Bioorganic & Medicinal Chemistry 17 (2009) 6340–6347

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Special feature of mixed phosphotriester derivatives of cytarabine

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ARTICLE INFO

Article history:
Received 9 March 2009
Revised 10 July 2009
Accepted 16 July 2009
Available online 23 July 2009

Keywords:
Cytotoxic nucleoside
araC
Prodrug
Biotransformation

ABSTRACT

Despite the unquestionable therapeutic interest of bis(SATE) pronucleotides, a presystemic metabolism preventing the delivery of the prodrugs in target cancer cells or tumours may constitute a limitation to the in vivo development of such derivatives. In order to overcome these drawbacks several strategies have been envisaged and we report herein the application of the *S*-acyl-2-thioethyl (SATE) phenyl pronucleotide approach to the well-known cytotoxic nucleoside cytosine-1-β-D-arabinofuranoside (cytarabine, araC). We describe modifications of the SATE moieties with the introduction of polar groups on the acyl residue, in order to study how these changes affect antitumoral activity and metabolic stability. Two different synthetic pathways were explored and lead to obtain the corresponding mixed derivatives in satisfactory yields. Cytotoxicity was studied in murine leukaemia cells L1210 as well as in cells derived from solid human tumours (Messa and MCF7). Biological evaluation of these compounds in cell culture experiments with nucleoside analogue-sensitive and resistant cell lines showed that the modified compounds were active at higher concentrations than unmodified cytarabine, yet were much able to partially reverse resistance due to deficient nucleoside transport or activation. These results can be correlated with an incomplete decomposition mechanism into the corresponding 5′-mononucleotide.

1. Introduction

Cytarabine (araC) is a cytotoxic nucleoside used clinically for the treatment of acute myelocytic leukaemia (AML). Like most nucleoside analogues, araC is inactive by itself and requires phosphorylation to the corresponding triphosphate (araCTP) to exert its antineoplastic activity by inhibition of nucleic acid biosynthesis. Variations in metabolic enzymes and membrane transporters have been shown to alter the cytotoxicity of araC, and different strategies allowing regaining activity have been developed.[1] During the last decade, we and other groups have investigated, especially in the field of antiviral nucleoside analogues,[2–5] the use of mononucleotide prodrugs (namely pronucleotides), bearing the *S*-acyl-2-thioethyl (SATE) biolabile phosphate protection.[6,7] Such entities were designed to give rise to the intracellular delivery of the 5′-mononucleotide (i.e., nucleoside 5′-monophosphate), which is further metabolized to the corresponding triphosphate analogue to exert activity. Such molecules should therefore be toxic on nucleoside analogue-resistant cancer cells with clinically relevant resistance mechanisms such as altered membrane transport or decreased deoxyribonucleoside kinase activity. Recently, the bis(SATE) approach was applied to araC and the resulting prodrug (Fig. 1, compound **1**) was able to partially reverse in vitro resistance.[8–10] Despite the unquestionable therapeutic interest of bis(SATE) pronucleotides,[11–13] a presystemic metabolism,[14,15] preventing the delivery of the prodrugs in target cancer cells or tumours, may constitute a limitation to the in vivo development of such derivatives. In order to overcome these drawbacks several strategies have been envisaged.[6] The first one consists in increasing the enzymatic stability of the esterase-labile phosphate protections by the introduction of various modifications on the *t*BuSATE group, especially polar functions.[16] Another possibility is to use a specific enzymatic activation process in order to target cells or tissues according to preferential transport and distribution or to obtain selective bio-activation.[17,18] Attempts illustrating these latter strategies have been reported in the literature and led to the design of mixed SATE phosphoesters.[6,19,20]

On the basis of these results, we investigated other series of prodrugs combining both approaches (i.e., introduction of polar function on the *t*BuSATE pro-moiety and involvement of two different enzymatic systems in the decomposition process, i.e., esterase activity and phosphodiesterase activity, Scheme 2), which have been firstly experimented on anti-retroviral nucleoside analogues such as 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine).[19,21]

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doi:10.1016/j.bmc.2009.07.038

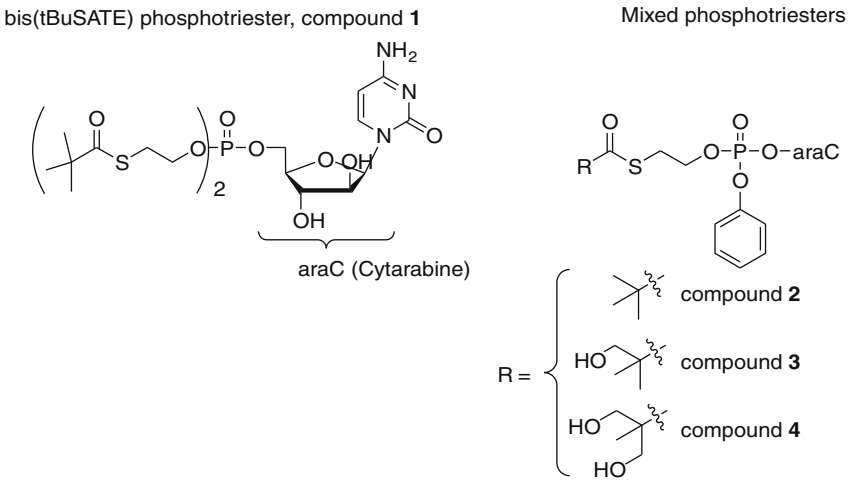


Figure 1. Chemical structures of araC prodrugs.

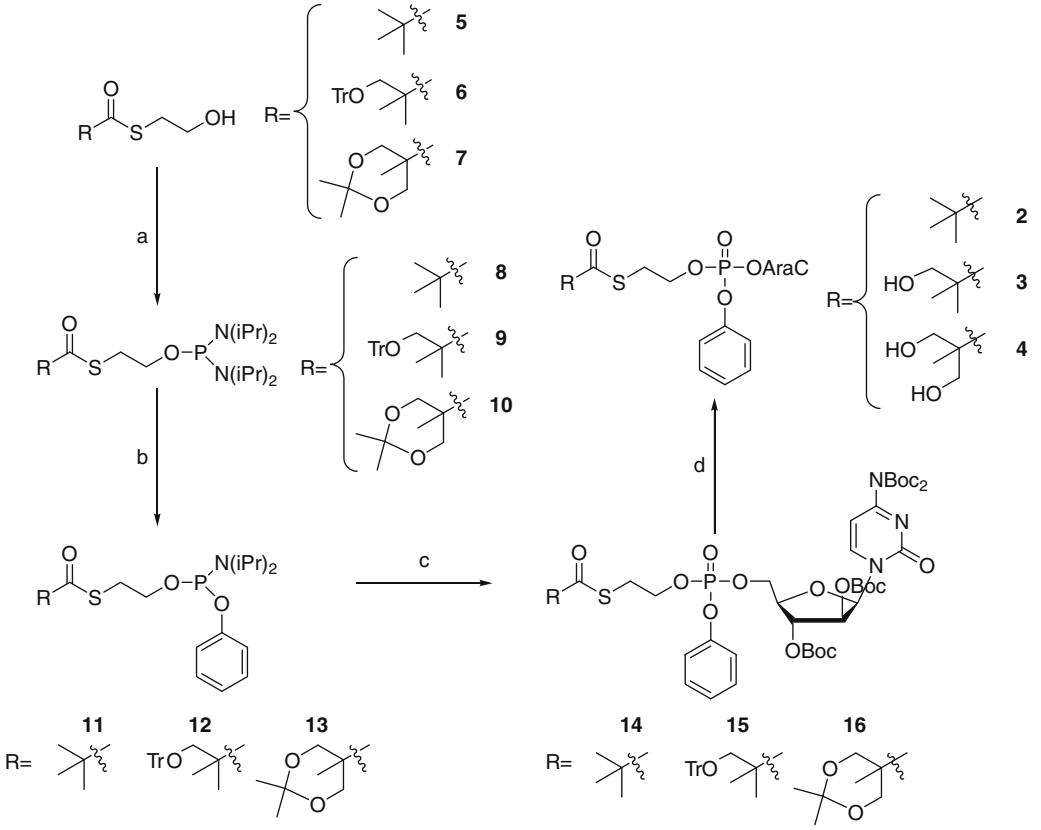
Thus, we have recently reported the enhancement of the biological profile of the corresponding phenyl SATE phosphotriester derivatives of AZT by modifications of the SATE moiety.[22] Here, we continue our search of biologically active pronucleotides by applying this approach to the cytotoxic nucleoside analogue araC (Fig. 1, compounds **2**–**4**).

2. Results and discussion

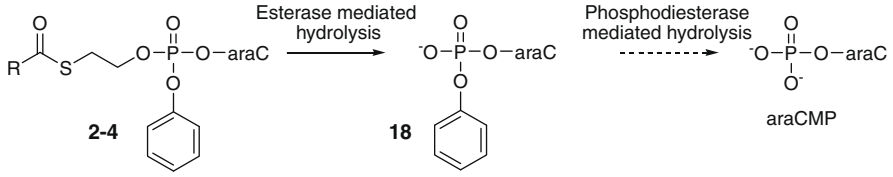
2.1. Chemistry

Synthesis of derivatives **2–4** (Fig. 1) may be performed using either P^V (phosphochloridate) or P^{III} (phosphoramidite) chemistry and both synthetic pathways were explored and compared. While prodrug formation with the two kinds of phosphorylating reagents (phosphochloridate[22] or phosphoramidate[21]) was observed, yields significantly decreased as the scale increased when using phosphochloridate intermediates. Thus, the use of phosphoramidite as phosphorylating reagents led to the desired derivatives in 19–29% overall yields, in four steps. Furthermore, the P^{III} approach allows introducing variable aryl substituents whereas the one using P^V is restricted to the use of the commercially available phenyl phosphorodichloridate.

Briefly, preparation of the required thioesters (compounds **5–7**, Scheme 1) was accomplished as previously described,[22] using



Scheme 1. Reagents and conditions: (a) Cl-P[N(*i*Pr)$_2$]$_2$, NEt$_3$, Et$_2$O, 0 °C, 2 h, 60–90%; (b) phenol, 1-*H*-tetrazole, HN(*i*Pr)$_2$, CH$_3$CN, 85%; (c) tetra(Boc)araC derivative, 1-*H*-tetrazole, CH$_3$CN, 2 h, then *t*BuOOH (1 M) in decan, 0 °C, 2 h, 42–56%; (d) TFA 25% in CH$_2$Cl$_2$, 0 °C then rt, 2 h, 55–90%.



Scheme 2. Expected decomposition pathways for SATE phenyl phosphotriester derivatives of araC **2**–**4** in biological media.

either pivaloyl chloride, 2,2-bis(hydroxymethyl)propionic acid or methyl-2, 2-dimethyl-3-hydroxypropionate as starting material.

Thus, phenyl SATE phosphotriester derivatives **2**–**4** could be accessible in four steps from the corresponding thioester precursors (Scheme 1). The phosphoramidite strategy has been often used for the synthesis of SATE pronucleotides.[19,21] It involves the preparation of the mixed phosphorylating agent bearing the two moieties, that is, the aryl and the SATE counter-parts. In this respect the bis(*N*,*N*-diisopropyl) phosphorochloridate was reacted with thioesters **5–7**, leading to the corresponding bis(*N*,*N*-diisopropyl) SATE phosphoramidite **8**–**10**, which are then coupled to phenol in presence of 1-*H*-tetrazole. Treatment of mixed phosphoramidites **11**–**13** with the protected cytarabine derivative (1-[4-*N*,*N*-2′,3′-*O*-tetra-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosyl] cytosine (abbreviated as tetra(Boc)-araC)[10] in presence of 1-*H*-tetrazole, followed by in situ oxidation of the resulting phosphite triester with *tert*-butyl hydroperoxide afforded after purification by column chromatography the desired protected phosphotriester derivatives **14**–**16** in moderate yields. Finally, removal of the protecting groups using a 25% solution of trifluoroacetic acid in dichloromethane furnished the targeted SATE phenyl phosphotriester derivatives of araC, **2**–**4**. Due to the chirality of the phosphorus centre, derivatives **14**–**16** and **2**–**4** were isolated as a mixture of two diastereoisomers (ratio: 1/1) as evidenced from their ^{31}P NMR spectra.

2.2. Cell-culture evaluation data

The cytotoxicity of mixed derivatives **2–4** was determined and compared to the activity of araC and compound **1** on nucleoside analogue-sensitive and resistant cells, using the MTT assay as described in Section 4 (Tables 1 and 2). Except for compound **2** on MCF7 cells, the pronucleotides were less active than araC in all cell lines. We confirmed the reversion of araC-resistance in dCK-deficient L1210 10K cells with compound **1**, and observed a slightly better activity of compound **2** than araC on these cells. On the human leukaemia cells CCRF-CEM, compound **2** presented a slightly better activity than araC and compound **1** on the nucleoside transporter-deficient cell line CEM/ARAC08. Compound **2** also showed interesting activity on Messa cells, as it was 7.6-fold less active than araC on parental Messa cells and 66-fold more active on dCK-deficient Messa 10K cells, resulting in a statistically significant 345-fold decrease in the resistance ratio as compared to araC. On MCF7 1K cells, in which the resistance to nucleoside analogues is due to increased ribonucleotide reductase (RNR) activity, compound **2** had approximately the same IC_{50} as araC. Finally, the mono- and dihydroxylated derivatives **3** and **4** were less active than araC and compound **2**, and did not show significant reversion of araC-resistance in any cell model studied.

These results showed some discrepancy with the previous data obtained with antiviral agents such as AZT containing SATE phenyl pronucleotides.[21] This lower anti-tumour activity observed in the cytarabine series may be due to the poor cell membrane penetration of the parent compounds **2–4**, a slow kinetic of decomposition, or an unexpected decomposition mechanism that hampered the intracellular formation of the 5′-mononucleotide (araCMP, Scheme 2). In order to explain our observations, Log *P* values were determined and stability studies in biological media were performed (Table 3). Concerning the Log *P* value which is commonly considered as a predictive factor for the passive diffusion of a compound through cell membranes, it should be in the range of 2–4. Thus, while the values obtained for compounds **1** and **2** (2.6 and 1.9, respectively) are in the required range, it is not the case of derivatives **3** and **4** (0.58 and −0.35, respectively) which appeared as hydrophilic compounds. This may reflect reduced cell penetration through a passive diffusion process of these two polar derivatives, resulting in a low cytotoxic activity as compared to the transporter-dependent penetration of cytarabine.

2.3. Stability studies and decomposition pathways

In view of the biological evaluation results, kinetics and decomposition pathways of compounds **1**–**4** were studied in total cell extracts (TCE) from L1210 cells. This would give indications on the intracellular behaviour of our compounds, and experiments were performed by LC/MS coupling.

Stability studies shown that all compounds lost the SATE group as a first step of the decomposition pathways leading to the expected phenylphosphodiester for derivatives **2**–**4** (Scheme 2), and that the presence of a polar group led to an increased stability of the parent phosphotriester towards esterase hydrolysis (Table 3). Thus, the mono- and dihydroxylated derivatives **3** and **4** are

Table 1
Sensitivity (IC_{50} in μM) for araC and compounds **1–4** in nucleoside analogue-sensitive and resistant cancer cell lines as determined by the cytotoxicity assay

Described mechanism of resistance	CCRF-CEM	CEM/ARAC8C Deficiency in nucleoside transport[26]	L1210 wt	L1210 10K Deficiency in dCK[9]	Messa wt	Messa 10K Deficiency in dCK[27]	MCF7 wt	MCF7 1K Increased expression and activity of RNR[28]
AraC	0.001 ± 0.000	100[c] ± 0	0.00040 ± 0.00015	103 ± 50	1.7 ± 0.3	6333[a] ± 2028	0.0040 ± 0.0000	0.040[b] ± 0.006
1	0.097 ± 0.055	77[b] ± 12	0.020 ± 0.006	4.7[b] ± 0.7	nd	nd	nd	nd
2	0.017 ± 0.003	55[a] ± 16	0.010 ± 0.000	43[b] ± 7	13 ± 8	93[b] ± 7	0.00032 ± 0.00024	0.083 ± 0.058
3	0.056 ± 0.012	77[a] ± 23	0.19 ± 0.07	>30	19 ± 7	>30	0.024 ± 0.014	0.37[a] ± 0.12
4	0.12 ± 0.04	77[a] ± 23	0.13 ± 0.03	>30	6 ± 3	>30	0.0049 ± 0.0027	0.23 ± 0.09

Data are mean values of three single experiments ± standard error. Nd stands for not determined.
[a] p <0.05.
[b] p <0.01.
[c] p <0.001 when compared to IC_{50} of the corresponding parental cell line with Student's t-test.

Table 2
Resistance ratios (RR) for araC and compounds **1**–**4** in nucleoside analogue-sensitive and resistant cancer cell lines

RR	CCRF-CEM	L1210	Messa	MCF7
AraC	100,000	257,500	3725	10
1	794	235	nd	nd
2	3235	4300	7.2	259
3	1375	>158	>1.6	15
4	641	>231	>5	47

RR corresponds to IC_{50} of resistant cells divided by IC_{50} of parental cells.

Table 3
Log *P* values[a] and calculated half-lives ($t_{1/2}$) of derivatives **1**–**4** in total cell extracts (TCE) from parental (WT) and resistant (10 K) L1210 cell lines[b]

Media/compound	**1**	**2**	**3**	**4**
Log *P*	2.6 ± 0.05	1.9 ± 0.01	0.58 ± 0.006	−0.35 ± 0.004
TCE from L1210 wt	3.9 h	8.3 h	3.2 d	10.7 d
TCE from L1210 10K	5.3 h	8.3 h	3.5 d	12.0 d

[a] Data are mean values of six single experiments ± standard error.
[b] All data represent an average values for three separate experiments and variability was lower than 10%.

exhibiting half-lives of 3–12 days in comparison to 4–8 h for the hydrophobic derivatives **1** and **2**.

The decomposition pathway of compound **1** differs from derivatives **2**–**4** after removal of the SATE moiety (Scheme 2). Indeed, prodrug **1** was metabolized into araCMP after esterase-mediated hydrolysis steps in TCE, whereas SATE phenyl phosphotriesters of araC evolved to a stable metabolite, identified as the corresponding phenyl phosphodiester derivative (compound **18**, Scheme 3).

These unexpected results prompted us to synthesize and to study the affinity of metabolite **18** towards purified snake venom phosphodiesterase (SVP) which is commonly used as representative for the type I phosphodiesterase family, and to compare its behaviour to the corresponding AZT analogue **19** as well as to the commercially available well-known SVP-substrate *p*-nitrophenyl thymidinyl phosphate.[23,24] Both phenyl phosphodiester derivatives of araC and AZT (respectively compounds **17** and **19**, Scheme 4) were obtained by coupling the commercial phenyl phosphorodichloridate with either AZT or protected araC (tetra-(Boc)araC) in presence of triethylamine and subsequent hydrolysis of the mixed phosphorochloridate.[25] Removal of protecting groups from **17** led to isolated **18** in high yields.

All three derivatives were incubated in presence of purified SVP at 37 °C and gave rise to the formation of the corresponding nucleoside 5′-monophosphate with half-lives of 4.6 h, 53 min and 43 min, respectively, for **18**, **19** and *p*-nitrophenyl thymidinyl phosphate. Thus, in agreement with literature data showing that 2′-deoxynucleotides were hydrolyzed faster than the corresponding ribonucleotides, the araC metabolite appeared as a poor substrate of SVP compared to AZT or thymidine.[23] Nevertheless, we observed the enzymatic conversion of phenyl phosphodiester of araC **18** into araCMP as we early hypothesized.

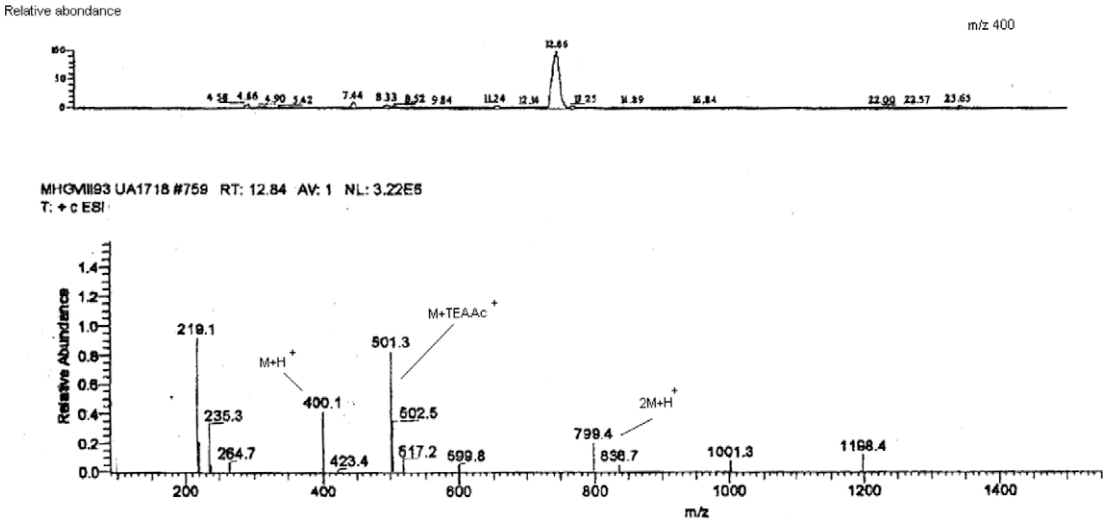
3. Conclusion

A new series of SATE phenyl phosphotriester derivatives of araC was synthesized. Their cytotoxicity as well as the kinetic and decomposition mechanism in biological media was determined. Conversely to what was observed using AZT as nucleosidic model, araC derivatives were active at significantly higher concentrations than the parental compound, but were much more efficient that the latter in lines deficient for nucleoside transport or activation. These results may be supported by an incomplete decomposition mechanism of the prodrugs, low diffusion through cell membrane and inadequate kinetics for cell-culture evaluation. However, the discrepancies of biological behaviour between araC and AZT derivatives suggest that in order to evaluate the potentiality of this prodrug-approach, it should be applied to another and less hydrophilic cytotoxic nucleoside analogue such as gemcitabine.

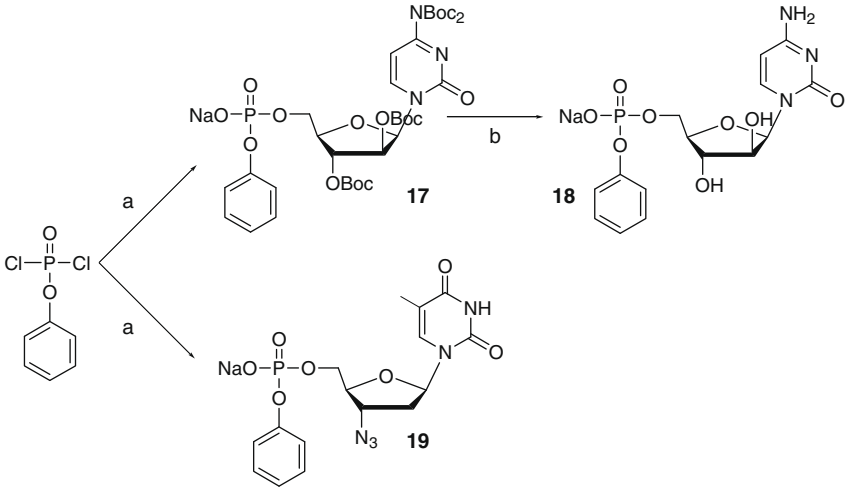
4. Experimental

4.1. Chemistry

Unless otherwise stated, ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 or 100 MHz with proton decoupling at 25 °C on a Bruker 300 Avance or DRX 400, respectively. Chemical shifts are given in δ values referenced to the residual solvent peak ($CDCl_3$ at 7.26 ppm and 77 ppm, DMSO-d_6 at 2.49 ppm and 39.5 ppm) relative to TMS. Deuterium exchange, decoupling and COSY experiments were performed in order to confirm proton assignments. Coupling constants, *J*, are reported in



Scheme 3. Typical extracted-ion chromatogram obtained after incubation of compound **3** in TCE for 30 h and corresponding positive-ion ESI spectra of the observed metabolite (MW = 399).



Scheme 4. Reagents and conditions: (a) AZT or tetra(Boc)araC, NEt_3, THF, −78 °C to rt, 4 h, then acetone, Dowex Na^+, 41–47%; (b) TFA 25% in CH_2Cl_2, 0 °C to rt, 1 h, then Dowex Na^+, 85%.

hertz. 2D ^1H–^{13}C heteronuclear COSY were recorded for the attribution of ^{13}C signals. Unless otherwise stated, ^{31}P NMR spectra were recorded at ambient temperature at 121 MHz with proton decoupling. Chemical shifts are reported relative to external H_3PO_4. FAB mass spectra were recorded in the positive-ion or negative-ion mode on a JEOL JMS DX 300 using thioglycerol/glycerol (1:1, v/v, G–T) as matrix. Melting points were determined in open capillary tubes on a Büchi-545 and are uncorrected. UV spectra were recorded in ethanol 95 on an Uvikon 931 (Kontron). Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). TLC was performed on precoated aluminium sheets of Silica Gel 60 F_{254} (Merck, Art. 9385), visualisation of products being accomplished by UV absorbance followed by charring with 5% ethanolic sulfuric acid with heating for nucleotides. Flash chromatography was carried out using 63–100 μm silica gel (Merck Art. No. 115101) otherwise 40–63 μm silica gel (Merck Art. No. 109385) was used. Thin layer chromatography was carried out using aluminium supported Silica Gel 60 plates (Merck Art. No. 105554). Solvents were reagent grade or purified by distillation prior to use, and solids were dried over P_2O_5 under reduced pressure at rt. Moisture sensitive reactions were performed under argon atmosphere using oven-dried glassware. All aqueous (aq) solutions were saturated with the specified salt unless otherwise indicated. Organic solutions were dried over Na_2SO_4 after workup and solvents were removed by evaporation at reduced pressure.

Analytical HPLC studies were carried out on an Alliance 2690 system (Waters, Milford, Massachusetts, USA) equipped with 996 photodiode detector and a Millennium data workstation. A reverse-phase analytical column (Hypersil BDS, C_{18}, 100 × 4.6 mm, 3 μm) equipped with a prefilter, and a precolumn (DeltaPak Waters, C_{18}, 5 μm) were used. Detection was monitored at 267 nm. Nucleotidic derivatives were eluted using a linear gradient of 0–80% acetonitrile in 20 mm triethylammonium acetate buffer (pH 6.9) over 20 min at 1 mL/min flow rate. Electrospray ionisation-mass spectrometry (ESI-MS) was performed using a SSQ 7000 single quadrupole mass spectrometer (Finnigan, San Jose, California, USA) in the negative-ion mode with a spray voltage at−4.5 kV. The capillary temperature was maintained at 250 °C. Nitrogen served both as sheath gas (operating pressure of 550 KPa) and as auxiliary gas with a flow rate of 103 KPa. Under these conditions, full scan data acquisition was performed from m/z 200 to 800 in centroïd mode and using a cycle time of 1.0 s. AraC was from Intsel Chimos; bis(*N,N*-diisopropyl) phosphorochloridate was purchased from Acros. Bis(*t*BuSATE) phosphotriester derivative of araC **1** and tetra (Boc) araC were synthesized following previously published procedures.

4.1.1. Standard procedure 1: preparation of SATE di(*N,N*-diisopropylamino) phosphoramidites (8–10)

To a solution of bis(*N,N*-diisopropyl) phosphorochloridate (1 equiv) in anhydrous diethyl ether (3 mL/mmol) at 0 °C, a solution of **5** (3.0 g, 18.77 mmol) or **6** (1.0 g, 2.38 mmol), or **7** (0.6 g, 2.38 mmol) and triethylamine (1.1 equiv) in anhydrous diethyl ether (2 mL/mmol) was added drop wise. The reaction mixture was stirred 2 h at room temperature, and then filtrated, and the filtrate was concentrated under vacuum. The corresponding oily residue was purified by column silica gel chromatography using cyclohexane/ethyl acetate/triethylamine (90/9/1, v/v/v) as eluent.

4.1.2. *S*-Pivaloyl-2-thioethyl di(*N,N*-diisopropylamino) phosphoramidite (8)

6.6 g (90%) of the desired compound were obtained as colourless oil.

R_f 0.7 (cyclohexane/AcOEt/Et_3N, 8/1/1, v/v). NMR ^1H (DMSO-d_6, 400 MHz) δ 3.55 (m, 2H, CH_2O), 3.52–3.42 (m, 4H, $(CH_3)_2CH$N), 3.02 (t, 2H, J = 6.2, SCH_2), 1.16 (1s, 9H, C(CH_3)$_3$), 1.11, 1.09 (2d, 24H, J = 6.5, $(CH_3)_2$CHN). NMR ^{13}C (DMSO-d_6, 100 MHz) δ 205.4 (C=O), 62.3 (d, *C*H_2O), 45.9 (*C*(CH$_3$)$_3$), 43.8 (d, $(CH_3)_2$*C*HN), 29.9 (d, SCH_2), 26.9 (C(CH_3)$_3$), 24.3, 23.5 (2d, $(CH_3)_2$CHN). NMR ^{31}P (DMSO-d_6, 81 MHz) δ 125.5. MS FAB >0 (GT) *m/z* 393 $(M+H)^+$, 145 $(t$BuCOSCH$_2$CH$_2)^+$, 85 $(t$BuCO$)^+$, 57 $(t$Bu$)^+$; FAB <0 (GT) *m/z* 247 $(M-t$BuSATE$)^-$, 117 $(t$BuCOS$)^-$. Anal. Calcd for $C_{19}H_{41}N_2O_2PS$: C, 58.12; H, 10.53; N, 7.14. Found: C, 57.99; H, 10.31; N, 7.32.

4.1.3. *S*-(2,2-Dimethyl-3-triphenylmethoxypropionyl)-2-thioethyl, di(*N,N*-diisopropylamino) phosphorobisamidite (9)

1.4 g (90%) of the desired compound was obtained as colourless oil.

R_f 0.8 (cyclohexane/AcOEt/Et_3N, 8/1/1, v/v). NMR ^1H (DMSO-d_6, 300 MHz) δ 7.29 (m, 15H, Ph), 3.59 (m, 2H, CH_2O), 3.48 (m, 4H, $(CH_3)_2CH$N), 3.05 (m, 4H, TrOCH_2, SCH_2), 1.40 (1s, 6H, C(CH_3)$_2$), 1.11, 1.09 (2d, 24H, $(CH_3)_2$CHN). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 204.0 (C=O), 128.2, 127.8, 127.0 (3s, Ph), 69.5 (TrO*C*H$_2$), 62.3 (d, *C*H_2O), 50.2 (*C*(CH$_3$)$_2$), 43.8 (d, $(CH_3)_2$CHN), 29.8 (SCH_2), 26.3 (C(CH_3)$_2$), 24.3, 23.5 (2d, $(CH_3)_2$CHN). NMR ^{31}P (DMSO-d_6, 121 MHz) δ 124.8. MS FAB >0 (GT) *m/z* 651 $(M+H)^+$, 243 $(Tr)^+$. Anal. Calcd for $C_{38}H_{55}N_2O_3PS$: C, 70.12; H, 8.52; P, 4.76. Found: C, 70.35; H, 8.66; P, 4.27.

4.1.4. *S*-[2-(2,2-Dimethyl-1,3-dioxan-5-yl)propionyl]-2-thioethyl, di(*N,N*-diisopropylamino) phosphorobisamidite (10)

0.67 g (61%) of the desired compound was obtained as colourless oil.

R_f 0.8 (cyclohexane/AcOEt/Et_3N, 8/1/1, v/v). NMR ^1H (DMSO-d_6, 300 MHz) δ 4.15 (d, 2H, J = 12.1, OCH_2), 3.61 (m, 4H, OCH_2, CH_2O), 3.42 (m, 4H, $(CH_3)_2CHN$), 3.10 (t, 2H, J = 6.2, SCH_2), 1.33, 1.31 (2s, 6H, $C(CH_3)_2$), 1.20 (s, 3H, CH_3), 1.10, 1.08 (2d, 24H, $(CH_3)_2CHN$). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 202.0 (C=O), 97.5 ($C(CH_3)_2$), 65.2 (OCH_2), 62.2 (d, CH_2O), 48.4 ($C(CH_3)$), 43.8 (d, $(CH_3)_2CHN$), 29.8 (d, SCH_2), 26.3, 25.3 (2s, $C(CH_3)_2$), 24.3, 23.5 (2d, $(CH_3)_2CHN$), 18.8 (CH_3). NMR ^{31}P (DMSO-d_6, 121 MHz) δ 124.3. MS FAB >0 (GT) m/z 465 $(M+H)^+$, 364 $(M-NiPr_2+H)^+$, 100 $(NiPr)^+$, 43 $(iPr)^+$, FAB <0 (GT) m/z 362 $(M-NiPr_2-H)^-$. Anal. Calcd for $C_{22}H_{45}N_2O_4PS$: C, 56.87; H, 9.76; N, 6.03. Found: C, 56.56; H, 9.72; N, 5.77.

4.1.5. Standard procedure 2: preparation of phenyl SATE (*N,N*-diisopropylamino) phosphoramidites (11–13)

To a solution of phenol (0.2 g, 2.55 mmol) in anhydrous acetonitrile (14 mL) at 0 °C, in presence of molecular sieve 3 Å (2.5 g), was successively added anhydrous diisopropylamine (0.72 mL, 5.10 mmol), 1-*H*-tetrazole (0.45 M solution in acetonitrile, 11.3 mL, 5.10 mmol) and the required bisphosphoramidite **8**, or **9**, or **10** (3.82 mmol). After 15 min at 0 °C, the reaction mixture was stirred for 2 h at room temperature and then 100 mL of ethyl acetate were added. The resulting solution was washed with brine and then water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography using cyclohexane/ethyl acetate/triethylamine (97/2/1, v/v/v) as eluent to give the mixed phenyl SATE (*N,N*-diisopropylamino) phosphoramidites **11**–**13** (≈85%) as colourless oil.

4.1.6. *O*-Phenyl *S*-pivaloyl-2-thioethyl *N,N*-diisopropylamino phosphoramidite (11)

R_f 0.8 (cyclohexane/AcOEt/Et_3N, 8/1/1, v/v/v). NMR ^1H (DMSO-d_6, 300 MHz) δ 7.29 (m, 2H, Ph), 7.00 (m, 3H, Ph), 3.70 (m, 4H, CH_2O, $(CH_3)_2CHN$), 3.09 (t, 2H, J = 6.4, SCH_2), 1.40 (s, 9H, *t*Bu), 1.17, 1.11 (2d, 12H, J = 6.8, $(CH_3)_2CHN$). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 205.3 (C=O), 154.1 (Ph ipso), 129.4, 122.3, 119.7, 119.6 (4s, Ph), 62.0 (d, CH_2O), 45.9 ($C(CH_3)_3$), 42.9 (d, $(CH_3)_2CHN$), 29.4 (d, SCH_2), 26.9, 26.8, 26.7 (3s, $C(CH_3)_3$), 24.3, 24.1 (2d, $(CH_3)_2CHN$). NMR ^{31}P (DMSO-d_6, 121 MHz) δ 145.9. MS FAB >0 (GT) m/z 243 $(M-tBuSATE+H)^+$, 145 $(tBuSATE)^+$, 57 $(tBu)^+$, FAB <0 (GT) m/z 383 $(M-H)^-$, 291 $(M-PhO-H)^-$. Anal. Calcd for $C_{19}H_{32}NO_3PS$: C, 59.20; H, 8.37; N, 3.63. Found: C, 59.30; H, 8.63; N, 3.51.

4.1.7. *O*-Phenyl *S*-(2,2-dimethyl-3-triphenylmethoxypropionyl)-2-thioethyl *N,N*-diisopropyl- aminophosphoramidite (12)

R_f 0.8 (cyclohexane/AcOEt/Et_3N, 8/1/1, v/v/v). NMR ^1H (DMSO-d_6, 300 MHz) δ 7.29 (m, 17H, Ph, Tr), 7.00 (m, 3H, Ph), 3.71 (m, 4H, CH_2O, $(CH_3)_2CHN$), 3.12 (t, 2H, SCH_2), 3.05 (s, 2H, $TrOCH_2$), 1.17 (s, 6H, $C(CH_3)_2$), 1.11, 1.09 (2s, 12H, $(CH_3)_2CHN$). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 205.3 (C=O), 155.7 (Ph ipso), 145.1, 131.1, 129.8, 129.4, 128.6 124.0, 121.4 (7s, Ph), 87.4 ($(Ph)_3C$), 71.2 ($TrOCH_2$), 63.7 (d, CH_2O), 51.9 ($C(CH_3)_2$), 44.5 (d, $(CH_3)_2CHN$), 30.9 (d, SCH_2), 25.9 (d, $(CH_3)_2CHN$), 24.0 ($C(CH_3)_2$). NMR ^{31}P (DMSO-d_6, 121 MHz) δ 145.9. MS FAB >0 (GT) m/z 643 $(M+H)^+$, 243 $(Tr)^+$, FAB <0 (GT) m/z 641 $(M-H)^-$.

4.1.8. *O*-Phenyl *S*-[2-(2,2-dimethyl-1,3-dioxan-5-yl)propionyl]-2-thioethyl *N,N*-diisopropyl-aminophosphoramidite (13)

R_f 0.8 (cyclohexane/AcOEt/Et_3N, 8/1/1, v/v/v). NMR ^1H(DMSO-d_6, 300 MHz) δ 7.29 (m, 2H, Ph), 7.00 (m, 3H, Ph), 4.08, 3.70 (2d, 4H, J = 12,1, OCH_2), 3.70 (m, 4H, CH_2CH_2O, $(CH_3)_2CHN$), 3.13 (t, 2H, J = 6.3, SCH_2), 1.37, 1.25 (2s, 6H, $C(CH_3)_2$), 1.19, 1.10 (2d, 12H, $(CH_3)_2CHN$). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 200.7 (C=O), 152.8 (Ph ipso), 128.2, 121.1, 118.5, 118.4 (4s, Ph), 96.3 ($C(CH_3)_2$), 63.9 (OCH_2), 60.6 (d, CH_2CH_2O), 47.2 ($C(CH_3)$), 41.7 (d, $(CH_3)_2CHN$), 28.1 (d, SCH_2), 24.2, 20.4 (2s, $C(CH_3)_2$), 23.0, 22.9 (2d, $(CH_3)_2CHN$), 17.5 (CH_3). NMR ^{31}P (DMSO-d_6, 121 MHz) δ 145.9. Anal. Calcd for $C_{22}H_{36}NO_5PS$: C, 57.75; H, 7.93; N, 3.06; P, 6.77; S, 7.01. Found: C, 58.14; H, 8.19; N, 3.16; P, 6.66; S, 7.36.

4.1.9. Standard procedure 3: preparation of phenyl SATE phosphotriesters (14–16)

To a solution of the protected nucleoside (1 equiv) in anhydrous acetonitrile in presence of molecular sieve 3 Å, were successively added 1*H*-tetrazole (4 equiv), the required mixed phosphoramidite (1.4 equiv), that is, compound **11** (0.5 g, 1.22 mmol), or **12** (0.9 g, 1.40 mmol), or **13** (0.6 g, 1.20 mmol). After one hour stirring at room temperature under an argon atmosphere, the reaction mixture was cooled down to 0 °C and a *tert*-butyl hydroperoxide solution (2.4 equiv, 5 M in decan) was added drop wise. The reaction proceeded for one hour at room temperature and then diluted in dichloromethane. The resulting organic layer was washed with an aqueous solution of $Na_2S_2O_3$ (10%, w/v), then brine and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified on silica gel column chromatography.

4.1.10. *O*-[4-*N,N*-2′,3′-*O*-Tetra-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosylcytosine]-*O*′-(*S*-pivaloyl-2-thioethyl)-*O*″-phenyl phosphate (14)

0.46 g (56%) of the fully protected phosphotriester **14** was obtained as yellow oil after purification on silica gel using a gradient of methanol (0–1%) in dichloromethane.

R_f 0.8 (CH_2Cl_2/AcOEt, 7/3, v/v). NMR $^{1H\ (DMSO\text{-}d_6}$, 400 MHz) δ 8.03 (d, 1H, J = 7.6, H-6), 7.43 (m, 2H, Ph), 7.39 (m, 3H, Ph), 7.25 (t, 1H, H-5), 6.27 (m, 1H, H-1′), 5.30 (m, 1H, H-2′), 5.15 (m, 1H, H-3′), 4.45 (m, 2H, H-5′, H-5″), 4.38 (m, 1H, H-4′), 4.18 (m, 2H, CH_2O), 3.15 (t, 2H, SCH_2), 1.49 (s, 18H, $C(CH_3)_3$, Boc), 1.45, 1.29 (2s, 18H, $C(CH_3)_3$, Boc), 1.15 (s, 9H, $C(CH_3)_3$, SATE). NMR ^{13}C (DMSO-d_6, 100 MHz) δ 205.0 (C=O, SATE), 161.8 (C-4), 152.6, 151.6, 150.8, 150.0 (4s, C-2 et C=O, Boc), 149.0 (C-6), 129.9 (d, Ph ipso), 125.7, 125.4, 121.5 (3s, Ph), 119.9 (d, Ph ortho), 95.2 (C-5), 84.7 (C-1′), 83.4, 83.3 (2s, $C(CH_3)_3$, Boc), 78.0 (C-4′), 77.5 (C-3′), 76.5 (C-2′), 67.1 (CH_2O), 66.5 (C-5′), 50.8 ($C(CH_3)_3$, SATE), 28.6 (SCH_2), 28.5, 28.4, 27.6, 27.4 (4s, $C(CH_3)_3$, Boc), 27.3 ($C(CH_3)_3$, SATE). NMR ^{31}P (DMSO-d_6, 100 MHz) δ −5.6, −5.7. MS FAB >0 (GT) m/z 944 $(M+H)^+$, 844 $(M-Boc+H)^+$, 744 $(M-2Boc+H)^+$, 644 $(M-3Boc+H)^+$. UV (EtOH 95%) λ_{max1} 294 nm (ε 4800), λ_{max2} 235 nm (ε 11,400).

4.1.11. *O*-[4-*N,N*-2′,3′-*O*-Tetra-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosylcytosine]-*O*′-[*S*-(2,2-dimethyl-3-triphenyl methoxypropionyl)-2-thioethyl)-*O*″-phenyl phosphate (15)

0.55 g (46%) of the fully protected phosphotriester **15** was obtained as yellow oil after purification on silica gel using a gradient of methanol (0–1%) in dichloromethane.

R_f 0.8 (CH_2Cl_2/AcOEt, 8/2, v/v). NMR ^1H (DMSO-d_6, 300 MHz) δ 8.02 (d, 1H, J = 7.4, H-6), 7.32–7.21 (m, 20H, Ph, $(Ph)_3C$), 6.83 (t, 1H, H-5), 6.25 (m, 1H, H-1′), 5.30 (m, 1H, H-2′), 5.15 (m, 1H, H-3′), 4.40 (m, 3H, H-4′, H-5′, H-5″), 4.16 (m, 2H, CH_2CH_2O), 3.19 (m, 2H, SCH_2), 3.05 (m, 2H, $TrOCH_2$), 1.59, 1.49, 1.42, 1.29 (4s, 36H, $C(CH_3)_3$, Boc), 1.15 (s, 6H, $C(CH_3)_2$, SATE). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 203.8 (C=O, SATE), 162.3 (C-4), 153.0, 152.0, 151.2, 150.4 (4s, C-2 et C=O, Boc), 149.4 (C-6), 147.2, 143.8 (2s, Ph), 128.6, 128.2, 127.5, 125.8 (4s, Ph), 120.3 (d, Ph ortho), 95.7 (C-5), 86.2 ($(Ph)_3C$), 85.7 (C-1′), 83.8, 83.7 (2s, $C(CH_3)_3$, Boc), 79.3 (C-4′), 78.0 (C-3′), 76.0 (C-2′), 69.9 ($TrOCH_2$), 67.0 (CH_2CH_2O), 66.9 (C-5′), 50.8 ($C(CH_3)_2$, SATE), 28.5 (SCH_2), 28.5, 27.8, 27.6, 27.3 (4s,

C(CH_3)$_3$, Boc), 22.7 (C(CH_3)$_2$, SATE). NMR ^{31}P (DMSO-d_6, 121 MHz) δ −6.62, −6.65. MS FAB >0 (GT) *m/z* 1202 (M+H)$^+$, 859 (M−Tr−Boc)$^+$, FAB >0 (NBA) *m/z* 1202 (M+H)$^+$, 1102 (M−Boc+H)$^+$, 1002 (M−2Boc+H)$^+$, 243 (Tr)$^+$, 77 (C_6H_5)$^+$, FAB <0 (NBA) *m/z* 1124 (M−C_6H_5)$^-$, 1024 (M−Boc−C_6H_5)$^-$, 798 (M−SATE)$^-$, 698 (M−Boc−SATE)$^-$, 575 (M−nu)$^-$. UV (EtOH 95%) λ_{max} 297 nm (ε 5200). Anal. Calcd for $C_6H_{76}N_3O_{18}PS$, 0.4 C_6H_{14}: C, 61.73; H, 6.41; N, 3.41; P, 2.51; S, 2.60. Found: C, 61.74; H, 6.71; N, 3.48; P, 2.43; S, 2.77.

4.1.12. *O*-[4-*N*,*N*-2′,3′-*O*-Tetra-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosylcytosine]-*O*′-[*S*-(2-(2,2-dimethyl-1,3-dioxan-5-yl)propionyl)-2-thioethyl]-*O*″-phenyl phosphate (16)

0.37 g (42%) of the fully protected phosphotriester **16** was obtained as yellow oil after purification on silica gel using a gradient of methanol (0–1%) in dichloromethane.

R_f 0.8 (CH_2Cl_2/AcOEt, 7/3, v/v). NMR ^{1}H (DMSO-d_6, 300 MHz) δ 8.02 (d, 1H, H-6), 7.40 (m, 2H, Ph), 7.20 (m, 3H, Ph), 6.83 (t, 1H, H-5), 6.27 (m, 1H, H-1′), 5.30 (m, 1H, H-2′), 5.15 (m, 1H, H-3′), 4.40 (m, 3H, H-4′, H-5′, H-5″), 4.20 (m, 2H, CH_2O), 4.08 (m, 2H, OCH_2), 3.71 (m, 2H, OCH_2), 3.20 (m, 2H, SCH_2), 1.49, 1.45 (2s, 27H, C(CH_3)$_3$, Boc), 1.39, 1.29 (2s, 6H, C(CH_3)$_2$), 1.28 (s, 9H, C(CH_3)$_3$, Boc), 1.05 (s, 3H, CH_3). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 201.7 (C=O, SATE), 161.8 (C-4), 152.6, 151.5, 150.8, 149.9 (4s, C-2 and C=O, Boc), 149.0 (C-6), 129.9 (Ph ipso), 125.4 (Ph), 119.9 (d, Ph ortho), 97.6 (C(CH_3)$_2$), 95.3 (C-5), 84.7 (C-1′), 83.4, 83.3 (2s, C(CH_3)$_3$, Boc), 79.0 (C-4′), 77.5 (C-3′), 76.5 (C-2′), 67.0 (CH_2CH_2O), 65.1 (C-5′), 48.5 (C(CH_3)), 27.9 (SCH_2CH_2), 27.1, 26.9 (2s, C(CH_3)$_3$, Boc), 25.6, 21.5 (2s, C(CH_3)$_2$), 18.6 (CH_3). NMR ^{31}P (DMSO-d_6, 121 MHz) δ −6.59, −6.64. MS FAB >0 (NBA) *m/z* 1015 (M)$^+$, 915 (M−Boc)$^+$, 815 (M−2Boc)$^+$, FAB <0 (NBA) *m/z* 798 (M−SATE)$^-$, 698 (M−Boc−SATE)$^-$. UV (EtOH 95%) λ_{max1} 294 nm (ε 6300), λ_{max2} 238 nm (ε 13,600).

4.1.13. Standard procedure 4: Preparation of phenyl SATE phosphotriester derivatives of araC (2–4)

To a solution of the phosphotriester derivative **15** (0.5 g, 0.53 mmol), or **16** (0.5 g, 0.46 mmol), or **17** (0.37 g, 0.36 mmol) in dichloromethane (10 mL/mmol), at 0 °C, was added drop wise a trifluoroacetic acid solution (50%, v/v) in dichloromethane (10 mL/mmol). The reaction mixture was stirred 15 min at 0 °C and then one hour at room temperature. Solvents were evaporated under vacuum and the crude was purified.

4.1.14. *O*-[β-D-Arabinofuranosylcytosine]-*O*′-(*S*-pivaloyl-2-thioethyl)-*O*″-phenyl phosphate (2)

0.19 g (67%) of targeted compound was obtained as a white powder after two columns chromatography; firstly on silica gel using a gradient of methanol (0–10%) in dichloromethane and then on reverse phase (RP 18, using a gradient of acetonitrile (0–40%) in water).

R_f 0.4 (CH_2Cl_2/MeOH, 85/15, v/v). NMR ^{1}H (DMSO-d_6, 300 MHz) δ 7.54 (t, 1H, *J* = 7.5, H-6), 7.41 (m, 2H, Ph), 7.24 (m, 3H, Ph), 7.10, 7.05 (2s, 2H, NH_2), 6.12 (m, 1H, H-1′), 5.63 (m, 3H, H-5, H-3′, OH), 4.35 (m, 2H, H-5′, H-5″), 4.19 (m, 2H, CH_2O), 3.93 (m, 3H, H-2′, H-4′, OH), 3.15 (m, 2H, SCH_2), 1.18 (s, 9H, C(CH_3)$_3$). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 205.0 (C=O, SATE), 165.6 (C-4), 155.0 (C-2), 142.7 (C-6), 129.9 (d, Ph ipso), 125.3 (Ph), 119.9 (d, Ph), 92.4 (C-5), 86.5 (C-1′), 82.5 (C-4′), 76.3 (C-3′), 74.0 (C-2′), 67.8 (C-5′), 66.3 (d, CH_2O), 46.0 (C(CH_3)$_3$, SATE), 28.0 (SCH_2), 26.8 (C(CH_3)$_3$, SATE). NMR ^{31}P (DMSO-d_6, 121 MHz) δ −6.4, −6.5. MS FAB >0 (GT) *m/z* 1087 (2 M+H)$^+$, 544 (M+H)$^+$, 112 (BH_2)$^+$, 57 (*t*Bu)$^+$. HR-MS ($C_{22}H_{31}N_3O_9PS$) calcd (M+H)$^+$: 544.1519; found: 544.1547. UV (EtOH 95%) λ_{max} 272 nm (ε 7400). Anal. Calcd for $C_{22}H_{30}N_3O_9PS$ (0.7 mol of H_2O): C, 47.51; H, 5.69; N, 7.56; P, 5.77; S, 5.57. Found: C, 47.16; H, 5.65; N, 7.46; P, 5.29; S, 5.55.

4.1.15. *O*-[β-D-Arabinofuranosylcytosine]-*O*′-[*S*-(2,2-dimethyl-3-hydroxypropionyl)-2-thioethyl]-*O*″-phenyl phosphate (3)

0.14 g (55%) of targeted compound was obtained as a white powder after two columns chromatography; firstly on silica gel using a gradient of methanol (0–10%) in dichloromethane and then on reverse phase (RP 18, using a gradient of acetonitrile (0–30%) in water).

R_f 0.4 (CH_2Cl_2/MeOH, 85/15, v/v). NMR ^{1}H (DMSO-d_6, 300 MHz) δ 7.55 (d, 1H, H-6), 7.42 (m, 2H, Ph), 7.23 (m, 3H, Ph), 7.10, 7.05 (2s, 2H, NH_2), 6.11 (d, 1H, H-1′), 5.61 (m, 3H, H-5, H-3′, OH), 4.94 (m, 1H, CH_2OH), 4.31 (m, 2H, H-5′, H-5″), 4.19 (m, 2H, CH_2O), 3.99 (m, 2H, H-2′, H-4′), 3.90 (m, 1H, OH), 3.42 (m, 2H, CH_2OH), 3.13 (m, 2H, SCH_2), 1.12 (s, 6H, C(CH_3)$_2$). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 203.9 (C=O, SATE), 165.6 (C-4), 155.0 (C-2), 142.8 (d, C-6), 129.9 (d, Ph ipso), 125.3 (Ph), 119.9 (d, Ph), 92.5 (C-5), 86.5 (C-1′), 82.5 (d, C-4′), 76.3 (C-3′), 74.0 (C-2′), 68.3 (CH_2OH), 67.9 (C-5′), 66.3 (d, CH_2O), 51.7 (C(CH_3)$_2$, SATE), 28.0 (d, SCH_2), 21.8 (C(CH_3)$_2$, SATE). NMR ^{31}P (DMSO-d_6, 121 MHz) δ −6.4, −6.5. MS FAB >0 (GT) *m/z* 1119 (2 M+H)$^+$, 560 (M+H)$^+$, FAB <0 (GT) *m/z* 558 (M−H)$^-$, 398 (M−SATE)$^-$. HR-MS ($C_{22}H_{31}N_3O_{10}PS$) (M+H)$^+$: 560.1468; found: 560.1479. UV (EtOH 95%) λ_{max1} 272 nm (ε 9800), λ_{max2} 229 (ε 12,400). Anal. Calcd for $C_{22}H_{30}N_3O_{10}PS$ (0.7 mol of H_2O): C, 46.18; H, 5.53; N, 7.34; P, 5.41. Found: C, 46.00; H, 5.47; N, 7.32; P, 5.19.

4.1.16. *O*-[β-D-Arabinofuranosylcytosine]-*O*′-[*S*-(2,2-hydroxymethyl)propionyl)-2-thioethyl]-*O*″-phenyl phosphate (4)

0.18 g (89%) of targeted compound was obtained as a white powder after chromatography on a reverse-phase column (RP 18), using a gradient of acetonitrile (0–20%) in water.

R_f 0.4 (CH_2Cl_2/MeOH, 85/15, v/v). NMR ^{1}H (DMSO-d_6, 300 MHz) δ 7.54 (t, 1H, H-6), 7.41 (m, 2H, Ph), 7.25 (m, 3H, Ph), 7.09, 7.04 (2s, 2H, NH_2), 6.11 (d, 1H, H-1′), 5.61 (m, 3H, H-5, OH-3′, OH-2′), 4.80 (m, 2H, CH_2OH), 4.40 (m, 2H, H-5′, H-5″), 4.15 (m, 2H, CH_2O), 3.99 (m, 2H, H-2′, H-3′), 3.90 (m, 1H, H-4′), 3.57, 3.47 (2 m, 4H, CH_2OH), 3.13 (m, 2H, SCH_2), 1.12 (s, 3H, CH_3). NMR ^{13}C (DMSO-d_6, 75 MHz) δ 202.5 (C=O, SATE), 165.6 (C-4), 155.0 (C-2), 142.8 (C-6), 129.9 (d, Ph ipso), 125.3 (Ph), 119.9 (d, Ph), 92.5 (C-5), 86.5 (C-1′), 82.5 (C-3′), 76.3 (C-4′), 74.0 (C-2′), 67.8 (C-5′), 66.3 (d, CH_2O), 64.3 (d, CH_2OH), 57.5 (C(CH_3)), 27.8 (SCH_2), 16.8 (CH_3). NMR ^{31}P (DMSO-d_6, 121 MHz) δ −6.38, −6.42. MS FAB >0 (GT) *m/z* 1151 (2 M+H)$^+$, 576 (M+H)$^+$, FAB <0 (GT) *m/z* 575 (M−H)$^-$. HR-MS ($C_{22}H_{31}N_3O_{11}PS$) (M+H)$^+$: 576.1417; found: 576.1414. UV (EtOH 95%) λ_{max} 274 nm (ε 10,700). Anal. Calcd for $C_{22}H_{30}N_3O_{11}PS$ (1 mol of H_2O): C, 44.52; H, 5.43; N, 7.08; P, 5.22; S, 5.40. Found: C, 44.21; H, 5.34; N, 6.95; P, 5.18; S, 5.41.

4.2. Biological methods

4.2.1. Cell culture

Human sarcoma cells Messa, human T-lymphoblastoid leukaemia cells CCRF-CEM and murine leukaemia cells L1210 were grown in RPMI media (Gibco, Cergy Pontoise, France) and human breast adenocarcinoma cells MCF7 were grown in DMEM media (Gibco, Cergy Pontoise, France), all containing L-glutamine, penicillin (200 UI/ml), streptomycin (200 μg/ml) (Gibco, Cergy Pontoise, France) and 10% foetal bovine serum (Gibco, Cergy Pontoise, France). All cells were grown at 37 °C in presence of 5% CO_2. Development and characterisation of deoxynucleoside analogue-resistant sub-cell lines of Messa (Messa 10K), CCRF-CEM (CEM/ARAC8C), L1210 (L1210 10K) and MCF7 (MCF7 1K) have been reported elsewhere.[26–28,29]

4.2.2. Cytotoxicity studies

For adherent cells (MCF7 and Messa), 3000 cells were plated per well in 96-well plates (Becton Dickinson) in a volume of 100 μl and

incubated for 24 h at 37 °C before drugs were added at different concentrations. For suspension cells (CCRF-CEM and L1210), 100 μl with 10,000 cells were added to each well of 96-well plates containing 100 μl media with different concentrations of drugs. After incubation at 37 °C for 3 days, MTT (100 μg, Sigma–Aldrich, St. Quentin Fallavier, France) was added and, after 2 h of incubation at 37 °C, the supernatant was replaced with 100 μl isopropanol/HCl/H_20 (v/v/v, 90/1/9) and spectrophotometric determination of optical density was performed using a micro plate reader (Labsystem Multiskanner RC). The inhibitory concentration 50 (IC_{50}) was defined as the concentration inhibiting proliferation to a level equal to 50% of that of controls and the resistance ratio (RR) was the ratio between the IC_{50} of the deoxynucleoside analogue-resistant cell line and the IC_{50} of the sensitive parental cell line. The statistical significance between IC_{50}s and RRs was determined using Student's *t*-test and Microsoft® Office Excel 2003 (Microsoft Corporation).

4.3. Stability studies

HPLC analyses were carried out using an improved 'On-line ISRP cleaning method'. Briefly, the crude sample (80 μl, initial concentration 50 μm) was injected onto the precolumn and eluted firstly with 20 mM triethylammonium acetate buffer (pH 6.9) for 5 min at a flow rate of 2 mL/min. Then a six port Rheodine valve was activated and a linear gradient from 0% to 80% acetonitrile over 20 min was run at 1 mL/min flow rate. In these conditions, analytes were back-flushed from the precolumn to the analytical column and eluted. The decomposition products were identified by HPLC/MS coupling after calibration and/or by co-injection with authentic samples (AraCMP, AZTMP, **18**, etc.). For each incubation time, the calculation of the relative concentration of each species was related to the peak areas, these data were considered as the experimental data. The rate constants of disappearance and the half-lives (kinetic data) of the phosphotriester and phosphodiester derivatives were calculated according to pseudo-first order kinetic models and optimized using mono- or poly-exponential regressions.

For stability studies in biological media; cell extracts were prepared and studies were performed according to published procedure.[29]

For stability studies with purified SVP from Sigma; the borate buffer (pH 9.0) was prepared following the procedure of Clark & Lub.[30] In a vial containing 950 μl of the SVP solution in the borate buffer (2.4 μg/mL) was added 50 μl of the studied compound in solution in water (1 mM initial concentration). The mixture was incubated at 37 °C. At the required times, aliquots (80 μl) were sampled and directly injected onto the previously described HPLC system.

Acknowledgments

We thank 'Association pour la Recherche contre le Cancer' (ARC, France), Astri og Birger Torsteds legat and Olav Raagholt og Gerd Meidel Raagholts stiftelse for forskning for funding. M.-H. Gouy is also grateful to MENRT (Ministère de l'Education Nationale, de la Recherche et des Technologies) for a PhD grant.

References and notes

1. Jordheim, L. P.; Dumontet, C. *Biochim. Biophys. Acta* **2007**, *1776*, 138.
2. Drontle, D. P.; Wagner, C. R. *Mini-Rev. Med. Chem.* **2004**, *4*, 409.
3. McGuigan, C.; Hassan-Abdallah, A.; Srinivasan, S.; Wang, Y.; Siddiqui, A.; Daluge, S. M.; Gudmundsson, K. S.; Zhou, H.; McLean, E. W.; Peckham, J. P.; Burnette, T. C.; Marr, H.; Hazen, R.; Condreay, L. D.; Johnson, L.; Balzarini, J. *J. Med. Chem.* **2006**, *49*, 7215.
4. Perrone, P.; Luoni, G. M.; Kelleher, M. R.; Daverio, F.; Angell, A.; Mulready, S.; Congiatu, C.; Rajyaguru, S.; Martin, J. A.; Levêque, V.; Le Pogam, S.; Najera, I.; Klumpp, K.; Smith, D. B.; McGuigan, C. *J. Med. Chem.* **2007**, *50*, 1840.
5. Meier, C.; Balzarini, J. *Antiviral Res.* **2006**, *71*, 282.
6. Jochum, A.; Schlienger, N.; Egron, D.; Peyrottes, S.; Perigaud, C. *J. Organomet. Chem.* **2005**, *690*, 2614.
7. Peyrottes, S.; Egron, D.; Lefebvre, I.; Gosselin, G.; Imbach, J. L.; Périgaud, C. *Mini-Rev. Med. Chem.* **2004**, *4*, 395.
8. Gröschel, B.; Cinatl, J.; Kotchetkov, R.; Köhl, U.; Rohrbach, E.; Kornhuber, B.; Bazzanini, R.; Périgaud, C.; Gosselin, G.; Imbach, J.-L.; Doerr, H. W.; Cinatl, J. J. In *Hematology and Blood Transfusion 39. Acute Leukemias VII. Experimental Approaches and Novel Therapies*; Hidderman, T., Ed.; Springer: Berlin, Heidelberg, 1998; p 628.
9. Jordheim, L. P.; Cros, E.; Gouy, M.-H.; Galmarini, C. M.; Peyrottes, S.; Mackey, J.; Perigaud, C.; Dumontet, C. *Clin. Cancer Res.* **2004**, *10*, 5614.
10. Bazzanini, R.; Gouy, M.-H.; Peyrottes, S.; Gosselin, G.; Périgaud, C. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 1635.
11. Placidi, L.; Faraj, A.; Loi, A. G.; Pierra, C.; Egron, D.; Cretton-Scott, E.; Gosselin, G.; Périgaud, C.; Martin, L. T.; Schinazi, R. F.; Imbach, J.-L.; El Kouni, M. H.; Bryant, M. L.; Sommadossi, J. P. *Antiviral Chem. Chemother.* **2001**, *12*, 99.
12. Périgaud, C.; Gosselin, G.; Girardet, J.-L.; Korba, B. E.; Imbach, J.-L. *Antiviral Res.* **1999**, *40*, 167.
13. Hantz, O.; Périgaud, C.; Borel, C.; Jamard, C.; Zoulim, F.; Trépo, C.; Imbach, J.-L.; Gosselin, G. *Antiviral Res.* **1999**, *40*, 179.
14. Tan, X.; Boudinot, F. D.; Chu, C. K.; Egron, D.; Perigaud, C.; Gosselin, G.; Imbach, J.-L. *Antiviral Chem. Chemother.* **2000**, *11*, 203.
15. Martin, L. T.; Cretton-Scott, E.; Placidi, L.; Faraj, A.; Loi, A. G.; Schinazi, R. F.; McClure, H. M.; Gosselin, G.; Imbach, J.-L.; Sommadossi, J. P. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 481.
16. Shafiee, M.; Deferme, S.; Villard, A.-L.; Egron, D.; Gosselin, G.; Imbach, J.-L.; Lioux, T.; Pompon, A.; Varray, S.; Aubertin, A.-M.; Van Den Mooter, G.; Kinget, R.; Périgaud, C.; Augustinjs, P. J. *J. Pharm. Sci.* **2001**, *90*, 448.
17. Boyer, S. H.; Sun, Z.; Jiang, H.; esterbook, J.; Gomez-Galeno, J. E.; Craigo, W.; reddy, K. R.; Ugarkar, B. G.; MacKenna, D.; Erion, M. D. *J. Med. Chem.* **2006**, *49*, 7711.
18. Sommadossi, J. P.; Gosselin, G.; Pierra, C.; Périgaud, C.; Peyrottes, S. US Patent WO 2008/082601, 2008.
19. Peyrottes, S.; Coussot, G.; lefebvre, I.; Imbach, J.-L.; Gosselin, G.; Aubertin, A.-M.; Périgaud, C. *J. Med. Chem.* **2003**, *46*, 782.
20. Egron, D.; Imbach, J.-L.; Gosselin, G.; Aubertin, A.-M.; Périgaud, C. *J. Med. Chem.* **2003**, *46*, 4564.
21. Schlienger, N.; Peyrottes, S.; Kassem, T.; Imbach, J.-L.; Gosselin, G.; Aubertin, A.-M.; Périgaud, C. *J. Med. Chem.* **2000**, *43*, 4570.
22. Villard, A.-L.; Coussot, G.; Lefebvre, I.; Augustijns, P.; Aubertin, A.-M.; Gosselin, G.; Peyrottes, S.; Périgaud, C. *Bioorg. Med. Chem.* **2008**, *16*, 7321.
23. Razzell, W. E.; Khorana, H. G. *J. Biol. Chem.* **1959**, *234*, 2105.
24. Lefebvre, I.; Beltran, T.; Peyrottes, S.; Périgaud, C. *Biomed. Chromatogr.* 2009, available online 2009 May 14.
25. Schlienger, N.; Peyrottes, S.; Aubertin, A.-M.; Gosselin, G.; Imbach, J.-L.; Périgaud, C. *Nucleosides Nucleotides* **1999**, *18*, 1025.
26. Ullmann, B. *Adv. Exp. Med. Biol.* **1989**, *253B*, 415.
27. Jordheim, L. P.; Galmarini, C. M.; Dumontet, C. *Cancer Chemother. Pharmacol.* **2006**, *58*, 547.
28. Jordheim, L. P.; Guittet, O.; Lepoivre, M.; Galmarini, C. M.; Dumontet, C. *Mol. Cancer Ther.* **2005**, *4*, 1268.
29. Lefebvre, I.; Périgaud, C.; Pompon, A.; Aubertin, A.-M.; Girardet, J.-L.; Kirn, A.; Gosselin, G.; Imbach, J.-L. *J. Med. Chem.* **1995**, *38*, 3941.
30. Bates, R. G.; Paabo, M. In *Handbook of Biochemistry. Physical and Chemical Data*; p J227.