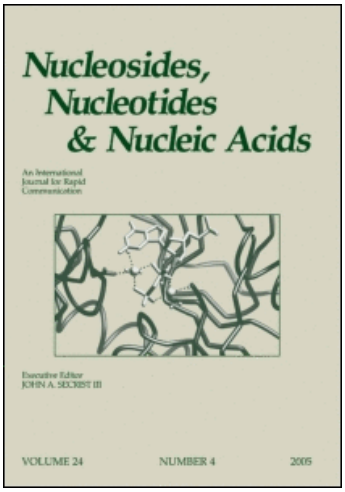


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# Synthetic Approaches to a Mononucleotide Prodrug of Cytarabine

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# SYNTHETIC APPROACHES TO A MONONUCLEOTIDE PRODRUG OF CYTARABINE

**R. Bazzanini, M.-H. Gouy, S. Peyrottes, G. Gosselin, and C. Périgaud** □ *UMR 5625 CNRS–UM II, Université Montpellier II, Montpellier, France*

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□ *Synthetic pathways to a mononucleotide prodrug of cytarabine (Ara-C) bearing S-pivaloyl-2-thioethyl (tBuSATE) groups, as biolabile phosphate protections, are reported. Using a common phosphoramidite approach, two different kinds of nucleoside protecting groups have been investigated. During this study, we observed an intermolecular migration of the Boc protecting group in the course of the tert-butyldimethylsilyl ether cleavage using tetrabutyl ammonium fluoride.*

**Keywords** Ara-C; Leukemia; Prodrug; Phosphotriester



## INTRODUCTION

The pyrimidine nucleoside analogue Ara-C (cytosine arabinoside, cytarabine, 1-$\beta$-D-arabinofuranosylcytosine; Scheme 1, compound **1**) is an effective anticancer drug used alone, or in combination, particularly in acute myeloid leukemia (AML).[1–3] Once inside the cells, Ara-C has to be converted to its cytotoxic metabolite cytosine arabinoside triphosphate (Ara-CTP)[4,5] via a sequence of kinase activations. Thus, incorporation of Ara-CTP into DNA and subsequent induction of DNA chain termination has been proposed to be the major mechanism by which Ara-C exerts cytotoxicity.[6,7]

In honor and celebration of the life and career of John A. Montgomery.

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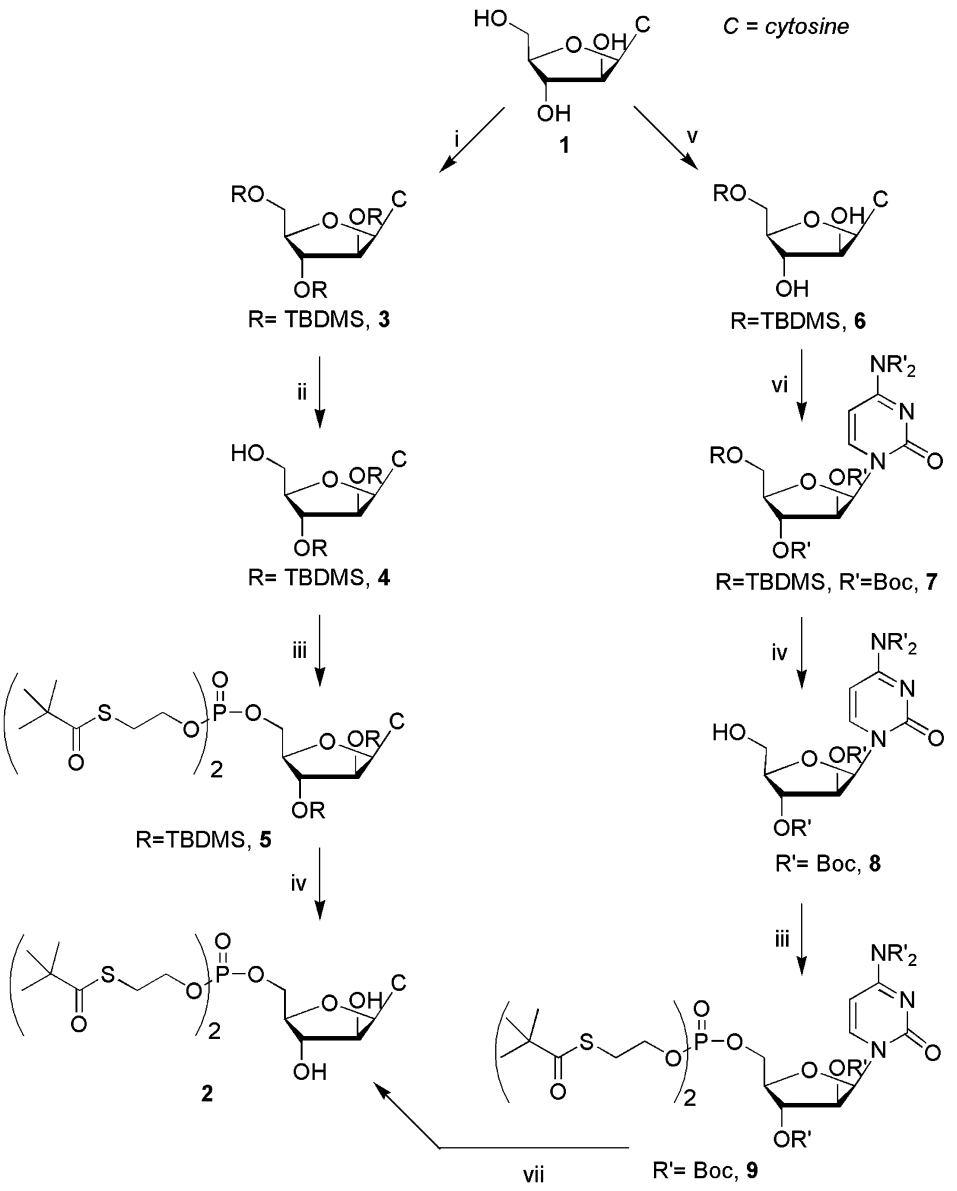



**SCHEME 1** Synthetic pathways to *bis*(*t*BuSATE) phosphotriester derivative of Ara-C, **2**. Reagents and conditions. (i) TBDMSCl, imidazole, DMAP, DMF, 72%; (ii) AcOHaq 80%, 60°C, 64%; (iii) *bis*(*t*BuSATE)$_2$PNiPr$_2$, *1*-H-tetrazole, THF, 79–84%; (iv) NEt$_3$•3HF in THF, 82% for **8** and 55% for **2**; (v) TBDMSCl, imidazole, pyridine, 95%; (vi) DBDC, dioxan, DMAP, TEA, 79%; (vii) HCl, Et$_2$O, quantitative.

Long-term chemotherapy with Ara-C results in the development of resistant phenomena, the principal cause of AML treatment failure,[8] and mainly associated to enzymatic activity alterations.[8] Within these various mechanisms, the most remarkable is reduction of deoxycytidine kinase (dCK)[9–11] and in a smaller increase of 5′-nucleotidases.[12] In addition, Ara-C has a number of limitations such as a rapid deactivation by cytidine deaminase to AraU, a very low lipophilicity, and it is quickly excreted.[5] To address these problems, extensive search is ongoing in the field of formulations[13] as well as in the design of Ara-C derivatives that will exhibit better pharmacokinetic parameters. In this respect, the use of mononucleotide prodrugs appeared as a valuable strategy to overcome some of the Ara-C limitations.[14] In fact, such compounds have been shown to bypass nucleoside kinase deficiency in different cell culture systems and the potentiality of few phosphorylated derivatives of Ara-C has already been reported.[15–19] Thus, we decided to apply the *bis* SATE (*S*-acyl-2-thioethyl) mononucleotide prodrug approach[20,21] to this nucleoside derivative. The enzyme-labile SATE phosphate protecting groups offered several advantages when applied to a wide variety of nucleoside analogues.[20,22–24] In our case, the use of *bis* (SATE) mononucleotide derivative of Ara-C may: (a) broaden the therapeutic spectrum of Ara-C toward kinase-deficient tumor cells; (b) protect Ara-C from deamination since the major substrate requirement of cytidine deaminase is a free 5′-hydroxyl group; and (c) increase the lipophilicity of the parent Ara-C.



Thus, the *bis* (*S*-pivaloyl-2-thioethyl) 5′-phosphotriester derivative of Ara-C (Scheme 1, compound **2**) has been synthesized[25] and evaluated in comparison to the parent nucleoside for growth inhibitory activity in wild-type and nucleoside kinase-deficient cell lines.[12,26] Whereas the prodrug is slightly less potent than Ara-C in the parental cell lines, it emerged as a potent growth inhibitor in dCK-deficient cells (RL-G and L1210/10K) with a 199-fold increased of resistance ratios.[12] Results of biological evaluation have shown the Ara-C prodrug potency in comparison to the parent nucleoside against dCK-deficient cell lines and strongly supported a decomposition mechanism leading to the intracellular delivery of Ara-C monophosphate. These preliminary results prompted us to synthesize larger quantities of the *t*BuSATE derivative **2** for its evaluation in animal models. Herein, we will describe different synthetic pathways to set an efficient method to obtain the target compound on a large scale, as well as the identification of byproducts.

## RESULTS AND DISCUSSION

In order to synthesize *bis* (SATE) mononucleotide prodrugs, we developed both PV[27] and PIII[28] approaches. Phosphoramidite chemistry

appeared as an advantageous strategy in terms of nucleoside phosphorylation yield, the introduction of the nucleoside analogue arising in the last step, and accessibility to a wide range of *bis* (SATE) phosphoramidite derivatives.[28] First attempts to carry out direct phosphytilation of Ara-C with bis (*t*BuSATE) phosphoramidite,[28] allowed us to obtain compound **2** (Scheme 1, route not shown) in moderate yield (53%, determined by UV/LC analysis of the crude, data not shown), contaminated by the 3′- and 5′,3′-phosphotriester derivatives, respectively, in 4% and trace. Amount of unreacted nucleoside was also observed, due to the low solubility of Ara-C in commonly used organic solvents. Furthermore, the separation of 5′- and 3′-isomers was not straightforward and required several steps of fastidious chromatographic purification, ending to isolate the desired compound in only 28% overall yield. Thus, protection of 3′-position of Ara-C was needed and we decided to explore synthetic pathways using in a first time protecting group with mild deprotection conditions, such as *tert*-butyldimethylsilyl (TBDMS), for the hydroxyl functions of the nucleoside (Scheme 1).

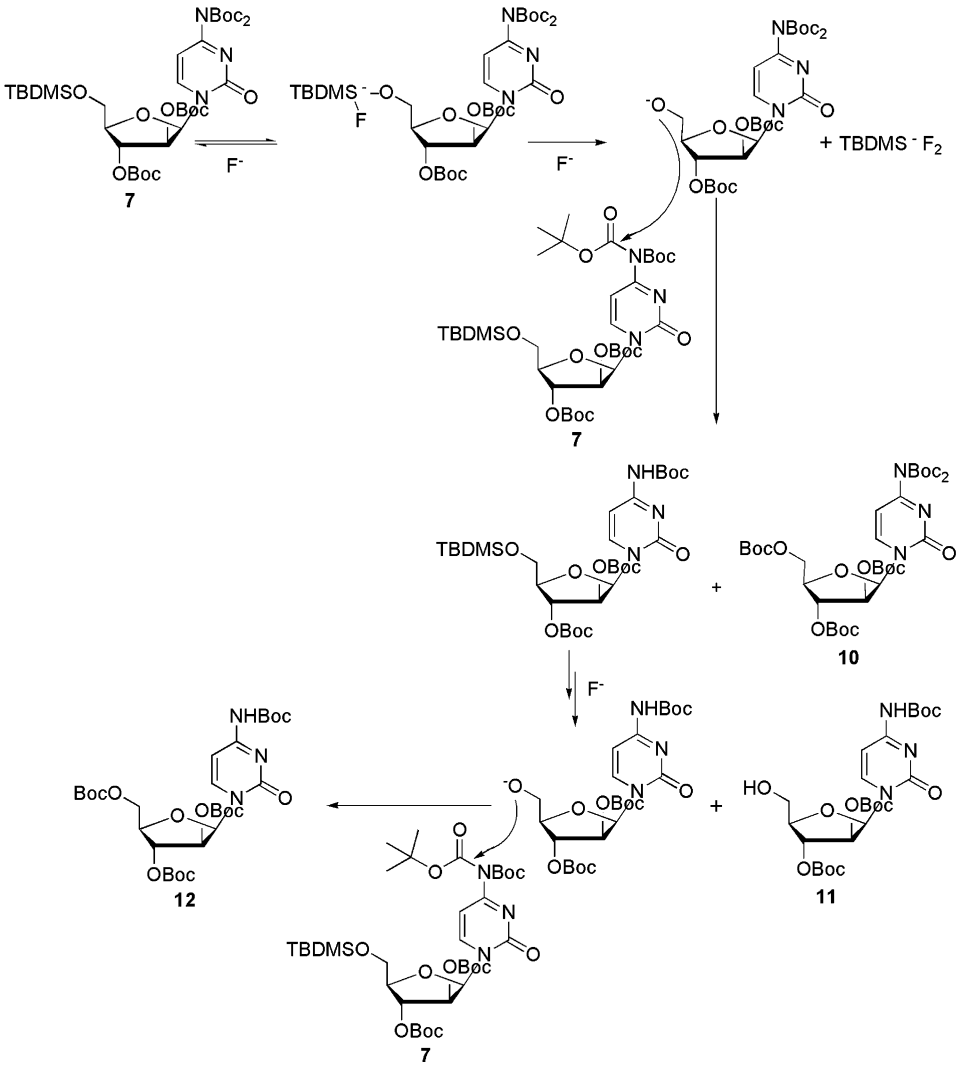
Therefore, Ara-C was persilylated on its sugar moiety using a large excess of TBDMSCl and imidazole in anhydrous dimethylformamide, in the presence of catalytic amount of *N*,*N*-dimethylaminopyridine.[29,30] Then, compound **3** was selectively deprotected at the 5′-position with 80% aqueous acetic acid, as previously reported by Gray et al.[31] to give **4** (in 64% yield), which could be directly engaged in the phosphitylation reaction. Coupling of the protected nucleoside **4** was accomplished according to Lefebvre and coworkers[28,32] using the *bis* (*t*BuSATE) *N*,*N*-diisopropylphosphoramidite and 1-*H*-tetrazole. Then, in situ oxidation of the resulting phosphite triester with *t*BuOOH led to the PV derivative **5** in good yield (84%). Initial attempts to remove TBDMS groups on the 2′- and 3′-positions of the protected phosphotriester **5** with $NH_4F$,[33] KF/crown ether[34] or TBAF[35] gave rise to the corresponding phosphodiester derivative due to the lability of the phosphotriester moiety. Then, the deprotection reaction was carried out with TBAF in the presence of acetic acid in anhydrous THF[36,37] and this degradation did not occur. However, the drawback of the last step of this synthetic approach resulted in the difficult separation of the final compound **2** from the tetrabutylammonium salts. Finally, the use of a weakly acidic fluoride reagent, such as $Et_3N{\bullet}3HF$ in tetrahydrofuran, allowed the deprotection of **5** under very mild conditions. No phosphotriester bond cleavage was detected and the excess of reagent was easily removed by column chromatography, in this last case the expected derivative **2** was obtained in moderate yield (55%). However, the overall yield (21%) of this approach (four steps) is still low.

In a second approach, we intended to use an acid labile protecting group such as the *tert*-butoxycarbonyl (*t*Boc) moiety. Introduction of *t*Boc

groups on amino- and 2′, 3′-hydroxyl functions was performed on the 5′-monosilylated derivative of Ara-C, **6**. Monosilylation of Ara-C was carried out using a small excess of TBDMSCl in presence of imidazole in pyridine. Then, compound **6** was treated by a large excess of di(*tert*)butyldicarbonate (DBDC) in presence of DMAP and triethylamine,[38] leading to the fully protected derivative **7** containing four *t*Boc groups, in 79% yield. At this stage, removal of the TBDMS group at the 5′-position was tedious. Indeed, during the course of these experiments we observed an intermolecular migration of the *t*Boc protecting group using tetrabutylammonium fluoride (TBAF) as fluoride source.[39] Treatment of compound **7** with only 1.15 eq of TBAF in THF (1.5 h at room temperature) did not allowed us to isolate **8**, but various derivatives of Ara-C bearing a *t*Boc group in the 5′-position (Scheme 2, compounds **10**, **12**) as well as compound **11**, which corresponds to a partially protected derivative bearing three *t*Boc groups. Analysis of the structures of the compounds obtained during this reaction, their ratio (50/25/25), as well as the literature[40] allowed us to propose the intermolecular mechanism depicted in Scheme 2 for the formation of derivatives **10-12**, which were fully characterized. Basicity of the fluorosilylated complex[39] formed during the course of the silyl deprotection reaction generates the 5′-alcoholate of Ara-C, which is then able to attack the carbonyl of one of the two *t*Boc groups borne by the exocyclic amine of cytosine. The migration mechanism was proposed in comparison with the migration of acyl group in the corresponding purine and pyrimidine derivatives which has been well document in the literature.[37,41,42] The first step in the hypothetical reaction series gave rise to the penta(*t*Boc) derivative **10** (50%) as well as an intermediate (not isolated) incorporating a remaining silyl group at the 5′-position and only one *t*Boc group on the aminoexocyclic function. This intermediate could undergo a similar sequence of reaction (removal of the 5′-TBDMS, formation of the 5′-alcoholate and nucleophilic attack on a *N*-*t*Boc group) and consequently generates the *N*,2′,3′-*O* tri(*t*Boc) derivative **11** and the *N*, 2′,3′,5′-*O* tetra(*t*Boc) derivative **12**, respectively, 25% of each.

From our previous expertise on TBDMS group removal, we carried out several attempts using other fluoride reagents such as ammonium fluoride ($NH_4F$)[33] and triethylamine trihydrofluoride complex ($Et_3N{\bullet}3HF$).[43–45] In these mild conditions, compound **8** was obtained in 55% and 82% yield, respectively.

Finally, the desired protected bis (*t*BuSATE) phosphotriester **9** was obtained in the coupling conditions previously used for derivative **5**, and removal of the hydroxyl and amino protecting groups (*t*Boc) was carried out in one step by treatment of compound **9** with an ethereal HCl saturated solution. The expected phosphotriester **2** was isolated quantitatively from **9** as its hydrochloride salt.




**SCHEME 2** Proposed mechanism for the formation of **10-12**.

## EXPERIMENTAL SECTION

### Starting Materials

Starting compound *bis* (*t*BuSATE) *N,N*-diisopropylphosphoramidite was prepared according to reported procedures.[28] Compounds **3**,[30,35] **4**,[35] and **6**[35,46] were prepared following slightly modified literature procedures. Ara-C was purchased from Intsel Chimos (Issy les Moulineaux, France).

$^1$H NMR spectra were recorded at 400 MHz and $^{13}$C NMR spectra at 100 MHz with proton decoupling at ambient temperature using a Bruker AM-400 spectrometer. Chemical shifts ($\delta$) are given in parts per million (ppm) referenced to the residual solvent peak (DMSO-$d_5$ 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 Hertz. 2D $^1$H-$^{13}$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 81 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 using thioglycerol/glycerol (1:1, v/v, GT) as matrix. Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). TLC was performed on precoated aluminum sheets of silica gel 60 $F_{254}$ (Merck, Art. 9385), visualization of products being accomplished by UV absorbance (at 254 nm) followed by charring with 5% ethanolic sulfuric acid with heating for nucleoside containing compounds; phosphorus derivatives were detected by spraying with Hanes molybdate reagent. Flash chromatography was carried out using 63–100 $\mu$m silica gel (Merck Art. No. 115101) otherwise 40–63 $\mu$m silica gel (Merck Art. No. 109385) was used.



**1-[2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-$\beta$-D-arabinofuranosyl]cytosine (3).** To a solution of Ara-C (2.44 g, 10 mmol) in anhydrous DMF (25 mL) was added TBDMSCl (6.08 g, 40 mmol), imidazole (2.72 g, 40 mmol), and DMAP (0.32 g, 2.5 mmol). The mixture was stirred overnight at room temperature, and then additional TBDMSCl (3.04 g, 20 mmol), imidazole (1.36 g, 20 mmol), and DMAP (0.32 g, 2.5 mmol) were added. After 60 h the solution was heated at 60°C for 2 h, and then the solvent was evaporated under high vacuum. The crude product was dissolved in $CH_2Cl_2$ (50 mL), and washed with saturated aqueous $NaHCO_3$, then brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation to dryness, the corresponding residue was purified by column silica gel chromatography (stepwise gradient of MeOH [0–3%] in $CH_2Cl_2$) to afford 4.23 g (72%) of **3** as white solid. Mp 214–215°C, ($CH_3CN$); $^1$H NMR (DMSO-$d_6$) $\delta$ 7.41 (d, 1H, H6, $J = 7.4$), 7.09 (d, 2H, $NH_2$), 6.00 (d, 1H, H1′, $J = 3.1$), 5.68 (d, 1H, H5, $J = 7.4$), 4.14 (brs, 1H, H3′), 4.02 (d, 1H, H2′, $J = 2.9$), 3.85–3.70 (m, 3H, H4′, H5′, and H5″), 0.87, 0.86, and 0.76 (s, 27H, $C(CH_3)_3$), 0.10, 0.05, −0.16, and −0.21 (s, 18H, $Si(CH_3)_2$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 165.6 (C2), 154.7 (C4), 142.0 (C6), 92.6 (C5), 86.6 (C1′), 85.8 (C4′), 77.8 (C3′), 76.0 (C2′), 62.3 (C5′), 25.6, 25.5, and 25.4 ($C(CH_3)_3$), 17.9, 17.5, and 17.4 ($SiC(CH_3)_3$), −4.8, −4.9, −5.5, −5.6, and −5.7 ($Si(CH_3)_2$); MS-FAB$^+$ *m/z*

1171 $(2M+H)^+$, 586 $(M+H)^+$, 112 $(BH2)^+$; UV (ethanol) $\lambda_{max}$ 270 nm ($\varepsilon$ 8500). Anal. calcd for ($C_{27}H_{55}N_3O_5Si_3$): C: 55.34%; H: 9.46%; N: 7.17%. Found C: 55.48%; H: 9.55%; N: 6.82%.

**1-[2′,3′-di-*O*-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl]cytosine (4).** Compound **3** (4 g, 6.82 mmol) was dissolved in 80% aqueous acetic acid (68.2 mL) and heated at 50–60°C for 10 h with stirring. When the reaction was complete, ethanol (50 mL) was added and the mixture was evaporated under high vacuum, co-evaporation was repeated several times. The resulting pale yellow solid was purified by silica gel column chromatography (stepwise gradient of MeOH [0–8%] in $CH_2Cl_2$) to afford 2.06 g (64%) of white solid. Mp 121–123°C ($CH_3CN$) lit. 114–116°C;[46] $^1$H NMR (DMSO-$d_6$): $\delta$ 7.48 (d, 1H, H6, $J = 7.4$), 7.08 (bd, 2H, $NH_2$), 5.98 (d, 1H, H1′, $J = 3.2$), 5.68 (d, 1H, H5, $J = 7.4$), 4.95 (sl, 1H, OH5′), 4.06 (s, 1H, H3′), 3.99 (dd, 1H, H2′, $J = 3.0$ and 1.0), 3.79 (t, 1H, H4′, $J = 6.8$), 3.60–3.50 (m, 2H, H5′, and H5″), 0.87 and 0.75 (s, 18H, $C(CH_3)_3$), 0.09, −0.01, and −0.22 (s, 12H, $Si(CH_3)_2$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 165.3 (C2), 154.5 (C4), 142.5 (C6), 92.6 (C5), 86.8 (C1′), 86.6 (C4′), 77.9 (C3′), 75.9 (C2′), 61.1 (C5′), 25.7, 25.6, and 25.5 ($C(CH_3)_3$), 17.5 and 17.4 ($SiC(CH_3)_3$), −4.8, −4.9, −5.4, and −5.7 ($Si(CH_3)_2$); MS-FAB$^+$ *m/z* 943 $(2M+H)^+$, 472 $(M+H)^+$, 112 $(BH2)^+$; MS-FAB$^-$ *m/z* 470 $(M-H)^-$, 110 $(B)^-$; UV (ethanol) $\lambda_{max}$ 272 nm ($\varepsilon$ 9000). Anal. calcd for ($C_{21}H_{41}N_3O_5Si_2$): C: 53.47%; H: 8.76%; N: 8.91%. Found C: 52.60%; H: 8.76%; N: 8.93%.

**1-[5′-*O*-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl]cytosine (6).** To a solution of Ara-C (3.5 g, 14.31 mmol) and imidazole (1.169 g, 17.17 mmol) in anhydrous pyridine (30 mL), TBDMSCl (2.588 g, 17.17 mmol) was added. The mixture was stirred at room temperature for 3 h and then methanol (10 mL) was added. Solvents were removed under reduced pressure and co-evaporation with toluene was repeated three times. Crystallization from acetonitrile gave **6** (5.351 g, 95%). Mp 147–145.5°C, lit. 203–205°C.[46] $^1$H NMR (DMSO-$d_6$): $\delta$ 7.51 (d, 1H, H6, $J = 7.5$), 7.32 (s, 2H, $NH_2$), 6.00 (d, 1H, H1′, $J = 4.7$), 5.65 (d, 1H, H5, $J = 13.9$), 3.96 (m, 1H, H2′), 3.83 (m, 1H, H3′), 3.69 (m, 3H, H4′, H5′ and H5″), 0.80 (s, 9H, $C(CH_3)_3$), 0.00 ppm (s, 6H, $Si(CH_3)_2$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 165.9 (C4), 155.6 (C2), 143.6 (C6), 93.4 (C5), 86.2 (C1′), 84.4 (C4′), 76.2 (C3′), 75.9 (C2′), 63.2 (C5′), 26.6 ($C(CH_3)_3$), 18.9 ($SiC(CH_3)_3$), −4.5 ($Si(CH_3)_2$); MS-FAB$^+$ *m/z* 358 $(M+H)^+$, 715 $(2M+H)^+$; MS-FAB$^-$ *m/z* 356 $(M-H)^-$, 713 $(2M-H)^-$; HR-MS calcd 358.1798. Found 358.1796; UV (ethanol): $\lambda_{max}$ 272 nm ($\varepsilon$ 9050). Anal. calcd for ($C_{15}H_{27}N_3O_5Si$): C: 50.40%; H: 7.61%; N: 11.65%. Found C: 50.95%; H: 7.52%; N: 11.62%.

**1-[4-*N*,*N*-2′,3′-*O*-Tetra-(*tert*-butyloxycarbonyl)-5′-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]cytosine (7).** To a stirred solution of **6** (5 g,

12.70 mmol) and DMAP (202 mg, 1.65 mmol) in dioxan (70 mL) and TEA (50 mL), DBDC (27.69 g, 127 mmol) was added. The reaction mixture was stirred at room temperature for 19 h. Solvents were evaporated under reduced pressure. The residue was treated with EtOAc (300 mL), washed with 5% $NaHCO_3$, then brine and dried over $Na_2SO_4$, then concentrated to dryness. Flash chromatography (stepwise gradient of EtOAc [0–15%] in hexane) gave **7** (7.564 g, 79%) as an oil. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.02 (d, 1H, H6, $J = 7.6$), 6.81 (d, 1H, H5, $J = 7.6$), 6.16 (d, 1H, H1′, $J = 5.4$), 5.31 (q, 1H, H2′), 5.06 (m, 1H, H3′), 4.08 (m, 1H, H4′), 3.85–3.69 (2m, 2H, H5′ and H5″), 1.41 (s, 18H, $C(CH_3)_3$, Boc), 1.34 and 1.22 (2s, 18H, $C(CH_3)_3$, Boc), 0.80 (s, 9H, $SiC(CH_3)_3$), 0.00 ppm (s, 6H, $Si(CH_3)_2$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 162.6 (C4), 149.9, 151.9, 152.5 and 153.6 (C2 and CO, Boc), 146.9 (C6), 96.1 (C5), 85.7 (C1′), 83.9 ($C(CH_3)_3$, Boc), 81.0 (C4′), 77.7 and 77.5 (C2′ and C3′), 62.2 (C5′), 27.9, 28.0, 28.2 ($C(CH_3)_3$, Boc), 26.5 ($SiC(CH_3)_3$), 18.8 ($SiC(CH_3)_3$), −4.4 ($Si(CH_3)_2$). MS-FAB$^+$ *m/z* 1515 $(2M+H)^+$, 758 $(M+H)^+$; MS-FAB$^-$ *m/z* 757 $(M-H)^-$, 656 (M-Boc-H)$^-$, 556 (M-2Boc-H)$^-$; UV (ethanol): $\lambda_{max}$ 293 nm ($\varepsilon$ 9100); Anal. ($C_{35}H_{59}N_3O_{13}Si$): Calcd C: 55.46%; H: 7.85%; N: 5.54%. Found C: 55.02%; H: 7.76%; N: 5.74%.

**1-[4-*N*,*N*-2′,3′,5′-*O*-Penta-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosyl] cytosine (10).** $^1$H NMR (DMSO-$d_6$): $\delta$ 8.04 (d, 1H, H6, $J = 7.5$), 6.90 (d, 1H, H5, $J = 7.5$), 6.27 (d, 1H, H1′, $J = 4.7$), 5.34 (m, 1H, H2′), 5.15 (brs, 1H, H3′), 4.36 (m, 1H, H4′), 4.34 (m, 2H, H5′ and H5″), 1.47 and 1.49 (2s, 18H, $C(CH_3)_3$), 1.53 (s, 18H, $C(CH_3)_3$), 1.34 (s, 9H, $C(CH_3)_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 162.9 (C4), 149.9 and 151.7, 152.4, 153.5 (C2 and CO, Boc), 146.9 (C6), 96.4 (C5), 85.7 (C1′), 80.0, 82.9, 84.2, and 84.3 ($C(CH_3)_3$, Boc), 79.2 (C4′), 78.8 (C3′), 77.5 (C2′), 66.1 (C5′), 27.9, 28.1, 28.2 ($C(CH_3)_3$, Boc); MS-FAB$^+$ *m/z* 1488 $(2M+H)^+$, 744 $(M+H)^+$; MS-FAB$^-$ *m/z* 743 $(M-H)^-$; HR-MS calcd 744.3555. Found 744.3557; UV (ethanol): $\lambda_{max}$ 295 nm ($\varepsilon$ 9300). Anal. calcd for ($C_{34}H_{53}N_3O_{15}$, 0.5 $CH_2Cl_2$) C: 53.06%; H: 6.87%; N: 5.30%. Found C: 52.99%; H: 7.01%; N: 5.61%.

**1-[4-*N*-2′,3′-*O*-Tri-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosyl]cytosine (11).** $^1$H NMR (DMSO-$d_6$): $\delta$ 10.45 (s, 1H, NH), 8.06 (d, 1H, H6, $J = 7.5$), 7.05 (d, 1H, H5, $J = 7.5$), 6.22 (d, 1H, H1′, $J = 4.7$), 5.25 (m, 1H, H2′), 5.20 (m, 1H, OH), 5.09 (m, 1H, H3′), 4.08 (m, 1H, H4′), 3.80–3.58 (2m, 2H, H5′, and H5″), 1.47 (2s, 18H, $C(CH_3)_3$), 1.30 (s, 9H, $C(CH_3)_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 164.1 (C4), 151.8, 152.5, 152.9, and 154.6 (C2 and CO, Boc), 145.9 (C6), 95.1 (C5), 84.7 (C1′), 82.0, 83.9 ($C(CH_3)_3$), 81.9 (C4′), 78.4 (C3′), 77.7 (C2′), 60.7 (C5′), 27.8, 28.1, 28.6 ($C(CH_3)_3$); MS-FAB$^+$ *m/z* 1087 $(2M+H)^+$, 544 $(M+H)^+$; MS-FAB$^-$ *m/z* 1085 $(2M-H)^-$, 542 $(M-H)^-$, 442 (M-Boc)$^-$, UV (ethanol): $\lambda_{max}$ 290 nm ($\varepsilon$ 10,700). Anal.

calcd for ($C_{24}H_{37}N_3O_{11}$): C: 53.03%; H: 6.86%; N: 7.73%. Found C: 52.79%; H: 6.94%; N: 7.61%.

**1-[4-*N*-2′,3′,5′-*O*-Tetra-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosyl]cytosine (12).** $^1$H NMR (DMSO-$d_6$): $\delta$ 10.45 (s, 1H, NH), 7.93 (d, 1H, H6, $J = 7.5$), 7.05 (d, 1H, H5, $J = 7.5$), 6.22 (d, 1H, H1′, $J = 4.7$), 5.28 (m, 1H, H2′), 5.12 (brs, 1H, H3′), 4.41 (m, 1H, H4′), 4.35 (m, 2H, H5′, and H5″), 1.50, 1.49, and 1.47 (3s, 27H, C($CH_3$)$_3$), 1.34 (s, 9H, C($CH_3$)$_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 164.2 (C4), 151.8, 152.5, 152.8, 153.6, and 154.5 (C2 and CO, Boc), 149.9 (C6), 95.2 (C5), 82.8, 84.1, 85.6, and 86.2 (*C*($CH_3$)$_3$), 82.1 (C1′), 78.9 (C4′), 78.7 (C3′), 7.77 (C2′), 60.6 (C5′), 27.8, 28.1, 28.2, and 28.6 (C(*C*$H_3$)$_3$); MS-FAB$^+$ *m/z* 1287 (2M+H)$^+$, 644 (M+H)$^+$; MS-FAB$^-$ *m/z* 1285 (2M−H)$^-$, 642 (M−H)$^-$, 542 (M-Boc)$^-$; UV (ethanol): $\lambda_{max}$ 292 nm ($\varepsilon$ 8700). Anal. calcd for ($C_{29}H_{45}N_3O_{13}$): C: 54.11%; H: 7.05%; N: 6.53%. Found C: 53.99%; H: 7.09%; N: 6.81%.

**1-[4-*N*,*N*-2′,3′-*O*-Tetra-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosyl]cytosine (8).** To a solution of **7** (500 mg, 0.66 mmol) in anhydrous THF (6.6 mL), was added triethylamine trihydrofluoride (532 mg, 3.3 mmol). After stirring at room temperature for 16 h, TLC analysis showed the reaction to be complete. EtOAc (60 mL) was added to the reaction mixture and the solution was washed successively with brine and water, dried over $Na_2SO_4$, and concentrated to dryness under reduced pressure. The residue was subjected to flash chromatography (stepwise gradient of EtOAc [0–50%] in hexane) to give **8** (275 mg, 82%). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.09 (d, 1H, H6, $J = 7.6$), 6.78 (d, 1H, H5, $J = 7.6$), 6.12 (d, 1H, H1′, $J = 4.8$), 5.21 (m, 1H, H2′), 5.10 (t, 1H, OH), 5.00 (m, 1H, H3′), 4.01 (m, 1H, H4′, $J = 4.8$), 3.70–3.45 (2m, 2H, H5′ and H5″), 1.41 (s, 18H, C($CH_3$)$_3$), 1.36 and 1.22 (2s, 18H, C($CH_3$)$_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 162.7 (C4), 149.9, 151.8, 152.5, and 153.6 (C2 and CO of Boc groups), 146.9 (C6), 96.2 (C5), 85.0 (C1′), 83.9, 84.0, and 85.7 (*C*($CH_3$)$_3$), 82.1 (C4′), 78.3 (C3′), 77.7 (C2′), 60.6 (C5′), 27.9, 28.1 (C(*C*$H_3$)$_3$); MS-FAB$^+$ *m/z* 644 (M+H)$^+$, 544 (M-Boc+2H)$^+$; MS-FAB$^-$ *m/z* 642 (M−H)$^-$, 542 (M-Boc)$^-$, 442 (M-2Boc+H)$^-$. UV (ethanol): $\lambda_{max}$ 295 nm ($\varepsilon$ 9500); Anal. calcd for ($C_{29}H_{45}N_3O_{13}$): C: 54.11%; H: 7.05%; N: 6.53%. Found C: 53.56%; H: 6.98%; N: 6.52%.

## General Procedure for Phosphytilation

1*H*-Tetrazole (3 mmol, 0.45 M solution in $CH_3CN$) was added to a stirred solution of the appropriate protected derivative of Ara-C (1.0 mmol) in anhydrous acetonitrile (3 mL) under argon atmosphere. At 0°C, the *bis* (*S*-pivaloyl-2-thioethyl) *N*,*N*-diisopropylphosphoramidite[28] (1.2 mmol) in anhydrous acetonitrile (2 mL) was added. After stirring for 2 h at room

temperature, the reaction mixture was cooled to 0°C and a 3 M solution of *tert*-butyl hydroperoxyde in anhydrous decan (3 mmol) was added drop wise; the mixture was then allowed to warm to room temperature over 1 h. EtOAc (100 mL) was added and the solution was washed with sodium sulphite (10% aqueous solution) to destroy the *tert*-butyl hydroperoxyde excess. The organic phase was separated and the aqueous layer washed twice with dichloromethane. The combined organic layers were washed with water, dried over anhydrous $Na_2SO_4$, filtered, and evaporated to dryness under reduced pressure.

**1-[2′,3′-di-*O*-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl]cytosine-5′-*bis*(*S*-pivaloyl-2-thioethyl)phosphate (5).** Purification on column chromatography (isocratic of ethyl acetate) afforded 0.70 g of compound **5** (84% yield) as a white foam. $^1$H NMR (DMSO-$d_6$) $\delta$ 7.57 (d, 1H, H6, $J = 7.4$), 7.12 (d, 2H, $NH_2$), 6.05 (d, 1H, H1′, $J = 3.0$), 5.67 (d, 1H, H5, $J = 7.4$), 4.20–4.15 (m, 2H, H3′ and H4′), 4.05–3.95 (m, 7H, H2′, H5′, H5″ and CH2O), 3.10 (t, 4H, CH2S, $J = 6.2$), 1.17 and 1.16 (s, 18H, CO-C($CH_3$)$_3$), 0.87 and 0.76 (s, 18H, SiC($CH_3$)$_3$), 0.11, −0.01, and −0.22 (s, 12H, Si($CH_3$)$_2$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 204.9 (COS), 165.6 (C4), 154.7 (C2), 142.3 (C6), 129.2, and 128.5 (*C*($CH_3$)$_3$, SATE), 92.7 (C5), 87.2 (C1′), 84.2 (C4′), 77.6; 75.3 (C3′, C2′), 66.6, 66.5 (C5′), 65.7 and 65.6 ($CH_2O$), 45.9 (Si*C*($CH_3$)$_3$), 28.2, 28.1, and 28.0 ($CH_2S$), 26.8 (C(*C*$H_3$)$_3$, SATE), 25.5 (SiC(*C*$H_3$)$_3$), −4.9, −5.0, −5.5, and −5.85 (Si(*C*$H_3$)$_2$). $^{31}$P NMR (DMSO-$d_6$) $\delta$ −0.49 (s); MS-FAB$^+$ *m/z* 840 (M+H)$^+$, 145 (*t*BuCOSCH$_{22}$CH$_2$)$^+$, 112 ($BH_2$)$^+$; MS-FAB$^-$ *m/z* 694 (M-*t*BuCOSCH$_2$CH$_2$)$^-$; UV (ethanol) $\lambda_{max}$ 270 nm ($\varepsilon$ 8500). Anal. calcd for ($C_{35}H_{66}N_3O_{10}PS_2Si_2$): C: 50.03%; H: 7.92%; N: 5.00%. Found C: 50.27%; H: 7.91%; N: 4.91%.

**1-[4-*N*,*N*, 2′,3′-*O*-Tetra-(*tert*-butyloxycarbonyl)-β-D-arabinofuranosyl] cytosine-5′-*bis*(*S*-pivaloyl-2-thioethyl)phosphate (9).** Flash chromatography (stepwise gradient of EtOAc [0–10%] in dichloromethane) gave the compound **9** (802 mg, 79%). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.00 (d, 1H, H6, $J = 7.6$), 6.86 (d, 1H, H5, $J = 7.6$), 6.20 (d, 1H, H1′, $J = 4.5$), 5.20 (m, 1H, H2′), 5.01 (m, 1H, H3′), 4.23 (m, 3H, H4′, H5′, and H5″), 3.98 (m, 4H, $CH_2O$), 3.02 (m, 4H, $CH_2S$), 1.40, 1.36, and 1.35 (3s, 27H, C($CH_3$)$_3$, Boc), 1.20 (s, 9H, C($CH_3$)$_3$, Boc), 1.10 ppm (s, 18 H, CO-C($CH_3$)$_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 205.8 (COS), 162.7 (C4), 151.8, 152.4, 153.5 (C2 and CO, Boc), 149.9 (C6), 96.1 (C5), 85.6 (*C*($CH_3$)$_3$, SATE), 85.0 (C1′), 83.9, 84.0, and 84.2 (*C*($CH_3$)$_3$, Boc), 79.9 (C4′), 78.3 (C3′), 77.3 (C2′), 66.8 (C5′), 66.7 and 66.5 ($CH_2O$), 28.9 and 29.0 ($CH_2S$), 27.7, 27.8, 27.9, and 28.0 (C(*C*$H_3$)$_3$, SATE and Boc). $^{31}$P NMR (DMSO-$d_6$): −0.50 (s). MS-FAB$^+$ *m/z* 1012 (M+H)$^+$, 1011 (M-Boc+2H)$^+$, 812 (M-2Boc+3H)$^+$, 712 (M-3Boc+4H)$^+$; MS-FAB$^-$ *m/z* 1011 (M−H)$^-$, 910 (M-Boc)$^-$; UV (ethanol): $\lambda_{max}$ 295 nm ($\varepsilon$ 8500). Anal. calcd

for ($C_{43}H_{70}N_3O_{18}PS_2$)C: 51.03%; H: 6.97%; N: 4.15%; S: 6.34%; P: 3.06%. Found C: 50.72%; H: 6.93%; N: 4.19%; S: 6.59%; P: 3.00%.

### Deprotection of Silyl Derivative 5

To a solution of the silylated nucleotide **5** in anhydrous THF (10 mL/mmol), $Et_3N{\bullet}3HF$ (10 equiv.) was added and the mixture was vigorously stirred under argon positive pressure at room temperature for 72 h. When the reaction was complete, the mixture was evaporated to dryness under vacuum. After purification on silica gel column chromatography (stepwise gradient of methanol [0–6%] in dichloromethane), traces of fluoride reagent in compound **2** were removed by reversed-phase column chromatography on RP-2 (stepwise gradient of methanol [0–80%] in water) afforded 0.25 g of white powder (55% yield).

### Deprotection of Boc Derivative 9

To a solution of **9** (118 mg, 0.117 mmol) in diethyl ether (2 mL), an ethereal saturated HCl solution (3 mL) was added. After stirring at 0°C for 1 h, the reaction mixture was concentrated to dryness to give the compound **2**, quantitatively.



**1-β-D-Arabinofuranosyl Cytosine-5′-*bis*(*S*-pivaloyl-2-thioethyl)phosphate (2).** $^1$H NMR (DMSO-$d_6$): $\delta$ 7.54 (d, 1H, H6, $J$ = 7.4), 7.07 (ld, 2H, $NH_2$), 6.09 (d, 1H, H1′, $J$ = 3.5), 5.63 (d, 1H, H5, $J$ = 7.4), 5.60 (m, 2H, OH and H3′), 4.20 (m, 2H, H5′, H5″), 4.00–3.95 (m, 6H, H4′ and H2′, $CH_2O$), 3.60 (sl, 1H, OH), 3.10 (t, 4H, $CH_2S$, $J$ = 6.2), 1.19 and 1.18 ppm (2s, 18H, $C(CH_3)_3$, SATE); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 205.0 (COS), 165.5 (C4), 154.9 (C2), 142.8 (C6), 92.4 (C5), 86.4 (C1′), 82.6 ($C(CH_3)_3$, SATE), 82.5 (C4′), 76.4 (C3′), 74.1 (C2′), 67.2 (C5′), 65.5 and 65.6 ($CH_2O$), 28.1 and 28.2 ($CH_2S$), 26.8 ($C(CH_3)_3$, SATE); $^{31}$P NMR (DMSO-$d_6$): $\delta$ −0.51 (s). MS-FAB$^+$ *m/z* 1223 $(2M+H)^+$, 612 $(M+H)^+$, 112 $(BH2)^+$; MS-FAB$^+$ *m/z* 1221 $(2M-H)^-$, 646 $(M+Cl)^-$, 610 $(M-H)^-$; HR-MS: calcd 612.1815. Found 612.1804; UV (ethanol): $\lambda_{max}$ 272 nm ($\varepsilon$ 9300). Anal. calcd for ($C_{23}H_{38}N_3O_{10}PS_2$, $H_2O$): C: 43.87%; H: 6.40%; N: 6.67%; S: 10.18%; P: 4.92%. Found C: 43.73%; H: 6.21%; N: 6.64%; S: 10.25%; P: 5.00%.

## CONCLUSION

In this study, we investigated the use of two different 2′,3′-hydroxyl protecting groups in order to obtain on large scale a bis (*t*BuSATE) phosphotriester derivative of Ara-C via the phosphoramidite approach. Direct phosphitylation of unprotected Ara-C or use of the *tert*-butyldimethylsilyl

protecting groups led to the desired phosphotriester **2** in low overall yields, respectively 28 and 21%. On the other hand, the use of an acid-labile protecting group, such as *tert*-butyloxycarbonyl, allowed us to isolated, despite five steps, the prodrug **2** in 48% overall yield. Scale-up of this last synthetic approach is currently in progress.

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