH3′); 6.14 (d, 1H, J = 5.4 Hz, H1′); 7.52 (s, 1H, H6); 11.32 (br s, 1H, NH). MALDI-TOFMS: *m*/*z* 385.4 Da $(M+H)^+$; 407.2 Da $(M+Na)^+$; 423.2 Da $(M+K)^+$. $C_{18}H_{28}N_2O_7$ requires 384.19. Anal. ($C_{18}H_{28}N_2O_7$) C, H, N.

5.4.9. 1-(2′-*O*-Decanoyl-β-D-arabinofuranosyl)thymine (8e). Yield 42%; white foam.

^{1}H NMR (DMSO) δ (ppm): 0.85 (t, 3H, J = 6.8 Hz, CH_3); 1.10–1.28 (m, 12H, $6\times CH_2$); 1.32–1.40 (m, 2H, CH_2CH$_2$CO); 1.77 (s, 3H, CH_3); 2.10–2.28 (m, 2H, CH_2CO); 3.58–3.70 (m, 2H, H5′, H5″); 3.74–3.78 (m, 1H, H4′); 4.11–4.15 (m, 1H, H3′); 5.06–5.09 (m, 1H, OH5′); 5.12–5.16 (m, 1H, H2′); 5.75–5.77 (m, 1H, OH3′); 6.14 (d, 1H, J = 5.2 Hz, H1′); 7.52 (s, 1H, H6); 11.37 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 435.2 Da $(M+Na)^+$; 451.3 Da $(M+K)^+$. $C_{20}H_{32}N_2O_7$ requires 412.22. Anal. ($C_{20}H_{32}N_2O_7$) C, H, N.

5.4.10. 1-(2′-*O*-Pentanoyl-β-D-arabinofuranosyl)thymine (8f). Yield 60%; pale yellow foam.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.79 (t, 3H, J = 7.1 Hz, CH_3); 1.09–1.42 (m, 4H, $2\times CH_2$); 1.77 (s, 3H, CH_3 heterocycle); 2.05–2.35 (m, 2H, CH_2CO); 3.58–3.81 (m, 3H, H5′, H5″, H4′); 4.09–4.23 (m, 1H, H3′); 5.01–5.23 (m, 2H, OH5′, H2′); 5.70–5.81 (m, 1H, OH3′); 6.14 (d, 1H, J = 5.4 Hz, H1′); 7.53 (d, 1H, J = 1 Hz, H6); 11.35 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 365.2 Da $(M+Na)^+$; 381.3 Da $(M+K)^+$. $C_{15}H_{22}N_2O_7$ requires 342.14. Anal. ($C_{15}H_{22}N_2O_7$) C, H, N.

5.4.11. 1-(2′-*O*-Dodecanoyl-β-D-arabinofuranosyl)thymine (8g). Yield 50%; yellow foam.

^{1}H NMR (DMSO-d_6) δ (ppm): 0.85 (t, 3H, J = 6.6 Hz, CH_3); 1.09–1.30 (m, 16H, $8\times CH_2$); 1.16–1.20 (m, 2H, CH_2CH$_2$CO); 1.77 (s, 3H, CH_3); 2.10–2.25 (m, 2H, CH_2CO); 3.56–3.71 (m, 2H, H5′, H5″); 3.46–3.71 (m, 1H, H4′); 4.50–4.85 (m, 1H, H3′); 5.06–5.09 (m, 1H, OH5′); 5.61–5.90 (m, 1H, H2′); 5.55–5.75 (m, 1H, OH3′); 6.14 (d, 1H, J = 5.2 Hz, H1′); 7.52 (s, 1H, H6); 13.33 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 463.9 Da $(M+Na)^+$; 379.9 Da $(M+K)^+$. $C_{22}H_{36}N_2O_7$ requires 440.25. Anal. ($C_{22}H_{36}N_2O_7$) C, H, N: H calcd 8.24; found 8.18.

5.5. General procedure for the synthesis of 2′-*O*-aminoacyl derivatives 9, 10

To a stirred suspension of compound **7h** or **7i** (0.5 mmol) in dry diethyl ether (20 mL), HCl was bubbled at 0 °C until disappearance of the starting material. The obtained white precipitate was collected by filtration and washed with a minimum amount of cold diethyl ether. The resulting sticky solid, in both cases obtained, was crystallized from CH_3OH and diethyl ether to give the derivatives **9** and **10**.

5.5.1. 1-[2′-*O*-(4-Aminobutanoyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil hydrochloride (9). Yield 60%; white hygroscopic solid (mp: 200–203 °C).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.64–1.72 (m, 2H, CH_2CH_2CO); 2.16–2.24 (m, 1H, C*H*H–N); 2.37–2.45 (m, 1H, CH*H*–N); 2.70–2.75 (m, 2H, CH_2CO); 3.61–3.72 (m, 2H, H5′ and H5″); 3.79–3.83 (m, 1H, H4′); 4.11–4.15 (m, 1H, H3′); 5.21–5.26 (m, 2H, H2′ and OH5′); 5.81 (d, 1H, J = 5.2 Hz, OH3′); 6.11 (d, 1H, J = 5.6 Hz, H1′); 6.88 (d, 1H, J = 13.6 Hz, vinyl); 7.25 (d, 1H, J = 13.6 Hz, vinyl); 7.76 (br s, 3H, NH_3^+); 8.05 (s, 1H, H6); 11.62 (s, 1H, NH).

MALDI-TOFMS: *m*/*z*: 435.5 Da $(M+H)^+$; 457.7 Da $(M+Na)^+$ $C_{15}H_{20}BrN_3O_7 \cdot HCl$ requires 433.05. Anal. ($C_{15}H_{20}BrN_3O_7 \cdot HCl$) C, H, N.

5.5.2. 1-[2′-*O*-(6-Aminohexanoyl)-β-D-arabinofuranosyl]-5(*E*)-(2-bromovinyl)uracil hydrochloride (10). Yield 90%; white hygroscopic solid (mp: 198–200 °C).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.17–1.48 (m, 6H, 3 × CH_2); 2.09–2.16 (m, 1H, C*H*H–CO); 2.23–2.30 (m, 1H, CH*H*–CO); 2.68–2.70 (m, 2H, CH_2–N); 3.57–3.67 (m, 2H, H5′ and H5″); 3.80–3.81 (m, 1H, H4′); 4.11–4.13 (m, 1H, H3′); 5.20–5.26 (m, 2H, H2′ and OH5′); 5.80 (br s, 1H, OH3′); 6.13 (d, 1H, J = 4.8 Hz, H1′); 6.91 (d, 1H, J = 13.2 Hz, vinyl); 7.26 (d, 1H, J = 13.2 Hz, vinyl); 7.81 (br s, 3H, NH_3^+); 7.98 (s, 1H, H6); 11.65 (s, 1H, NH).

MALDI-TOFMS: *m*/*z* 463.4 Da $(M+H)^+$; 485.5 Da $(M+Na)^+$. $C_{17}H_{24}BrN_3O_7 \cdot HCl$ requires 461.08. Anal. ($C_{17}H_{24}BrN_3O_7 \cdot HCl$) C, H, N.

5.5.3. 1-(3′,5′-*O*-Tetrahydropyran-2-yl-β-D-arabinofuranosyl)thymine (13). Compound **12**[26] (316 mg, 1.32 mmol) was dissolved in dry CH_3CN (12 mL) and dihydropyrane (DHP, 1.14 mL, 13.2 mmol) and *p*-toluenesulfonic acid monohydrate (*p*-TsOH, 24 mg, 0.13 mmol) were added. After 3 h at room temperature the solvent was evaporated and the resulting residue, dissolved in CH_2Cl_2 (10 mL), was washed with saturated $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4) and evaporated to give a crude residue (700 mg) which was dissolved in a solution of 0.1 M KOH in 95% EtOH (12 mL); then the obtained mixture was heated at reflux conditions for 4 h. The solvent was then evaporated and the residue, dissolved in EtOAc (20 mL), was washed with brine (20 mL), dried over Na_2SO_4, filtered, and evaporated. The resulting crude yellow oil was purified by silica gel column chromathography (eluent: petroleum ether/EtOAc, 70/30 → 0/100, v/v) to give **13** (420 mg, overall yield 75%) as yellow oil.

^{1}H NMR ($CDCl_3$) δ (ppm): 1.42–1.75 (m, 12H, 6 × CH_2 THP); 1.87 (s, 3H, CH_3); 3.40–3.55 (m, 2H, H5′, H5″); 3.60–3.90 (m, 4H, 2 × CH_2O THP); 3.92–4.29 (m, 3H, H4′, H3′, H2′); 4.62–4.90 (m, 2H, 2 × CHO THP); 5.65–5.77 (m, 1H, OH2′); 5.97 (d, 1H, J = 6.0 Hz, H1′); 7.35 (s, 1H, H6); 11.31 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 449.5 Da $(M+Na)^+$; 465.4 Da $(M+K)^+$. $C_{20}H_{30}N_2O_8$ requires 426.20.

5.5.4. 1-(2′-*O*-Benzyl-β-D-arabinofuranosyl)thymine (15). To a solution of compound **13** (100 mg, 0.23 mmol) in anhydrous THF (3 mL) 60% NaH (27 mg, 0.58 mmol) and, after 10 min, benzyl bromide (70 μL, 0.58 mmol) were added and the mixture was stirred at room temperature for 16 h under argon atmosphere. Then the solvent was evaporated and the residue dissolved in CH_2Cl_2 (20 mL) was washed with aqueous saturated NH_4Cl solution (10 mL) and H_2O (10 mL). The organic layer, dried (Na_2SO_4) and evaporated to dryness, gave a crude residue that was used for the next step, without any further purification. The above mentioned residue was dissolved in CH_3OH (4 mL) and *p*-TsOH monohydrate (115 mg, 0.514 mmol) was added and the mixture was vigorously stirred at room temperature for 4 h. After the evaporation the crude residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 98/2 → 90/10, v/v) to give **15** (42 mg, overall yield 52%) as a yellow oil.

^{1}H NMR (DMSO-d_6) δ (ppm): 1.74 (s, 3H, CH_3); 3.55–3.73 (m, 3H, H5″, H5′, H4′); 4.04–4.10 (m, 2H, H3′, H2′); 4.41, 4.58 (d, AB system, 2H, J = 11.9 Hz, CH_2–Ph); 5.02–5.11 (m, 1H, OH5′); 5.56–5.59 (m, 1H, OH3′); 6.17 (d, 1H, J = 5.3 Hz, H1′); 7.10–7.45 (m, 5H, Ph); 7.62 (s, 1H, H6): 11.33 (br s, 1H, NH).

MALDI-TOFMS: *m*/*z* 371.2 Da $(M+Na)^+$; 387.4 Da $(M+K)^+$. $C_{17}H_{20}N_2O_6$ requires 348.13. Anal. ($C_{17}H_{20}N_2O_6$) C, H, N.

5.5.5. N^3-Octyl-1-(β-D-arabinofuranosyl)thymine (16). Compound **13** (120 mg, 0.28 mmol) was dissolved in a 1:1 mixture of anhydrous THF–DMSO (3 mL) and to the stirred solution octyl bromide (67 μl, 0.33 mmol) and K_2CO_3 (46 mg, 0.33 mmol) were added. The reaction mixture was kept at room temperature under argon atmosphere for 6 h, then the mixture was diluted with H_2O, neutralized with 2 N HCl (30 mL), and extracted with EtOAc. The organic phase, dried (Na_2SO_4) and evaporated to dryness, gave a crude residue that was used, without any further purification, for the next step.

The above mentioned residue (100 mg) was dissolved in CH_3OH (4 mL) and *p*-TsOH monohydrate (115 mg, 0.514 mmol) was added. The mixture was vigorously stirred at room temperature for 4 h. After evaporation the crude residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 98/2 → 94/6, v/v) to give **16** (60 mg, overall yield 58%) as a yellow oil.

^{1}H NMR (DMSO-d_6) δ (ppm): 0.85 (t, 3H, J = 6.0 Hz, CH_3); 1.12–1.34 (m, 10H, 5 × CH_2); 1.40–1.59 (m, 2H, CH_2CH_2N); 1.80 (s, 3H, CH_3); 3.58–3.82 (m, 4H, H5′, H5″, CH_2N); 3.86–4.19 (m, 3H, H4′, H3′, H2′); 5.05–5.13 (m, 1H, OH5′); 5.41–5.58 (m, 2H, OH3′, OH2′); 6.01 (d, 1H, J = 4.6 Hz, H1′); 7.57 (s, 1H, H6).

MALDI-TOFMS: *m/z* 471.9 Da $(M+H)^+$; 393.2 Da $(M+Na)^+$; 409.4 Da $(M+K)^+$. $C_{18}H_{30}N_2O_6$ requires 370.21. Anal. ($C_{18}H_{30}N_2O_6$) C, H, N.

5.5.6. 1-(2′-*O*-Octyl-β-D-arabinofuranosyl)thymine (17) and N^3-octyl-1-(2′-*O*-octyl-β-D-arabinofuranosyl)thymine (18). To a stirred solution of compound **13** (188 mg, 0.44 mmol) in anhydrous THF (2.5 mL) 60% NaH (70 mg, 1.75 mmol) was added at room temperature under argon atmosphere. After 10 min a solution of freshly prepared octyl iodide (425 mg, 1.75 mmol) in anhydrous THF (2.5 mL) was added, and the mixture was heated at 70 °C for 24 h. The solvent was evaporated and the residue, dissolved in CH_2Cl_2 (20 mL), was washed with aqueous saturated NH_4Cl solution (10 mL), and H_2O (10 mL). The organic phase was then dried (Na_2SO_4) and evaporated to dryness to give crude residue that was used, for the next step, without any further purification. The above mentioned residue (110 mg) was dissolved in CH_3OH (8 mL) and treated with *p*-TsOH monohydrate (196 mg, 0.88 mmol), and the mixture was vigorously stirred at room temperature for 4 h. After evaporation of the solvent the resulting crude residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 98/2 → 90/10, v/v) to give a mixture of the expected hydroxyl derivative (**17**), together with the N^3, 2′-O-bis-alkylated product (**18**) (molar ratio **17/18** = 3:1).

5.5.7. 1-(2′-*O*-Octyl-β-D-arabinofuranosyl)thymine (17). Yield 55% with respect to **13**; yellow oil.

^{1}H NMR (DMSO-d_6) δ (ppm): 0.84 (t, 3H, J = 6.4 Hz, CH_3); 1.05–1.42 (m, 12H, 6 × CH_2); 1.75 (s, 3H, CH_3); 3.11–3.70 (m, 5H, H5″, H5′, CH_2O, H4′); 3.82–3.91 (m, 1H, H3′); 3.93–4.07 (m, 1H, H2′); 4.09–5.08 (m, 1H, OH5′); 5.51–5.59 (m, 1H, OH3′); 6.12 (d, 1H, J = 5.5 Hz, H1′); 7.48 (s, 1H, H6); 11.31 (br s, 1H, NH).

MALDI-TOFMS: *m/z* 393.3 Da $(M+Na)^+$; 409.2 Da $(M+K)^+$. $C_{18}H_{30}N_2O_6$ requires 370.21. Anal. ($C_{18}H_{30}N_2O_6$) C, H, N.

5.5.8. N^3-Octyl-1-(2′-*O*-octyl-β-D-arabino-furanosyl)thymine (18). Yield 17% with respect to **13**; yellow oil.

^{1}H NMR (DMSO d_6) δ (ppm): 0.79–0.90 (m, 6H, 2 × CH_3); 1.05–1.34 (m, 22H, 11 × CH_2); 1.40–1.59 (m, 2H, CH_2CH_2O); 1.80 (s, 3H, CH_3); 3.11–3.35 (m, 2H, CH_2N); 3.52–3.83 (m, 5H, H4′, H5″, H5′, CH_2O); 3.90–4.07 (m, 2H, H3′, H2′); 5.02–5.11 (m, 1H, OH5′); 5.53–5.60 (m, 1H, OH3′); 6.15 (d, 1H, J = 5.5 Hz, H1′); 7.58 (s, 1H, H6).

MALDI-TOFMS: *m/z* 505.3 Da $(M+Na)^+$; 521.8 Da $(M+K)^+$. $C_{26}H_{46}N_2O_6$ requires 482.34. Anal. ($C_{26}H_{46}N_2O_6$) C, H, N.

5.5.9. 1-(2′-*O*-Octyl-3′,5′-*O*-tetrahydropyran-2-yl-β-D-arabinofuranosyl)uracil (19). To a stirred solution of compound **14**[28] (465 mg, 1.13 mmol) in anhydrous THF (9.5 mL), 60% NaH (125 mg, 5.2 mmol) was added at room temperature under argon atmosphere. After 10 min a solution of freshly prepared octyl iodide (324 mg 1.35 mmol) in anhydrous THF (2.5 mL) was added and the mixture was heated at refluxing conditions for 24 h. The solvent was evaporated and the residue dissolved in CH_2Cl_2 (20 mL), washed with aqueous saturated NH_4Cl solution (10 mL) and H_2O (10 mL). The organic phase, dried over $MgSO_4$ and evaporated to dryness, gave a crude residue that was purified by silica gel column chromatography (eluent: petroleum ether/EtOAc, 90/10 → 0/100, v/v) to give **19** (295 mg, 50%) as a thick white oil.

^{1}H NMR (DMSO-d_6) δ: 0.83 (t, 3H, J = 6.0 Hz, CH_3); 1.15–1.30 (m, 12H, 6 × CH_2); 1.32–1.79 (m, 12H, 6 × CH_2 THP); 3.22–3.64 (m, 4H, H5″, H5′, CH_2O); 3.66–4.32 (m, 7H, 2 × CH_2O THP, H4′, H3′, H2′); 4.59–4.88 (m, 2H, 2 × CHO THP); 5.56–5.64 (m, 1H, H5); 6.18–6.20 (m, 1H, H1′); 7.52–7.58 (m, 1H, H6); 11.40 (br s, 1H, NH).

MALDI-TOFMS: *m/z* 561.9 Da $(M+Na)^+$; 577.8 Da $(M+K)^+$. $C_{27}H_{44}N_2O_8$ requires 524.31.

5.5.10. 4-(1,2,4-Triazol-1-y1)-1-(2′-*O*-octyl-3′,5′-*O*-tetrahydropyran-2-yl-β-D-arabinofuranosyl)uracil (20). 1,2,4-Triazole (355 mg, 5 mmol) and anhydrous $POCl_3$ (79 μl, 0.86 mmol) were dissolved, under argon atmosphere, in dry CH_3CN (2 mL) and to the mixture, cooled at 0 °C, was dropwise added dry triethylamine (TEA, 0.6 mL, 4.29 mmol). Compound **19** (150 mg, 0.286 mmol) dissolved in dry CH_3CN (1.5 mL) was added to the above prepared mixture and the obtained suspension was stirred at room temperature for 15 h. The solvent was then evaporated to dryness and the residue dissolved in CH_2Cl_2 (20 mL) washed with $NaHCO_3$ (20 mL), and brine (20 mL). After drying (Na_2SO_4), the organic layer was evaporated, and the obtained crude residue was purified by silica gel column chromatography (eluent: petroleum ether/Et_2O, 50/50 → 20/80, v/v) to give **20** (81 mg, 50%) as a thick white oil. The compound has been used immediately for the next preparation due to its relative instability.

^{1}H NMR ($CDCl_3$) δ: 0.78–0.88 (m, 3H, CH_3); 1.11–1.30 (m, 12H, 6 × CH_2); 1.61–1.82 (m, 12H, 6 × CH_2 THP); 3.25–3.63 (m, 4H, H5″, H5′, CH_2O); 3.60–4.39 (m, 7H, 2 × CH_2O THP, H4′, H3′, H2′); 4.62–4.88 (m, 2H, 2 × CHO THP); 6.35 (m, 1H, H1′); 6.98–6.03 (m, 1H, H5); 8.13 (s, 1H, H3 triazole); 8.33–8.48 (m, 1H, H6); 8.50 (s, 1H, H5 triazole).

MALDI-TOFMS: *m/z* 598.9 Da $(M+Na)^+$; 414.8 $(M+K)^+$. $C_{29}H_{45}N_5O_7$ requires 575.33.

5.5.11. 1-(2′-*O*-Octyl-β-D-arabinofuranosyl)cytosine (21). To a stirred solution of compound **20** (120 mg, 0.2 mmol) in dioxane (4 mL), 4 mL of 30% NH_4OH was added at room temperature. After 20 h the solvent was evaporated and the crude residue (about 110 mg) without any further purification, was dissolved in 4 mL CH_3OH and to the resulting solution, *p*-toluensulfonic acid monohydrate (70 mg, 0.368 mmol) was

added at room temperature. After 4 h the solvent was evaporated and the crude residue, dissolved in H_2O, was treated with Dowex® OH^- form 1×2–400 resin up to pH 7. The filtered solution was then evaporated and the resulting yellow residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 95/5 → 90/10, v/v) to give compound **21** (32 mg, overall yield 46%) as a white solid (mp: 89–92 °C).

^{1}H NMR (DMSO-d_6) δ: 0.84 (t, 3H, J = 6.0 Hz, CH_3); 1.14–1.30 (m, 12H, $6 \times CH_2$); 3.12–3.16 (m, 2H, CH_2O); 3.50–3.56 (m, 2H, H5″, H5′); 3.65–3.68 (m, 1H, H4′); 3.77–3.81 (m, 1H, H3′); 3.94–3.96 (m, 1H, H2′); 4.88 (t, 1H, J = 4.0 Hz, OH5′); 5.48 (d, 1H, J = 2.0 Hz, OH3′); 5.66 (d, 1H, J = 7.4 Hz, H5); 6.12 (d, 1H, J = 6.0 Hz, H1′); 7.05–7.12 (m, 2H, NH_2); 7.51 (d, 1H, J = 7.4 Hz, H6).

MALDI-TOFMS: m/z 356.4 Da $(M+H)^+$; 378.9 Da $(M+Na)^+$; 394.2 $(M+K)^+$. $C_{17}H_{29}N_3O_5$ requires 355.21. Anal. ($C_{17}H_{29}N_3O_5$) C, H, N.

5.5.12. 1-[2′-Amino-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (23). A solution of **22**[30] (100 mg, 0.16 mmol) in dry methanol (5 mL) was vigorously stirred in a hydrogen atmosphere with 10% palladium-on-carbon catalyst for 2 h. The mixture was then filtered on Celite pad and evaporated under vacuo. The crude residue was purified by silica gel column chromatography (eluent CH_2Cl_2/CH_3OH, 99/1, v/v) to give the expected product **23** as a white foam (32 mg, 40%).

^{1}H NMR (DMSO) δ (ppm): 0.97–1.07 (m, 28H, TIPDS); 1.73 (s, 3H, CH_3); 3.56 (t, 1H, J = 7.6 Hz, H3′); 3.66–3.69 (m, 1H, H4′); 3.90–4.20 (m, 3H, H5′, H5″, H2′); 5.90 (d, 1H, J = 6.8 Hz, H1′); 7.27 (d, 1H, J = 1.1 Hz, H6); 9.18 (br s, 2H, NH_2); 11.30 (br s, 1H, NH).

MALDI-TOFMS: m/z 501.0 Da $(M+H)^+$; 522.6 Da $(M+Na)^+$; 538.8 $(M+K)^+$. $C_{22}H_{41}N_3O_6Si_2$ requires 499.25.

5.5.13. 1-[2′-*N*-Nonylcarboxamido-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]thymine (24). A mixture of decanoic acid (96 mg, 0.56 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (130 mg, 0.67 mmol), and 1-hydroxybenzotriazole hydrate (HOBT) (83 mg, 0.62 mmol) in 20 mL of dry CH_2Cl_2 was stirred at room temperature for 1 h. In a separate flask, compound **21** (280 mg, 0.56 mmol) was dissolved in 10 mL of dry CH_2Cl_2 and the resulting solution was added to the stirred active ester mixture. After 24 h at room temperature the organic layer was washed with saturated NH_4Cl (4×20 mL) and then dried over Na_2SO_4. The solvent was evaporated to dryness, and the obtained residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 99/1, v/v) to give the expected compound **24** (200 mg, 54%) as a white foam.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.87 (t, 3H, J = 7.2 Hz, CH_3); 0.94–1.24 (m, 40H, TIPDS, $6 \times CH_2$); 1.35–1.41 (m, 2H, $COCH_2CH_2$); 1.77 (s, 3H, CH_3 heterocycle); 1.98 (t, 2H, J = 7.6 Hz, $COCH_2$); 3.78–3.81 (m, 1H, H4′); 3.94, 4.05 (part of ABX system, 2H, J_{AB} = 12.8 Hz, J_{AX} = 2.8 Hz, J_{BX} = 3.2 Hz, H5′, H5″); 4.18–4.23 (m, 1H, H3′); 4.63–4.69 (m, 1H, H2′); 5.98 (d, 1H, J = 7.2 Hz, H1′); 7.22 (s, 1H, H6); 8.02 (d, 1H, J = 8.8 Hz, NH); 11.25 (br s, 1H, NH).

MALDI-TOFMS: m/z 676.8 Da $(M+Na)^+$; 692.2 $(M+K)^+$. $C_{32}H_{59}N_3O_7Si_2$ requires 653.39.

5.5.14. 1-(2′-*N*-Nonylcarboxamido-2′deoxy-β-D-arabinofuranosyl)thymine (25). The TIPDS cleavage of the compound **24** was carried out as previously described for the compounds **7** and **8**.

Yield 80%; white foam.

^{1}H NMR (DMSO) δ (ppm): 0.85 (t, 2H, J = 7.6 Hz, CH_3); 1.08–1.28 (m, 12H, $6 \times CH_2$); 1.74 (s, 3H, CH_3 heterocycle); 1.95 (t, 2H, J = 7.6 Hz, CH_2); 3.55–3.82 (m, 3H, H4′, H5′, H5″); 4.06 (m, 1H, H3′); 4.31 (m, 1H, H2′); 5.29 (br s, 1H, OH5′); 5.57 (d, 1H, J = 5.6 Hz, OH3′); 6.03 (d, 1H, J = 6.4 Hz, H1′); 7.48 (s, 1H, H6); 7.94 (d, 1H, J = 8.0 Hz, NH); 11.16 (s, 1H, NH heterocycle).

MALDI-TOFMS: m/z 434.3 Da $(M+Na)^+$; 450.8 $(M+K)^+$. $C_{20}H_{33}N_3O_6$ requires 411.24. Anal. ($C_{20}H_{33}N_3O_6$) C, H, N.

5.5.15. N^2-(Dimethylamino)methylen-9-[3′,5′- *O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-guanine (27). To a stirred suspension of compound **26**[31] (315 mg, 0.6 mmol), in dry CH_3CN (5 mL), *N*,*N*-diisopropylethylamine (DIPEA, 300 μL, 1.8 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (DMFDA, 240 μL, 1.8 mmol) were added under argon atmosphere. After 15 h the yellow solution was evaporated to dryness and the crude residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 98/2 → 95/5, v/v) to give the expected product **27** (243 mg, 70%) as a colorless solid (mp: 221–225 °C).

^{1}H NMR (DMSO) δ (ppm): 0.98–1.18 (m, 28H, TIPDS); 3.02 (s, 3H, N–CH_3); 3.14 (s, 3H, N–CH_3); 3.73–3.81 (m, 1H, H4′); 3.78–4.07 (m, 2H, H5′, H5″); 4.25–4.35 (m, 1H, H3′); 4.41–4.52 (m, 1H, H2′); 5.78–5.81 (m, 1H, OH2′); 6.12 (d, 1H, J = 6.6 Hz, H1′); 7.71 (s, 1H, CH–N); 8.56 (s, 1H, H8); 11.31 (br s, 1H, NH).

MALDI-TOFMS: m/z 581.4 Da $(M+H)^+$; 603.5 $(M+Na)^+$; 619.3 Da $(M+K)^+$. $C_{25}H_{44}N_6O_6Si_2$ requires 580.29.

5.5.16. N^2-(Dimethylamino)methylen-9-[2′-*O*-octanoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]guanine (28a). Compound **28a** was obtained from the compound **27** adapting the procedure described in the literature.[35] Compound **27** (200 mg, 0.35 mmol) was dissolved in dry pyridine (4 mL) and to the stirred solution TEA (125 μL, 0.09 mmol) and octanoyl chloride (175 μL, 0.63 mmol) were added.

The solution was heated at 50 °C for 4 h. The solvent was then evaporated and the crude residue dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$ (2 × 20 mL) then dried ($MgSO_4$) and evaporated to dryness. The resulting crude residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 95/5, v/v) to give the expected product **28a** (151 mg, 61%) as a yellow foam.

^{1}H NMR ($CDCl_3$) δ (ppm): 0.82–0.92 (m, 3H, CH_3); 1.00–1.32 (m, 38H, TIPDS, 5 × CH_2); 1.80–2.08 (m, 2H, CH_2CO); 3.10 (s, 3H, N–CH_3); 3.20 (s, 3H, N–CH_3); 3.81–4.18 (m, 3H, H5′, H4′, H5″); 4.23–4.67 (m, 1H, H3′); 5.51–5.58 (m, 1H, H2′); 6.40 (d, 1H, J = 6.34 Hz, H1′); 7.90 (1H, CH=N); 8.61 (s, 1H, H8); 9.53 (br s, 1H, NH).

MALDI-TOFMS: m/z 707.4 Da $(M+H)^+$; 729.4 $(M+Na)^+$; 745.3 Da $(M + K)^+$. $C_{33}H_{58}N_6O_7Si_2$ requires 706.39.

5.5.17. N^2-(Dimethylamino)methylen-9-[2′-*O*-pentanoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]guanine (28b). Compound **27** (200 mg, 0.35 mmol) was dissolved in dry DMF (5 mL) and to the stirred solution 4-DMAP (10 mg, 0.02 mmol) and valeric anhydride (92 μL, 0.49 mmol) were added. The solution was reacted at room temperature for 5 h. The solvent was then evaporated and the crude residue dissolved in Et_2O and washed with saturated H_2O (2 × 20 mL) then dried ($MgSO_4$) and evaporated to dryness. The resulting crude residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 97/3, v/v) to give the expected product **28b** (173 mg, 77%) as a yellow foam.

^{1}H NMR (DMSO) δ (ppm): 0.64–0.72 (m, 3H, CH_3); 0.08–1.20 (m, 30H, TIPDS, CH_2CH_3); 1.78–2.23 (m, 4H, CH_2CH_2CO); 3.03 (s, 3H, N–CH_3); 3.14 (s, 3H, N–CH_3); 3.93–4.02 (m, 2H, H5′, H4′); 4.09–4.20 (m, 1H, H5″); 4.56–4.64 (m, 1H, H3′); 5.56–5.64 (m, 1H, H2′); 6.31 (d, 1H, J = 6.1 Hz, H1′); 7.79 (s, 1H, CH=N); 8.49 (s, 1H, H8); 11.41 (br s, 1H, NH).

MALDI-TOFMS: m/z 665.8 Da $(M+H)^+$; 687.3 $(M+Na)^+$; 703.5 Da $(M + K)^+$. $C_{30}H_{52}N_6O_7Si_2$ requires 664.34.

5.5.18. N^2-(Dimethylamino)methylen-9-(2′-*O*-acyl-β-D-arabinofuranosyl)guanine. General procedure. (Compounds 29a and b). The TIPDS cleavage of compounds **28a** and **b** was carried out as previously described for the compounds **7** and **8**.

5.5.19. N^2-(Dimethylamino)methylen-9-(2′-*O*-octanoyl-β-D-arabinofuranosyl)guanine (29a). Yield 40%; yellow foam.

^{1}H NMR (DMSO) δ (ppm): 0.83 (t, 3H, J = 6.5 Hz, CH_3); 0.90–1.28 (m, 10H, 5 × CH_2); 1.80–2.20 (m, 2H, CH_2CO); 3.03 (s, 3H, N–CH_3); 3.16 (s, 3H, N–CH_3); 3.55–3.85 (m, 3H, H5′, H4′, H5″); 4.36–4.44 (m, 1H, H3′); 5.03–5.10 (m, 1H, H2′); 5.25–5.31 (m, 1H, OH5′); 5.82–5.84 (m, 1H, OH3′); 6.30 (d, 1H, J = 6.1 Hz, H1′); 7.93 (1H, CH–N); 8.54 (s, 1H, H8); 11.34 (br s, 1H, NH).

MALDI-TOFMS: m/z 465.4 Da $(M+H)^+$; 487.2 $(M+Na)^+$; 503.4 Da $(M+K)^+$. $C_{21}H_{32}N_6O_6$ requires 464.24.

5.5.20. N^2-(Dimethylamino)methylen-9-(2′-*O*-pentanoyl-β-D-arabinofuranosyl)guanine (29b). Yield 56%; yellow foam.

^{1}H NMR (DMSO) δ (ppm): 0.69 (t, 3H, J = 7.1 Hz, CH_3); 1.05–1.25 (m, 4H, 2 × CH_2); 1.85–2.18 (m, 2H, CH_2CO); 3.03 (s, 3H, N–CH_3); 3.16 (s, 3H, N–CH_3); 3.59–3.94 (m, 3H, H5′, H4′, H5″); 4.38–4.50 (m, 1H, H3′); 5.04–5.10 (m, 1H, H2′); 5.25–5.31 (m, 1H, OH5′); 5.81–5.84 (m, 1H, OH3′); 6.30 (d, 1H, J = 4.4 Hz, H1′); 7.94 (1H, CH–N); 8.54 (s, 1H, H8); 11.34 (br s, 1H, NH).

MALDI-TOFMS: m/z 423.3 Da $(M+H)^+$; 445.4 $(M+Na)^+$; 461.5 Da $(M+K)^+$. $C_{18}H_{26}N_6O_6$ requires 422.19.

5.5.21. 9-(2′-*O*-Octanoyl-β-D-arabinofuranosyl)guanine (30a). Compound **29a** (80 mg, 0.17 mmol) was dissolved in 410 μL of a 2:1 CH_3OH: H_2O mixture and to the stirred solution 44 μL of TFA (0.57 mmol) was added at room temperature. After 14 h half of the volume of the solution was evaporated in vacuo and to the solution were added a 2:1 mixture EtOH/H_2O and 17 mg (0.21 mmol) NH_4HCO_3. The mixture was then evaporated to dryness and the crude residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/CH_3OH, 98/2 → 90/10, v/v) to give the expected product **30a** (46 mg, yield 66%) as a yellow foam.

^{1}H NMR (DMSO) δ (ppm): 0.83 (t, 3H, J = 7.2 Hz, CH_3); 1.05–1.29 (m, 10H, 5 × CH_2); 1.89–2.18 (m, 2H, CH_2CO); 3.58–3.87 (m, 3H, H5″, H4′, H5′); 4.28–4.40 (m, 1H, H3′); 5.00–5.06 (m, 1H, H2′); 5.18–5.25 (m, 1H, OH5′); 5.80–5.83 (m, 1H, OH3′); 6.16 (d, 1H, J = 5.8 Hz, H1′); 6.50 (br s, 2H, NH_2); 7.78 (s, 1H, H8); 10.60 (br s, 1H, NH).

MALDI-TOFMS: m/z 410.3 Da $(M+H)^+$; 432.2 Da $(M+Na)^+$; 448.2 Da $(M+K)^+$. $C_{18}H_{27}N_5O_6$ requires 409.20. Anal. ($C_{18}H_{27}N_5O_6$) C, H, N.

5.5.22. 9-(2′-*O*-Pentanoyl-β-D-arabinofuranosyl)guanine (30b). Compound **29b** was deprotected with TFA in a 2:1 CH_3OH/H_2O mixture, as described for compound **30a**.

Yield 97%; yellow foam.

^{1}H NMR (DMSO) δ (ppm): 0.73 (t, 3H, J = 7.2 Hz, CH_3); 0.92–1.33 (m, 4H, 2 × CH_2); 1.89–2.21 (m, 2H, CH_2CO); 3.60–3.92 (m, 3H, H5′, H5″, H4′), 4.30–4.42 (m, 1H, H3′); 5.05 (br s, 1H, OH5′); 5.20–5.28 (m, 1H, H2′); 5.80 (br s, 1H, OH3′); 6.16 (d, 1H, J = 5.8 Hz, H1′); 6.53 (br s, 2H, NH_2); 7.79 (s, 1H, H8); 8.85 (br s, 1H, NH).

MALDI-TOFMS: *m/z* 368.4 Da $(M+H)^+$; 390.6 Da $(M+Na)^+$; 406.7 Da $(M+K)^+$. $C_{15}H_{21}N_5O_6$ requires 367.15 Da. Anal. ($C_{15}H_{21}N_5O_6$) C, H, N.

5.5.23. N^2-Octanoyl-9-[2′-*O*-octanoyl-(3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]-guanine (31). Compound **31** was obtained from the compound **26** using the general acylation method previously described for compounds **5** and **6**.

Yield 53%; pale yellow oil.

^{1}H NMR (DMSO) δ (ppm): 0.75–0.87 (m, 6H, $2\times CH_3$); 0.90–1.40 (m, 44H, TIPDS, $8\times CH_2$); 1.55–1.89 (m, 4H, $2\times CH_2CH_2CO$); 2.35–2.50 (m, 4H, $2\times CH_2CO$); 3.85–3.97 (m, 1H, H4′); 4.08–4.20 (m, 2H, H5′, H5″); 4.61–4.83 (m, 1H, H3′); 4.45–4.55 (m, 1H, H2′); 6.27 (d, 1H, J = 6.3 Hz, H1′); 7.74 (s, 1H, H8); 8.86 (br s, 1H, NHCO); 11.21 (br s, 1H, NH).

MALDI-TOFMS: *m/z* 778.5 Da $(M+H)^+$; 800.4 $(M+Na)^+$; 816.5 Da $(M+K)^+$. $C_{38}H_{67}N_5O_8Si_2$ requires 777.45.

5.5.24. N^2-Octanoyl-9-(2′-*O*-octanoyl-β-D-arabinofuranosyl)guanine (32). The cleavage of TIPDS of the compound **31** (320 mg, 0.4 mmol) was carried out as previously described for compounds **7** and **8**, to give compound **32** (86 mg, 40%) as a pale yellow oil.

^{1}H NMR (DMSO) δ (ppm): 0.77–0.92 (m, 6H, $2\times CH_3$); 1.01–1.38 (m, 16H, $8\times CH_2$); 1.49–1.69 (m, 4H, $2\times CH_2CH_2CO$); 1.83–2.25 (m, 4H, $2\times CH_2CO$); 3.43–3.90 (m, 3H, H5′, H4′, H5″); 4.32–4.46 (m, 1H, H3′); 5.09–5.18 (m, 1H, OH5′); 5.31–5.47 (m, 1H, H2′); 5.87–5.92 (m, 1H, OH3′); 6.26 (d, 1H, J = 5.96 Hz, H1′); 8.14 (s, 1H, H8); 8.75 (br s, 1H, NHCO).

MALDI-TOFMS: *m/z* 558.6 $(M+Na)^+$; 574.3 Da $(M+K)^+$. $C_{26}H_{41}N_5O_7$ requires 535.30. Anal. ($C_{26}H_{41}N_5O_7$) C, H, N.

6. Biological materials and methods

6.1. Enzyme assays

The radiolabeled substrate [methyl-^{3}H]dThd (70 Ci/mmol) was obtained from Amersham Pharmacia Biotech. The cDNAs of TK1, TK2, *Dm*-dNK, and HSV-1 TK were inserted in the pGEX-5X-1 vector, expressed as fusion proteins to glutathione *S*-transferase, and purified. The activity of the purified recombinant nucleoside kinases was assayed in a 50 μl reaction mixture containing 50 mM Tris–HCl, pH 8.0, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate), 3 mg/mL bovine serum albumin, 2.5 mM ATP and 0.25 μCi [methyl-^{3}H]dThd. The samples were incubated at 37 °C for 30 min in the presence or absence of different concentrations of the test compounds. Aliquots of 45 μl of the reaction mixtures were spotted on Whatman DE-81 filter paper disks. The filters were washed 3 times for 5 min in 1 mM ammonium formate, once for 1 min in H_2O, and once for 5 min in ethanol. The radioactivity was determined by scintillation counting.

6.2. Tissue cultures

Primary fibroblasts were derived from forearm skin biopsies from healthy volunteers (with informed consent). Cells were grown in a permissive medium: DMEM (Dulbecco's modified Eagle's medium) with 4.5 g/l glucose, 10% fetal calf serum in the presence of 50 μg/ml uridine and 110 μg/mL (DMEM UP) or in a restrictive medium: glucose free RPMI (Roswell Park Memorial Institute) supplied with 50 μM galactose and 10% dialyzed fetal calf serum (GAL) at 37 °C in 5% CO_2.[33] Viability was assessed by trypan blue exclusion.

6.3. Mitochondrial isolation

Mitochondria were isolated from fibroblasts homogenized by nitrogen cavitation followed by differential centrifugation in sucrose buffer.[36]

6.4. Enzymatic assays in fibroblast mitochondria

Cytochrome *c* oxidase was measured by spectrophotometer, monitoring the rate of oxidation of cytochrome *c* at 550 nm and succinate dehydrogenase by following phenazine methosulfate-mediated reduction of dichlorophenolindophenol at 600 nm.[37,38]

TK2 activity in isolated fibroblast mitochondria was determined by radiochemical methods measuring the phosphorylation of [methyl-^{3}H]dThd and [^{3}H]dCyd while dGK was measured with [^{3}H]dado.[38,2]

6.5. Real time PCR

Mitochondrial DNA was quantified by real-time quantitative PCR using Taqman probes from Applied Biosystems UK. The mtDNA to nuclear DNA ratio was calculated and compared to control samples.[39]

Acknowledgments

A part of this work was supported by research grants from the Israeli Academy of Sciences No. 406/02 and Israeli Ministry of Health No. 5307 (A.S.) and by the University of Ferrara (FAR 2005-2006, project CAN2005-2006) Also, the European Commission (QLRT-2001-01004) (J.B. and A.K.) and the Flemish 'Fonds voor Wetenschappelijk Onderzoek' (FWO G-0267.04) (J.B.) provided financial support to J.B. and A.K.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.01.049.

References and notes

1. Reichard, P. *Ann. Rev. Biochem.* **1988**, *57*, 349.
2. Arner, E. S. J.; Eriksson, S. *Pharmacol. Ther.* **1995**, *67*, 155.
3. Zhu, C. Y.; Johansson, M.; Permert, J.; Karlsson, A. *J. Biol. Chem.* **1998**, *273*, 14707.
4. Hatzis, P.; Al-Madhoon, A. S.; Jullig, M.; Petrakis, T. G.; Eriksson, S.; Talianidis, I. *J. Biol. Chem.* **1998**, *273*, 30239.
5. Saada, A. *DNA Cell Biol.* **2004**, *23*, 797.
6. Saada, A.; Shaag, A.; Mandel, H.; Nevo, Y.; Eriksson, S.; Elpeleg, O. *Nat. Genet.* **2001**, *29*, 342.
7. Mandel, H.; Szargel, R.; Labay, V.; Elpeleg, O.; Saada, A.; Shalata, A.; Anbinder, Y.; Berkowitz, D.; Hartman, C.; Barak, M.; Eriksson, S.; Cohen, N. *Nat. Genet.* **2001**, *29*, 337.
8. Johansson, M.; Van Rompay, A. R.; Degreve, B.; Balzarini, J.; Karlsson, A. *J. Biol. Chem.* **1999**, *274*, 23814.
9. Balzarini, J.; Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E. *Biochem. Pharmacol.* **1988**, *37*, 2847.
10. Balzarini, J.; Herdewijn, P.; De Clercq, E. *J. Biol. Chem.* **1989**, *264*, 6127.
11. De Clercq, E.; Degreef, H.; Wildiers, J.; Dejonge, G.; Drochmans, A.; Descamps, J.; Desomer, P. *Br. Med. J.* **1980**, 1178.
12. Wang, J.; Eriksson, S. *Antimicrob. Agents Chemother.* **1996**, *40*, 1555.
13. Wildiers, J.; De Clercq, E. *Eur. J. Cancer Clin. Oncol.* **1984**, *20*, 471.
14. Colacino, J. M. *Antiviral. Res.* **1996**, *29*, 125.
15. Lewis, W.; Dalakas, M. C. *Nat. Med.* **1995**, *1*, 417.
16. Blaese, R. M.; Ishiimorita, H.; Mullen, C.; Ramsey, J.; Ram, Z.; Oldfield, E.; Culver, K. *Eur. J. Cancer* **1994**, *30*, 1190.
17. Freeman, S. M.; Whartenby, K. A.; Freeman, J. L.; Abboud, C. N.; Marrogi, A. J. *Semin. Oncol.* **1996**, *23*, 31.
18. Hernandez, A.-I.; Balzarini, J.; Roche, P.; Esnouf, R.; Karlsson, A.; Camarasa, M. J.; Perez-Perez, M. J. *Mol. Pharmacol.* **2003**, *63*, 263.
19. Hernandez, A.-I.; Balzarini, J.; Karlsson, A.; Camarasa, M. J.; Perez-Perez, M. J. *J. Med. Chem.* **2002**, *45*, 4254.
20. Hernandez, A.-I.; Balzarini, J.; Rodriguez-Barrios, F.; San-Felix, A.; Karlsson, A.; Gago, F.; Camarasa, M. J.; Perez-Perez, M. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3027.
21. Balzarini, J.; Zhu, C.-Y.; De Clercq, E.; Perez-Perez, M. J.; Chamorro, C.; Camarasa, M. J.; Karlsson, A. *J. Biochem.* **2000**, *351*, 167.
22. Balzarini, J.; Degreve, B.; Zhu, C.; Durini, E.; Porcu, L.; De Clercq, E.; Karlsson, A.; Manfredini, S. *Biochem. Pharmacol.* **2001**, *61*, 727.
23. Manfredini, S.; Baraldi, P. G.; Durini, E.; Porcu, L.; Angusti, A.; Vertuani, S.; Solaroli, N.; De Clercq, E.; Karlsson, A.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1329.
24. Manfredini, S.; Baraldi, P. G.; Bazzanini, R.; Bortolotti, F.; Vertuani, S.; Assida, N.; Machida, H. *Antiviral Chem. Chemother.* **1998**, *9*, 25.
25. Vorbrüggen, H.; Krolikiewicz, K.; Bennue, B. *Chem. Ber.* **1981**, *114*, 1234.
26. Vorbrüggen, H.; Bennue, B. *Chem. Ber.* **1981**, *114*, 1279.
27. Schinazi, R. F.; Chen, S. M.; Prusoff, W. H. *J. Med. Chem.* **1979**, *22*, 1273.
28. M Manfredini, S.; Baraldi, P.-G.; Durini, E.; Vertuani, S.; Balzarini, J.; De Clercq, E.; Karlsson, A.; Buzzoni, V.; Thelander, L. *J. Med. Chem.* **1999**, *42*, 3243.
29. Reese, C. B.; Ubasawa, A. *Tetrahedron Lett.* **1980**, *21*, 2265.
30. Matsuda, A.; Yasuoka, J.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 999.
31. Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron* **1984**, *40*, 125.
32. Davisson, V. J.; Davis, D. R.; Dixit, V. M.; Poulter, C. D. *J. Org. Chem.* **1987**, *52*, 1794.
33. Munch-Petersen, B.; Cloos, L.; Tyrsted, G.; Eriksson, S. *J. Biol. Chem.* **1991**, *266*, 9032.
34. Robinson, B. H. *Methods Enzymol.* **1996**, *264*, 454.
35. Kawasaki, A. M.; Casper, M. D.; Freier, S. M.; Lesnik, E. A.; Zounes, M. C.; Cummins, L. L.; Gonzales, C.; Dan Cook, P. *J. Med. Chem.* **1993**, *36*, 831.
36. Saada, A.; Shaag, A.; Elpeleg, O. *Mol. Genet. Metab.* **2003**, *79*, 1.
37. Wharton, D. C.; Tzagoloff, A. *Methods Enzymol.* **1967**, *10*, 245.
38. Fischer, J. C.; Ruitenbeek, W.; Trijbels, J. M.; Veerkamp, J. H.; Stadhouders, A. M.; Sengers, R. C.; Janssen, A. J. *Clin. Chim. Acta* **1986**, *155*, 263.
39. Pogozelski, W. K.; Hamel, C. J.; Woeller, C. F.; Jackson, W. E.; Zullo, S. J.; Fischel-Ghodsian, N.; Blakely, W. F. *Mitochondrion* **2003**, *2*, 415.